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YEARLY REVIEW

THE ACCEPTOR SIDE OF PHOTOSYSTEM II IN PHOTOSYNTHESIS*

Introduction

Light absorbed by PS II† of green plants leads to the oxidation of water to O_2 and the reduction of the PQ pool. In this review, we shall restrict our discussion to the most important observations on the acceptor (quinone) side of PS II, the part of the electron transport chain between the PS II reaction center Chl *a*, P680, and the PQ pool (Fig. 1). This review will begin with a discussion of the primary electron acceptor of PS II, pheophytin, followed by a discussion of the heterogeneity in the next-electron carrier, the quinone Q—the possible relationships between various suggested kinetic species of Q (Q_1, Q_2, Q_x, Q_β, Q_L and Q_H) will be included; details of the back reaction of Q^- with $P680^+$ will not be reviewed here (see e.g. Lavorel *et al.* [98], DeVault [41] and Govindjee and Jursinic [57]). We shall then present a review of: a 32 kD protein associated with Q and with the next two-electron carrier the plastoquinone B; the actions of herbicides and of HCO_3^- on electron transport in the Q/B region; action of some of the new electron transport inhibitors (diphenylamines, UHDBT, dinitrophenylethers of phenols and mellitin); and the proposed electrogenic loop (the 'Q' cycle) at the PQ level. Finally, we shall present a short discussion dealing with certain electron carriers beyond PQ (the Rieske FeS center, cytochromes and ferredoxin) as techniques used or the ideas evolved are of relevance to the discussion of the acceptor side of PS II.

The primary electron acceptor of PS II

Until a few years ago, Q, a one-electron acceptor, was considered to be the 'primary electron acceptor' of PS II, i.e. Q was assumed to be reduced by P680 directly. However, Gläser *et al.* [55] observed that a flash of much higher intensity was required for a maximal oxidation of the PS II reaction center Chl, P680 (as measured by absorption changes at 685 nm) than for maximal reduction of Q. This was interpreted as indicating the existence of an intermediate electron acceptor between P680 and Q. Furthermore, experiments by van Best and Duysens [189] on Chl *a* luminescence (delayed light emission, DLE) from a green alga also suggested that an intermediate ('W') exists prior to Q: the amplitude of a 1 μs DLE component was enhanced when the system was excited in the $P680 \cdot Q^-$ state. The authors [189] suggested the following scheme to explain these data:

$$\begin{aligned} P680 \cdot W \cdot Q^- &\overset{h\nu}{\rightleftharpoons} (P680)^* \cdot W \cdot Q^- \\ &\rightleftharpoons P680^+ \cdot W^- \cdot Q^- \\ &\rightleftharpoons (P680)^* \cdot W \cdot Q^- \\ &\rightleftharpoons P680 \cdot W \cdot Q^- + h\nu(DLE), \end{aligned}$$

where $(P680)^*$ or $^1(P680)$ is the singlet excited state of P680. These luminescence measurements have been extended recently by using chloroplasts with prereduced Q [168–170]: DLE consisted of a ~1 μs and a ~150 ns lifetime component; if 'W' would not exist between P680 and Q, charge separation could not have occurred because Q was prereduced, and thus, no DLE (caused by charge recombination) would have been observed. At low temperatures ($T < 200$ K), only the 150 ns component was detected and its amplitude was increased by the presence of a magnetic field. This might be explained by a magnetic field induced increase in the ratio of $^1(P680^+ \cdot W^-)/^3(P680^+ \cdot W^-)$, leading to a higher concentration of $(P680)^*$, and, thus, light emission [168, 170].

*This review is dedicated to the memory of Bessel Kok.

†*Abbreviations and symbols:* ΔA_{515}: electrochromic change detected at 515 nm; ANT2p: 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene; B (≡R): second quinone-type two-electron PS II acceptor; C (=X_2): a postulated one-electron acceptor from U; C400: a one-electron acceptor with a midpoint potential of ~ +400 mV; Car: carotenoid; Chl: chlorophyll; cyt (b_{563}; *f*): cytochrome (b_{563}; *f*); DAD: diaminodurene; DBMIB: 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP: 2,6-dichlorophenolindophenol; DLE: delayed light emission; DNOC: 4,6-dinitro-*o*-cresol; DNP-INT: dinitrophenylether of iodonitrothymol; DPC: diphenylcarbazide; E_h: redox potential; E_m: midpoint potential; EPR: electron paramagnetic resonance; F_0: initial fluorescence yield; F_{var}: variable fluorescence yield; FeCy: ferricyanide ($Fe(CN)_6^{3-}$); LHCP: light harvesting Chl *a*/*b* protein complex; MV: methyl viologen; MW: molecular weight; P680: PS II reaction center Chl *a*; Pheo: pheophytin; PQ: plastoquinone; PS II (I): photosystem II (I); Q(=Q_1): first quinone-type PS II acceptor; Q_2: another postulated form of Q; Q_x: first quinone-type PS II acceptor in 'α centers'; Q_β: first quinone-type PS II acceptor in 'β centers'; Rieske FeS: Rieske iron-sulphur center; SiMo: silicomolybdate ($SiMo_{12}O_{40}^{4-}$); U: specialized plastoquinone molecule; UHDBT: 5-n-undecyl-6-hydroxy-4,7-dioxobenzothiazole; V: an electron acceptor involved in a possible proton-pumping loop around plastoquinone; W: proposed intermediate between P680 and Q; and X_2: proposed intermediate between P680 and Q.

Flash-induced absorption changes at 690 nm, supposed to monitor P680 oxidation and rereduction, are observed even when Q is in the reduced form [45, 46]. This also indicates that P680 can be oxidized by an intermediate (labelled as X_4) other than Q.

In papers contemporary to that of van Best and Duysens [189], Klevanik *et al.* [86] and Klimov *et al.* [87] suggested that Pheo might act as an electron carrier between the reaction center Chl and Q, as demonstrated in photosynthetic bacteria (see [136]): the difference (light minus dark) spectrum of absorption changes in sub-chloroplast particles enriched in PS II ('PS II particles'), when Q was chemically prereduced ($E_h \sim -300$ mV), indicated that light caused Pheo reduction. Further evidence that a Pheo molecule acts as a direct electron acceptor from P680 was obtained by EPR (electron paramagnetic resonance) and absorption change measurements. When Q was chemically reduced and the PS II particles subsequently illuminated, resulting in an accumulation of the state P680·Pheo$^-$·Q$^-$, an EPR signal developed with $g = 2.0035$ and ΔH (line width) = 12.5 G [89]; this radical is assumed to be Pheo$^-$ because it is similar in its characteristics to that of the monomeric Pheo$^-$ measured in solutions [52]. Also the E_m (midpoint potential) of the Pheo/Pheo$^-$ redox couple (~ -610 mV, as measured by the disappearance of the light-induced 685 nm absorbance change [90], or by the light-induced spin-polarized reaction center triplet EPR signal [156]) is in agreement with the value obtained for monomeric Pheo [52]. The EPR signal ascribed to Pheo$^-$ was also observed [124] in chloroplasts from a PS I-deficient barely mutant zb^{63} [63] after they were frozen under illumination (freezing to approx. 80 K is necessary for a clear observation of the signal).

The equivalence of Pheo with W or X_4 is uncertain since the kinetics of P680$^+$·Pheo$^-$ → P680·Pheo appears to be faster (2–6 ns) than that of the back reaction between P680$^+$ and the reduced W (for a discussion, see [168]) or X_4.

When reaction centers are in the state P680·Pheo·Q$^-$ before illumination, then charge separation between P680 and Pheo will occur upon illumination, yielding P680$^+$·Pheo$^-$. This radical pair is likely to undergo a back reaction, yielding (P680)*·Pheo. However the spin of one of the unpaired electrons on P680$^+$ or Pheo$^-$ might reverse. Then, upon reduction of P680$^+$ by Pheo$^-$, a triplet state is formed on P680 (radical pair mechanism). If this spin 'flip-over' can occur in the primary reactions of PS II, then a high triplet yield would be expected upon illumination of a system which is in the P680·Pheo·Q$^-$ form. Triplet energy transfer from Chl to Car is an efficient process in most photosynthetic systems, and, therefore, a relationship between the redox state of Pheo and Q and the formation of ^{3}Car would be expected to exist when the radical pair mechanism occurs; however, if for some reason the rate of triplet energy transfer from P680 to Car is much lower than from the antenna Chl to Car, this relationship is no longer valid. The formation of ^{3}Car was found to be independent of the redox state of the PS II centers and, thus, this observed triplet was interpreted as resulting from intersystem crossing in singlet excited molecules of antenna Chl followed by energy transfer to Car [107]. Later, it was shown that the quantum yield of Car triplet formation was not significantly coupled to the yield of P680 photooxidation when Q was prereduced [95]. Satoh and Mathis [161] observed that a gradual reduction of Q (as measured by increase in fluorescence yield) resulted in a gradual increase in the Car triplet yield. These authors concluded that the Car triplet state did not result from a back reaction between Pheo$^-$ and P680$^+$ after reversal of the spin of one of the unpaired electrons, but from intersystem crossing from a singlet excited state of Chl *a*; as [Q$^-$] increases and thus, the lifetime of excited state increases, there is more time to form triplets in the antenna. However, in our opinion, the existence of a radical pair mechanism of triplet formation is equally plausible: if Q is reduced, then Pheo$^-$ cannot transfer its electron to Q and a back reaction with P680$^+$ may occur, which can lead to a 'radical pair' triplet formation. The more Q is reduced, the more triplets are formed. There are many other observations that show a triplet formation by a radical pair mechanism in the primary PS II reactions analogous to that reported in bacterial reaction centers [137]: In PS II preparations the quantity of ^{3}Car was found to be dependent on the redox state of Q and Pheo, implying that at least part of the ^{3}Car is formed by a back reaction between P680$^+$ and Pheo$^-$ [92]. However, Shuvalov *et al.* [165] showed the absence of a relationship between the redox state of Pheo and ^{3}Car. A triplet state was observed by EPR at 4.2 K showing the same polarization pattern as reported for the spin-polarized triplet state of the primary donor in purple photosynthetic bacteria. This polarization pattern was interpreted to be indicative of the triplet formation from a radical pair (P680$^+$·Pheo$^-$). Moreover, an increase in the extent of the triplet was observed when Q was reduced and a decrease when Pheo was reduced [157].

