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A DYNAMIC INTERACTION BETWEEN THE BICARBONATE LIGAND AND PHOTOSYSTEM II REACTION CENTER COMPLEXES IN CHLOROPLASTS *

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Summary

The binding of HCO_3^- to Photosystem II reaction center complexes in broken chloroplasts requires the presence of the oxidized form of the negative charge accumulator 'B'. HCO_3^- which is already bound to the reaction center II complex can escape if chloroplasts are illuminated with saturating light in the presence of a Hill oxidant and high concentrations of sodium chloride and sodium formate. These observations are explain in terms of a cyclic dark binding of HCO_3^- to the reaction center II complex and a subsequent release in the light.

Introduction

Maximum rates of Photosystem II-driven electron flow depend on the presence of HCO_3^- [1—3]. The HCO_3^- which controls electron flow is a small pool, tightly bound to the reaction center complex [4]. One of the effects of this pool is to increase the number of reaction centers evolving oxygen [3,5]. A second effect is to speed the electron transfer rate from the primary electron acceptor Q, to the charge accumulator 'B' [6] (denoted also 'R', see ref. 7) and also to speed the transfer rate from B to plastoquinone (PQ) [5,8]. It was reported previously that when chloroplast fragments are depleted of bicarbonate, darkness is required after readdition of HCO_3^- in order for reactivation to take place [2]. The present work shows that the binding of HCO_3^- to the reaction center complex depends on the oxidation state of B. It was also reported previously that the Hill reaction can be made dependent on exogenous HCO_3^- if grana are illuminated in the presence of high salt concentrations (0.25 M

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NaCl, 0.04 M sodium acetate) and a Hill oxidant [9]. Studies reported here indicate that the HCO_3^- ligand is momentarily released as the reaction center II complex transfers two electrons from B^{2-} to PQ, but is immediately recomplexed with oxidized B. The conditions required to block the recomplexing of HCO_3^- are also reported here.

Methods

Maize (*Zea mays* L.) plants were grown and chloroplasts were isolated from them as described elsewhere [2,4]. Only chloroplasts subjected to an osmotic shock during isolation were used in these experiments. The procedure required to deplete chloroplasts of endogenous bound HCO_3^- was previously described in detail [10].

Ferricyanide-supported oxygen evolution was monitored with a Rank Brothers, Clark-type electrode. The light source was a General Electric Quartzline 300 W lamp. The focused beam passed through a Corning 3-66 orange cut-off filter. The incident intensity was 440 $W \cdot m^{-2}$, unless otherwise specified.

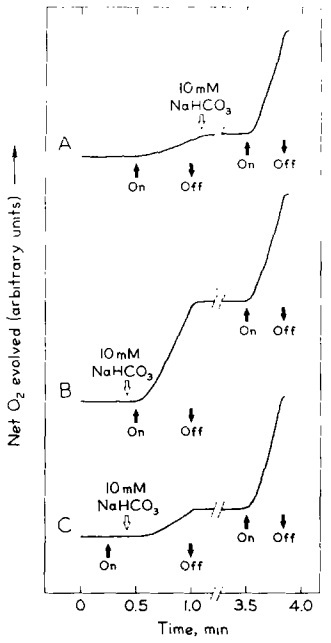
In experiments employing flashing light, the source was a General Radio Stroboslave type 1539-A.

Chloroplast samples labeled with $H^{14}CO_3^-$ were processed and counted as described elsewhere [4].

Results and Discussion

Dark reactivation of HCO_3^--depleted chloroplasts. When HCO_3^--depleted chloroplast fragments are given exogenous HCO_3^-, full reactivation takes place only in the dark (Fig. 1). The control sample (trace A) was illuminated for 30 s, then given 10 mM $NaHCO_3$. After 2.5 min dark, the chloroplasts were reilluminated. A nearly 10-fold increase in the rate of oxygen evolution was observed. When HCO_3^- is given to a similar dark-adapted sample (trace B), 10 s before the first illumination, about 80% recovery is observed. A subsequent dark period of 2.5 min restores full activity. In contrast, if the grana are illuminated with continuous light for 15 s before the additon of HCO_3^- (trace C), and illuminated continuously thereafter, oxygen-evolving ability barely exceeds the control (given no HCO_3^-). Only after illumination has ceased for several minutes is full activity once again observed. Clearly, light prevents HCO_3^- from restoring normal rates of electron flow. These data confirm earlier observations [2].

