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ON THE ACTIVE SPECIES IN BICARBONATE STIMULATION OF HILL REACTION IN THYLAKOID MEMBRANES

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Summary

At 5°C, addition of CO_2 or HCO_3^- to $CO_2(HCO_3^-)$-depleted thylakoids (containing 100 mM formate) initiates, within 10 s, the activation of the Hill reaction in light. In contrast to HCO_3^- addition, where there is a lag of 6—8 s, the activation by CO_2 addition is almost instantaneous. With CO_2, prior addition of carbonic anhydrase produces a lag of about 6 s that approaches the lag observed by the HCO_3^- addition. These data suggest that CO_2 is an active species involved in stimulating the Hill reaction. Binding of $CO_2(HCO_3^-)$ to a component on the external side of the thylakoid membranes is supported by the present study.

The major role of CO_2 in photosynthesis is its fixation into organic matter [1]. The other roles of $CO_2(HCO_3^-)$ are: the activation of reaction center II complex [2]; the activation of ribulose-bisphosphate carboxylase [3]; the stimulation of photophosphorylation [4]; and the stimulation of electron flow between the two photoreactions [5]. The mechanism of $CO_2(HCO_3^-)$ action on the electron flow between the two photoreactions is the focus of our present study (for a recent review, see Ref. 6). Good [7] showed stimulation of electron transport by $CO_2(HCO_3^-)$ at pH $\geqslant$ 6.5. Also, he found that this $CO_2(HCO_3^-)$ dependence was greater in the presence of high concentrations of

Abbreviation: DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea.

NaCl and sodium formate in the $CO_2(HCO_3^-)$ depletion medium. On the basis of these and other results, he implied that HCO_3^-, not CO_2 is the important species. Khanna et al. [8] reported the largest stimulation of the ferricyanide Hill reaction by non-saturating amounts of $CO_2(HCO_3^-)$ in the pH range of 6—7 quite close to the pK_a of CO_2/HCO_3^-. This could suggest that both CO_2 and HCO_3^- are important. In the 6—7 pH range, the HCO_3^- is the predominant species, but it remains so even at a higher pH (8.0) where the stimulatory effect declines; since at pH 6.5, the $[CO_2]$ is higher than at pH 8.0, a role of CO_2 could be implicated. In this report we present data showing that CO_2 is an active species involved in the stimulation of Hill reaction in thylakoid membranes. Whether it is only the species that diffuses to the site of binding or is also the species that actively binds to the membrane remains to be proven.

Thylakoid membranes, isolated from pea leaves, were depleted of CO_2-(HCO_3^-) as described earlier [9,10] except that the $CO_2(HCO_3^-)$ depletion was carried out at 4°C. The rate of oxygen evolution during ferricyanide Hill reaction was measured by a Clark type oxygen electrode at an irradiance of 100 mW · cm^{-2} at 5°C. The low assay temperature was used to slow down the interconversion of CO_2 and HCO_3^- and an alkaline pH (7.3) to obtain a high (>10) ratio of HCO_3^-/CO_2 at equilibrium. Thylakoids were illuminated for 1—2 min, and then CO_2 or HCO_3^- was added. A mixture of 17 μl of 200 mM $NaHCO_3$ and 7 μl of H_2O (pH 8.6) provided 2 mM HCO_3^- when injected into 1.7 ml sample. Similarly, a mixture of 17 μl of 200 mM $NaHCO_3$ and 7 μl of 0.55 N HCl (pH 2.0) provided 2 mM CO_2. The injected CO_2 or HCO_3^- is expected to last long enough in the system to allow us to distinguish the effects of the two species. (We note that the steady state pH of the reaction mixture did not significantly change upon the injection of CO_2 or HCO_3^-.)

The electron transport rates in formate containing $CO_2(HCO_3^-)$-depleted thylakoids at 5°C are very low, approaching zero (Fig. 1D). When 2 mM $NaHCO_3$ is added, a clear lag lasting for 8—9 s is seen before O_2 evolution starts (Fig. 1A). Addition of 2 mM CO_2 ($NaHCO_3$ and HCl mixed in a syringe and injected) to the $CO_2(HCO_3^-)$-depleted formate containing thylakoids leads, within 2 s, to O_2 evolution (Fig. 1A). The lag of approx. 2 s observed upon CO_2 injection may represent an instrumental lag as (a) the addition of ferricyanide to illuminated CO_2-sufficient thylakoids (Fig. 1C), or (b) illumination of CO_2-sufficient thylakoids containing ferricyanide also gives the same lag. Thus, CO_2 may initiate the stimulation of Hill reaction almost instantaneously; the HCO_3^- addition would then show a lag of approx. 6 s (Fig. 1B).