The above data indicate that under appropriate conditions at least a large part of the formed triplets is the result of charge separation and recombination between P680 and Pheo. Until now, no satisfying explanation for some discrepancy in results between various laboratories has been offered; it might be possible that the triplet transfer from ^{3}Chl to Car is not very efficient under certain conditions; obviously, more experiments are needed before a more definitive conclusion concerning this problem can be drawn.

In bacterial photosynthetic reaction centers, where Q is associated with an Fe-atom, a characteristic split signal of the Pheo$^-$ radical has been observed at 4–15 K; this signal arises from exchange interaction between Pheo$^-$ and Q$^-$ coupled to Fe [131, 180, 212]. At 6–7 K, TSF -II *a* particles from

spinach (highly enriched in PS II reaction center components [82]), under reducing conditions, showed not only a light-induced EPR singlet signal due to $Pheo^-$, but also a split signal, centered at $g = 2.00$ with a splitting of ~55 G. Extraction of Q (or Fe) from the TSF-II *a* particles resulted in the elimination of the EPR doublet, but not of the singlet $Pheo^-$ signal [91]. It has been suggested that in higher plants Q might be in close contact with Fe (or another transition-metal ion) just as in photosynthetic bacteria [91, 93]. The hypothesis that Q may be an Fe–Q complex was supported by results of Nugent *et al.* [123] on PS II particles from a *Chlamydomonas* mutant [42]; at 5 K, these preparations, which lacked B, revealed in their light minus dark difference spectra a signal with $g \sim 1.84$; this signal is similar to the semiquinone–iron signal in photosynthetic bacteria. A similar signal was also observed in PS II particles when Q was chemically reduced in the dark; illumination of this sample at 5 K resulted in the development of a radical signal at $g = 2.00$ attributed to $Pheo^-$ [123] showing a clear distinction between the singlet and the doublet signal.

A problem that is not yet solved is the origin of variable Chl *a* fluorescence. In the classical model, the yield of fluorescence, coming from antenna Chl, is determined directly by the presence or absence of the fluorescence quenchers Q [44] and $P680^+$ [28, 132]. However, Klimov *et al.* [87] and Klevanik *et al.* [86] proposed that the variable PS II Chl *a* fluorescence (F_{var}) might be due to charge recombination between $Pheo^-$ and $P680^+$, resulting in (P680)*, followed by transfer of the excited state to antenna Chl. This suggestion explains why the fluorescence yield is high if Q is in the reduced form: $Pheo^-$ cannot transfer its electron to Q^-, and might back react with $P680^+$, yielding (P680)*; (P680)* can transfer its energy to the antenna and the antenna may fluoresce. Moreover, it explains why the state $P680^+ \cdot Pheo \cdot Q^-$ has a low fluorescence yield: no charge separation and, thus, no back reaction can occur between $P680^+$ and Pheo.

At low redox potentials ($E_h < -300$ mV), when Q is prereduced, a decrease in fluorescence yield is observed during illumination [101] presumably due to the accumulation of $P680 \cdot Pheo^- \cdot Q^-$. There is, however, a problem in explaining this observation: when P680* is created by $P680^+ \cdot Pheo^-$ recombination, it is highly fluorescent, but, when it is created by direct excitation of $P680 \cdot Pheo^-$, it is weakly fluorescent; the assumption that $Pheo^-$ acts as a quencher as it may be a non-radiative energy sink due to its broad absorption in the long wavelength region (P. Mathis, 5th Int. Congr. Photosyn, Greece, 1980) provides a plausible explanation for this discrepancy. However, the fluorescence yield of PS II under 'normal' circumstances ($E_h > +100$ mV) in the presence of DCMU, which blocks Q^- reoxidation by B, has often been shown not to decrease much in time while the chloroplast suspension is illuminated. This may mean that no $P680 \cdot Pheo^-$ accumulates under these circumstances.

Klimov *et al.* [88] observed that the variable fluorescence of PS II showed an activation energy of 0.075 eV in chloroplasts and considered this as the activation energy for the $P680^+ \cdot Pheo^- \rightarrow (P680)^* \cdot Pheo$ reaction. This observation might also be interpreted in terms of the classical scheme: the activation energy might be the energy necessary for 'uphill' excitation transfer from (P680)* to antenna Chl (the absorption spectrum of P680 is shifted to the red compared to antenna Chl (~10 nm), indicating that the energy gap between S^0 and S^1 is smaller in P680 than in antenna Chl). However, an absorption peak difference between P680 and antenna Chl of approx. 30 nm (instead of the assumed 10 nm) is necessary to generate a difference of 0.075 eV in the energy gap between S^0 and S^1 in P680 and antenna Chl. Therefore, in our opinion, the 0.075 eV activation energy in variable fluorescence is explained much better by the theory that F_{var} is due to a back reaction between $Pheo^-$ and $P680^+$ [86, 87] than by the classical theory. In conclusion, we can say that Pheo has been shown to act as an intermediate between P680 and Q and that the variable fluorescence is possibly due to a back reaction between $Pheo^-$ and $P680^+$, forming (P680)*; the energy is transferred from (P680)* to the antenna Chl.

A possible heterogeneity in Q and in reaction centers

The existence of more than one type of Q has often been suggested. Joliot and Joliot [75] observed that in the presence of DCMU an absorption change at 550 nm (C550), probably caused by a blue shift of Pheo absorbance by reduced Q [86], reached its maximal amplitude already after one saturating flash whereas it took several flashes before the maximal fluorescence yield was reached. Therefore, the existence of 2 Q's was proposed, Q_1 and Q_2 (Q and Q_2 in Fig. 1), in which Q_1 is responsible (indirectly) for the C550 absorption change and for the major part of fluorescence quenching, whereas Q_2 is less efficient and becomes predominant at the end of the fluorescence induction curve. It seems that the physical locations of Q_1 and Q_2 are not identical because only the reduction of Q_1, but not of Q_2, leads to the formation of a transmembrane potential (measured as ΔA_{515}) [75]. Furthermore, the reoxidation of Q_2 in the presence of DCMU and NH_2OH was reported to be faster than that of Q_1: Q_2 was reoxidized in a biphasic fashion with $t_{1/2} = 5$ s and 2 min whereas the $t_{1/2}$ of Q_1 reoxidation was 20–30 min [76] or even longer (P. Joliot, personal communication). Cyt b_{563} was suggested to be the electron acceptor of Q_2 because a DCMU-insensitive cyt b_{563} reduction was observed which is supposedly connected to PS II [76]. The observation of a component in DLE (presumably caused by a back reaction between Q^- and $P680^+$) that is insensitive to the transmembrane potential [80] might be explained by a back reaction between Q_2^- and $P680^+$. It is assumed [76] that the

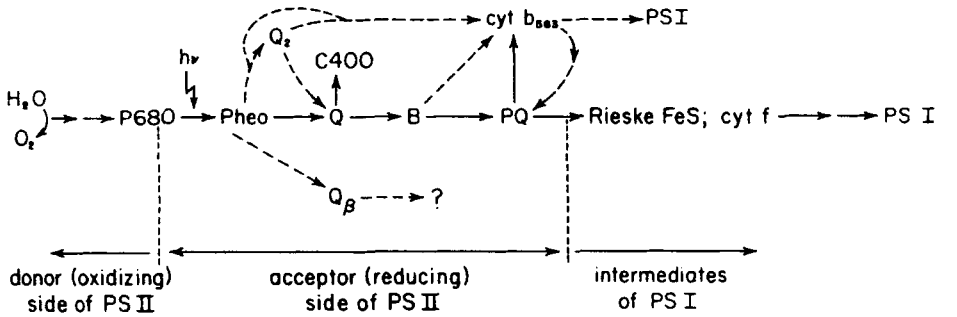

Figure 1. Electron transport scheme of the reducing side of PS II. The solid lines indicate reasonably unequivocal pathways, the dashed lines less certain pathways. Due to space limitations, not all proposed intermediates in the PQ region could be incorporated into the scheme (see also Fig. 2). For an explanation, see text.

path of electron flow is from P680 to Q_2 and Q_1 and that all PS II centers include the same primary donor (P680) and acceptor (Q_2) but Q_2 is suggested not to be connected to Q_1 in all the centers; when Q_2 is connected to Q_1, the latter accepts electrons efficiently from Q_2^-; in centers where Q_2 is not connected to Q_1, Q_2^-, proposed to be stable only in the absence of Q_1^-, is suggested to reduce only cyt b_{563} [76]. Q_2 might be identical to the intermediate X_a. (Reduction of X_a also does not lead to a ΔA_{515} that exists for longer than a few μs [45].) Further experiments are needed to test this possibility. The physiological importance for this postulated heterogeneity in electron acceptors, if any, remains to be established.

Another type of heterogeneity in PS II has also been postulated: one type of PS II unit has a large antenna in which energy transfer between antenna Chl of more than one reaction center is possible (α centers), and the other type has a small antenna in which no energy transfer from one antenna to the other can take place (β centers) [110, 113]. This results in a rather fast, sigmoidal rise (due to α centers) in the Chl *a* fluorescence induction curve in the presence of DCMU, followed by a slower rise (due to β centers) [111, 112].

In the absence of Mg^{2+} or other divalent cations, the Chl *a* fluorescence induction curve was found to be nearly monophasic [24]; the number of β centers decreased, and the number of α centers increased upon the addition of divalent cations. It was suggested that the α centers are located in grana thylakoids (absence of Mg^{2+} causes unstacking) whereas β centers are in the stroma thylakoids [113]. In the presence of divalent cations, a decrease of the redox potential to -80 mV leads to the elimination of the β component without significantly affecting the α component; this suggests that the E_m of Q is different for α and β centers [109] and might imply that the two types of centers have different types of Q. However, the Q's in α and β centers (Q_α and Q_β, respectively; Q and Q_β in Fig. 1) have nearly identical absorption spectra in the near-UV (260–340 nm) [110] and both lead to an absorption change in the 550 nm region [114] which might imply that both are closely linked to Pheo [86]. Therefore, no significant difference in the molecular nature of Q_α and Q_β is expected. The observation that reduction of Q in both α and β centers leads to an absorption change in the 550 nm region excludes the possibility that Q_α and Q_β are identical to Q_1 and Q_2 postulated by Joliot and Joliot [75, 76] because only Q_1 reduction results in an absorption change at 550 nm.

