Biochimica et Biophysica Acta, 634 (1981) 105—116

BBA 47985

EVIDENCE FOR A CLOSE SPATIAL LOCATION OF THE BINDING SITES FOR CO_2 AND FOR PHOTOSYSTEM II INHIBITORS

RITA KHANNA, KLAUS PFISTER, ÁRON KERESZTES *, JACK J.S. VAN RENSEN ** and GOVINDJEE ***

Departments of Physiology and Biophysics, and Botany, University of Illinois, Urbana, IL 61801 (U.S.A.)

(Received July 23rd, 1980)

Key words: Photosystem II inhibitor; Atrazine; Bicarbonate effect; CO_2-binding site; Hill reaction; Electron flow; Electron microscopy; Membrane structure

Summary

1. CO_2-depletion of thylakoid membranes results in a decrease of binding affinity of the Photosystem II (PS II) inhibitor atrazine. The inhibitory efficiency of atrazine, expressed as I_{50}-concentration (50% inhibition) of 2,6-dichlorophenolindophenol reduction, is the same in CO_2-depleted as well as in control thylakoids. This shows that CO_2-depletion results in a complete inactivation of a part of the total number of electron transport chains.

2. A major site of action of CO_2, which had previously been located between the two electron acceptor quinone molecule B (or R) and plastoquinone, appears to be in the same membrane protein which binds the Photosystem II inhibitor atrazine as suggested by the following observations: (a) CO_2-depletion results in a shift of the binding constant (k_b) of [^{14}C]atrazine to thylakoid mebranes indicating a decreased affinity of atrazine to the membrane; (b) trypsin treatment, which is known to modify the Photosystem II complex at the level of B, strongly diminishes CO_2 stimulation of electron transport reactions in CO_2-depleted membranes; and (c) thylakoids from atrazine-resistant plants, which contain a Photosystem II complex modified at the inhibitor binding site, show an altered CO_2-stimulation of electron flow.

3. CO_2-depletion does not produce structural changes in enzyme complexes involved in Photosystem II function of thylakoid membranes, as shown by freeze-fracture studies using electron microscopy.

* On leave from the Department of Plant Anatomy, Eötvös Loránd University, Budapest, Hungary.

** On leave from the laboratory of Plant Physiological Research, Agricultural University, Wageningen, The Netherlands.

*** Correspondence should be addressed to: Professor Govindjee, Department of Botany, 289 Morrill Hall, University of Illinois, Urbana, IL 61801, U.S.A.

Introduction

A major site of CO_2 effect in the electron transport from water to nicotinamide adenine dinucleotide phosphate in thylakoid membranes has been shown to be between the two electron carrier quinone B (or R) and plastoquinone (for a review, see Govindjee and Van Rensen [1]). We do not yet know how CO_2 affects this reaction. It is plausible that this effect is through the binding of CO_2 to a protein that affects the function of B. Recent experiments (see Pfister and Arntzen [2]; and Arntzen et al. [3]) have indicated that several Photosystem II (PS II) herbicides (e.g., atrazine) inhibit electron flow by binding to a protein close to 'B'. Thus, in order to test whether CO_2 also binds to the same protein, we have measured the binding of [^{14}C]atrazine to CO_2-depleted thylakoid membranes and to CO_2-depleted membranes resupplied with CO_2. In addition, we report the effect of CO_2 on electron flow in (a) thylakoids from plants that are resistant to atrazine and have their 'B-protein' affected [2]; and (b) thylakoids treated with trypsin which is known to affect the B-region of the Photosystem II complex (Trebst [4], Renger [5]). The results of these experiments suggest that CO_2 binds close to the binding site of the Photosystem II inhibitor atrazine, which binds close to or at the 'B-protein' [3].

When thylakoids are depleted of CO_2, different degrees of CO_2-stimulation are obtained depending on how well the depletion had taken place. Usually only partial, although large, depletion is achieved. We were interested in determining whether the individual membranes are partially inactivated or whether some membranes are unaffected and the CO_2-depleted ones are totally inactivated. If the former were true, then a 50% inhibition of the Hill reaction in CO_2-depleted thylakoids would need a different concentration of atrazine than in the control. However, if the depletion process totally inactivates the CO_2-depleted membranes, then the 50% inhibition would occur at the same concentration of atrazine as in the control. Results reported in this paper support the latter case.

Since CO_2 depletion requires the suspension of thylakoids in a low pH buffer, it seemed important to test whether this treatment affects structurally the thylakoid membranes. In this paper, we report on the freeze-fracture electron microscopy of CO_2-depleted and control thylakoid membranes. Our results indicate that the CO_2-depletion procedure does not alter the size and the distribution of membrane particles.

