

Characterization of Glow Peaks of Chloroplast Membranes: Part III— Effect of Bicarbonate Depletion on Peaks I and II Associated with Photosystem II

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Depletion of thylakoid membranes of bicarbonate (HCO_3^-) in the presence of 100 mM sodium formate changes the relative intensity of glow peak I (appearing at 237K) and peak II (appearing at 261K): peak I decreases and peak II increases. This effect is similar to the one observed upon the addition of DCMU (3-(3',4'-dichlorophenyl)-1, 1-dimethylurea) to thylakoid membranes. Considering the relationship of peaks I and II with the "S" states of the water oxidizing complex and with the primary and secondary quinone acceptors Q_A and Q_B (which provide reducing equivalents for the production of these peaks), it is concluded that HCO_3^- depletion does not affect the formation of S_2 state from S_1 but decreases electron flow from Q_A^- to Q_B^- .

The delayed light emission (sec. to min components) has recently been correlated with the glow peaks from photosynthetic membranes¹⁻³. The relationship of glow peaks with the "S" states⁴⁻⁶ and with electron transport components on the acceptor side⁷⁻¹⁰ of photosystem II (PS II) has been established. Of the different glow peaks, peak I appearing at 237K has been shown to be unrelated to the "S" states; it has been suggested to arise from a recombination of Z^+ with Q_B^- where Q_B is a secondary quinone acceptor of PS II^{9,10}. On the other hand peak II (appearing at 261K) appears from a recombination of the S_2 state with Q_A^- , where Q_A is the primary quinone acceptor of PS II⁸⁻¹⁰. In view of the above and the reported site(s) of action of HCO_3^- ^{11,12}, we studied the effect of depletion of bicarbonate and its readdition on peak I and II in order to check, by the independent technique of thermoluminescence, if the effect of HCO_3^- is (a) on the electron acceptor side of PS II (before or after the site of diuron (DCMU) action) or (b) on the functioning of oxygen evolving complex, i.e. the "S" states.

The data presented in this communication show that HCO_3^- addition to HCO_3^- depleted membranes, at least in the presence of sodium formate (present in all our samples), enhances peak I production; however, peak II is slightly depressed. The addition of diuron (DCMU) that blocks electron flow from Q_A^- to Q_B^- gives results qualitatively similar to that of HCO_3^- depletion. Thus, these results suggest that the

conversion of S_1 to S_2 , that occurs in the presence of diuron, also takes place in the absence of HCO_3^- , but the electron flow from Q_A^- to Q_B^- is suppressed under similar conditions.

Materials and Methods

Chloroplasts were isolated according to Sane *et al.*¹³. CO_2^- depletion was done as described by Vermaas and Govindjee¹⁴. Low pH and formate were used to drive off CO_2 and to prevent the binding of CO_2 to thylakoids. The CO_2^- depleted thylakoids were finally suspended in CO_2^- free buffer (pH 6.5) containing 50 mM sodium phosphate, 100 mM sodium formate, 100 mM sodium chloride, and 5 mM MgCl_2 .

Thermoluminescence was measured as described earlier¹⁵. Bicarbonate-depleted thylakoids were either frozen as such to 77K under illumination (saturating white light) or they were incubated with 10 mM HCO_3^- in the dark for 1 min prior to freezing to 77K in light. Diuron, when used, was added to a final concentration of 10 μM . For Hill reaction studies, CO_2^- depleted samples with and without 10 mM HCO_3^- were taken in a 3 ml cuvette containing HCO_3^- free 2,6 dichlorophenol-indophenol (DCPIP) to give a final concentration of 0.1 mM. The change in optical density (absorbance) at 600 nm was monitored after illumination for 30 sec. The rates of electron transport from water to DCPIP were stimulated 3-4 fold in most experiments by the addition of HCO_3^- to HCO_3^- free samples.

Results and Discussion

The glow curve pattern of HCO_3^- depleted thylakoids (Fig.1, labelled as CO_2^-) is characterized

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* CO_2 and HCO_3^- are interchangeably used in this paper without any implication(s) of the active species involved.