The absorption cross-section per reaction center has been calculated to be three times larger for centers in the statistical pigment bed (α centers) than for centers in separate PS II units (β centers) [110]. Melis and Homann [111] observed that the ratio of the rates of Q_α and Q_β reduction increased with increasing light intensity. This ratio is, however, expected to be independent of light intensity. Thus, the proposal of α and β centers might not explain all data.

Recent work on α and β centers has been performed using chloroplasts of wild type tobacco and the mutant Su/su, var. Aurea. The mutant has much less grana than the wild type; it has a higher amount of β centers (up to 70%) in contrast to the wild type which has only 30% β centers. This strengthens the hypothesis that β centers are located on stroma thylokoids [115].

Thielen *et al.* [179] calculated from chloroplast absorption spectra at 4 K that the spectra are different for α and β centers: an absorption peak at 672 nm and a shoulder at 650 nm were found for PS II_α and a peak at 683 nm for PS II_β. This suggests that the Chl *a* and *b* containing light-harvesting complex is connected to α centers but not to β centers [179].

The most significant difference between α and β centers is in the midpoint potential of Q_α and Q_β. As mentioned above, Q_β appears to have a higher midpoint potential than Q_α: at -80 mV Q_β was reduced while Q_α was not [109]. The reduction of Q as a function of redox potential occurs in two steps: one pool, Q_L (L = low), appears to have a $E_{m,7}$ (midpoint potential at pH 7) of ~ -220 mV while the other, Q_H (H = high), titrates at -30 mV [30, 56, 67, 69]. The

$E_{m,7}$ of Q_H is pH-sensitive at physiological pH: -60 mV/pH unit, indicating the involvement of 1 H^+ per e^- [56, 94]:

The pH sensitivity was found to disappear at pH values above 8.9; this indicates that the pK_a value of QH is about 8.9 [94]. This means that at physiological pH Q^- becomes protonated. However, Horton and Croze, observing a pH dependence of the Q_L midpoint potential, could not confirm a pH dependence for Q_H [69]. van Gorkom [190] concluded from the Q/Q^- difference spectrum that Q^- is not protonated on the time scale of many seconds. After treatment of chloroplasts with trypsin, known to remove (parts of) the protein associated with Q and B (see below), no protonation of Q^- was observed on a ms timescale [152]. The redox titration of Q, in which a protonation is observed, takes a much longer time because the chloroplasts are equilibrated at a certain E_h for at least a couple of minutes. During the redox titration, a slow protonation of Q^- (in the order of min) may occur with a rate dependent on the chloroplast preparation, thus explaining the apparent disagreement.

Measurements by Golbeck and Kok [56] implied that two electrons are involved in the reduction of Q_H. This seems surprising because Q is reduced by only one electron (see the discussion by Thielen and van Gorkom [178] for a possible explanation). Other results point clearly to the involvement of only one electron in Q_H reduction as would be expected [69]. Also, one electron is involved in Q_L reduction [69]. Now the question arises as to what causes the difference between Q_L and Q_H, and whether this heterogeneity is related to the postulated α and β centers. At -80 mV, only Q_L is oxidized; therefore, one could suggest that Q_L represents α centers and Q_H β centers. If plants are grown under an intermittent light regime, the chloroplasts lack LHCP [2] and grana stacking [1]. Absence of grana stacking might imply a large number of β centers [115], and therefore, such chloroplasts would be expected to have a large amount of Q_H, if Q_H represents Q_β. Indeed, plants grown under intermittent light do not have Q_L; after several hours of continuous illumination, Q_L develops [70]. In order to ascertain that grana stacking, but not the presence of LHCP, is the important factor in the development of Q_L, a redox titration of Q in chloroplasts from a mutant lacking most components of LHCP but still retaining grana stacking was performed. This mutant had a Q_H/Q_L ratio comparable to that in the wild type [70]. This observation strengthened the hypothesis that Q_H would be Q_β. Recently, however, this hypothesis has been shown to be wrong [66]: at $+50$ mV the slow phase in the fluorescence transient, corresponding to the β component, disappeared, even though Q_H was nearly completely oxidized. At $+190$ mV, both the fast and slow phases were present in the fluorescence induction curve. The β phase had an $E_{m,7} = +120$ mV ($n = 1$ transition) whereas the $E_{m,7}$ for Q_H was approx. -20 mV [66]. At $+50$ mV, the initial level of fluorescence was only slightly increased compared to $+190$ mV [66]. This increase might be due just to a partial reduction of Q_H. If the slow phase in the fluorescence induction curve is due to the existence of β centers, reduction of Q_β should result in an appropriate increase in the initial fluorescence yield (F_0). Although the slow phase was found to represent 35% of the total amplitude of the variable fluorescence, the slow phase could be removed by reducing only 10% of Q_H [66]; furthermore, a clear increase in the F_0/F_{max} ratio with $E_m = +120$ mV could not be observed upon lowering the E_h [56, 69, 70]. This also casts some doubt on the interpretation that the slow and fast phase in the fluorescence induction curve are caused by different types of PS II centers. The above-stated measurements were confirmed by Thielen and van Gorkom [178]; however, a reasonable increase in the instantaneous fluorescence yield relative to the maximal fluorescence yield was observed upon reduction of the component responsible for the slow phase, which might indicate that the slow and fast phase in the fluorescence induction curve are due to two types of PS II centers.

Q_β was found not be be related to a two-electron gate: pre-illumination with flashes followed by addition of DCMU and fluorescence induction measurements revealed no oscillations in the amplitude of the β phase, but a fixed amount of 20% reduced Q_β after one or more pre-illuminating flashes. Furthermore, the $E_{m,7}$ of Q_β is higher than that of the PQ pool (the latter has an E_m of $+106$ mV at pH 7.2 [56]) indicating that Q_β cannot transfer electrons effectively via PQ [192]. In view of the absence of a connection of Q_β with a two-electron gate or with PQ, Q_β cannot be a part of the 'normal' electron transport system [192]. Thus, it is highly surprising, in our opinion, that DCMU blocks electron transport in the β centers: the proteinaceous environment of Q_β is probably different from that of Q_α; the Q/B apoprotein that bears the DCMU binding site may not include Q_β; furthermore, the environment of B is presumably different from the environment of the Q^--oxidizing-intermediate related to β centers. So, why should DCMU prevent reoxidation of Q_β^- with approximately identical efficiency as it does in α-centers? In our opinion, then, the assignment of Q_α and Q_β to different centers is not as logical as it was originally suggested.

The appearance of Q_H was found to be strongly dependent on the redox state of B and on the presence of DCMU [178]: when most of the PS II chains are in the state $Q \cdot B^-$, more Q_H is found than if the chains are in the state $Q \cdot B$; in the presence of DCMU, Q_H may disappear. If DCMU is added to the chloroplast suspension when, starting from state $Q \cdot B$, the redox potential of the system is brought to -100 or -200 mV, reduced Q_H is reoxidized and a low instantaneous fluorescence yield is obtained. It was proposed that under these conditions DCMU is able to shift the equilibrium of $Q^-B^- \rightleftharpoons QB^{2-}$ to the

right, explaining the above data [178]. The midpoint potential of the B/B^- couple was earlier suggested [203] to decrease in the presence of DCMU. On the other hand, the shift in the $Q^-B^- \rightleftharpoons QB^{2-}$ equilibrium points to an increase in the E_m of the B^-/B^{2-} couple by DCMU. This might indicate that DCMU does not influence the B/B^{2-} midpoint potential ($= 1/2[E_m(B/B^-) + E_m(B^-/B^{2-})]$) but that it only 'destabilizes' the B^- state. This apparent instability of B^- might be due to a competition of DCMU with B for a common binding site near Q [201]: B and B^{2-} (or, rather, BH_2) might be able to exchange with the PQ pool while B^- might be tightly bound to a site near Q [201]; then the reduction of Q by B^- upon addition of DCMU can be written as: $Q \cdot B^- + DCMU \rightleftharpoons Q^- \cdot DCMU + PQ$ and the oxidation of Q^- by B^- as: $Q^- \cdot B^- + DCMU \rightleftharpoons Q \cdot DCMU + PQ^{2-}$ (for details, see [178]). There is another indication that a semiquinone radical is not easily replaced from the binding site whereas the fully oxidized and reduced forms are: Lavergne [97] suggested that externally added benzoquinone accepts its first electron from B, yielding benzosemiquinone, in a DCMU-sensitive fashion, but benzosemiquinone oxidizes Q^- in a DCMU-insensitive fashion. We interpret Lavergne's data by assuming that benzoquinone (BeQu) oxidizes B^-, but we suggest that the now generated B is free to leave the binding site and might be replaced by the benzosemiquinone that is formed. This benzosemiquinone ($BeQu^-$) does not seem to be easily replaced by DCMU (half time, several min [97]) possibly due to the $DCMU + Q \cdot BeQu^- \rightleftharpoons Q^- \cdot DCMU + BeQu$ being much more to the left than the $DCMU + Q \cdot B^- \rightleftharpoons Q^- \cdot DCMU + B$ equilibrium. After oxidation of Q^- by $BeQu^-$, $BeQuH_2$ is formed (or initially, perhaps, $BeQu^{2-}$), which easily dissociates from the binding site. The above suggestions might shed a completely new light on the mode of action of herbicides.