Materials and Methods

Isolation of thylakoid membranes. Thylakoids were prepared from *Amaranthus hybridus* (pigweed) grown as described by Arntzen et al. [6] or from pea-leaves grown in a growth chamber. Thylakoid membranes (prepared according to Stemler et al. [7]) were suspended in a medium containing 50 mM phosphate (pH 5.0), 100 mM sodium formate and 100 mM NaCl ([Chl], 50 μg/ml). N_2 gas was passed over the suspension while it was shaken gently for about 15 min in the dark at 23°C (also see Stemler et al. [7]). The suspension was transferred with the help of a syringe into cold screw-capped tubes previously flushed with N_2 gas. After centrifugation, these tubes were stored on ice until use. The

supernatant was discarded just before the assay and the thylakoid membranes were suspended in a buffer containing 50 mM phosphate (pH 6.8), 100 mM sodium formate and 100 mM NaCl.

Electron transport rates. The steady-state O_2 evolution rate of thylakoid membranes under continuous light was measured with a Pt/Ag-$AgCl_2$ Clark electrode, using a oxygen monitor (Model 53) and an Esterline Angus recorder (Model E11015). The sample chamber (2 ml) was temperature-regulated at 25°C by circulating water from a constant temperature bath through the water jacket surrounding the reaction vessel. Saturating continuous illumination (250 mW/cm^2) was provided by light from an incandescent lamp passed through a Corning CS3-71 glass filter and an additional 2-inch water filter. Thylakoids were suspended at a chlorophyll concentration of ~30 μg/ml in the appropriate buffer with 0.5 mM $[Fe(CN)_6]^{3-}$ as an electron acceptor. In experiments on atrazine resistant and susceptible thylakoids, 1 mM NH_4Cl was also included in the reaction mixture.

Photosystem II (PS II)-mediated 2,6-dichlorophenolindophenol (DCIP) reduction was measured at 580 nm using a Hitachi Model 100-60 spectrophotometer. The sample, containing 5 μg chlorophyll/ml, was illuminated from the side through a red Corning CS2-58 filter. A blue Corning CS4-96 filter was placed in front of the photomultiplier to protect it from the scattered actinic light. Electron transport rates were calculated from direct recordings of the absorbance change using the extinction coefficient of DCIP as described by Armstrong [8].

Herbicide-binding studies. For herbicide-binding analysis, thylakoid membranes were isolated in phosphate buffer and depleted of bicarbonate. Binding studies were conducted in the assay medium (50 mM phosphate (pH 6.8), 100 mM formate and 100 mM NaCl) according to the procedure described by Pfister et al. [9] and Tischer and Strotmann [10]. Suspension medium (1 ml) for binding experiments contained 2—10 μl of ^{14}C-labelled atrazine (5.37 Ci/mol) and 50 μg chlorophyll/ml. Atrazine was dissolved in methanol and the final methanol concentration in the incubation medium was less than 1%. After 2 min incubation under dim room light at 22°C, the samples were centrifuged for 3 min at 12 000 × g in an Eppendorf 5415 centrifuge. Aliquots (0.8 ml) of the clear supernatant were removed and added to 9 ml of Phase Combining System-scintillation fluid (Amersham-Buchler). Radioactivity of the samples was measured by a liquid scintillation counter (Searle Analytic Inc., Model Delta 300). The amount of bound inhibitor was calculated from the difference between the total inhibitor added to the thylakoids and the amount of free inhibitor found in the supernatant after centrifugation. A plot of the inverse of the concentration, $1/b$, of bound inhibitor in nM per mg chlorophyll as a function of the inverse of the concentration $1/f$, of free inhibitor in solution permitted a calculation of the concentration, X_I, of the inhibitor binding sites, and of the binding constant, K_b, from the relationship:

$$\frac{1}{b} = \left(\frac{K_b}{X_I}\right)\frac{1}{f} + \frac{1}{X_I} \qquad (1)$$

Trypsin treatment. Trypsin treatment of thylakoid membranes was done

according to the method of Renger [11]. Thylakoid membranes were depleted of bicarbonate and suspended (40 μg chlorophyll/ml) in a medium containing 50 mM phosphate (pH 6.8), 100 mM NaCl and 100 mM sodium formate. Trypsin from bovine pancreas, Type XI (8675 units/mg protein) was added to a final concentration of 40 μg/ml and incubated at room temperature (25°C) for different time periods (1—5 min). The typsin action was stopped by adding a 20-fold excess of trypsin inhibitor (from soybean, Type I-S, Sigma).

