

Control of Photosynthesis by CO₂: Evidence for A Bicarbonate-Inhibited Redox Feedback in Photosystem II¹

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Abstract

The role of bicarbonate or CO₂ as a regulator of electron flow was studied in spinach chloroplasts. It was found that bicarbonate inhibited a proposed cyclic pathway around Photosystem II while stimulating the forward flow of electrons toward Photosystem I. Such behavior would result in increased rates of ATP and NADPH formation by the green plant. It was also found that in isolated chloroplasts other compounds, such as chelators, uncouplers, or any inhibitor of silicomolybdic acid reduction in a DCMU-insensitive pathway around PS II would increase the forward flow of electrons to Photosystem I, provided that the compound itself was not inhibitory of ferricyanide or dimethylbenzoquinone reduction in Photosystem II, or inhibited the H₂O → methyl viologen pathway which covers both photosystems. Bathocuproine in low concentrations (10-15 μg/ml) turned out to be a model compound in all respects.

Introduction

Stimulation of photosynthetic reactions by CO₂ was discovered by Boyle (7) but investigated in greater detail by Warburg and Krippahl (13), Good (9), and many others. Until recently, conflicting reports were obtained. In 1973, Stemler and Govindjee (11) discovered that the bicarbonate ion, not CO₂, was a critical factor in photosynthetic O₂ evolution. They used bicarbonate-depleted chloroplasts to show up to five-fold increases in Hill reaction rates with added bicarbonate. Bicarbonate also increases the amount of delayed light emission from chloroplasts and has an effect on chlorophyll a fluorescence transients (12). In conclusion, bicarbonate affects oxygen evolution on the oxidizing side of Photosystem II and also has an effect on the reducing side of PS II in isolated chloroplasts (14).

Based on Govindjee and associates' discoveries about the effect of CO₂, we in this paper would like to propose an alternative hypothesis to explain the known facts. The role of CO₂ or bicarbonate in photosynthesis may be that of a regulator: shutting off a cyclic flow of electrons around PS II and stimulation of the Hill reaction in a forward flow of electrons toward PS I resulting in increased NADPH production.

Materials and Methods

Market spinach was used for making chloroplasts, using Jagendorf and Avron's method (10). The grinding and suspension medium was 0.4 M sucrose with 0.05 M NaCl. Chlorophyll was determined according to Arnon (1).

All PS I and II assays were done polarographically measuring either O₂ uptake or evolution with a Clark-type electrode attached to

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a Yellow Springs Instrument Oxygen Monitor and recorded with a Honeywell recorder.

The reaction mixture for measuring oxygen evolution associated with ferricyanide reduction by PS II in presence of DBMIB contained in 1.5 ml total volume: chloroplasts (50 μ g), Trizma-Mes, 37.5 μ moles, 3 μ moles NH_4Cl , 3 μ moles MgCl_2 , 2.5 μ moles $\text{K}_3\text{Fe}(\text{CN})_6$, pH 7.0, and 0.06 μ g DBMIB. The $\text{H}_2\text{O} \rightarrow \text{DMBQ}$ reaction medium in presence of DBMIB contained all of the above ingredients except 2.5 μ moles DMBQ in place of ferricyanide. The $\text{H}_2\text{O} \rightarrow \text{MV}$ reaction medium contained the same ingredients except 5 μ moles MV in place of ferricyanide. No DBMIB was present in this case. The $\text{H}_2\text{O} \rightarrow \text{SM}$ reaction contained the same amount of buffer with NH_4Cl and MgCl_2 but 0.2 mg SM in place of ferricyanide and 0.3 μ moles DCMU substituted for DBMIB. The dark preincubation period for each assay was 2 min. The reaction rates are expressed as μ equivalents/mg chlorophyll/hr.

Bicarbonate for adding to assays was made fresh daily. All other additives—chelators, uncouplers, quinone analogs—were dissolved in ethanol (sometimes heated on waterbath) and added to reaction mixtures not to exceed 25 μ l/1.5 ml. In Fig. 1-11 the concentration of additives is expressed as μ moles or μ g/1.5 ml.