Returning to the Q_H and Q_L problems, the above-cited data might indicate that 'Q_H' (the component that is reduced with $E_{m,7} \sim -30$ mV and that, in its reduced form, leads to a high instantaneous fluorescence yield) is related directly to a change in the redox state of B, the B^-/B^{2-} transition, and only indirectly to a change in the redox state of Q itself: the $Q \cdot B^{2-} \rightleftharpoons Q^-B^-$ equilibrium is suggested to have an equilibrium constant (K) of approx. 1 (i.e. the equilibrium is 'in the middle') whereas the $Q \cdot B^- \rightleftharpoons Q^- \cdot B$ equilibrium is far to the left ($K \sim 0.1$) [178]. This would imply that approx. 50% of Q is reduced if the redox potential is low enough to form B^{2-}: $Q \cdot B^{2-}$ and $Q^- \cdot B^-$ are present in equal amounts. In this model, one would expect that Q^- is formed (in the dark) when B becomes doubly reduced until the Q^-/Q ratio is approx. 1. Therefore, it might be suggested that the partial reduction of Q when lowering the redox potential to -50 mV ('Q_H') is due to the reduction of $Q \cdot B^-$ to $Q \cdot B^{2-}$; $Q \cdot B^{2-}$ is in equilibrium with $Q^- \cdot B^-$ and this is seen as an approx. 50% reduction of the Q pool. Thus, the 'Q_H' would not represent a different Q-pool than 'Q_L', but 'Q_H' would reflect the reduction of B^- to B^{2-} whereas 'Q_L' would monitor the reduction of Q to Q^-, regardless of the redox state of B. However, there are thermodynamic problems with such an interpretation: if the equilibrium constant of $Q^- + B^- \rightleftharpoons Q + B^{2-}$ is approx. 1, as suggested by Thielen and van Gorkom [178], then the $[Q^-]/[Q]$ should be of the same order of magnitude as $[B^-]/[B^{2-}]$; this would imply that the midpoint potentials of the Q/Q^- couple and the B^-/B^{2-} couple are nearly identical. This is not observed. Thielen and van Gorkom [178] propose another scheme, in which the reduction of Q_H represents a 2-electron reduction of the $Q \cdot B$ pair and the reduction of Q_L represents the addition of one electron to the doubly reduced $Q \cdot B$ pair. (For a further discussion of this model, see Thielen and van Gorkom [178].) Our discussion thus far has ignored the protonation of Q^-. Bowes and Crofts [19] have pointed out that $E_{m,7}$ of couple Q_H/Q_H^- (-30 mV) is the potential of importance under equilibrium dark conditions but the physiologically important $E_{m,7}$ is that of the Q_H/Q_H^- redox couple which is 100 mV lower. These considerations, however, do not solve any of the above stated problems. It is clear that many more experiments have to be performed before an unambiguous solution for the proposed heterogeneity in Q can be expected.

The influence of herbicides on electron transport: the 32 kD protein

The mode of action of many herbicides (e.g. DCMU, atrazine, ioxynil, bromonitrothymol) is to block electron transport between Q and B by binding to a protein related to Q and B [185]. Although the chemical structures of DCMU or atrazine-type and phenolic herbicides are entirely different and the mechanism of binding probably not identical [193, 197], metribuzin [182], a triazinone herbicide, or atrazine [141] replaces urea (e.g. DCMU), and triazine herbicides as well as phenolic herbicides like ioxynil or bromonitrothymol [147, 184]. This suggests that the binding sites of these herbicides are located very close to each other but are not completely identical.

First, some recent results concerning the protein(s) to which herbicides might bind are summarized. Mild trypsin treatment makes Q accessible to ferricyanide [149, 184, 194] and appears to decrease the E_m of cyt b_{559HP} [71]. Furthermore, this treatment causes the release of herbicides from the membrane [139, 172]. This implies that trypsin is able to change the Q/B apoprotein which is supposed to bind herbicides [149]. If PS II particles are subjected to trypsin treatment, the DCMU sensitivity of electron transport from diphenylcarbazide (DPC) to 2,6-dichlorophenolindophenol (DCPIP) decreases. (In comparison to chloroplasts, these particles are less sensitive to DCMU even before trypsin treatment: 10 μM

DCMU causes only 60% inhibition of electron flow.) Polyacrylamide gel electrophoresis of denatured proteins extracted from the particles before and after trypsin treatment reveals that two protein bands, with apparent MW = 27 and 32 kD, are lost after trypsin treatment while a 17 kD component band appears [35]. This suggests that (one of) these proteins are (is) involved in 'covering' Q in binding of herbicides. However, when gel patterns of the protein components of atrazine susceptible and resistant chloroplast membranes were compared, then differences in the 18–20 kD bands were observed suggesting that the lower MW protein is involved in herbicide binding [138]. Recent evidence, though, points to an involvement of the 32 kD component, instead of the 18–20 or 27 kD components, in electron flow and herbicide binding [108, 120]. The assignment of the 32 kD protein as the herbicide-binding protein is confirmed by the observation that chloroplasts from a maize mutant lacking the 32 kD polypeptide are not able to bind [^{14}C]-DCMU [99]. If *Spirodela* thylakoids are selectively depleted of the rapidly turning over 32 kD protein by blocking protein synthesis prior to the isolation of thylakoids, then the FeCy Hill reaction is inhibited whereas the SiMo Hill reaction (i.e. electron transfer to Q [54, 214]) and electron transport from DCPIP/ascorbate or DAD/ascorbate to MV (methylviologen) (i.e. PS I electron transport) is not affected. This suggests that a 32 kD protein is necessary for effective electron transport between Q and PQ [108]. Since it is not known what fraction of the electron transport chains still contains a 32 kD protein after depletion, it is not clear whether electron transfer is completely blocked or is less effective in the absence of the 32 kD protein. The trypsin concentration dependence of the disappearance of the 32 kD protein parallels the disappearance of DCMU sensitivity of the FeCy Hill reaction [108]. Trypsin treatment converts the 32 kD protein into a 31.3 kD, then into a 19.5 kD and later into a 17 kD unit. This 17 kD unit, while in the membrane, is not further attacked by trypsin, but is readily digested if it is extracted by detergents. Therefore, it was suggested that this 17 kD unit, also observed by Croze *et al.* [35], serves as a hydrophobic 'anchor' of the protein in the membrane [108]. Note that the conversion of the 32 kD protein into a 31.3 kD component (just the removal of a <1 kD peptide) by mild trypsinization seems to be able to make Q accessible to external redox reagents such as FeCy [108]. However, when thylakoids are treated with glutaraldehyde, then no DCMU-insensitive FeCy Hill reaction occurs after trypsin treatment. Furthermore, after glutaraldehyde fixation the amplitude of the 334 nm absorption change, due to reduction of Q (see e.g. [190]), is reduced to approx. 60% of the original amplitude [153] indicating that a part of stable charge separation between P680 and Q is no longer possible. Chl *a* fluorescence induction curves before and after the addition of DCMU show that the glutaraldehyde fixation does not significantly block electron transport between Q and PQ [153]. Therefore, glutaraldehyde might affect one part of the Q/B apoprotein (responsible for inhibition of trypsin action) but not (or only to a smaller extent) the part that is necessary for efficient $Q \rightarrow PQ$ electron transport. Glutaraldehyde might react with the free $-NH_2$ groups of lysine [135] while trypsin attacks lysine or arginine. For this reason, we speculate that trypsin-induced relief of DCMU sensitivity of the FeCy Hill reaction is due to trypsin attack at a lysine residue in the Q/B apoprotein.

Trypsin has multiple effects on the herbicide binding protein depending on incubation conditions (after a short incubation, a small portion of the protein molecule is removed; longer incubation leads to a larger disintegration of the protein [108]). It was initially implied [146] that trypsin treatment of isolated thylakoids results in herbicide-insensitivity of electron transport through the normal electron transport chain. Later, it turned out that trypsin treatment makes Q accessible to FeCy and blocks electron transport beyond Q in the normal chain [13, 140, 149, 150, 172, 184]. The effects of trypsin treatment on herbicide binding are complicated: trypsin treatment leads both to an increase in the binding constant (a decrease in the affinity of the herbicide for the site) and to a decrease in the number of sites [140, 172]. The binding constant seems to be more rapidly affected than the number of sites, and, therefore, it is possible that the protein is attacked by trypsin in two or more steps [172]; the latter may be the reason for the observation of the gradual disintegration of the 32 kD protein [108]. Tischer and Strotmann [183] observed an increase, although small, in the dissociation constant for [^{14}C]-metribuzin upon trypsin treatment. In contrast, phenolic herbicides like dinoseb and bromonitrothymol appear to have an increased affinity for the binding site after mild trypsin treatment; after longer periods of trypsin incubation, this increase in affinity is reversed [172]. DCMU- or atrazine-type herbicides do not show such a drop in I_{50} [172]. These observations might be related to the high sensitivity of atrazine-resistant chloroplasts to phenolic herbicides [7]; we note that the DCMU sensitivity in atrazine resistant plants is nearly equal to that in susceptible plants; we consider it likely that the 32 kD protein in atrazine-resistant plants has the same properties as the 32 kD protein in susceptible plants after removal of a <1 kD peptide by trypsin.

It is somewhat surprising that the 32 kD protein has turned out to be so important for herbicide binding and electron flow because this rapidly turning-over polypeptide, whose precursor is a 33.5 kD protein [47], did not seem to be involved in light-mediated CO_2 fixation: removal of up to 80% of the 32 kD protein did not decrease the CO_2 fixation rate [209] and, therefore, it was initially concluded that this protein was not directly involved in photosynthesis. The 33.5 kD polypeptide is probably coded on chloroplast DNA because it is uniparentally inherited

through the female parent: this parent determines the susceptibility or resistance for atrazine [36, 171]. Furthermore, the structural gene for a 34 kD polypeptide, which might be the 33.5 kD precursor of the 32 kD protein, has been localized on chloroplast DNA in maize [10].

A 33 kD protein can easily be isolated from spinach chloroplasts [96]. This protein is associated with PS II as it is present in PS II particles but absent in PS I particles [96]. Approximately one such protein molecule per 300 Chl molecules is found in thylakoids (N. Murata, unpublished), which might indicate a 1:1 PS II reaction center/33 kD protein ratio. However, this 33 kD protein is not necessarily the herbicide binding protein: von Wettstein [208] proposed that one of the Chl *a/b* proteins near P680 is a 32 kD protein. This suggests that a 32 kD protein is located in the middle or inner part of the thylakoid membrane. Kuwabara and Murata [96], however, reported that 'their' 33 kD protein was easily released from the thylakoid membrane by EDTA treatment or by sonic oscillation, which suggests a location on the outer side of the membrane. Thus, the 33 kD protein, reported by Kuwabara and Murata, is probably not a Chl *a/b* protein, but may be the herbicide binding protein.

The 32 kD herbicide binding protein is present not only in eukaryotes but also in the prokaryotic Cyanobacteria: in *Aphanocapsa* 6714, a good correlation between DCMU sensitivity and the presence of a 33 kD polypeptide has been reported [8]. If DCMU is added to the growth medium of a mutant of *Aphanocapsa*, then the production of a 33 kD protein seems to stop and electron transport in such cells becomes DCMU insensitive. Furthermore, if this 33 kD protein is selectively removed from the membrane, the DCPIP Hill reaction becomes DCMU insensitive. Therefore, the authors [8] concluded that this polypeptide is not necessary for electron transport but only facilitates it. However, it is equally possible, in our opinion, that in the absence of the 33 kD protein DCPIP is able to accept electrons from Q, which would be a DCMU-insensitive electron transport and, thus, the 32 kD protein might be a requirement for the normal electron flow from Q to B to PQ. However, no unambiguous proof exists to show that the absence of the 33 kD protein inhibits electron transport from Q to PS I.