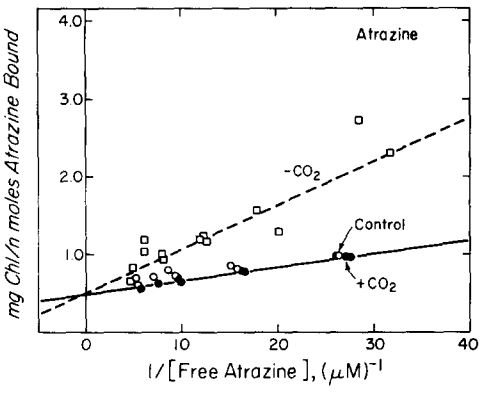
Freeze-fracture electron microscopy. Freeze-fracturing was performed on unfixed isolated thylakoids without addition of glycerol in order to have the same conditions as those of the photochemical assays. In spite of the lack of cryoprotectant, most preparations were free of ice crystals, except occasional crystals in the thylakoid lumen rather than in the medium. Replicas were prepared on a Balzers BAF 301 freeze-etching apparatus as previously described [12]. After cleaning in bleach and 70% H_2SO_4, replicas were examined in a Hitachi 300 electron microscope. Particle diameters were measured on electron micrographs using a magnifier fitted with a micrometer scale calibrated to 0.1 mm.

Results and Discussion

Herbicide binding to bicarbonate-depleted pea thylakoid membranes

Binding of the radioactive PS II-inhibitor atrazine was studied in normal, bicarbonate-depleted ($-CO_2$) and reconstituted ($+CO_2$) thylakoids. The amount of [^{14}C]atrazine bound to thylakoid membranes increased with increasing amounts of atrazine (0.053 to 0.26 nmol) added to the suspension ([Chl], 50 μg). We analyzed the binding process in a double reciprocal plot (mg chlorophyll/nmol bound atrazine ($1/b$) vs. $1/\mu$M free atrazine ($1/f$)). From this plot (Eqn. 1 and Fig. 1) two parameters were obtained: (1) the intercept on the ordinate ($1/X_I$) that gives the number of chlorophyll molecules per binding site; and (2) the intercept on the abscissa ($-1/K_b$) that is a measurement of the affinity of the inhibitor towards its receptor site at the membrane. In these double reciprocal plots for control, $-CO_2$, and $+CO_2$ samples, the intercepts on the ordinate were approximately the same indicating that the number of binding sites is not affected by the depletion procedure. Binding for the three samples (control, $-CO_2$ and $+CO_2$) is saturated at 2 nmol/mg Chl, i.e., 1 binding site per 500 chlorophyll molecules. This ratio agrees well with earlier reported values [9].

From the double reciprocal plots, the K_b values for control, bicarbonate-depleted and reconstituted samples were calculated as $3.4 \cdot 10^{-8}$, $1.2 \cdot 10^{-7}$ and $4.9 \cdot 10^{-8}$ M, respectively. This implies that in the bicarbonate-depleted sample the affinity of atrazine to its binding site is reduced. It appears that the absence of bicarbonate somehow changes the 'conformation' of the herbicide-binding protein located near the reducing site of PS II, and, this change may lead to a change in the affinity of the herbicide for the protein. Upon the addition of bicarbonate to CO_2-depleted thylakoids, the binding affinity for atrazine is restored and becomes comparable with that of the control. The binding sites for CO_2 and for Photosystem II inhibitors are, in all likelihood, at different locations on the same membrane protein.



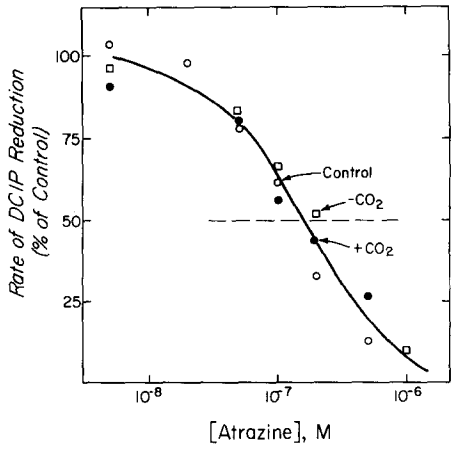


Fig. 1. Binding of [^{14}C]atrazine to control, CO_2-depleted and reconstituted (+20 mM $NaHCO_3$) pea thylakoids. Double reciprocal ([mg Chl]/[nM bound atrazine]) vs. 1/[μM free atrazine]) plots are shown. Thylakoid membranes were incubated at 23°C with various concentrations of ^{14}C-labelled atrazine. The amount of bound inhibitor was calculated from the difference between the total radioactivity added to the thylakoid suspension and the amount of free inhibitor found in the supernatant after centrifugation.

Fig. 2. Inhibition of 2,6-dichlorophenolindophenol (DCIP) reduction by atrazine in control, CO_2-depleted and reconstituted (+20 mM $NaHCO_3$) pea thylakoids at 23° C. Thylakoids were suspended, at a concentration of 5 μg Chl/ml, in a medium containing 50 mM phosphate (pH 6.8), 100 mM NaCl, 100 mM sodium formate, 0.05 mM DCIP, 1 mM NH_4Cl, and 1 μM gramicidin. All values without atrazine were normalized to 100; control thylakoids had a rate of 100—120 μmol O_2/mg Chl per h without atrazine.