Restoration of electron flow as a function of flash number before readdition of HCO_3^-. The previously discussed results raise the question of how much light is necessary to prevent restoration of normal electron flow by added HCO_3^-. HCO_3^--depleted grana were suspended in reaction mixture and dark adapted for 5 min. The chloroplasts were then exposed to up to 3 strobe light flashes spaced 1 s apart. Immediately after the last flash, HCO_3^- was injected to a final concentration of 10 mM and after 10 s, saturating continuous light was applied. After a 30-s assay period, the grana were kept in the dark for 2.5 min to allow full reactivation, then reilluminated. The slope of the oxygen trace observed in the first illumination, divided by that observed after full recovery (in the second illumination), multiplied by 100, gave the percent recovery; this percent



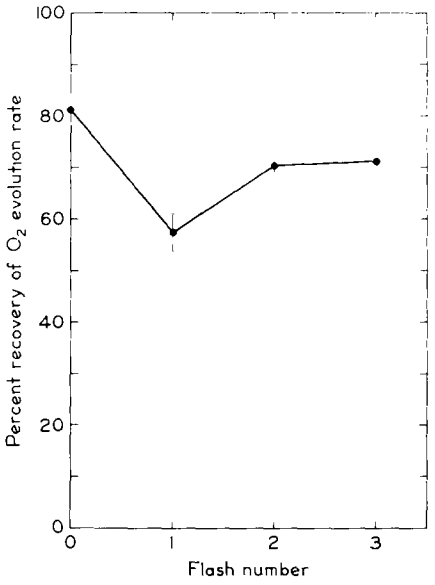


Fig. 1. Dark-only restoration of oxygen evolution with added HCO_3^-. HCO_3^--depleted chloroplasts were suspended in reaction mixture which contained 0.1 M sodium phosphate (pH 6.8), 0.175 M NaCl, 0.1 M sodium formate, 15 μg chlorophyll · ml^{-1}. $K^3Fe(CN)_6$ was injected into all samples at 0.5 min to a final concentration of 2 mM. The light intensity was 440 W · m^{-2}. The temperature was 24° C. Trace A represents the control; HCO_3^- was injected in the dark after the first light period. Trace B, HCO_3 was injected in the dark 15 s before the first light period. Trace C, HCO_3^- was injected 15 s after the beginning of the first light period.

Fig. 2. Restoration of oxygen evolution rate as a function of flash number prior to addition of HCO_3^-. The HCO_3^--depleted chloroplasts were suspended in the chamber of a Clark-type oxygen electrode containing the reaction mixture described in Fig. 1. Just before addition of HCO_3^- to a concentration of 10 mM the grana were subjected to a varying number of saturating light flashes, spaced 1 s apart, from a strobe lamp. 10 s after addition of HCO_3^-, $K^3Fe(CN)_6$ was injected (final concentration 2 mM) and, simultaneously, the chloroplasts were given continuous saturating light. After 30 s of illumination, the chloroplasts were allowed to reactivate completely in a subsequent 2.5 min dark period. The fully reactivated chloroplasts were given a second period of illumination. The percent recovery is the initial slope of the oxygen trace observed during the first period of continuous illumination divided by that observed during the second period (after complete recovery), magnified by 100. Each point expresses the mean of at least 5 measurements.

was plotted as a function of flash number in Fig. 2. 10 s after addition of HCO_3^- to dark-adapted chloroplasts, about 80% recovery of the oxygen evolution rate was observed, compared to full recovery seen after a subsequent 2.5 min dark. A single saturating flash prior to addition of HCO_3^- reduced recovery to about 58%. The most surprising observation was made following 2 saturating flashes. In this case recovery increased again to about 70%. Recovery following a third flash was about the same as that following the second. What one sees, then, in these results is the first cycle of an oscillation in recovery with flash number. The cycle has a period of 2. This result immediately suggests that the charge accumulator on the reducing side of Photosystem II, B, is involved.

