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 (HCO₃⁻) 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 Q₁ and Q₂ (which provide reducing equivalents for the production of these peaks), it is concluded that HCO₃⁻ depletion does not affect the formation of S₂ state from S₁ but decreases electron flow from Q₁ to Q₂.

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 recombinations of Z⁻ with Q₂⁻ where Q₂ is a secondary quinone acceptor of PS II³⁻¹⁰. On the other hand peak II (appearing at 261K) appears from a recombinations of the S₂ state with Q₁⁻, where Q₁ is the primary quinone acceptor of PS II³⁻¹⁰. In view of the above and the reported sita(s) of action of HCO₃⁻¹¹⁻¹², 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 HCO₃⁻ 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 HCO₃⁻ addition to HCO₃⁻ 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 Q₁⁻ to Q₂ gives results qualitatively similar to that of HCO₃⁻ depletion. Thus, these results suggest that the conversion of S₁ to S₂, that occurs in the presence of diuron, also takes place in the absence of HCO₃⁻, but the electron flow from Q₁⁻ to Q₂ is suppressed under similar conditions.

Materials and Methods
Chloroplasts were isolated according to Sane et al.¹³, CO₂⁻ depletion was done as described by Vermaas and Govindjee¹⁴. Low pH and formate were used to drive off CO₂ and to prevent the binding of CO₂ to thylakoids. The CO₂-depleted thylakoids were finally suspended in CO₂-free buffer (pH 6.5) containing 50 mM sodium phosphate, 100 mM sodium formate, 100 mM sodium chloride, and 5 mM MgCl₂.

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 HCO₃⁻ 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, CO₂⁻ depleted samples with and without 10 mM HCO₃⁻ were taken in a 3 ml cuvette containing HCO₃⁻ 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 HCO₃⁻ to HCO₃⁻ free samples.

Results and Discussion
The glow curve pattern of HCO₃⁻ depleted thylakoids (Fig.1, labelled as CO₂⁻) is characterized

*CO₂ and HCO₃⁻ are interchangably used in this paper without any implication(s) of the active species involved.
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\textsubscript{3}\textsuperscript{-} was added shows (Fig. 1, labelled as CO\textsubscript{2}\textsuperscript{+}) that HCO\textsubscript{3}\textsuperscript{-} enhances peak I and decreases, to some extent, peak II. The pattern obtained in the presence of 10 mM HCO\textsubscript{3}\textsuperscript{-} + 10 \mu M diuron (Fig. 1, labelled as DCMU + CO\textsubscript{2}\textsuperscript{+}) is similar to the one obtained for a sample depleted of CO\textsubscript{2}. Diuron has been shown to compete with the quinones\textsuperscript{16}; it has been suggested to block electron flow from the bound primary quinone Q\textsubscript{A} to the bound secondary quinone Q\textsubscript{B} by physically displacing the latter\textsuperscript{17}. Since the effect of diuron and the HCO\textsubscript{3}\textsuperscript{-}-depletion is similar as regards its effect of peak I is concerned, we conclude that the absence of HCO\textsubscript{3}\textsuperscript{-} blocks electron flow from Q\textsubscript{A} to Q\textsubscript{B} supporting the earlier conclusions of Jurisic et al.\textsuperscript{18} which were based on Chlorophyll \textit{a} fluorescence decay measurements.

Of the two peaks seen in Fig. 1 (CO\textsubscript{2}\textsuperscript{+}), the peak II (261 K) is dependent on the formation of “S” states of the oxygen evolving complex (see Inoue and Shibata, 1982)\textsuperscript{6}. This conclusion is supported by the observations\textsuperscript{9} that peak II is lost on alkaline Tris-Treatment\textsuperscript{19} or upon treatment with tetranitromethane\textsuperscript{20}: both these treatments prevent electron flow from water to PS II reaction centre chlorophyll \textit{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” states\textsuperscript{9}. The observation that HCO\textsubscript{3}\textsuperscript{-} depletion increases the intensity of peak II (associated with the “S” states) suggests that HCO\textsubscript{3}\textsuperscript{-} 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\textsuperscript{10,15} to involve the reducing entities located beyond the site of diuron action. Thus the loss of peak I by HCO\textsubscript{3}\textsuperscript{-} depletion as well as by the addition of diuron leads us to suggest that the plastoquinone pool cannot be reduced in the CO\textsubscript{2}-depleted thylakoids. This conclusion is in agreement with that obtained from chlorophyll \textit{a} fluorescence transients measurements of Vermaas and Govindjee\textsuperscript{14}.

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\textsubscript{2} show much decreased peak IV even after the addition of HCO\textsubscript{3}\textsuperscript{-}. 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\textsubscript{3}\textsuperscript{-} 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\textsubscript{2} have not allowed us to study the effects of HCO\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-}. The qualitative changes, however, are clearcut and show, by independent thermoluminescence methods that (a) the site of HCO\textsubscript{3}\textsuperscript{-} action is similar to that of DCMU and (b) HCO\textsubscript{3}\textsuperscript{-} depletion does not interfere with the formation of “S\textsubscript{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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References