Upon bicarbonate depletion, a fraction of the thylakoid membranes remains unaffected, i.e., a fraction still shows normal electron flow. From the herbicide-binding studies it appears that bicarbonate depletion changes the affinity of the herbicide to its binding site. We were interested in determining whether all thylakoid membranes are partially affected or only a fraction of the membranes is completely inactivated. This was tested by measuring the concentration dependence of the inhibitory effect of atrazine on the rate of PS II electron transport (Fig. 2). From the data shown in Fig. 2, I_{50} values (concentration of herbicide giving half-maximal inhibition of electron transport) were calculated to be ~0.2 μM for all three cases: control, —CO_2 and +CO_2. If bicarbonate depletion affects the affinity of atrazine for the total population of thylakoids, we would expect to see a different I_{50} value for the depleted (—CO_2) thylakoids. However, the I_{50} value of thylakoids did not change upon CO_2-depletion suggesting that the membranes which do not undergo depletion have the same affinity for atrazine as the control or reconstituted thylakoids. Therefore, the difference in binding affinities (as calculated from herbicide-binding experiments, Fig. 1) must be due to a change in the affinity of only those thylakoid membranes which were inactivated completely by depletion of CO_2. (Thus, it seems that the binding affinity (K_b) we have measured in CO_2-depleted samples is that of a mixed population of CO_2-depleted and control membranes. This indicates that the true K_b for a completely CO_2-depleted population of thylakoids is much higher than the $1.2 \cdot 10^{-7}$ M reported above.)

We conclude that bicarbonate depletion of a single electron transport chain results in the complete loss of activity of that particular chain, and in addition, the inhibitor affinity is drastically changed. This conclusion is not in contradiction to previous findings [1,7,13,14,15] showing changes in kinetics of electron flow in the Q-B-PQ region as a result of CO_2-depletion because in these experiments an average (or mixed) kinetics of active and inactivated electron transport chain is measured.

The bicarbonate effect in atrazine-resistant and -susceptible Amaranthus hybridus thylakoids

The properties of atrazine-resistant and -susceptible *A. hybridus* chloroplasts were extensively studied by several investigators (for a review, see Ref. 2). It appears from this work that in these chloroplasts the inhibitor-binding site (the B-protein) is slightly modified, which results in a high degree of resistance towards several PS II inhibitors. We were interested in studying whether this modification of the herbicide-binding protein (the B-protein) might also affect the bicarbonate binding. Although the bicarbonate stimulation of Hill reaction was observed on CO_2-depleted thylakoids from both the resistant and susceptible plants, there was a much larger effect (Fig. 3) in the thylakoids from the atrazine-resistant ones because it was easier to deplete them of CO_2 (Table I). Thus, it seems that in resistant *A. hybridus* thylakoids bicarbonate is more loosely bound (lowered affinity) and is more easily removed. This is supported by the experiment (Fig. 3) in which we measured reactivation of the Hill reaction after addition of various concentrations of bicarbonate to CO_2-depleted chloroplasts of atrazine-resistant and susceptible pigweed. In the Lineweaver-Burk plot (Fig. 4) the intercept on the abscissa yields the inverse of the Michaelis constant K_m for CO_2-binding. The K_m is 1.02 mM in susceptible chloroplasts, close to the value of 1 mM which can be calculated from the data of Khanna et al. [13]. In the resistant chloroplasts the K_m appears to be 1.82 mM, i.e., the affinity of the CO_2-binding protein is lower in resistant chloroplasts. A change in the herbicide-binding protein also changes the binding of CO_2 indicating a close relationship between the binding of CO_2 and of PS II inhibitor atrazine.

Bicarbonate effect in trypsin-treated pea thylakoid membranes

The proteolytic enzyme trypsin is described as a modifying agent of thyla-

TABLE I

BICARBONATE EFFECT ON THE HILL REACTION IN ATRAZINE-SUSCEPTIBLE AND ATRAZINE-RESISTANT *AMARANTHUS* THYLAKOIDS

Hill reaction rates are expressed in μmol O_2/mg Chl per h; average control rate of susceptible chloroplasts was 90 μmol O_2/Chl per h; and that of resistant chloroplasts was 75 μmol O_2/mg Chl per h. The number of experiments is 6.