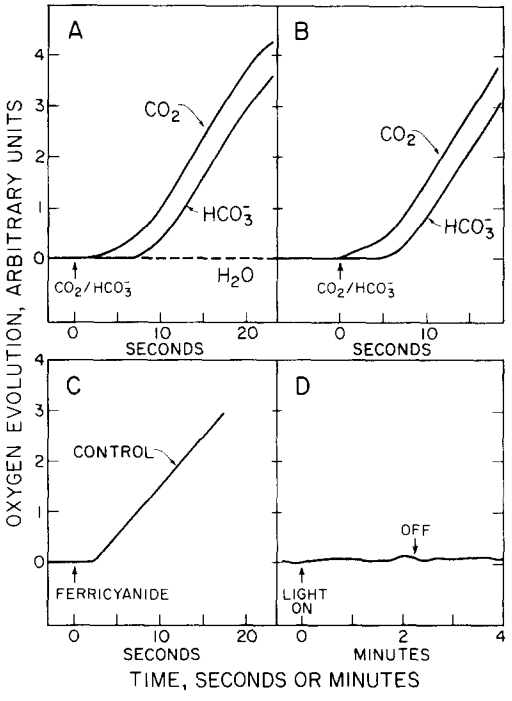
The initial rate of O_2 evolution upon CO_2 addition to formate-containing thylakoids is in the range of 5 to 7 μmol O_2 · mg^{-1} Chl · h^{-1}. However, when equilibrium is reached, several-fold higher rates (15 to 30 μmol O_2 · mg^{-1} Chl · h^{-1}) of Hill reaction are observed whether CO_2 or HCO_3^- is the injected species. If, however, sodium formate is removed from the assay medium, addition of CO_2 leads to monophasic kinetics with the higher rate suggesting that CO_2 and formate may compete for the same binding site.

The observed differences between CO_2 and HCO_3^- effects are not artifacts of measurements because addition of CO_2 or HCO_3^- to thylakoids containing 5 μM DCMU gave no change (data not shown).

The above data suggest that CO_2 is an active species involved in the stimula-

tion of the Hill reaction. Indeed, when carbonic anhydrase (210 units) is added to the illuminated CO_2 (HCO_3^-)-depleted thylakoids and CO_2 is injected in the reaction mixture after 2—4 min of the enzyme addition, the observed lag time increases from 2 to 6—7 s (Fig. 2A); addition of 105 or 210 units of the enzyme give similar results. The shift from 2 to 6—7 s occurs because the conversion of CO_2 to HCO_3^- by the enzyme decreases the availability of CO_2 for the initial stimulation of the Hill reaction. At 25°C, however, the equilibrium between HCO_3^- and CO_2 is reached at a faster rate in the presence as well as the absence of carbonic anhydrase, and, thus, no significant differences are observed here (Fig. 2B); the observed lag of 2—3 s is independent of the presence of 105 or 210 units of the enzyme.

It is clear from the above data that CO_2 is involved in stimulating the Hill reaction. Whether CO_2 is only the species that diffuses to the site of binding or is also the species that actively binds to the membrane remains to be investi-