To explain the results, it is necessary to postulate that HCO_3^- binds to the reaction center II complex only when B is in the oxidized state. It is known

that in dark-adapted chloroplasts about 70% of B exists in the oxidized state, while 30% exists as singly reduced B, i.e. as B^- [11]. Adding HCO_3^- to dark-adapted grana produces a rapid recovery of electron flow rate of about 80% (Fig. 2), consistent with the idea that only oxidized B permits HCO_3^- binding.

After a single flash, all B should be converted to B^- and all B^- should be converted first to B^{2-}, then to B following dark reoxidation by PQ. Thus, a single flash should, in principle, reverse the initial relative proportions of B and B^-. If HCO_3^- binds only to B, one might therefore expect a single flash to reduce the recovery of electron flow rate from 70 to about 30%. A reduction of this magnitude is not observed (Fig. 2) for the following reasons: About 10% 'recovery' is expected even without addition of HCO_3^- (Fig. 1, trace A). This residual rate in HCO_3^--depleted chloroplasts was not subtracted. Secondly, HCO_3^--depleted chloroplasts experience a high 'miss' rate [3]. A miss, first proposed to explain damping in oxygen yield oscillations in flashing light [12] is defined as the failure of a reaction center, after receiving a photon, to perform a photochemical reaction leading to stable products. The miss rate is about 11% in normal chloroplasts [12] but is higher in HCO_3^--depleted chloroplasts [3]. Because of the miss parameter, probably about 15% of the initial population of oxidized B will not be reduced by the first flash, but instead will remain oxidized and allow HCO_3^- to complex. Finally, in the 10 s between injection of HCO_3^- and assay for recovery, some dark reversion of B^- to B might very well take place, increasing the percent recovery. Thus, after a single flash one must still expect a minimum of 50% recovery, even though HCO_3^- only complexes with oxidized B.

A second flash should in principle reverse the relative proportions of B and B^- so that B again predominates. Indeed, the percent recovery of oxygen evolution rate does go up following a second flash. A third flash allows about the same recovery as two flashes so that a true oscillatory pattern is already completely damped, probably because of the high miss parameter and dark reversions of B^- to B.

An additional proposal must be made to explain the lack of recovery of electron flow rates when HCO_3^- is added to chloroplasts while they are being illuminated with continuous saturating light (Fig. 1, trace C). Under this condition, electron flow rate is limited by the electron transfer reaction between B^{2-} and plastoquinone [8]. This reaction was shown by Siggel et al. [8] to have a half-time greater than 100 ms in HCO_3^--depleted chloroplasts. In saturating continuous light, therefore, the charge accumulator will spend nearly all its time in the form B^{2-}. The data suggest that HCO_3^- will not bind to the reaction center II complex when B is in this doubly reduced form, just as it does not bind when B is singly reduced.

Binding of $H^{14}CO_3^-$ to thylakoid membranes as a function of flash number. Variation in the binding of HCO_3^- to reaction center II complexes can be measured directly with $H^{14}CO_3^-$. HCO_3^--depleted chloroplast fragments were suspended in reaction mixture, dark-adapted for 5 min and then exposed to up to 3 strobe light flashes. After the last flash, $H^{14}CO_3^-$ (final concn. 0.2 mM) was injected into the suspension. The grana were incubated for 15 s, then unlabeled $NaHCO_3^-$ was injected to a concentration of 100 mM. This addition effectively stopped further binding of labeled HCO_3^-. The grana were then washed 3 times

by centrifugation and resuspension in buffered solution to remove unbound label. The $H^{14}CO_3^-$ remaining with the membranes was then assayed (see ref. 4 for detailed methods). The results are plotted in Fig. 3. Maximum binding of $H^{14}CO_3^-$ was observed in the dark-adapted samples. A single flash resulted in a significant drop in the amount of $H^{14}CO_3^-$ bound. The effects of a second and third flash are not as certain because of the scatter in the data. It is clear, however, that the second flash produces no noticeable decrease in binding compared to the first flash, and may result in increased binding.