	Susceptible			Resistant		
	$-CO_2$	+10 mM CO_2	Ratio $+CO_2/-CO_2$	$-CO_2$	10 mM CO_2	Ratio $+CO_2/-CO_2$
Mean (±σ)	14.5 (±3.3)	32.4 (±5.2)	2.3 (±0.35)	7.1 (±1.4)	33.5 (±6.8)	5.1 (±2.0)

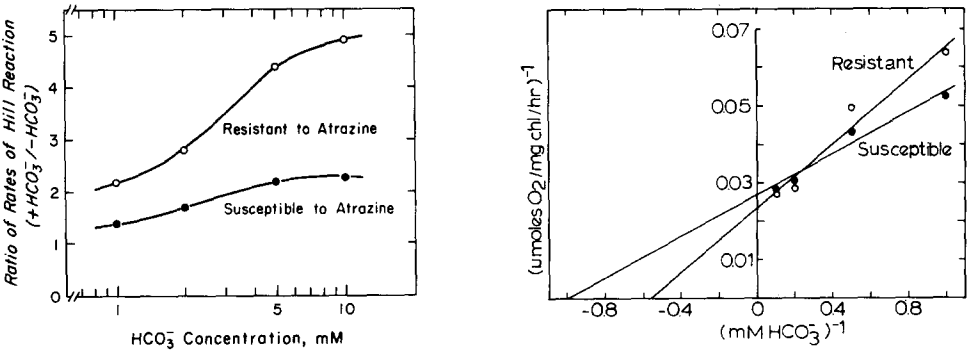


Fig. 3. Ratio of electron transport in the presence of various bicarbonate concentrations over that in its absence in atrazine-resistant and susceptible *Amaranthus hybridus* thylakoids. See Materials and Methods for details.

Fig. 4. Double reciprocal plots of electron transport rate (H_2O to ferricyanide) vs. bicarbonate concentrations in CO_2-depleted atrazine-resistant and susceptible *A. hybridus* chloroplasts (also see Fig. 3).

koid membranes. One very specific effect of trypsin is the digestion of a protein of the PS II complex [5]. There is now evidence that during this process trypsin inactivates the function of the PS II acceptor B as well as the binding sites of the PS II inhibitors (see e.g., Ref. 3). Govindjee et al. [14] and Siggel et al. [15] showed that CO_2 depletion results in an inactivation of the electron transfer from reduced B to plastoquinone. Thus, it seems reasonable that bicarbonate might be acting via the same protein which is proposed to be the target of the PS II inhibitors on the reducing side of PS II.

Fig. 5 demonstrates the bicarbonate effect on the Hill reaction in trypsin treated thylakoid membranes. After bicarbonate depletion thylakoid membranes were subjected to trypsin treatment for varying time periods. As the time of trypsin treatment was increased from 1 min to 5 min the stimulation of

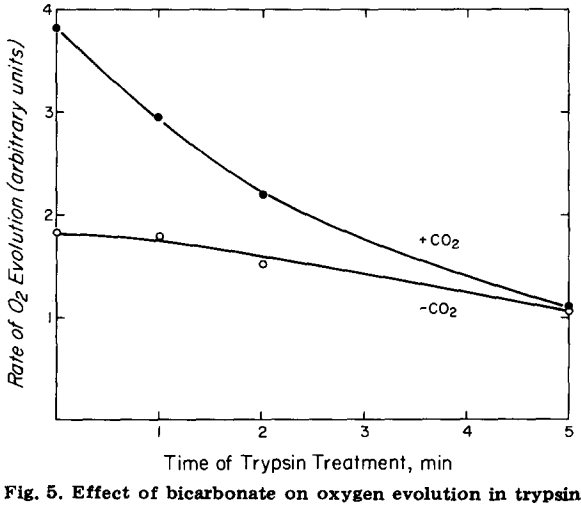


Fig. 5. Effect of bicarbonate on oxygen evolution in trypsinated pea thylakoid membranes at 25°C. Pea thylakoids were depleted of bicarbonate and treated with trypsin (40 μg/ml) at a chlorophyll concentration of 40 μg/ml for different time periods (1—5 min). Upper curve (filled circles), with 10 mM $NaHCO_3$; lower curve (open circles), without $NaHCO_3$. $[Fe(CN)_6]^{3-}$, 0.5 mM.