Results and Discussion

The aim of this study was to show that an alternative interpretation of the CO_2 or bicarbonate effect on photosynthesis as proposed by Govindjee and associates (11, 12, 14) is also possible. Assuming a cyclic flow of electrons around PS II, as is known to occur in PS I (2) all that is needed for the stimulation of forward electron flow in the electron transport chain of chloroplasts is a regulator to inhibit the PS II cycle. We can show that bicarbonate among other substances (Table 1, Fig. 1 and 2) inhibits silicomolybdic acid reduction in a DCMU-insensitive manner in the region of Q, the primary acceptor, in PS II (6, 8). This results in increased rates of other PS II reactions, such as ferricyanide or dimethyl-benzoquinone reduction. The $\text{H}_2\text{O} \rightarrow$

TABLE 1. Factors Affecting Silicomolybdic Acid Reduction by Chloroplast Photosystem II.

Additions	Stimulation or Inhibition of O_2 Evolution (% of Control)	
	$\text{H}_2\text{O} \rightarrow \text{SM}$	$\text{H}_2\text{O} \rightarrow \text{ferricyanide}$
DCMU (4.5 μ moles) -----	-8	-100
Ethanol (50 μ l) -----	-52	-19
Bathophenanthroline (25 μ g) -----	-20	+24
Orthophenanthroline (5 μ g) -----	+29	-100
Sodium bicarbonate (100 μ g) -----	-50	-12
Potassium permanganate (25 μ moles) -----	-75	-20
Tertoctyleatechol (80 μ moles) -----	-63	+234

Reaction conditions as in Methods.

The control rates for the $\text{H}_2\text{O} \rightarrow \text{SM}$ (+ DCMU) reaction varied from 252-312 μ equiv./mg chlorophyll/hr, for the $\text{H}_2\text{O} \rightarrow \text{FeCy}$ (+ DBMIB) reaction from 250-360. + indicates stimulation.

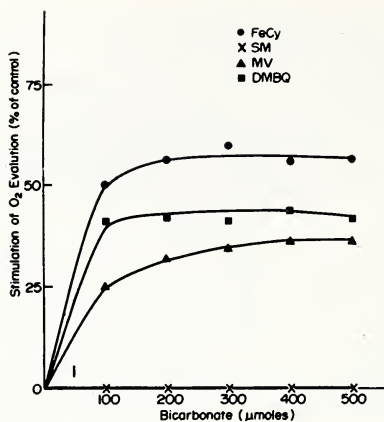


FIGURE 1. The effect of bicarbonate on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide ($\bullet$), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone ($\blacksquare$) in bicarbonate-depleted chloroplasts. Reaction conditions stated in Materials and Methods. The control rate for $H_2O \rightarrow FeCy$ was 203 μ equiv./mg chlorophyll/hr, for $H_2O \rightarrow SM-O_2$, for $H_2O \rightarrow MV-519$, and for $H_2O \rightarrow DMBQ-361$.

methyl viologen reaction which encompasses both photosystems is also stimulated (Fig. 1, 2). By definition this makes CO_2 or bicarbonate the regulator mechanism of electron flow in chloroplasts. Subsequently, the forward flow of electrons results in more efficient energy conservation by the chloroplasts and the whole green plant.

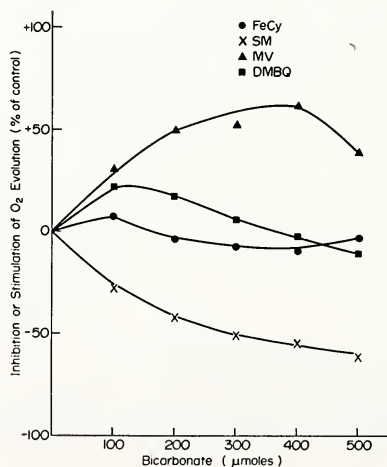


FIGURE 2. The effect of bicarbonate on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide ($\bullet$), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone ($\blacksquare$) in sucrose-sodium chloride chloroplasts in presence of NH_4Cl . Reaction conditions as in Fig. 1. The control rate for $H_2O \rightarrow FeCy$ was 305 μ equiv./mg chlorophyll/hr, for $H_2O \rightarrow SM-214$, for $H_2O \rightarrow MV-158$, for $H_2O \rightarrow DMBQ-$

