

Stimulation of Electron Transport from Photosystem II to Photosystem I in Spinach Chloroplasts¹

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Abstract

Electron transport from Photosystem II to Photosystem I of spinach chloroplasts can be stimulated by bicarbonate and various carbonyl or carboxyl compounds. Monovalent or divalent cations, which have hitherto been implicated in the energy distribution between the two photosystems, i.e., "spillover" phenomena at low light intensities, show a similar effect under high light conditions employed in this study. A mechanism for this stimulation of forward electron transport from Photosystem II to Photosystem I could involve inhibition of two types of Photosystem II partial reactions, which may involve cycling of electrons around Photosystem II. One of these is the DCMU-insensitive silicomolybdate reduction, and the other is ferricyanide reduction by Photosystem II at pH 8 in the presence of dibromothymoquinone. Greater stimulation of forward electron transport reactions is observed when both types of Photosystem II cyclic reactions are inhibited by bicarbonate, carbonyl and carboxyl-type compounds, or by certain mono- or divalent cations.

Introduction

Bicarbonate stimulation of Photosystem II in bicarbonate-depleted chloroplasts is well known [1-5]. Its main site of action is located between the two photosystems, i.e., between the DCMU inhibition site and the plastoquinone A site [6-8]. According to Stemler's studies with $\text{H}^{14}\text{CO}_3^-$ [9, 10], there is a small, high-affinity HCO_3^- binding pool located before the DCMU inhibition

¹Abbreviations used: DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea; DCIP, 2,6-dichloroindophenol; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; FeCN, potassium ferricyanide; MV, methylviologen; PS I, photosystem I; PS II, photosystem II; SM, silicomolybdate acid.

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site, and a larger pool with lower affinity for HCO_3^- binding, located after the DCMU inhibition site. Crane and Barr [11] and Barr and Crane [12] have shown that stimulation of forward electron transport reactions can be obtained by compounds other than bicarbonate or CO_2 , and that the mechanism of this stimulation may result from inhibiting cyclic electron flow around PS II, which preferentially prevented electrons from flowing towards PS I. This is a continuation of our search for a chemical "key" to control electron flow in PS II in chloroplasts.

Materials and Methods

Chloroplasts were made from market spinach as previously described [12]. The grinding and suspension medium was 0.4 M sucrose with 0.05 M NaCl. Chlorophyll was determined according to Arnon [13].

All photosystem I and II reactions were measured polarographically with a Clark-type electrode attached to a Yellow Springs Instrument oxygen monitor. Rates were recorded with a Sargent-Welch SRG recorder. Reaction mixtures for various reactions are provided in the figure legends. Reactions were uncoupled by 5 mM NH_4Cl . Light intensity for illumination of reaction mixtures was $\approx 4 \times 10^5$ ergs \cdot cm² \cdot sec.⁻¹, provided by a specially built lamp.

Dimethyl carbonate, propylene carbonate, and thiosalicylic acid were obtained from the Aldrich Chemical Co.

Results and Discussion

As shown by Govindjee and associates [1-10], bicarbonate stimulates electron transport up to 10 times at a site between the DCMU inhibition site and the plastoquinone pool in bicarbonate-depleted chloroplasts. As previously shown by Crane and Barr [11, 12], and as seen in Fig. 1A, bicarbonate can stimulate the forward electron transport reactions, such as the $\text{H}_2\text{O} \rightarrow \text{MV}$ (pH 7) and ferricyanide reduction at pH 6, if no other ions are present except 5 mM NH_4Cl as an uncoupler. Under these conditions the DCMU-insensitive silicomolybdate reduction by PS II and ferricyanide reduction in presence of dibromothymoquinone at pH 8 are inhibited by bicarbonate concentrations from 10-50 mM. Various carbonyl compounds, such as dimethyl carbonate (Fig. 1B) or propylene carbonate (Fig. 1C), behave in a similar manner, except that higher concentrations of them are required for stimulation of forward electron transport in chloroplasts isolated from summer spinach than previously reported from winter spinach [11]. Monovalent cations such as KCl (Fig. 1D) or divalent cations like MgCl_2