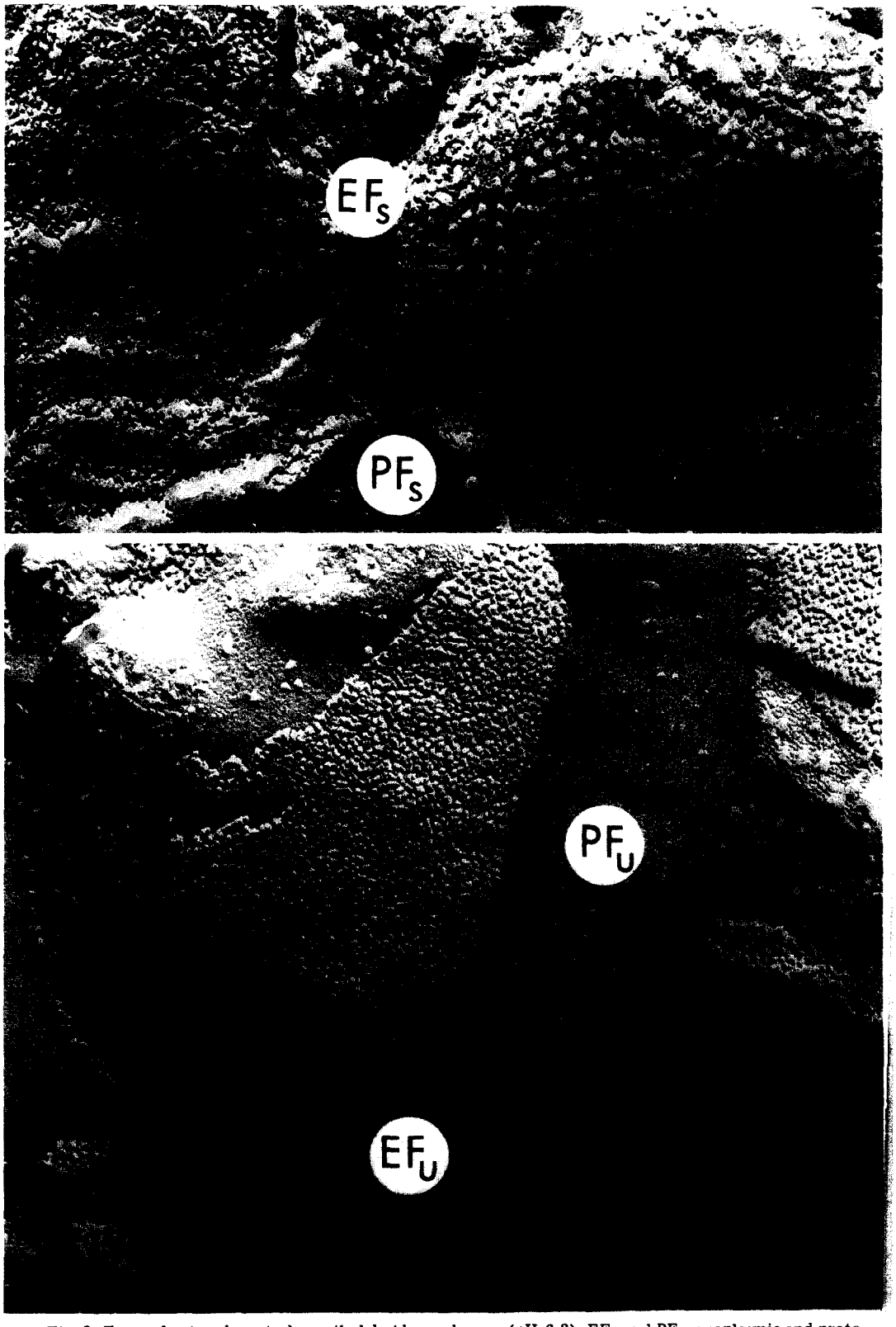


Fig. 6. Freeze-fractured control pea thylakoid membranes (pH 6.8). EF_s and PF_s, exoplasmic and protoplasmic fracture faces of stacked thylakoids; EF_u and PF_u, the same for unstacked thylakoids. The arrow in the right lower corner shows the direction of shadowing. Magnification: 110 000×.

electron flow by bicarbonate was reduced (Fig. 5). Without trypsin treatment 10 mM bicarbonate showed a 2-fold stimulation in these particular preparations. After a 1-min trypsin treatment, bicarbonate stimulated O_2 evolution by only 1.6-fold. Trypsin treatment for 5 min completely eliminated any effect of added bicarbonate.

The data of Fig. 5 show that with an increase in the time of trypsin treatment there is a decrease in the rate of electron flow both in CO_2-depleted and reconstituted samples. This may be due to an inactivation of the electron transport chain at the level of B. The decrease in electron flow by trypsin treatment is different for the two cases, i.e., 33% and 68% for CO_2-depleted and reconstituted samples, respectively. Since CO_2-depletion results in a complete inactivation of a single chain (Fig. 2), a further effect of trypsin on this chain cannot be seen. This may explain the comparatively lower effect of trypsin on CO_2-depleted chloroplasts or the lower degree of bicarbonate stimulation of the electron transport in trypsin treated chloroplasts. Although the present data do not provide any evidence for a mechanism, we may speculate that bicarbonate functions as an allosteric regulator of the electron flow from the electron acceptor Q (or X 320) to the secondary electron acceptor B (also a quinone) and finally from B^{2-} to the PQ pool. Bicarbonate binding to the protein component (close to or at the B protein) may place it in a proper orientation thereby making the functional connection between B and the PQ pool. Trypsin treatment may alter the bicarbonate binding site in such a way that bicarbonate can no longer bind to it. Since trypsin treatment also alters the binding site for the herbicide diuron (DCMU) or other PS II inhibitors (e.g., atrazine [3]), a very close spatial relationship between the bicarbonate-binding site and the site of action of diuron is suggested. This conclusion is consistent with the binding studies done by Stemler [16]. He observed that the removal of bound bicarbonate by silicomolybdate is inhibited if diuron (DCMU) is added prior to washing with silicomolybdate, suggesting a close spatial relationship between the binding sites of bicarbonate and diuron (DCMU). However there is no direct competition between bound atrazine and added bicarbonate or formate (data not shown).