An adaptation of unicellular organisms to inhibitors as found by Astier and Joset-Espardellier [8] seems to be rather common: Urbach *et al.* [188] reported that in the green alga *Ankistrodesmus* electron transport is inhibited by ioxynil in the first minutes but soon becomes insensitive to the inhibitor. If after this 'adaptation' DBMIB is added, then also no inhibition of electron transport is observed. However, DCMU is an active inhibitor in 'ioxynil-adapted' algae [188]. Ioxynil and DBMIB probably have different binding sites: ioxynil inhibits electron transport between Q and B whereas DBMIB, at low concentrations, blocks between PQ and PS I; we refrain from speculating on the mechanism of adaptation mentioned here.

The identification of the 32 kD protein as the herbicide binding protein has been confirmed by experiments in which [^{14}C]-azidoatrazine, which behaves like atrazine, was covalently bound to the chloroplast membrane by UV-irradiation (photoaffinity labelling [140, 141]). After protein extraction and SDS–PAGE the radioactivity was found to be in a band which corresponded to the 32 kD protein [53, 140, 141]. Pfister *et al.* [140, 141] observed also a minor incorporation of label into 16 and 25 kD protein bands.

The techniques of photoaffinity labelling were also used to identify the phenolic-herbicide binding site using [^{3}H]-i-dinoseb in which a -NO_2 group was replaced by a -N_3 (azide) group [128]. The modified i-dinoseb bound covalently to polypeptides of about 40 and 15 kD. Furthermore, a 25 kD protein complex, presumably a part of the light-harvesting complex, was unspecifically labelled. Oettmeier *et al.* [128] suggested that the 40 kD protein is the only specific i-dinoseb binding protein that is involved in electron transport. Experiments with unmodified [^{3}H]-i-dinoseb show that DCMU-type inhibitors interfere noncompetitively with the 'specific binding' of i-dinoseb whereas phenolic herbicides show a competitive interaction [126]. DBMIB, on the other hand, does not interfere with i-dinoseb binding. Trypsin treatment does not change the 'specific binding' of i-dinoseb [126]. However, there is a problem in the interpretation of the data: the binding of i-dinoseb, defined as 'specific binding', has a binding constant (K_b) of 6.9×10^{-8} M ($pK_b = 7.2$) whereas the pI_{50} (i.e. the negative log of the concentration that is necessary for 50% inhibition of electron transport) of i-dinoseb is about 5.5. Therefore, the K_b and I_{50} differ by a factor of ~40 [126] while these values should be identical (at least, when extrapolated to [Chl] = 0 [182]). Even if the values are corrected for the non-zero Chl concentration the K_b and I_{50} still differ by a factor of ~25. This discrepancy might indicate that the K_b of 'specific binding' is not the K_b of i-dinoseb binding to the site which affects electron transport but the K_b of binding to a site that does not influence electron transport. We suggest that the observed interaction between binding of i-dinoseb and DCMU or ioxynil is not due to an interaction at the binding site that affects electron flow, but to an interaction at a site to which i-dinoseb binds strongly but which does not influence electron flow. In support of the latter we note that there is only one high-affinity i-dinoseb binding site per 3 PS II reaction centers [126], whereas for DCMU type inhibitors [182] or ioxynil [129] binding there is one site per PS II reaction center. Thus, for stoichiometric reasons, it is almost impossible that the electron flow inhibiting DCMU binding site and the high affinity i-dinoseb binding site are related. These considerations imply that the use of radioactively labelled i-dinoseb to study the binding to the electron flow inhibiting site is not as

straightforward as the use of [^{14}C]-DCMU, metribuzin, or atrazine is. When using i-dinoseb, we must distinguish between a large amount of 'non-specific' binding and a relatively small amount (probably $<5\%$) of binding related to regulation of electron flow. However, the 40 kD protein that had specifically bound modified i-dinoseb may represent the small fraction related to electron transport and indeed be involved in regulation of electron flow because a large amount of [^{3}H]-azido-i-dinoseb (probably $>90\%$ of the total bound ^{3}H) was bound to low MW molecules, possibly to lipids [128]. The absence of labelling of a 32 kD protein suggests that the phenolic herbicides binding protein is not identical to the DCMU/atrazine binding protein. Indeed, PS II particles depleted of 32 kD polypeptide are still largely sensitive to dinoseb [120]. Recently, Oettmeier *et al.* [129] reported results obtained with ioxynil and 2-iodo-4-nitro-6-isobutylphenol, phenolic herbicides, in which the binding constant and I_{50} are nearly identical. Use of these compounds that bind specifically to the site that influence photosynthetic electron transport might prove to be much more fruitful in studies of the protein that binds phenolic herbicides than the rather unspecific i-dinoseb.

Another interesting topic which has developed in the last few years is the appearance of a resistance to a certain herbicide in various plants. The resistance for a particular herbicide is very specific [7, 138]; for instance, atrazine-resistant *Senecio vulgaris* chloroplasts are only slightly less susceptible to DCMU compared to the normal susceptible type and the number of DCMU binding sites is almost equal in the two [139]; furthermore, atrazine resistant plants are more sensitive to phenolic herbicides than the atrazine sensitive ones [7, 138]. Such statements are in apparent contradiction with the observation that atrazine- and DCMU-type inhibitors replace each other on the binding site [139, 182]. A plausible explanation for this contradiction is to assume that herbicide binding requires not only the 'essential element' (a N–C= group bound to a lipophilic alkyl- or aryl-group [26, 185]), but other regions of the structure as well. These 'other regions' are assumed to bind to domains other than where the 'essential element' does. This 'other' binding is suggested to be to different domains for different groups of herbicides. This also explains why the DCMU binding constant remains nearly unchanged even when the atrazine affinity is altered [138, 139]: the 'other' binding region for atrazine is changed such that atrazine can no longer bind whereas the 'other' binding region for DCMU is not significantly altered.

Atrazine resistance leads to a severe decrease in the rate of electron flow from Q to B; in normal (atrazine susceptible) chloroplasts, the decay of the variable fluorescence yield (indicating the decay of the Q^- concentration after a flash) is rather fast: after 200–700 μs *half of* Q^- *is reoxidized*; the oxidation of Q^- by B is faster than its oxidation by B^-, yielding a periodicity of two with flash number in the decay rates of Q^- [19]. In atrazine resistant chloroplasts the oxidation of a fraction of Q^- by B is ~ 10 times slower than in normal chloroplasts; the other fraction of Q^- decays very slowly: at 100 ms after a flash 30–60% of Q^- is not reoxidized; the binary oscillation in the rate of Q^- oxidation is of opposite phase compared to that in normal (susceptible) chloroplasts [22]. One of the possibilities to explain these data is that the B/B^- and, possibly, also the B^-/B^{2-} redox potentials are lowered in resistant compared to susceptible chloroplasts. Whatever the precise cause of atrazine resistance, it seems to result from a change in the 32 kD polypeptide that influences binding of herbicides and the kinetics of Q^- oxidation.

Possible modes of action of herbicides

Velthuys and Amesz [203] proposed that DCMU acts by lowering the midpoint potential of B. This view is generally accepted, but the question as to the molecular mechanism involved remains unanswered. There are several possibilities [164]: herbicide binding (1) might reduce the magnitude of an anion-stabilizing electric field across B or (2) could inhibit the conformational relaxation or protonation of the protein in response to reduction of B to B^-, or (3) might lead to a displacement of the quinone head of B from its binding site (as mentioned earlier). It was also proposed [164] that arginine might be involved in the binding of hydrophilic sites of herbicides and/or the stabilization of B^- relative to B. The lipophilic parts of the herbicides then would serve to increase the lipid solubility or to fit the hydrophobic surfaces of the herbicide binding site. However, experimental proof for such hypotheses is still lacking.

Although studies have been made on the relationship between the binding constant and the structure of the herbicides, yet it has been difficult to relate the physical and chemical properties of the herbicides to their activity (see e.g. [43]); the efficiency of phenols is correlated to the size of the substituents but not to the pK_A of the OH-group or the lipophilicity of the compound [185, 187]. One of the factors affecting the binding of herbicides to their binding site, directly or indirectly, seems to be the mobility of the membrane lipids; the temperature dependence of the dissociation constant K_d for metribuzin almost disappears at $T < 14$ °C; the bend at 14 °C in the Arrhenius plot (ln K_d vs. $1/T$) may be caused by a phase transition of the lipid phase [183].

Effects of HCO_3^- *or* CO_2

It is known that the absence of HCO_3^- or CO_2 inhibits electron transport between Q and PQ. HCO_3^- or CO_2 probably binds to or near the Q/B apoprotein and changes its conformation such that efficient electron transport from Q^- to B and from B^{2-} to PQ is allowed [58, 85, 205, 206]. Since it is not known whether binding of CO_2 or HCO_3^- leads to efficient electron transport, we will use the description

'HCO_3^-*' to indicate the HCO_3^- or CO_2 bound to the membrane at the active site. The oxidation of Q^- is slowed down by a factor of 10 or more in the absence of HCO_3^-* [79, 81] whereas the B^{2-} oxidation is probably completely blocked [206].

Stemler [173] proposed a direct interaction of HCO_3^- with B because the rate of HCO_3^-* binding to CO_2-depleted chloroplasts shows a binary oscillation with a period of 2; the rate appears to be faster after zero or an even number of flashes than after an odd number; the oscillation is, however, not dramatic. An interaction of HCO_3^-* with B would imply a close relationship of the herbicide and HCO_3^-* binding sites. Indeed, a fully competitive interaction between HCO_3^-* and the phenolic herbicide DNOC has been shown [195]. Furthermore, there is a difference in affinity of [^{14}C]-atrazine to the membrane in CO_2-depleted chloroplasts and those to which HCO_3^-* has been resupplied [85]. DCMU seems to show a partial competition towards HCO_3^-* binding [195].

To elucidate if CO_2 or HCO_3^- is involved in this HCO_3^-* action, reactivation of electron transport by HCO_3^- addition at various pH values has been measured. The maximum effect is observed at pH 6.5–6.8 [83, 207], close to the pK_a of CO_2, which is 6.4 at 25°C. This may be interpreted to suggest that both CO_2 and HCO_3^- are involved in the HCO_3^-* action.