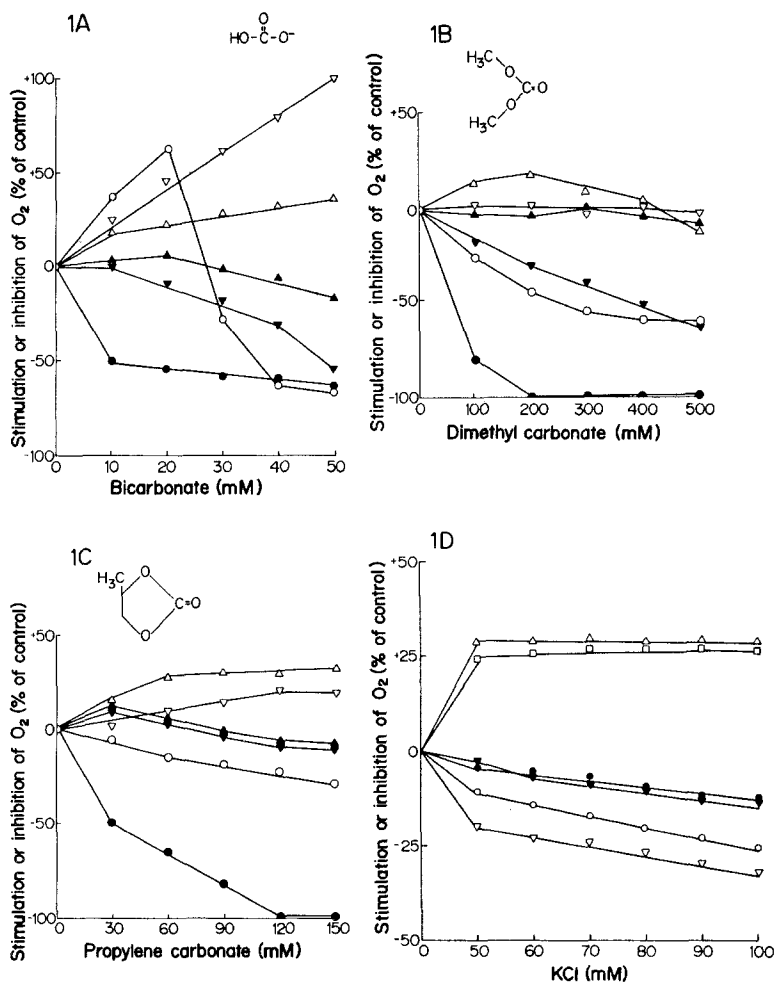


Fig. 1. Stimulation or inhibition of electron transport in spinach chloroplasts by various compounds. (A) Bicarbonate; (B) dimethyl carbonate; (C) propylene carbonate; (D) KCl. Control rates ($\mu\text{equiv./mg chl} \cdot \text{h}$) for $\text{H}_2\text{O} \rightarrow \text{MV}$, pH 7 (Δ), were: (A) 603; (B) 761; (C) 719; (D) 885; pH 8 (\blacktriangle): (A) 581; (B) 784; (C) 862; for $\text{H}_2\text{O} \rightarrow \text{FeCN}$, pH 6 (∇): (A) 209; (B) 265; (C) 237; (D) 316; pH 8 (\blacktriangledown): (A) 136; (B) 214; (C) 152; (D) 146; for $\text{H}_2\text{O} \rightarrow \text{SM}$ (+DCMU), pH 6 (\circ): (A) 276; (B) 226; (C) 220; (D) 293; pH 8 (\bullet): (A) 90; (B) 169; (C) 146; (D) 192; for $\text{H}_2\text{O} \rightarrow \text{DCIP}$, pH 7: (\square): (D) 704. The reaction mixture for the $\text{H}_2\text{O} \rightarrow \text{MV}$ reaction contained chloroplasts ($50 \mu\text{g}$ chlorophyll), 25 mM Tris-Mes buffer, pH 7 or 8, 5 mM NH_4Cl , 0.5 mM Na azide, and 0.5 mM MV. The reaction mixture for $\text{H}_2\text{O} \rightarrow \text{FeCN}$ contained chloroplasts as above, 25 mM Tris-Mes, pH 6, 5 mM NH_4Cl , and 0.25 mM FeCN. The reaction mixture for $\text{H}_2\text{O} \rightarrow \text{FeCN}$ (+DBMIB) contained chloroplasts as above, 25 mM Tris-Mes, pH 8, 2 μM DBMIB, 5 mM NH_4Cl and 0.5 mM FeCN. The reaction mixture for $\text{H}_2\text{O} \rightarrow \text{SM}$ (+DCMU) contained chloroplasts as above, 25 mM Tris-Mes, pH 6 or 8, 5 mM NH_4Cl , 2 μM DCMU, and 85 μM silicomolybdc acid at pH 6, 0.25 mM SM at pH 8. The reaction mixture for $\text{H}_2\text{O} \rightarrow \text{DCIP}$ contained chloroplasts above, 25 mM Tris-Mes, pH 7, 5 mM NH_4Cl , and 0.5 mM DCIP. + indicates stimulation; - indicates inhibition of rate in relation to control.