Freeze-fracture electron microscopy of bicarbonate-depleted pea thylakoids

Fracture faces of CO_2-depleted thylakoids (at pH 5.0) showed no visible differences as compared to those of the control (pH 6.8) when examined by freeze-fracture analysis. The only exceptions were small areas where the membranes transformed into appressed, particle-free myelin-like structures (Figs. 6 and 7). (Sometimes, these areas increased dramatically when the thylakoids became damaged during isolation and/or depletion steps.) Quantitative evaluation (see Table II) of fracture faces revealed no significant differences between the control and CO_2-depleted or reconstituted samples (using the *t*-test analysis), if we express 5 Å as the limit of accuracy. This suggests that the membrane structure does not change significantly with a marked pH-decrease and addition of formate, included during CO_2-depletion, provided the thylakoids are intact.

A working model

Fig. 8 summarizes our view regarding the site of CO_2 binding; CO_2 is sug-

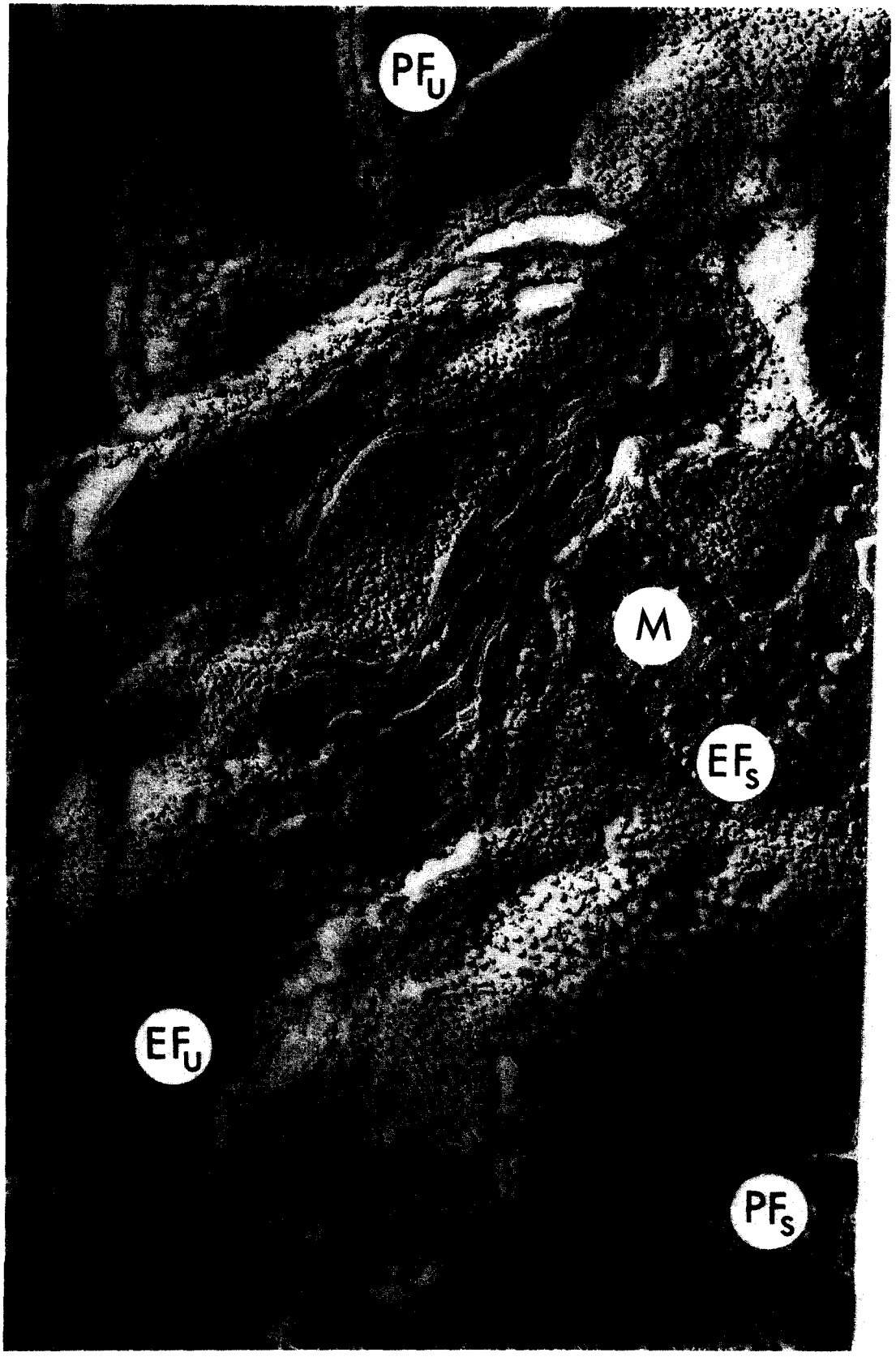

PFU
M
EFS
EFU
PFS

TABLE II

AVERAGE DIAMETER OF FREEZE-FRACTURE PARTICLES OBSERVED IN ISOLATED THYLAKOID PREPARATIONS

EF_s and PF_s, exoplasmic and protoplasmic fracture faces of stacked thylakoids; EF_u and PF_u, the same for unstacked thylakoids.