Formate (HCO_2^-), which is, probably, a competitive inhibitor of HCO_3^-* binding [83, 207], affects strongly the binding of HCO_3^-*: in the absence of formate, addition of much less HCO_3^- is able to restore electron transport than in the presence of formate [83]; furthermore, during illumination, in the presence of formate no significant restoration of electron flow in CO_2-depleted chloroplasts is observed upon HCO_3^- addition [173, 207], whereas in the absence of formate restoration of electron flow occurs under the same conditions [207]. A model was proposed [207] in which a binding site that could bind HCO_3^- is located just below the thylakoid surface; this site can only be reached by passing through a 'channel' surrounded by negative charges; only CO_2 would be able to diffuse through this channel in the light. That CO_2 may be the species diffusing to the binding site is supported by data on restoration of the Hill reaction in CO_2-depleted thylakoids upon the injection of HCO_3^- or a mixture of HCO_3^- and CO_2 [160].

Some experimental data seem to suggest a site of HCO_3^-* action on the donor or oxidizing (water) side of PS II: for instance, (1) the MV-mediated Mehler reaction (monitoring non-cyclic electron transfer through PS II and PS I) in thylakoids treated with NH_2OH (blocking $P680^+$ reduction by the physiological donor Z and reducing $P680^+$ via an unknown donor D [40]) seems to be relatively insensitive to HCO_3^-* [49]; (2) the intrathylakoidal rather than the external pH has been suggested to govern the rate of HCO_3^-* binding [174]; (3) formate effects, that were assumed to be related to HCO_3^-* effects, were observed specifically on the S_2 and S_3 states of the oxygen evolving system [175]; and (4) the kinetics of flash-induced O_2 evolution at low pH were reported to be slightly faster with than without HCO_3^- [176]. However (1; same numbering as above) it is not known whether NH_2OH in the above experiments had effects on photosynthetic electron transport other than on the donor side of PS II; moreover, under other conditions, significant HCO_3^-* effects have been observed even with NH_2OH as a donor (see [213]); (2) the time used in [174] to distinguish between the conditions for intrathylakoidal pH and external pH seems to be inappropriate (see [205]); (3) formate may have effects other than those related to the bicarbonate effect and the observed effects may be at locations other than the O_2 evolving system itself; and (4) the observed differences in O_2 kinetics are too small to be of much significance, and they could not be confirmed by the present authors (unpublished). For details on some of these criticisms, see [205]. Furthermore, the electron flow from H_2O to Q measured through the FeCy Hill reaction in trypsin-treated chloroplasts [85, 195] and the SiMo Hill reaction [54, 214] in CO_2-depleted chloroplasts are fully insensitive [83, 196] to HCO_3^- addition, implying that HCO_3^-* does not significantly affect the oxidizing (donor) side of PS II. In agreement with this statement is the observation [84] that proton translocation through the PQ pool was highly sensitive to CO_2 depletion whereas proton extrusion into the intrathylakoidal space by the oxygen-evolving system is much less influenced by CO_2 depletion. Although Metzner *et al.* [116] reported a small difference in $^{18}O_2$ evolution in the presence and the absence of $HC^{18}O_3^-$, Radmer and Ollinger [145] showed that no significant amount of $^{18}O_2$ was evolved when $HC^{18}O_3^-$ was added to CO_2-depleted chloroplasts; the latter observation supports the idea that CO_2 is not involved in O_2 evolution. Although small effects of HCO_3^- on the oxidizing (donor) side cannot be ruled out at the moment, we propose that the most important effect of HCO_3^-* on electron transport is near Q, B and PQ. A direct involvement of HCO_3^-* in O_2 evolution is still highly speculative; we believe that no solid experimental basis for this proposal is available at the moment.

HCO_3^-* appears to have some additional effects on electron transport near Q: in the absence of HCO_3^-* no electron transport from Q to C400 seems to occur [145]. C400 is a component ($E_{m,7} \sim 350$ mV) that is rapidly reduced by Q^- ($t_{1/2} \sim 130\,\mu$s; cf. [204]) but is very slowly reoxidized (takes minutes [19, 21]). (In the green alga *Chlorella*, a C400-like component appears to have a much lower $E_{m,7}$ than +350 mV [100].) Furthermore, in the presence of DCMU the Chl *a* fluorescence rise kinetics are approx. 2 times faster in the absence than in the presence of HCO_3^-* [177, 206]; this effect is probably caused by an

Table 1. Inhibition of electron transport on the acceptor side of PS II

Site	Conditions	Remarks
Q → Donor side of PS II	+ Diphenylamines	
Q → B	+ DCMU or analogs + Atrazine or analogs + Phenolic herbicides + UHDBT + DBMIB + Trypsin	High [DBMIB] required
B → PQ	$-HCO_3^-$ or CO_2 + Melittin	
PQ → FeS/cyt *f*	+ DBMIB + UHDBT + Bathophenanthroline	High [UHDBT] required

influence of HCO_3^- on the kinetics of the oxidation of $Pheo^-$ combined, perhaps, with an effect on the reoxidation of Q^- by $P680^+$; it has been shown not to involve the O_2-evolving system [206]. Therefore, most reactions that involve Q^- oxidation are slowed down in the absence of HCO_3^-* whereas the rate of B^{2-}- oxidation is dramatically decreased or even blocked [206]. For this reason, the modes of action of the known herbicides and lack of HCO_3^-* are not quite comparable; HCO_3^-* fulfils a unique role in regulating photosynthetic electron transport at the quinone level.

Some new electron transport inhibitors

(a) *Diphenylamines.* Diphenylamines are inhibitors with a pI_{50} value of 4–6. Most of the diphenylamine derivatives do not replace metribuzin [125], which, as mentioned before, binds partially to the same site as DCMU and atrazine do. Diphenylamines do not inhibit at the DBMIB site either, but they all function as ADRY-type reagents [127] (ADRY- acceleration of deactivation reactions of the watersplitting system Y): these reagents destabilize the states S_2 and S_3 of the O_2-evolving system 'Y' [148]. Furthermore, the inhibition of the FeCy Hill reaction by most diphenylamines is not relieved by trypsin treatment. Oettmeier and Renger suggested [127] that the main site of diphenylamine action is the protein moiety of cyt b_{559}; cyt b_{559} is known to interact with ADRY reagents [62, 106]. If one assumes that cyt b_{559} is located close to the Q/B apoprotein, then an influence on both cyt b_{559} and the Q/B apoprotein might be expected for some diphenylamine derivatives [127]..

(b) *UHDBT.* UHDBT, known to inhibit the oxidation of ubiquinol in bacterial photosynthesis [23, 61], was shown to act at low concentrations between Q and PQ in chloroplasts [130]; the inhibitor is rather efficient as the pI_{50} is 7.6. Unlike DBMIB, UHDBT replaces [^{14}C]-metribuzin [130]; thus, UHDBT may act at the same site as DCMU and metribuzin. However, electron transport with duroquinol (which donates directly to PQ [73, 210]) is not completely insensitive to UHDBT: the I_{50} of this inhibition is approx. 5×10^{-6} *M*. Thus, UHDBT also acts as a DBMIB analog at higher concentrations [130]; it might interact with the Rieske FeS center (J. Whitmarsh and coworkers, personal communication). The fact that the major site of UHDBT is located before PQH_2 oxidation has been confirmed by Malkin *et al.* [105]: PS I reactions with duroquinol or DAD/ascorbate as electron donors (donating to the PQ pool) are insensitive to up to 1 μM UHDBT whereas the I_{50} for the FeCy Hill reaction is approx. 10^{-7} *M*. DBMIB is, as expected, a potent inhibitor of PS I reactions with quinone-type electron donors. This confirms the difference in the site of action between UHDBT and DBMIB [105]. However, higher concentrations of DBMIB are able to block electron flow from Q to PQ [39, 60, 187]. This was confirmed by Bowes and Crofts [20]: the Q^- oxidation decreased drastically in the presence of 2–20 μM reduced DBMIB. The binary oscillation in Q^- oxidation was reversed in the presence of DBMIB compared to the control: after the first flash a slower Q^- reoxidation was observed than after the second one. These results might be interpreted as suggesting that B is in dynamic equilibrium with its binding site on the Q/B apoprotein, and that reduced DBMIB competes for this site. Only B^- seems to have a high affinity for the site relative to DBMIB [20, 202], just as was suggested before for B/DCMU interactions.

Qualitatively, no difference in the site of action appears to exist between DBMIB and UHDBT; quantitatively, DBMIB is a more effective inhibitor in the oxidation of PQH_2 whereas UHDBT is a better inhibitor of the Q^- oxidation by B.

(c) *Dinitrophenylethers of phenols.* It is generally known that various phenolic compounds act as inhibitors of electron flow between Q and B as mentioned above. However, if a phenolic herbicide such as bromonitrothymol is converted into its dinitrophenylether, then an inhibition of electron flow in a DBMIB-like fashion is observed [186]. Examples of relatively effective dinitrophenyl(DNP)-ether com-

pounds (pI_{50} between 5 and 6.5) are: the DNP-ether of ioxynil, the DNP-ether of bromonitrothymol and the DNP-ether of iodonitrothymol [184].

(d) *Melittin.* Melittin, a lytic peptide isolated from the venom of the honey bee, inhibits photosynthetic electron transport in the PQ region; more specifically, it seems to inhibit the reduction of PQ [12]. Although melittin does not have a straightforward blocking action and although it may have more than one mode of action, it seems to have the interesting property of inhibiting, at low concentrations, electron transport with DAD_{ox} as acceptor (which is supposed to accept electrons from PQ) whereas electron transport with quinones as acceptors remains unaffected [12]. (One side-effect of melittin action is probably the uncoupling of phosphorylation [37].) Surprisingly, the DCMU-insensitive SiMo reduction is inhibited by melittin whereas the DCMU-sensitive quinone reduction is not affected (A. M. Haller and S. P. Berg, personal communication). In spite of the complexities mentioned above, melittin or other small peptides (e.g. dipeptides with hydrophobic groups on the C- and N-termini [164]) might prove to be useful inhibitors of electron transport in the future.