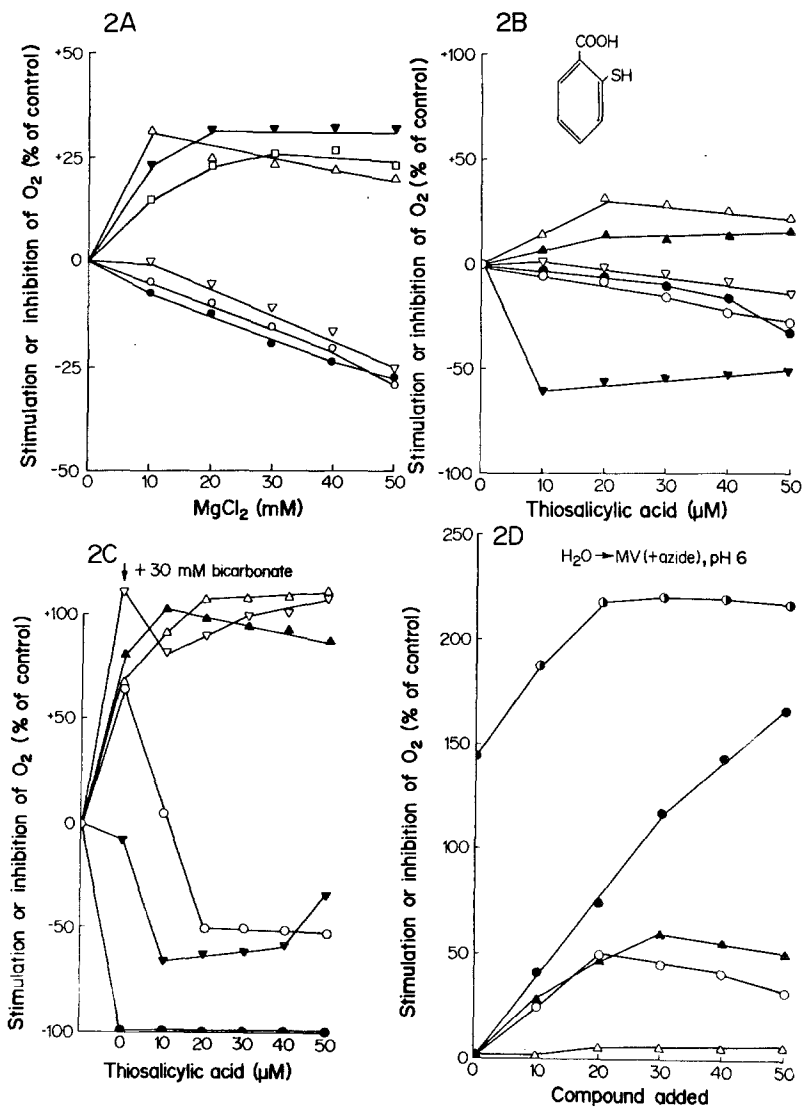


Fig. 2. Stimulation or inhibition of electron transport in spinach chloroplasts by various compounds or combinations of various stimulators and inhibitors. (A) $MgCl_2$; (B) thiosalicylic acid; (C) thiosalicylic acid plus 30 mM bicarbonate; (D) thiosalicylic acid, bicarbonate, and propylene carbonate. Control rates ($\mu\text{equiv./mg chl} \cdot \text{h}$) for $H_2O \rightarrow MV$, pH 7 (Δ) were: (A) 964; (B) 948; (C) 902; pH 8 (\blacktriangle): (B) 902; (C) 790; for $H_2O \rightarrow FeCN$, pH 6 (∇): (A) 282; (B) 158; (C) 152; pH 8 (\blacktriangledown): (A) 73; (B) 118; (C) 68; for $H_2O \rightarrow SM (+DCMU)$, pH 6 (\circ): (A) 293; (B) 158; (C) 96; pH 8 (\bullet): (A) 704. The control rate for $H_2O \rightarrow MV$, pH 6, in Fig. 2D was 271 without additions. Symbols in Fig. 2D designate various additions as follows: \circ , thiosalicylic acid (10–50 μM); \bullet , bicarbonate (10–50 μM); Δ , propylene carbonate (30–150 mM); \blacktriangle , propylene carbonate (90 mM) + thiosalicylic acid (10–50 μM). Reaction mixtures for various reactions are in Fig. 1. + indicates stimulation; – indicates inhibition of rate in relation to control.