	EF_s	PF_s	EF_u	PF_u
Control	156 ± 29	116 ± 24	129 ± 19	113 ± 30
CO_2-depleted	144 ± 22	108 ± 19	118 ± 24	112 ± 14
Reconstituted	150 ± 19	110 ± 14	117 ± 27	113 ± 22

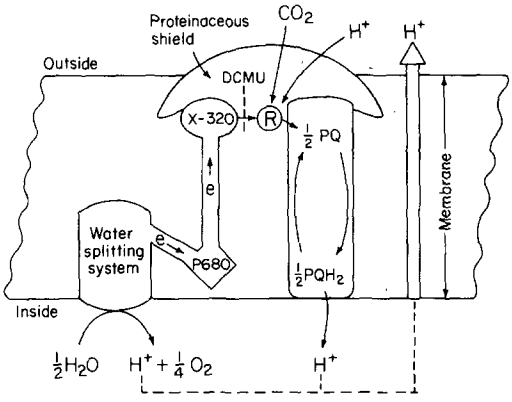


Fig. 8. A hypothetical model of G. Renger [5,11] for the components of Photosystem II modified to include the postulated site of CO_2 binding on the protein to which the 2-electron acceptor R is attached. R is equivalent to B in the text; *P*-680 is the reaction center chlorophyll *a*, *X*-320 is equivalent to the quinone Q, DCMU (3-(3,4-Dichlorophenyl)1,1-dimethylurea) is equivalent to diuron, and PQ stands for plastoquinone. The species that arrives at the binding site is CO_2, not HCO_3^- [17].

gested to bind to the protein to which B (or R) is attached. It is the same protein to which various herbicides (atrazine, DCMU, etc.) bind at other sites [3,4,5]. The model is that of Renger [5,11] with the modification that the postulated site of CO_2 binding is shown. Further experiments are needed to test this hypothesis.

Acknowledgements

We acknowledge financial assistance from USDA (590140) and NSF (24532). We are grateful to Professor Charles Arntzen for laboratory facilities, for discussions, and for a critical reading of this manuscript. One of us (K.P.) was supported by a travel grant from the Deutsche Forschungs Gemeinschaft (DFG).

Fig. 7. Freeze-fractured pea thylakoid membranes after CO_2-depletion (pH 5.0). M, myelin-like structure. For other marks, see Fig. 6. Magnification: 110 000X.

References

1 Govindjee and van Rensen, J.J.S. (1978) Biochim. Biophys. Acta 505, 183—213
2 Pfister, K. and Arntzen, C.J. (1979) Z. Naturforsch. 34C, 996—1009
3 Arntzen, C.J., Pfister, K. and Steinback, K.E. (1980) in Herbicide Resistance in Plants (LeBaron, H and Gressel, J., eds.), Wiley, New York, in the press
4 Trebst, A. (1979) Z. Naturofrsch. 34C, 986—991
5 Renger, G. (1979) Z. Naturforsch. 34C, 1010—1014
6 Arntzen, C.J., Ditto, C.L. and Brewer, P.E. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 278—282
7 Stemler, A., Babcock, G.T. and Govindjee (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 4679—4683
8 Armstrong, J.M. (1964) Biochim. Biophys. Acta 86, 194—197
9 Pfister, K., Radosevich, S.R. and Arntzen, C.J. (1979) Plant Physiol. 64, 995—999
10 Tischer, W. and Strotmann, H. (1977) Biochim. Biophys. Acta 460, 113—125
11 Renger, G. (1976) Biochim. Biophys. Acta 440, 287—300
12 Arntzen, C.J. and Burke, J.J. (1980) Methods Enzymol. 69c, 520—538
13 Khanna, R., Govindjee and Wydrzynski, T. (1977) Biochim. Biophys. Acta 462, 208—214
14 Govindjee, Pulles, M.P.J., Govindjee, R., van Gorkom, H.J. and Duysens, L.N.M. (1976) Biochim Biophys. Acta 449, 602—605
15 Siggel, U., Khanna, R., Renger, G. and Govindjee (1977) Biochim. Biophys. Acta 462, 196—207
16 Stemler, A. (1977) Biochim. Biophys. Acta 460, 511—522
17 Sarojini, G. and Govindjee (1980) Biochim. Biophys. Acta, in the press