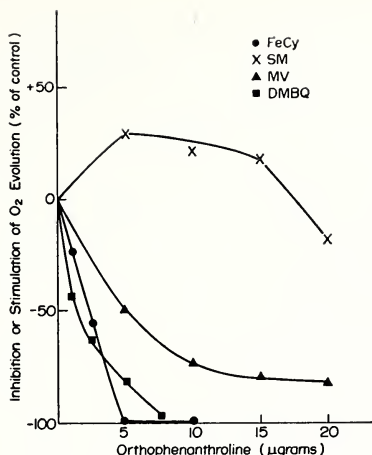


FIGURE 3. The effect of orthophenanthroline on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide (●), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone (■) in sucrose-sodium chloride chloroplasts. Reaction conditions as in Fig. 1.

Another way of testing the proposed model of bicarbonate acting as a regulator of electron flow in chloroplasts is to inhibit the forward flow of electrons toward PS I and observe what effect this has on the cyclic system around PS II. The assumption is that under these conditions increased rates of silicomolybdic acid reduction might be

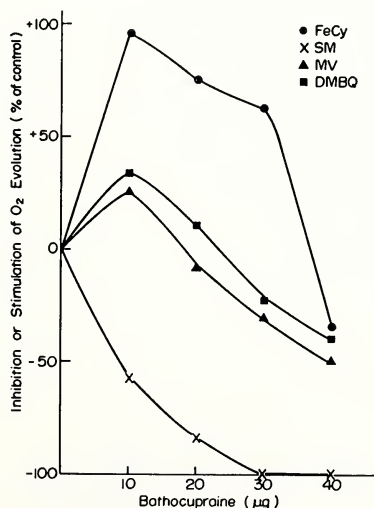


FIGURE 4. The effect of bathocupraine on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide (●), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone (■) in sucrose-sodium chloride chloroplasts. Reaction conditions as in Fig. 1.

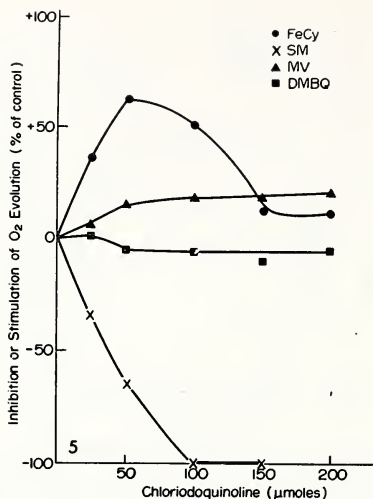


FIGURE 5. The effect of chloridoquinoline on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide (●), $H_2O \rightarrow$ silicomolybdic acid (X) $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone (■) in sucrose-sodium chloride chloroplasts. Reaction conditions as in Fig. 1.

observed. This is indeed the case, as seen in Fig. 3 using orthophenanthroline, a chelator, to inhibit ferricyanide and dimethylbenzoquinone reduction in PS II and the $H_2O \rightarrow$ methyl viologen reaction which covers both photosystems. Orthophenanthroline is known to inhibit electron

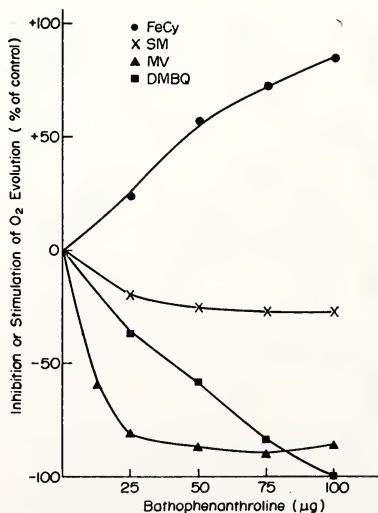


FIGURE 6. The effect of bathophenanthroline on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide (●), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone (■) in sucrose-sodium chloride chloroplasts. Reaction conditions as in Fig. 1.