(Fig. 2A) also stimulate the $\text{H}_2\text{O} \rightarrow \text{MV}$ pathway by about 25%, and indophenol reduction in PS II is inhibited. Ferricyanide reduction in this case appears to respond differently to monovalent than to divalent cations, when assayed at pH 6 or 8: MgCl_2 induced stimulation of ferricyanide reduction in the presence of dibromothymoquinone at pH 8, whereas KCl inhibited both types of ferricyanide reduction, either at pH 6 or 8. Ferricyanide reduction in the presence of dibromothymoquinone, when assayed at pH 8, has been previously shown by Sireci et al. [14] to involve a cyclic pathway around PS II, which can be inhibited by thiols. Inhibition of this pathway by low concentrations of thiosalicylic acid is shown in Fig. 2B. In the present communication we would again like to emphasize that several options are open to electrons arising from water oxidation in the light in isolated spinach chloroplasts: (1) they can proceed directly from PS II to PS I, as measured on the $\text{H}_2\text{O} \rightarrow \text{MV}$ pathway; (2) if an inhibitor such as dibromothymoquinone or DCMU prevents the transfer of electrons from PS II or PS I, they can cycle around PS II and can be measured as ferricyanide reduction in the presence of dibromothymoquinone at pH 8 or as silicomolybdic acid reduction in the presence of DCMU at pH 6 or 8.

With the above assumptions in mind, we propose that stimulation of forward photosynthetic electron transport is observed when the cycling of electrons around PS II is inhibited by bicarbonate or other ions and by carbonyl or carboxyl compounds, as shown by the data presented, according to the pathways outlined in Fig. 3. The role of mono- and divalent cations in the so-called "spillover," or the redistribution of excitation energy between the two photosystems described in detail by Gross and associates [15–18], can also be compared in terms stimulation of PS I reactions, when PS II cyclic reactions are inhibited by ions, although "spillover" occurs only under low light conditions, and would not be applicable to the present situation.

Theoretically, stimulation of forward electron transport should be at a maximum when all types of PS II cyclic reactions are inhibited. To test such a hypothesis, a number of partial reactions were tested in the presence of 30 mM bicarbonate to inhibit the DCMU-insensitive silicomolybdate reduction, with varying concentrations of thiosalicylic acid added to inhibit the dibromothymoquinone-insensitive ferricyanide reduction in PS II. With two possible cyclic pathways around PS II inhibited, the $\text{H}_2\text{O} \rightarrow \text{MV}$ reaction should be stimulated over the rate of stimulation obtained with each individual stimulator. Figures 2C and 2D show that this was the case. The double-inhibitor-induced stimulation on the $\text{H}_2\text{O} \rightarrow \text{MV}$ pathway was greater at pH 6 than at pH 7, possibly because at low pH the chloroplasts were partially bicarbonate-depleted and could, therefore, respond to added bicarbonate in a more spectacular way. Thus, the double-inhibitor studies confirm the hypothesis that inhibition of wasteful cycling of electrons around PS II could lead to

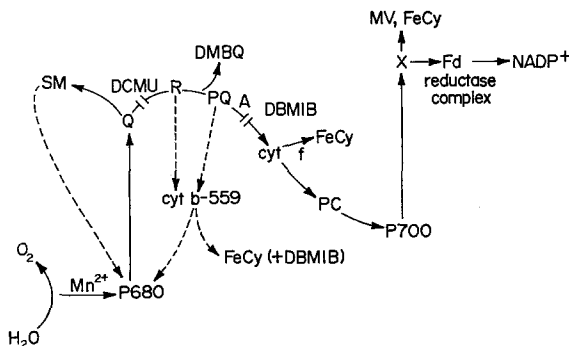


Fig. 3. Scheme for electron transport in chloroplasts, showing possible cyclic pathways around Photosystem II. Note that greatest stimulation of forward electron transport is expected when the two presumed cyclic pathways around PS II ($\text{H}_2\text{O} \rightarrow \text{SM}$ in presence of DCMU and $\text{H}_2\text{O} \rightarrow \text{FeCN}$ in presence of DBMIB) are jointly inhibited by bicarbonate and thiosalicylic acid (Fig. 2C).

stimulation of electron transport from PS II to PS I, with concomitant benefits of stimulated electron flow leading to increased photophosphorylation and, ultimately, to greater crop yields.

Acknowledgments

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