

REVERSIBLE ANION INTERACTIONS BETWEEN Q_A AND Q_B AND BETWEEN Z (or D) and Q_A IN LEAVES AND GREEN ALGAE

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Abstract- The requirement of HCO_3^- (or CO_2) for stimulating the electron flow at the acceptor side of photosystem II (which is a different role from that in CO_2 fixation) in spinach leaf discs and in *Chlamydomonas* cells, both pretreated with formate, is presented. Short formate-treatment produces a quenching of the maximum chlorophyll a fluorescence and decreases the rate of O_2 evolution; addition of HCO_3^- reverses both inhibitions. The same fluorescence quenching is observed in the presence of hydroxylamine as an electron donor suggesting that this site of anion action is between the electron donor "Z" and the primary plastoquinone Q_A . Long formate-treatment slows down the decay of variable fluorescence which is similar to that caused by DCMU-treatment; HCO_3^- addition accelerates this decay suggesting that this site of anion action is between Q_A and the plastoquinone (PQ) pool. Furthermore, addition of HCO_3^- to HCO_2^- treated samples produces 4 fold stimulation of the Hill reaction in the presence of DDMIB that inhibits electron flow between the two photosystems and, thus, CO_2 fixation. We suggest that, in spinach leaves and *Chlamydomonas*, HCO_3^- regulates the reactions of PS II from Z (or D) to Q_A and from Q_A to the PQ pool.

1. INTRODUCTION

Bicarbonate (or CO_2) has been shown to stimulate the electron flow from Q_A , the primary plastoquinone to Q_B , the secondary plastoquinone of Photosystem II (PS II) and, then, to the intersystem plastoquinone pool in formate-treated thylakoids (1, 2). We call this the "bicarbonate effect". The site of action of these anions appears to be located on the electron acceptor side in PS II; no evidence exists for its action on the oxygen evolving complex (3).

Ireland et al. (4) have presented data suggesting that this bicarbonate effect exists in leaves. (For data on green alga *Scenedesmus*, see ref. 2.) On the basis of experiments on the alga *Chlamydomonas*, Mende and Wiessner (5) concluded that bicarbonate-depletion affects both the oxygen evolving as well as the electron acceptor side of PSII. In this paper, this anion effect on PS II was tested in spinach leaves as well as in green alga *Chlamydomonas*. We show here that in addition to the effect on Q_A to Q_B electron flow, another site of action exists between "Z" and Q_A . These roles of HCO_3^- are different from that in CO_2 fixation.

2. MATERIALS AND METHODS

Formate treatment was carried out with formate incubation by

either the infiltration method (6) for spinach leaf discs or under nitrogen gas for *Chlamydomonas* cells (7). Simultaneous measurements of oxygen evolution rate and Chl *a* fluorescence were made by the Walker instrument (8). Fluorescence transient was measured by a home-built fluorometer (9). The Q_A^- decay of Chl *a* fluorescence was measured by the double flash technique (10).

3. RESULTS AND DISCUSSION

Depending on the infiltration time in different media, two sites of anion action on PS II of spinach leaf discs were observed. Fig. 1 shows the effect of short anion treatment (5 s) on simultaneous measurements of the O_2 evolution rate and Chl *a* fluorescence level.

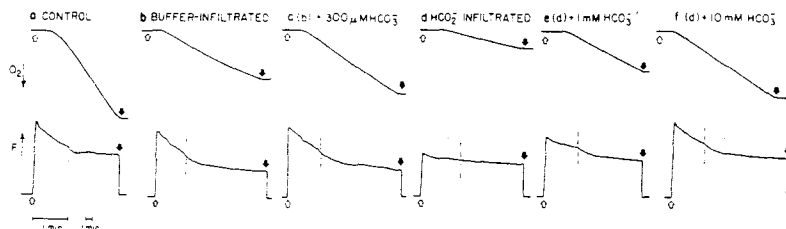


Figure 1. Parallel measurements on Chlorophyll *a* fluorescence (bottom curves) and on oxygen evolution in spinach leaf discs (pH 5.8).

Infiltration in non-bicarbonate containing media produced a quenching of the maximum fluorescence and a decrease of the oxygen evolution rate (cf. panels (b) and (d) with (a)). Addition of HCO_3^- reversed both inhibitions; it enhanced the rate of oxygen evolution and restored the maximum fluorescence (see panels (c), (e) and (f)).

To examine the inhibition observed above, 15mM hydroxylamine (pH 7.2) was used as an artificial electron donor to PSII. A comparison of trace 2 (short formate-treatment) with trace 1 (control) in Fig. 2 shows quenching of fluorescence in heated (45°C, 3min) and hydroxylamine-treated *Chlamydomonas* cells. Trace 3 shows restoration of fluorescence by 10mM bicarbonate addition. The same fluorescence quenching was observed in the presence of hydroxylamine as in its absence also in spinach leaf discs. These data suggest that the inhibition of the electron flow is located between the site of electron donation by hydroxylamine (Z or D) and Q_A . We call this site 1.