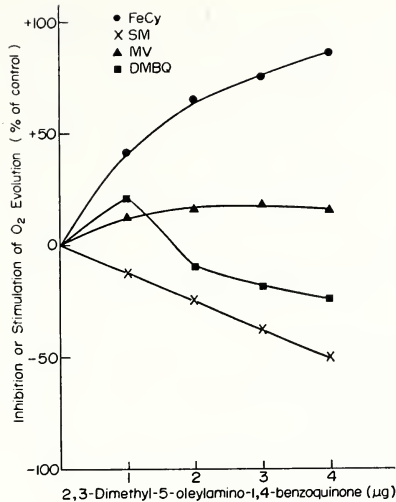


FIGURE 7. The effect of 2,3-dimethyl-5-oleylamino-1,4-benzoquinone on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide (●), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone (■) in sucrose-sodium chloride chloroplasts. Reaction conditions as in Fig. 1.

flow close to DCMU, although not at the same site (3), but it stimulates silicomolybdic acid reduction in low concentrations (Fig. 3). A similar effect, namely, lowered rates of forward electron transport toward PS I have been shown in absence of bicarbonate in bicarbonate-depleted chloroplasts by Stemler and Govindjee (11). The reverse

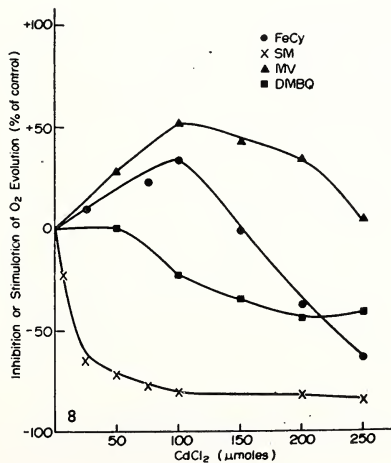


FIGURE 8. The effect of $CdCl_2$ on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide (●), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone (■) in sucrose-sodium chloride chloroplasts. Reaction conditions as in Fig. 1.

effect, i.e. stimulation of silicomolybdic acid reduction in bicarbonate-depleted chloroplasts is difficult to show because the depletion process inactivates silicomolybdic acid reduction (Fig. 1). This inactivation may not be due to the low pH used (pH 5-6) but to the high salt (0.25 M NaCl) concentration recommended by Stemler and Govindjee (11) for the depletion of CO_2 in chloroplasts. However, the inability to show stimulation of silicomolybdic acid reduction in isolated bicarbonate-depleted chloroplasts by CO_2 or bicarbonate does not invalidate the proposed hypothesis that agents which inhibit cyclic electron flow around PS II stimulate the forward flow of electrons which leads to increased PS I rates and, subsequently, increased ATP production by plants.

Best experimental proof for the hypothesis of CO_2 or bicarbonate as regulators of electron flow in photosynthesis comes from using other types of compounds, especially chelators and uncouplers. In this study 3 known chelators—bathocuproine, bathophenanthroline and chloriodoquinoline—were used. Bathocuproine (Fig. 4) which strongly inhibits silicomolybdic acid reduction in presence of DCMU (5) stimulates ferricyanide and dimethylbenzoquinone reduction by PS II and the flow of electrons from $\text{H}_2\text{O} \rightarrow$ methyl viologen. Chloriodoquinoline gave similar results (Fig. 5). Bathophenanthroline, another lipophilic chelator like bathocuproine, stimulates only ferricyanide reduction but inhibits all the other reactions studied. Bathophenanthroline-sensitive sites were found in the region between DCMU and plastoquinone by Barr and Crane (4). 2-3-Dimethyl-5-oleylamino-1,4-benzoquinone, a plastoquinone analog, acted in a similar manner (Fig. 7). Structurally, this quinone may also act as a chelator, so that it is not surprising to see an effect as with bathophenanthroline. Bathophenanthroline is pre-