In other experiments, $H^{14}CO_3^-$ was given to broken chloroplasts previously depleted of HCO_3^-, while they were being illuminated by high intensity continuous light. The results are shown in Table I. Control chloroplasts (line 4), given unlabelled HCO_3^- (final concentration, 100 mM) in the dark 1 min prior to addition of $H^{14}CO_3^-$, bound about 1000 dpm · mg^{-1} chlorophyll. This amount of binding represents the HCO_3^- taken up by the low-affinity pool of HCO_3^- binding sites which was described previously [4]. This non-specifically bound HCO_3^- appears not to have a physiological role, at least in Photosystem II functioning.

Chloroplasts given $H^{14}CO_3^-$ in the dark (Table I, line 1) 15 s prior to addition of unlabelled HCO_3^- (to 100 mM) bound nearly 5 times more label than the control. A single saturating light flash given immediately before addition of $H^{14}CO_3^-$, (line 2) reduced the amount of bound label to about 4 times more than the control. If the $H^{14}CO_3^-$ was given to the chloroplasts while they were

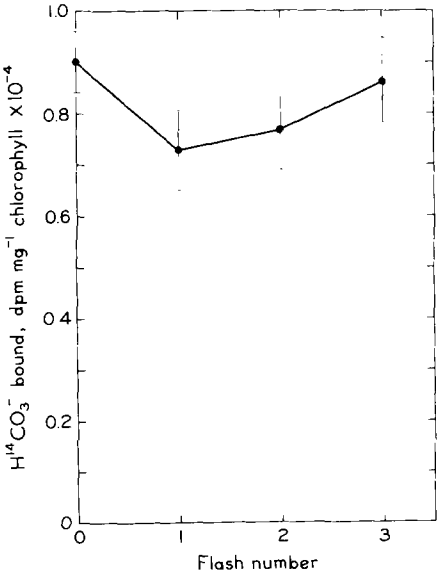


Fig. 3. The binding of $H^{14}CO_3^-$ to chloroplast grana as a function of flash number prior to addition of the $H^{14}CO_3^-$. The HCO_3^--depleted grana (50 μg chlorophyll · ml^{-1}) were suspended in reaction mixture containing 0.1 M sodium phosphate, pH 6.8 and 10 mM NaCl. The dark-adapted grana were subjected to a varying number of strobe light flashes spaced 1 s apart. Immediately after the last flash, $H^{14}CO_3^-$ was injected to a final concentration of 100 μM (6 μCi · ml^{-1}). Binding of label was stopped 15 s later by the addition of unlabeled HCO_3^- to a concentration of 100 mM. The grana were washed 3 times by centrifugation and resuspension in buffered solution to remove unbound $H^{14}CO_3^-$ and then measured for radioactivity (see ref. 4 for procedural details). Each point expresses the mean of at least 7 measurements.

TABLE I

THE BINDING OF $H^{14}CO_3^-$ TO CHLOROPLAST GRANA IN THE DARK, AFTER A SINGLE LIGHT FLASH, AND DURING CONTINUOUS ILLUMINATION

The reaction mixture and conditions are described in the legend, Fig. 3, except for a shorter (12 s) incubation time here. The control chloroplasts were given unlabelled HCO_3^- (final concentration, 100 mM) in the dark 1 min before addition of $H^{14}CO_3^-$. The incident light intensity was $1.6 \cdot 10^3$ W · m^{-2}. Each value expresses the mean of at least 6 measurements.

Incubation condition	$H^{14}CO_3^-$ bound (dpm · mg chlorophyll^{-1})
(1) Darkness	4861 ± 137
(2) Darkness after 1 saturating light flash	3930 ± 415
(3) Continuous high intensity illumination	2526 ± 118
(4) Control	1023 ± 51

being continuously illuminated, the amount of bound label was reduced even more, to about 2.5-fold compared to the control.

It is clear from these results (Fig. 3 and Table I) that the binding of HCO_3^- to thylakoid membranes is correlated with restoration of normal electron flow rates (Figs. 1 and 2). They are also consistent with the hypothesis that HCO_3^- binds to the reaction center II complex only when B is in the oxidized state.