Plastoquinone: an electrogenic loop

It is generally believed that as a consequence of PS II reactions PQ is reduced and protonated, forming PQH_2. The protonation occurs by proton uptake from the medium on the outer side of the thylakoid membrane. PQH_2 is oxidized, probably by the Rieske FeS center and or cyt *f* (see below), and the protons are extruded into the intrathylakoidal space, giving rise to a proton gradient, which can be used for ATP systhesis [74, 77]. We also note that PQ is not only in contact, directly or indirectly, with cyt *f* or the Rieske FeS center, but is also the electron donor to (a part of) cyt b_{559HP} [31].

Plastoquinones seem to be able to fulfil key roles in (1) electron transport, (2) the activation of a protein kinase and (3) providing the link between photosynthesis and respiration in cyanobacteria. (1) PQ acts both in cyclic electron transport around PS I and in non-cyclic electron flow; furthermore, PQ is the proton translocator. Electron transport in this crucial PQ region is specifically influenced by the fluidity of the membrane lipids whereas other parts of the electron transport chain remain unaffected by a phase transition [65]. (2) The other role of PQ in photosynthesis is the activation of a protein kinase, which catalyses phosphorylation of LHCP [11]; a reduced PQ pool leads to an activation of a kinase [68]. The LHCP phosphorylation appears to improve redistribution of excitation energy to PS I, resulting in a slow decrease in fluorescence yield [11]; a decrease in fluorescence yield with time of illumination is a well-known phenomenon (see e.g. [25]). The influence of PQH_2 on energy (re)distribution between PS II and PS I results in prevention of over-excitation of PS II and in stimulation of electron flow involving PS I, which indicates an important regulatory function for PQ [72]. (3) In a thermophilic cyanobacterium, PQ was shown to function in respiratory electron transport and, here, the PQ pool forms a link between photosynthesis and respiration [64]. Of course, in higher plants and eukaryotic algae such a direct link is not possible since respiration and photosynthesis occur in different organelles.

One would expect the proton uptake by PQ to oscillate with a period of 2 with flash number because the B/B^- ratio is >1 under normal conditions after dark adaptation. This is indeed observed [51]. However, the amplitude of the oscillation in proton uptake from the outside is smaller than the amplitude of proton release by PQH_2 into the inside [51]. Using cresol red as indicator of pH changes in the external phase no oscillation is observed [50]. Furthermore, Förster *et al.* [50] observed full proton uptake after the first flash in the presence of DCMU. In contrast, Velthuys [198] found H^+ uptake by PS II in the first flash to be fully inhibited by DCMU. Neither Q^- nor B^- appear to be protonated on a short time scale [144, 190–192]. Thus, Q^- cannot be directly responsible for the observed proton uptake in the presence of DCMU. It was suggested that a proton is taken up if the Q^- or B^- state is formed to serve as a specific counter ion without binding to the latter; for instance, semiquinone formation might induce the protonation of a neighboring proteinaceous group [50] as suggested earlier in bacterial systems [212].

Another problem is the stoichiometry of the number of protons taken up by PQ and the number of electrons transported through the electron transport chain. For photosynthetic bacteria, electron transport of one electron results in transport of two protons at the quinone level (e.g. see [212]). In a Q-cycle [119], an electron acceptor of the proton-translocating quinone is able to reduce, directly or indirectly, this quinone again, resulting in another proton translocation over the membrane. In this way, two protons per electron are translocated at the quinone level. If a Q-cycle type mechanism does exist in chloroplasts, then a H^+/e^- ratio of 3 would be expected for non-cyclic electron flow (1 H^+ per e^- produced at the oxygen-evolving site, 2 at the PQ level) whereas PS I cyclic electron flow would result in a H^+/e^- ratio of 2 (2 H^+ per e^- translocated at the PQ level). A ratio of 1:1:1 was reported [181] for external H^+ uptake, electron translocation through the PQ pool and internal H^+ release by PQH_2 in (presumably) non-cyclic electron flow using repetitive flashes. This observation suggests strongly that, at least in non-cyclic electron flow, no Q cycle (or a modification of it) occurs; earlier data also pointed to a $H^+ e^-$ ratio of 2 for non-cyclic electron flow [9, 159]. However, experiments by Velthuys [198, 199], Bouges-Bocquet [17] and Crowther *et al.* [32, 34] suggest that it might be wrong to conclude that a (modified) Q cycle does not exist at all in chloroplasts: a slow phase ($t_{1/2} \sim 10$ ms) in the rise of

the flash-induced change in absorbance in the 515 nm region is observed if the PQ pool is prereduced. The absorption spectrum for this slow phase matches that of the fast ΔA_{515}; the 515 nm change is due to an electric-field induced shift of the absorption spectrum of certain Chl *b* and Car molecules, and is assumed to be proportional to the electric field near these molecules [38, 78]. Therefore, the slow phase of ΔA_{515} may also be related to a change in transmembrane potential. Moreover, proton uptake from the external medium appears to be related to this slow phase [198]. Addition of DCMU after the reduction of the PQ pool does not inhibit the slow 515 nm change. This implies that the slow absorption change might be generated in PQH_2 oxidation by PS I. As mentioned before, in the presence of DCMU, a proton uptake from the outside is indeed observed; this uptake is probably due to a neutralization of charges at the quinone level [50]. Thus, one has to separate carefully this proton uptake from the 'electrogenic' proton uptake, i.e. the proton uptake due to a Q-cycle mechanism. The slow phase in the 515 nm absorption change (phase b) is inhibited by DBMIB, suggesting that quinones and/or Rieske FeS centers are involved in the pathway generating phase b [16, 32, 166]. (Phase 'a' refers to the fast phase in ΔA_{515}.) The slow phase in the electrochromic change can be produced by both PS II and PS I acting together [17]. However, Slovacek *et al.* [167] suggested that the slow phase in intact chloroplasts is related to cyclic electron transport around PS I only, as in the presence of MV, using repetitive flash illumination, no phase b is observed. However, under these experimental conditions, the PQ pool was partly oxidized, and, thus, the lack of phase b (mentioned above) does not necessarily indicate that the slow phase is only caused by cyclic PS I electron transport. The observation of a slow phase in the flash-induced ΔA_{515} only after the reduction of the PQ pool [198] does not necessarily mean that it is absent if PQ is oxidized: perhaps, phase b becomes slower because of less PQH_2 and, then, the rise could be masked by the decay of the fast component in the ΔA_{515} (see also [200]).

A DCMU-sensitive cyt b_{563} reduction is observed if dark-adapted chloroplasts are subjected to light flashes; this reduction oscillates in synchrony with PQH_2 formation [199]. Therefore, cyt b_{563} may function not only in cyclic [166] but also in non-cyclic electron transport. After comparing cyt b_{563} reduction and oxidation kinetics with those of cyt *f*, one of the electron acceptors of PQH_2, a scheme was presented [199] in which cyt b_{563} accepts one of the electrons from PQH_2 while the other one is accepted by a 'regular' electron acceptor like cyt *f* or the Rieske FeS center. Two reduced cyt b_{563} (or one reduced cyt b_{563} and another one-electron acceptor in the reduced form) might be able to reduce PQ again, two protons are taken up from the outside and PQH_2 is formed ('electrogenic loop'). This scheme results in a doubling of the H^+/e^- ratio at the PQ site [199]. The scheme implies that the two reductants (two cyt b_{563} molecules or one cyt b_{563} and one other molecule) are physically close together.

A DCMU-sensitive cyt b_{563} reduction by PS II has been observed by Böhme [14, 15]. However, this reaction is DBMIB-insensitive [14]; this casts doubt on the proposal that cyt b_{563} may accept electrons, together with cyt *f* or the Rieske center, from PQH_2 as this oxidation of PQH_2 by PS I is DBMIB-sensitive. However, if this sensitivity is due to the binding of DBMIB to the Rieske FeS center as EPR data may suggest [29, 143], then cyt b_{563} reduction that is not coupled to the reduction of the Rieske FeS center need not be DBMIB-sensitive. We cannot distinguish, at the moment, between a direct reduction of cyt b_{563} by B^{2-}, an electron transfer from PQH_2 to cyt b_{563} (coupled or not to the reduction of cyt *f* or the Rieske FeS center), or another mechanism of DCMU-sensitive cyt b_{563} reduction. It should be pointed out that the cyt b_{563} turn-overs mentioned here are not related to the PS II-induced cyt b_{563} reduction proposed by Joliot and Joliot [75, 76] because the latter is DCMU-insensitive. Olsen *et al.* [134] reported that the reductions of cyt *f* and cyt b_{563} occur at the same rate, and both are inhibited by bathophenanthroline, which inhibits electron transfer to cyt *f*, to the same extent. These observations are in full agreement with Velthuy's hypothesis. However, other data suggest a difference between cyt b_{563} and cyt *f* kinetics [32]. Furthermore, Olsen *et al.* [134] showed that only one H^+ per e^- is extruded inside the thylakoid by the PQ pool whether cyclic or non-cyclic electron flow is measured. In contrast to these data, Velthuys [200] showed that the proton release per electron by the PQ pool is about twice as high as the proton release by water oxidation, suggesting that there is an electrogenic loop connected to the PQ pool. The problem becomes even more confusing when considering that in intact *Chlorella* cells cyt b_{563} does not show absorbance changes related to phase b [17]; this suggests that cyt b_{563} is not involved in the electrogenic reaction [17]. However, absorption changes indicating the reduction of a component C [17] or X_2 [18], possibly a FeS center, are correlated with phase b of ΔA_{515}. C or X_2 is not the Rieske FeS center as the former has an E_m lower than +100 mV, whereas the latter has an E_m of +290 mV.