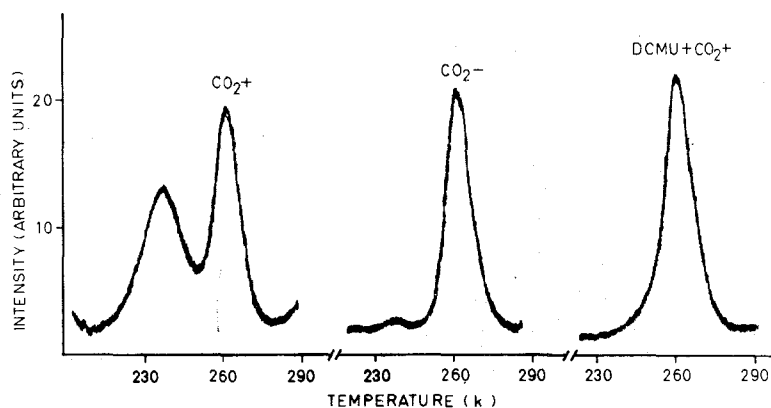


Fig. 1—Effect of bicarbonate addition on the glow peak pattern of bicarbonate-depleted thylakoids from spinach. Chloroplasts were isolated and thylakoids from them were depleted of CO_2 as described by Vermaas and Govindjee¹⁴. In CO_2^+ , the CO_2 -depleted thylakoids were first treated with 10 mM HCO_3^- , incubated in dark for 1 min, and then frozen to 77 K in light. In CO_2^- , the CO_2 -depleted thylakoids were frozen to 77 K without the addition of HCO_3^- . In $\text{DCMU} + \text{CO}_2^+$, DCMU (= diuron) to a final concentration of 10^{-6} M was added to thylakoids treated with 10 mM HCO_3^- and then frozen to 77 K in light

by a relatively high peak II (261 K) in contrast to a low peak I (237 K). The glow curve pattern of a sample to which 10 mM HCO_3^- was added shows (Fig. 1, labelled as CO_2^+) that HCO_3^- enhances peak I and decreases, to some extent, peak II. The pattern obtained in the presence of 10 mM $\text{HCO}_3^- + 10 \mu\text{M}$ diuron (Fig. 1, labelled as $\text{DCMU} + \text{CO}_2^+$) is similar to the one obtained for a sample depleted of CO_2 . Diuron has been shown to compete with the quinones¹⁶; it has been suggested to block electron flow from the bound primary quinone Q_A to the bound secondary quinone Q_B by physically displacing the latter¹⁷. Since the effect of diuron and the HCO_3^- -depletion is similar as regards its effect of peak I is concerned, we conclude that the absence of HCO_3^- blocks electron flow from Q_A^- to Q_B supporting the earlier conclusions of Jursinic *et al.*¹⁸ which were based on Chlorophyll *a* fluorescence decay measurements.

Of the two peaks seen in Fig. 1 (CO_2^+), the peak II (261 K) is dependent on the formation of "S" states of the oxygen evolving complex (see Inoue and Shibata, 1982)⁶. This conclusion is supported by the observations⁹ that peak II is lost on alkaline Tris-Treatment¹⁹ or upon treatment with tetranitromethane²⁰: both these treatments prevent electron flow from water to PS II reaction centre chlorophyll *a* and, thus, the formation of the higher "S" states. These treatments, however, do not interfere with the appearance of peak I (237 K) suggesting that this peak does not involve "S" states⁹. The observation that HCO_3^- depletion increases the intensity of peak II (associated with the "S" states) suggests that HCO_3^- depletion could not be interfering with the formation of "S" states or the oxygen evolving complex. On the other hand, peak I has been suggested^{10,15} to involve

the reducing entities located beyond the site of diuron action. Thus the loss of peak I by HCO_3^- depletion as well as by the addition of diuron leads us to suggest that the plastoquinone pool cannot be reduced in the CO_2 -depleted thylakoids. This conclusion is in agreement with that obtained from chlorophyll *a* fluorescence transients measurements of Vermaas and Govindjee¹⁴.

Chloroplasts, isolated in phosphate buffer, normally show dominant peaks I and IV. However, chloroplasts that have been treated with 100 mM formate to drive off CO_2 show much decreased peak IV even after the addition of HCO_3^- . In addition to the reversible effects discussed in this paper, the depletion procedure apparently also changes irreversibly the luminescence characteristics of the membrane. Even after HCO_3^- addition the peak II does not completely disappear. This also indicates that our reconstituted thylakoids are different from the untreated chloroplasts. Some of the irreversible changes introduced by the procedures, used here, for driving off CO_2 have not allowed us to study the effects of HCO_3^- depletion on peaks III and IV; furthermore, it was not possible to show an equivalent loss of peak II with a concomitant appearance of peak I with equal yield. The quantitative studies must await development of milder procedures to deplete membranes of HCO_3^- . The qualitative changes, however, are clearcut and show, by independent thermoluminescence methods that (a) the site of HCO_3^- action is similar to that of DCMU and (b) HCO_3^- depletion does not interfere with the formation of " S_2 " state.

After the completion of this work Govindjee *et al.* using milder depletion procedure than used here, have

extended these studies which will be reported elsewhere (Govindjee, H Y Nakatani, A W Rutherford and Y Inoue, submitted for publication).

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