Conditions required to induce HCO_3^- dependence in the light. When chloroplast grana are illuminated under the usual Hill reaction conditions, the rate of oxygen evolution does not become increasingly dependent on added HCO_3^-. The endogenous HCO_3^-, bound to the reaction center II complexes, seems to remain bound as the system cycles repeatedly [4]. On the other hand, if grana are illuminated in reaction mixtures containing high concentrations of chloride and acetate (or formate), the rate of oxygen evolution slowly becomes dependent on added HCO_3^- [9]. A reinvestigation of the conditions required to induce HCO_3^- dependent in the light was done in order to define the relationship between HCO_3^- and the charge accumulator B.

Chloroplast fragments, not previously depleted of HCO_3^-, were suspended in reaction mixture containing high salt concentrations (see legend, Table I). Samples were given various pretreatments, then their ability to evolve oxygen in the absence and then in the presence of 10 mM $NaHCO_3^-$ was measured. The results appear in Table II.

Chloroplasts kept in the dark for 3 min (with B predominantly in the oxidized state) showed no dependence on exogenous HCO_3^-, whether or not ferricyanide was present during pretreatment (Table II, lines 1 and 2). When samples were given strobe light flashes every 15 s for 3 min, a regime chosen to maintain a polulation of B in the singly reduced state, no dependence on HCO_3^- developed (line 3). When the grana were given high intensity continuous light for 3 min in the absence of a Hill oxidant (to maintain a large population of B^{2-}), only a slight HCO_3^- dependence was observed (line 4). It is clear from these results that keeping the charge accumulator B in any particular oxidation state does very little, in itself, to free bound HCO_3^- from the reaction center II complex.

In contrast, if the grana are illuminated for 3 min with saturating light in the presence of ferricyanide, a very noticeable HCO_3^- dependence develops (line

TABLE II

THE ABILITY OF VARIOUS PRETREATMENTS TO INDUCE DEPENDENCE OF THE HILL REACTION ON EXOGENOUS HCO_3^-

Chloroplast grana (15 μg chlorophyll · ml^{-1}), not previously HCO_3^--depleted, were suspended in HCO_3^- free reaction mixture containing 0.1 M sodium phosphate, pH 7.0, 0.175 M NaCl, 0.1 M sodium formate and, where indicated, 4 mM $K_3Fe(CN)_6$. After the indicated pretreatment, all samples were illuminated with saturating light (440 W · m^{-2}) in the presence of ferricyanide. Oxygen evolving ability was measured first in the absence, then in the presence of 10 mM $NaHCO_3$. The temperature was 22 C. Each value expresses the mean of at least 4 measurements.

Pretreatment	(μmol O_2 · mg^{-1} chlorophyll · h^{-1})		
	$-HCO_3^-$	$+HCO_3^-$	$\frac{+HCO_3^-}{-HCO_3^-}$
(1) 3 min dark, minus ferricyanide	47.0 ± 1.6	50.8 ± 1.76	1.08
(2) 3 min dark, plus ferricyanide	52.5 ± 2.7	53.2 ± 3.7	1.01
(3) 1 strobe flash every 15 s for 3 min, minus ferricyanide	45.2 ± 1.8	50.1 ± 1.1	1.11
(4) 3 min continuous saturating light, minus ferricyanide	34.5 ± 1.1	45.2 ± 0.84	1.31
(5) 3 min continuous saturating light, plus ferricyanide	7.62 ± 1.4	35.2 ± 2.2	4.62
(6) 3 min continous saturating light, plus ferricyanide plus 10 mM methylamine	7.6 ± 0.7	35.2 ± 4.0	4.63
(7) 3 min continuous light (about 50% saturating), plus ferricyanide	35.7 ± 1.6	53.6 ± 1.5	1.50

5). After such pretreatment, the rate of oxygen evolution in the absence of HCO_3^- is less than 25% of that observed after readdition of HCO_3^-. This result implies that HCO_3^- is lost from the reaction center II complex only when the center is turning over, that is, while B is making a transition from one oxidation state to another. Apparently one of the electron transfer reactions involving B can result in the liberation of the HCO_3^- ligand.

Since HCO_3^- can be washed from chloroplasts in the dark by suspending them in a solution which contains high salt concentrations at pH 5.0 [4] it seemed possible that HCO_3^- might be liberated from thylakoids in the light indirectly, as a consequence to the establishment of a low internal pH. However, 10 mM methylamine added to the reaction mixture to inhibit the formation of a pH gradient, did not prevent the development of HCO_3^- dependence in the light (line 6). A low internal pH, therefore, is not cause in itself for the liberation of HCO_3^-.