It has been suggested that C or X_2 is reduced by the semiquinone form ($\dot{U}H$) of a bound PQ called U ([18]; see Fig. 2). This $\dot{U}H$ might be formed, in turn, by the oxidation of UH_2 by the Rieske FeS center. In addition to these components, two other components have been proposed to exist: one labelled as V and another that has an E_m of −55 mV at pH 8.1 [32]. This −55 mV component might be involved in the slow electrochromic shift as phase b of ΔA_{515} is present only when this component is reduced. It is possible that this −55 mV component might be identical to C, X_2 or V, which may also be reduced by $\dot{U}H$. This −55 mV component is not identical to U: Bou-

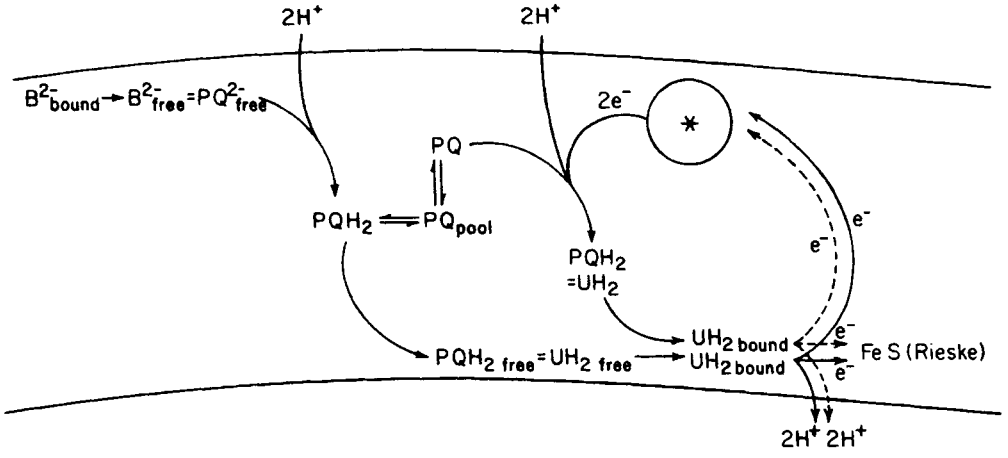

Figure 2. Proposed mechanism for an electrogenic loop in chloroplasts. In this scheme, B is a normal PQ molecule, but is bound to the Q/B apoprotein. B^{2-}, when formed, is released from its binding site on the Q/B apoprotein, and is protonated, resulting in PQH_2. Alternatively, it may be the quinol rather than the electrically charged form of the quinone that is able to leave its binding site [201]. The formed PQH_2 may or may not exchange with a molecule from the 'bulk' PQ pool; and PQH_2 is bound to a site on the Rieske FeS protein. This bound PQH_2 we identify as UH_2. Therefore, U is nothing more than a bound PQ. UH_2 is oxidized by the Rieske FeS center, yielding ŮH; ŮH is oxidized, directly or indirectly, by a pool of components indicated by ⊛, which contains C, X_2, V and/or cyt b_{563}. The two protons resulting from the UH_2 oxidation are released into the inside of the thylakoid membrane (bottom of figure). It is assumed that ⊛ has one stored electron before one more electron is transported from ŮH to it. With the two electrons in ⊛ after oxidation of ŮH, it may transfer these two electrons to a PQ molecule, which is protonated by protons from the outside of the thylakoid membrane (top of figure). The formed PQH_2 may bind to the site near the Rieske FeS center, and transfer one electron to this center and one electron to ⊛, bringing ⊛ back to its original state. This results in an overall translocation of 4 protons per turnover of B (2 electrons). (See text.)

ges-Bocquet [18] has calculated that the E_m of U-oxidized U-reduced (probably U UH_2) is ~40 mV higher than the E_m of PQ-oxidized/PQ-reduced (which is +100 mV, pH 7.2 [56]); thus, E_m of U/UH_2 is in the +140 mV range, which is very different from −55 mV.

A scheme that accounts for most of the data described above is given in Fig. 2 (see its legend for explanation). This figure, adapted and extended from schemes proposed by Velthuys [199] and Crowther and Hind [33], describes how flow of two electrons through B and the Rieske FeS center can give rise to a translocation of 4 protons at the PQ level.

The 'electrogenic loop' seems to be one of the areas where different laboratories using almost the same techniques have obtained contradictory results, especially concerning the oxidation/reduction kinetics of cyt b_{563} and concerning the number of protons, released from the PQ pool, per electron.

We have assumed, thus far, that phase b in the ΔA_{515} is due to the 'electrogenic loop'. This assumption is indeed reasonable: this slow phase is decelerated considerably, both in its rise and decay kinetics, when ^2H_2O replaces ^1H_2O in the medium [48]; therefore, protons may be involved in this phase. However, there may be other interpretations for a slow change in ΔA_{515}: in intact chloroplasts, under certain conditions, a much slower rise ($t_{1/2}$ ~ 100–150 ms) of a phase in ΔA_{515} is observed [163]. This phase, probably, cannot be ascribed to a proton translocation because such a phase is not observed if the transmembrane potential is measured by a microelectrode [27]. This 'very slow' phase has been interpreted to reflect a slow intramembrane structural change induced by field-dependent charge displacement in the vicinity of the molecules which undergo a shift in absorption spectrum giving rise to the ΔA_{515}. This very slow phase can be activated by PS I alone [162]. Although it is clear that this very slow rise in ΔA_{515} is not identical to phase b because of the difference in $t_{1/2}$ (150 and 15 ms, respectively), some conformational changes in the membrane may yet be related to phase b. Another hypothesis to explain phase b in terms other than an 'electrogenic loop' was proposed by Olsen and Barber [133]. Assuming a large distance between the site of charge separation and of the molecules responsible for the electrochromic shift (P515 molecules), the electric field at the site of the P515 molecules is expected to be small. When proton translocation through the PQ pool occurs yielding a free-moving OH^- at the outside and a free-moving H^+ at the inside, the 'charge separation' is no longer localized and the electric field over the membrane becomes homogeneous. This might result in an increase in the electric field at the site of the P515 molecules, and, thus, in an increase in the ΔA_{515} reflecting the proton

translocation through the PQ pool. However, such a model does not account for the disappearance of the slow phase if the PQ pool becomes oxidized [16, 198] because proton translocation is still possible under these conditions.

Related intermediates

As defined above, the acceptor (reducing) side of PS II includes electron flow from P680 to the PQ pool (Fig. 1). We have discussed already the reactions up to the PQ level. In the following, we include a brief description of the Rieske FeS center, which, as stated before, might be an electron carrier between PQ and cyt *f*. Also included is a description of two related areas: use of EPR in the study of cytochromes and ferredoxin reduction. Although ferredoxin is on the reducing side of PS I, it is mentioned here because some of the ideas might become relevant to the acceptor side of PS II.

(a) *The Rieske FeS center.* An FeS center has been detected in chloroplasts at cryogenic temperatures [29, 103] with an EPR signal ($g = 1.90$ and 2.03 [143]) analogous to the Rieske protein observed in mitochondria [155]. It is probably a 2Fe2S center [158]. It has been shown that this FeS center can be photooxidized by PS I and photoreduced by PS II indicating that the center is located between the two photosystems [211]. The photoreduction of the center is DCMU-sensitive, and the photooxidation is DCMU-resistant [102] indicating a location between B and P700. A site of location of the FeS center between PQ and cyt *f* has been proposed [103]. Furthermore, DBMIB, which blocks PQH_2 oxidation, has been shown to change the EPR spectrum of the Rieske center: only a $g = 1.95$ signal has been observed after the addition of DBMIB [29, 143]. This is strong evidence that DBMIB interacts with the FeS center directly in such a way that the center no longer can be reduced by PQH_2. However, Rich *et al.* [154] were unable to detect a change in the EPR spectrum of the Rieske center by DBMIB.

(b) *Use of EPR to detect cytochromes.* Studies of cytochrome redox reactions by means of absorbance changes are often complicated because of the overlapping spectra of the various cytochromes involved in photosynthesis. Therefore, Malkin and Vänngård [104] attempted to detect cytochrome Fe(III)-EPR signals at cryogenic temperatures. Cyt b_{559} was detected with a *g*-value of 2.9–3.0 whereas the cyt *f* signal showed a *g*-value of 3.5 [104]. Similar EPR signals attributed to oxidized cyt *b* were later observed by Nugent and Evans [122]. High-spin haem iron(III)-EPR signals at approx. $g = 6$ were also reported by both groups [104, 122]. The use of a partially purified cyt *b/f* complex and selective reduction of cytochromes confirmed that the $g = 3.0$ signal is due to cyt b_{559} and the $g = 3.5$ signal to cyt *f*. The redox potential had to be decreased rather dramatically (to -100 mV) before the high-spin haem signals disappeared. Therefore, these high-spin signals were tentatively assigned to cyt b_{563} [154]. Although with the EPR method well-separated signals for cytochromes are obtained, the measurements have to be performed at cryogenic temperatures, making kinetic measurements of cytochrome redox changes virtually impossible.

(c) *Ferredoxin reduction.* Arnon and coworkers claimed that Fd (ferredoxin) photoreduction (detected by EPR at low temperature), normally thought to occur only on the reducing side of PS I, takes place in a reaction in the presence of DBMIB [4] and DNP-INT [6] that are known to block electron flow between the two photosystems. However, this reaction is blocked by DCMU [5]. It is possible that Fd is reducible by PS II, perhaps, via the PQ pool? The so-called 'PS II-Fd' may not be different from the PS I reducible Fd, although differences in reoxidation of PS I and 'PS II-reduced' Fd have been claimed [4]: a PS II-induced cyt b_{563} reduction is possible, as we have seen before. Furthermore, Fd is involved in cyclic electron transport around PS I [3]. If we assume that the jump in the actual redox potential between cyt b_{563} and Fd is not too large, then Fd might be reduced in a DCMU-sensitive reaction from H_2O via PQ and cyt b_{563} to Fd; the last part of this pathway would then be a reversed electron flow. This would be an inefficient way of reducing Fd and would require energy input. (Compare with an old scheme [59].) Before we can determine whether the proposed Fd reduction by PS II only is of any significance, quantum yield and action spectra measurements should be performed at room temperature. Although there is no acceptable evidence for the reduction of Fd by PS II, the following reaction occurs: a Fd-dependent reduction of PQ and, probably, also of a part of Q by NADPH in the dark [117, 118].

Conclusions and Summary

(1) There is overwhelming evidence now that Pheo is an intermediate in electron transport from the reaction center P680 to the quinone Q.

(2) There is heterogeneity in electron transport chains; the latter may differ from each other at the Q level. Possibly, there is also heterogeneity in the size or the composition of the PS II units.

(3) Herbicides that block reoxidation of Q^- might do so by replacing the two-electron gate quinone B.

(4) Both Q and B appear to be embedded in a 32 kD protein.

(5) HCO_3^- or CO_2 fulfils a unique role in allowing electron transport at the quinone level.

(6) Plastoquinone (PQ) plays a key-role in photosynthetic electron transport; it acts as a proton translocator; its reoxidation is the rate limiting step of the electron transport chain; and, its redox state may influence the energy distribution between the two photosystems.

(7) The existence of an 'electrogenic loop' in chloroplasts at the PQ level, involving cyt b_{563}, an FeS

center and/or specialized PQ molecules, is still uncertain.

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