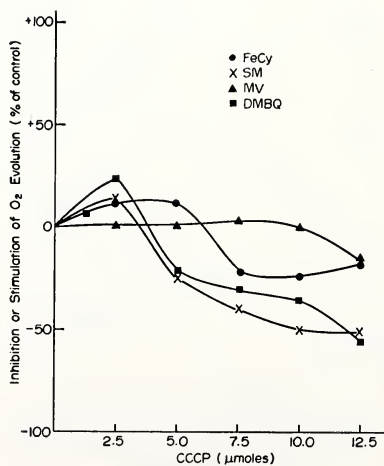


FIGURE 9. The effect of CCCP on 4 chloroplast reactions: $\text{H}_2\text{O} \rightarrow$ ferricyanide (●), $\text{H}_2\text{O} \rightarrow$ silicomolybdic acid (X), $\text{H}_2\text{O} \rightarrow$ methyl viologen (Δ), and $\text{H}_2\text{O} \rightarrow$ dimethylbenzoquinone in sucrose-sodium chloride chloroplasts in presence of NH_4Cl . Reaction conditions as in Fig. 1.

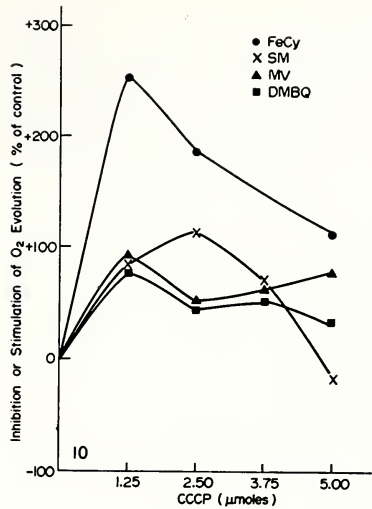


FIGURE 10. The effect of CCCP on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide (●), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone in sucrose-sodium chloride chloroplasts in absence of NH_4Cl . Reaction conditions as in Fig. 1 except no NH_4Cl present.

sumably acting on a metalloprotein complex, such as a non-heme iron, close to the phosphorylation site of PS II.

Acting in a different manner but giving the postulated results is cadmium chloride (Fig. 8). Its mode of action is unknown but the fact that it inhibits dimethylbenzoquinone reduction by PS II in higher

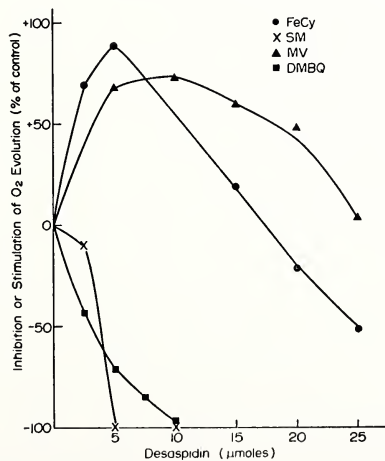


FIGURE 11. The effect of desaspidin on 4 chloroplast reactions: $H_2O \rightarrow$ ferricyanide (●), $H_2O \rightarrow$ silicomolybdic acid (X), $H_2O \rightarrow$ methyl viologen (Δ), and $H_2O \rightarrow$ dimethylbenzoquinone (■) in sucrose-sodium chloride chloroplasts. Reaction conditions as in Fig. 1.

concentrations suggests its closeness to the phosphorylation site in PS II.

An uncoupler like CCCP in presence of NH_4Cl shows only a slight stimulation of all 4 partial electron transport reactions studied (Fig. 9) but in absence of NH_4Cl greater stimulation is seen (Fig. 10). Silicomolybdic acid reduction is inhibited at a lower concentration (5 μmoles) while the other reactions still show varying degrees of stimulation due to uncoupling of electron transport from photophosphorylation. Desaspidin, another uncoupler, inhibits silicomolybdic acid reduction while stimulation of ferricyanide and the $\text{H}_2\text{O} \rightarrow$ methyl viologen reaction occurs (Fig. 11). Dimethylbenzoquinone reduction is not stimulated according to predictions because there may be an inhibition site in its pathway.

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