The most puzzling result was observed when the preillumination light intensity was reduced from a saturating level to a level to about 50% saturation. Only a small HCO_3^- dependence was induced by this pretreatment (line 7). This phenomenon will be explored in detail in the following section.

HCO_3^- dependence induced by saturating and sub-saturating illumination. Chloroplast grana, not previously HCO_3^--depleted, were suspended in reaction mixture containing 0.1 M sodium phosphate, pH 6.8, high salt concentrations (0.175 M NaCl, 0.1 M sodium formate) and ferricyanide as a Hill oxidant. One sample was illuminated at an intensity well above saturation for 2 min, then assayed for oxygen-evolving ability in the absence, then in the presence of

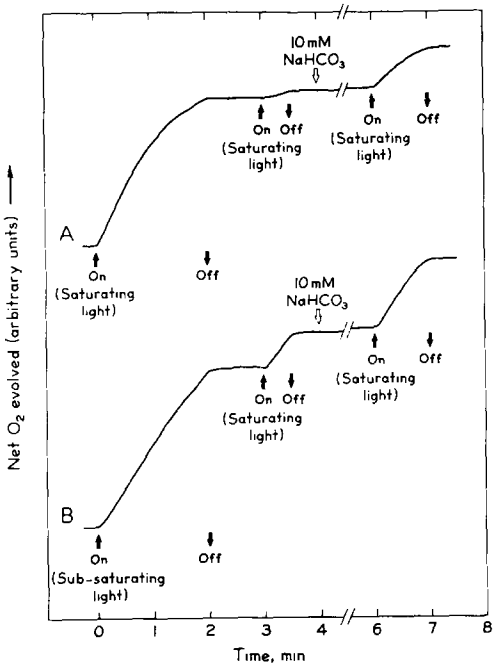


Fig. 4. Increasing dependence of oxygen evolution on added HCO_3^- in chloroplasts illuminated with saturating or slightly sub-saturating light. Chloroplast grana (15 μg chlorophyll · ml^{-1}), not previously HCO_3^- depleted, were suspended in reaction mixture containing 0.1 M sodium phosphate, pH 7.0, 0.175 M NaCl, 0.1 M sodium formate and 4 mM $K^3Fe(CN)_6$. The intensity of saturating light was 880 W · m^{-2}. Sub-saturating intensity was obtained by passing saturating light through a Balzers 5% neutral density filter. Sample A received saturating light throughout. Sample B received sub-saturating light during the first 2 min illumination period and saturating light thereafter. The temperature was 22° C.

10 mM $NaHCO_3$. The results are shown in Fig. 4, trace A. A second sample was treated similarly, except that the light intensity during the first illumation period only (0—2 min) was slightly below saturation (Fig. 4, trace B). The two samples show marked differences in induced dependence on HCO_3^-. Sample A, which received saturating light during the first 2 min of illumination, showed a higher initial rate of oxygen evolution, but the rate declined rapidly. These chloroplasts could give off little oxygen during a second illumination period (3—3.5 min). Adding HCO_3^- subsequently stimulated the rate of oxygen evolution more than 4-fold. Clearly this sample, illuminated at a saturating light intensity became increasingly dependent on HCO_3^- during the first illumination period (0—2 min).

Sample B, which received sub-saturating light intensity during the first illumination period only, showed a slightly lower initial rate of oxygen evolution (about 70%). Significantly, however, the rate did not decline but remained almost linear throughout the first illumination period, thus allowing sample B to give off as much net oxygen as sample A. When sample B was illuminated a second time (3—3.5 min) with saturating light, a high rate of oxygen evolution was observed, even in the absence of exogenous HCO_3^-. Adding HCO_3^- subsequently did not, in fact, significantly increase the rate of oxygen evolution in this sample. Clearly sample B, illuminated at sub-saturating light intensity did not become dependent on exogenous HCO_3^-.

The conclusion drawn from this experiment is that the rate at which Photosystem II reaction centers are forced to turn over is a more critical factor in allowing HCO_3^- to escape than is the actual number of turnovers.