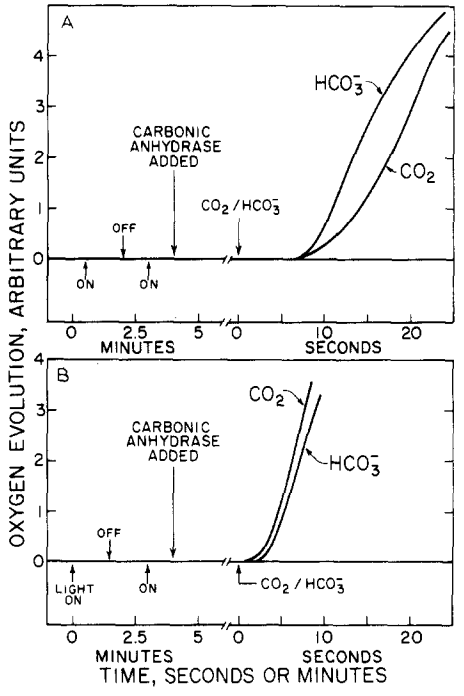


Fig. 1. Rate of oxygen evolution as a function of time in CO_2(HCO_3^-)-depleted pea thylakoids and those supplied with CO_2 or HCO_3^- at 5°C. (A) The kinetics of the Hill reaction when 2 mM CO_2 or HCO_3^- is injected into the illuminated thylakoid suspension containing 100 mM sodium formate. (B) The same as (A) except that an instrumental lag period of 2 s has been subtracted from it. (C) The kinetics of the Hill reaction in illuminated CO_2-sufficient thylakoids when 2 mM ferricyanide is injected into it; the same lag is observed when suspensions containing ferricyanide are illuminated. (D) Oxygen evolution traces in CO_2(HCO_3^-)-depleted thylakoids. [Chl], 50 μg/ml; [ferricyanide], 2 mM. Controls containing thylakoids with 5 μM DCMU showed no significant O_2 signals whether CO_2 or HCO_3^- is injected. Other details as in the text.

Fig. 2. Rate of oxygen evolution as a function of time in CO_2(HCO_3^-)-depleted pea thylakoids and those supplied with CO_2 or HCO_3^- in the presence of carbonic anhydrase at two temperatures. (A) At 5°C. (B) At 25°C. Where indicated, 210 units of carbonic anhydrase were added to illuminated thylakoids followed by injection of CO_2 or HCO_3^-. Other details as in the text.

gated. We shall, however, continue to assume, in the discussion that follows, that CO_2 is the species that binds to the membrane although the similarity between HCO_3^- and formate anions supports the idea that HCO_3^- is the species that binds to the membrane. Khanna et al. [8,11] have suggested that CO_2 initiates the activation of Hill reaction probably by binding to a protein on the outersurface of the thylakoid [12]; this protein is close to or is associated with the two electron carrier B (or, R) of Photosystem II (PS II). A close relationship of CO_2 binding to the functioning of B was already suggested by the early experiments of Govindjee et al. [13], Siggel et al. [5] and Stemler [14]. Binding of CO_2 to this protein may place B into an appropriate conformation such that B can efficiently accept electrons from Q (electron acceptor of PS II) and subsequently donate electrons to the plastoquinone pool. In view of the rapid CO_2 effect, observed in the present study, it seems logical that CO_2 may beind to a component close to the outer side of the membrane. The half-time for $CO_2(HCO_3^-)$ to cross the thylakoid membrane is approximately 15 s at 21°C (Ort, D., personal communication), and, this time may be even longer at 5°C. Thus, it seems unlikely that CO_2 binds to a component on the inner side of the membrane (e.g., the O_2 evolving enzyme).

After the present work was completed and submitted for publication a recent paper by Stemler [15] on the nature of the active species involved in bicarbonate stimulation became available. Based on a different type of experiment, Stemler also concludes that CO_2 is an active species involved in bicarbonate stimulation.

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References

1 Bassham, J. and Buchanan, R. (1981) in Photosynthesis (Govindjee, ed.), Academic Press, N.Y., in the press
2 Jursinic, P., Warden, J. and Govindjee (1976) Biochim. Biophys. Acta 440, 322—330
3 Lorimer, G.H., Badger, M.R. and Andrews, T.J. (1976) Biochem. 15, 529—536
4 Cohen, W.S. and Jagendorf, A.T. (1974) Plant Physiol. 53, 220—223
5 Siggel, U., Khanna, R., Renger, G. and Govindjee (1977) Biochim. Biophys. Acta 462, 196—207
6 Govindjee and van Rensen, J.J.S. (1978) Biochim. Biophys. Acta 505, 183—213
7 Good, N.E. (1963) Plant Physiol. 38, 298—304
8 Khanna, R., Govindjee and Wydrzynski, T. (1977) Biochim. Biophys. Acta 462, 208—214
9 Stemler, A. and Govindjee (1973) Plant Physiol. 52, 119—123
10 Stemler, A., Babcock, G.T. and Govindjee (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 4679—4683
11 Khanna, R., Pfister, K., Keresztes, A., van Rensen, J.J.S. and Govindjee (1981) Biochim. Biophys. Acta 634, 105—116
12 Renger, G. (1979) Z. Naturforsch. 34c, 1010—1014
13 Govindjee, Pulles, M.P.J., Govindjee, R., van Gorkom, H.J. and Duysens, L.N.M. (1976) Biochim. Biophys. Acta 449, 602—605
14 Stemler, A. (1979) Biochim. Biophys. Acta 545, 36—45
15 Stemler, A. (1980) Plant Physiol 65, 1160—1165