To locate the other site of anion action the samples were incubated for longer time in formate medium. Long formate-treatment of *Chlamydomonas* cells (3 h under N_2 gas) slowed down the decay of Q_A^- , as monitored by the decay of Chl *a* variable fluorescence. The similarity between the decay of Q_A^- of both formate and DCMU-treated cells, after the third actinic flash, is shown in Fig. 3. This shows that formate, like DCMU, slows down the Q_A^- reoxidation by the secondary plastoquinone Q_B (or Q_B^-). HCO_3^- addition accelerates this decay suggesting the oxidation of Q_A^- . We call this site 2. This is the first measurement of bicarbonate-reversible formate effect on Q_A to Q_B reaction in an intact eukaryotic cell.

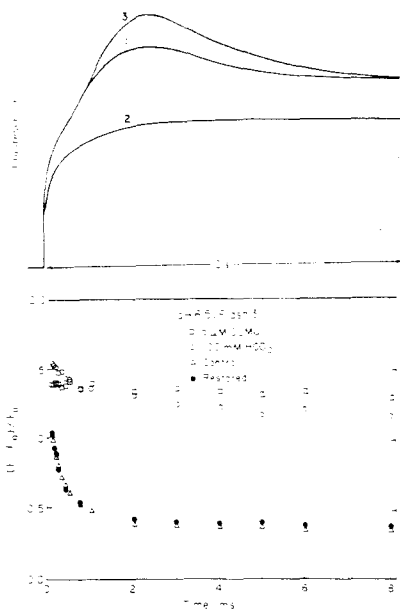


Figure 2. Chlorophyll *a* fluorescence transient in heated and hydroxylamine-treated *Chlamydomonas* cells. 1: control; 2: formate-treated; 3: 2 + HCO₃⁻ (Shintinawy, C. Xu and Govindjee, 1989, unpublished).

Figure 3. Variable chlorophyll *a* fluorescence decay in *Chlamydomonas* cells. F = fluorescence yield at time *t* after an actinic flash; F₀ = fluorescence yield before actinic flash (Shintinawy, C. Xu and Govindjee, 1989, unpublished).

The inhibition of the electron flow at site 2 is also shown by analyzing the data of Chl *a* fluorescence induction curves. The initial slope of Chl *a* fluorescence (upto 1 s of illumination) that reflects the reduction of Q_A, in spinach leaf discs as a function of [HCO₂⁻] concentration was accelerated (data not shown). These measurements indicate the inhibitory effect of HCO₂⁻ on the electron flow of PS II. Addition of bicarbonate reversed the HCO₂⁻ effect; it slowed down the rise of the fluorescence (up to one second of illumination) and decreased the ratio of variable to constant fluorescence as shown in Fig. 4. This further confirms the role of HCO₃⁻ in reversing the

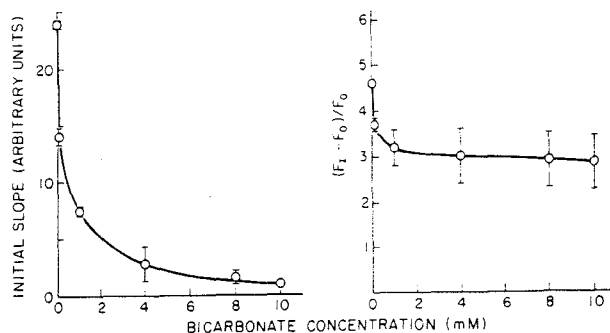


Figure 4. Initial slope and the ratio of variable to constant Chl *a* fluorescence in formate-treated spinach leaf discs as a function of bicarbonate concentration.

inhibition of the electron flow caused by formate-treatment in leaf discs. This stimulatory function of HCO₃⁻, which is unique among all monovalent anions, is located at the Q_AQ_B complex of PS II.

Furthermore, addition of HCO_3^- to formate-treated *Chlamydomonas* cells produced 4 fold stimulation of the Hill reaction (Table 1) in the presence of the inhibitor DEMIB that inhibits electron flow between the two photosystems and, thus, CO_2 fixation.

Table 1. Effect of HCO_3^- depletion and restoration on O_2 evolution in *Chlamydomonas* cells (Shintinawy, C. Xu and Govindjee, unpublished).

Rate of oxygen evolution ($\mu\text{mol O}_2$ (mg Chla) $^{-1}\text{h}^{-1}$)			Ratio $+\text{HCO}_3^-/-\text{HCO}_3^-$	
A	B	C	C-B	C-B/A
$-\text{HCO}_3^-$	$+\text{HCO}_3^-$ +(20mM)	$+\text{HCO}_3^-$ +(20mM)		
+DMQ (1mM)	-DMQ	+DMQ (1mM)		
+ $\text{K}_3\text{Fe}(\text{CN})_6$ (1mM)	- $\text{K}_3\text{Fe}(\text{CN})_6$	+ $\text{K}_3\text{Fe}(\text{CN})_3$ (1mM)		
58.9 ± 0.7	100 ± 0.7	358 ± 7	258	4.4
	+ 0.5 μM DEMIB			
57 ± 2	0 ± 0	245 ± 4	245	4.3

DEMIB= 2,5-dibromo-6-isopropyl-p-benzoquinone; DMQ=dimethylquinone

In conclusion, HCO_3^- has two sites of action: one between the electron donor Z (or D) and Q_A and the other between Q_A and the PQ pool in spinach leaf discs and *Chlamydomonas* cells.

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