A dynamic model. The observations reported here can be explained simply by proposing that the relationship between the HCO_3^- ligand and the Photosystem II reaction center (via the intermediate B) is a dynamic one. As a working hypothesis, the following reactions are proposed:

$$B + HCO_3^- \xrightarrow{\text{dark}} [B - HCO_3^-] \quad (1)$$

$$[B - HCO_3^-] \xrightarrow{Q^- \rightarrow Q} [B - HCO_3^-]^- \quad (2)$$

$$[B - HCO_3^-]^- \xrightarrow{Q^- \rightarrow Q} [B - HCO_3^-]^{2-} \quad (3)$$

$$[B - HCO_3^-]^{2-} \xrightarrow{PQ \rightarrow PQ^{2-}} B + HCO_3^- \quad (4)$$

B forms a complex with HCO_3^- in a 'dark' reaction 1. The resulting complex is reduced twice by reactions with Q^- (reactions 2 and 3). (The Q^- is, of course, produced in 2 separate Photosystem II light reactions.) When this charge-accumulating complex is doubly reduced, electrons are transferred to PQ and the complex is split, yielding B and free HCO_3^- (reaction 4). Under normal circumstances these two products, B and HCO_3^-, instantly reform a complex as proposed in reaction 1, thus establishing a cyclic process in which HCO_3^- plays a 'catalytic' role.

The conditions required for loss of bound HCO_3^- in the light are the presence of an electron acceptor, high salt concentrations and high light intensity. The electron acceptor is necessary to keep plastoquinone oxidized, thus allowing reaction 4 to proceed and actually free HCO_3^-. High salt concentrations, particularly HCO_3^- analogues such as formate or acetate, inhibit reaction 1, probably by a simple competitive mechanism. High light intensity is required to maximize the probability that B, after release of HCO_3^- in reaction 4, is reduced before reaction 1 takes place. If B is reduced before it can complex with HCO_3^-, the complex will not form at all (see discussion of Figs. 2 and 3). There will then be a high probability that HCO_3^- will be lost to the system. A high concentration of exogenous HCO_3^- (plus darkness) will be needed to reestablish the cycle.

Concluding remarks

One impression resulting from the data presented here and elsewhere is that the binding site for HCO_3^- appears to be in an inaccessible region of the thylakoid membrane. HCO_3^- which is bound there has a very low probability of escaping and exogenous HCO_3^- has little chance to enter as indicated by the high (larger than mM) concentrations required to allow entry of the catalytic amounts needed to restore electron flow rates [2,4].

Throughout the discussion, HCO_3^- has been assumed to be the 'active' form of carbon dioxide. In fact it is not certain what form is initially bound or what

form may be released. The assignment of HCO_3^-, therefore, must remain tentative until clear evidence is obtained.

The reason for the apparent dynamic nature of HCO_3^- binding also remains speculative. There is evidence that the negative charge accumulator B may be involved in proton pumping across the thylakoid membrane [11]. It is possible that HCO_3^-, which seems to complex with B and which can be reversibly protonated within a physiologically reasonable pH range, is a necessary component of the proton pump. A second possibility (not necessarily exclusive of the first) is that HCO_3^- controls the oxidation-reduction potential of B and in this way controls electron transfer reactions.

In some respects a dynamic role for catalytic amounts of HCO_3^- seems consistent with the model of oxygen evolution proposed by Metzner [13,14] whereby HCO_3^- is the immediate source of photosynthetically evolved oxygen. The site of action of HCO_3^- on the reducing side of Photosystem II appears to preclude HCO_3^- from the role proposed by Metzner. However, it is not yet definite that HCO_3^- acts only on the reducing side of Photosytem II. It is not clear why, for example, HCO_3^- increases the number of active Photosystem II reaction centers. This increase is apparent when measuring flash yields of oxygen [3] and the magnitude of both *P*-680 absorbance changes (Döring, G., quoted by Jursinic et al. [5]) and ESR signal II_{vf} [5]. This latter evidence indicates that HCO_3^- can influence reactions on the oxidizing side of Photosystem II. Although it does not prove a direct role for HCO_3^- in the oxygen evolving process, such evidence leaves open the possibility.

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