

Phosphate Stimulates or Inhibits Silicomolybdate Reduction in Spinach Chloroplasts

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Abbreviations used: ADP-adenosine phosphate dinucleotide; DCMU-3-(3,4-dichlorophenyl)-1,1 dimethylurea; MV-methylviologen; PS I - Photosystem I; PS II - Photosystem II.

Introduction

Silicomolybdic acid as an electron acceptor in chloroplasts was first described by Giaquinta *et al.* (7) and Barr *et al.* (4). Two sites were found in PS II: one, which was sensitive to DCMU, another, which was DCMU-insensitive. Berg and Izawa (5) showed that the DCMU-insensitive site could phosphorylate with a P/e_2 ratio of 0.2 in presence of protective agents, although Giaquinta and Dilley (6) had shown that no H^+ were transported in that portion of the electron transport chain from the water oxidation site to Q, the primary electron acceptor in PS II. After Barr and Crane (3) found 2 DCMU-insensitive silicomolybdate reduction sites in PS II of spinach chloroplasts, depending on the pH of the reaction medium, it became mandatory to describe all possible differences between these 2 sites. The present study was undertaken with the purpose of finding out, how anions affected silicomolybdate reduction in PS II.

Materials and Methods

Chloroplasts were prepared from market spinach in 0.4 M sucrose and 0.05 M NaCl as previously described (12). Chlorophyll was determined according to Arnon (1). Oxygen evolution or uptake was measured with a Clark-type electrode in illuminated chloroplasts according to Troxel *et al.* (12). Reaction mixtures for assays are given in figure legends. Reaction rates were recorded with a Sargent-Welch SRG recorder.

The anions used in this study were prepared as 0.25 M stock solutions of the sodium salts of phosphate, arsenate or sulfate. The pH of phosphate solutions was adjusted to 6 or 8 with an Orion pH meter, using mono and divalent phosphate salts; the pH of arsenate solutions was adjusted to pH 6 or 8 with HCl. Sulfate was used without adjustment of pH, the pH of Na sulfate solutions being ≈ 6.5 .

Results and Discussion

In order to find differences between the 2 DCMU-insensitive silicomolybdate reduction sites first described by Barr and Crane (3), hitherto separated on the basis of pH and various inhibitors, the effect of anions on these 2 sites was investigated in this study.

Previous studies, too numerous to discuss in detail here, have shown that ions can affect electron transport in chloroplasts in various ways, including a direct effect on the light harvesting pigment-protein complex (11), an effect on CF_1 (11), the coupling factor, or an effect on grana stacking (11). Alteration of local surface charges by ions (9) also have been investigated. In this study we have examined the effect of 3 anions—phosphate, arsenate, and sulfate—on electron transport under phosphorylating and under uncoupled conditions, in presence or absence of additional buffers. Figures 1A and 1B show that electron transport through PS I and II in a buffered system is only slightly stimulated by phosphate

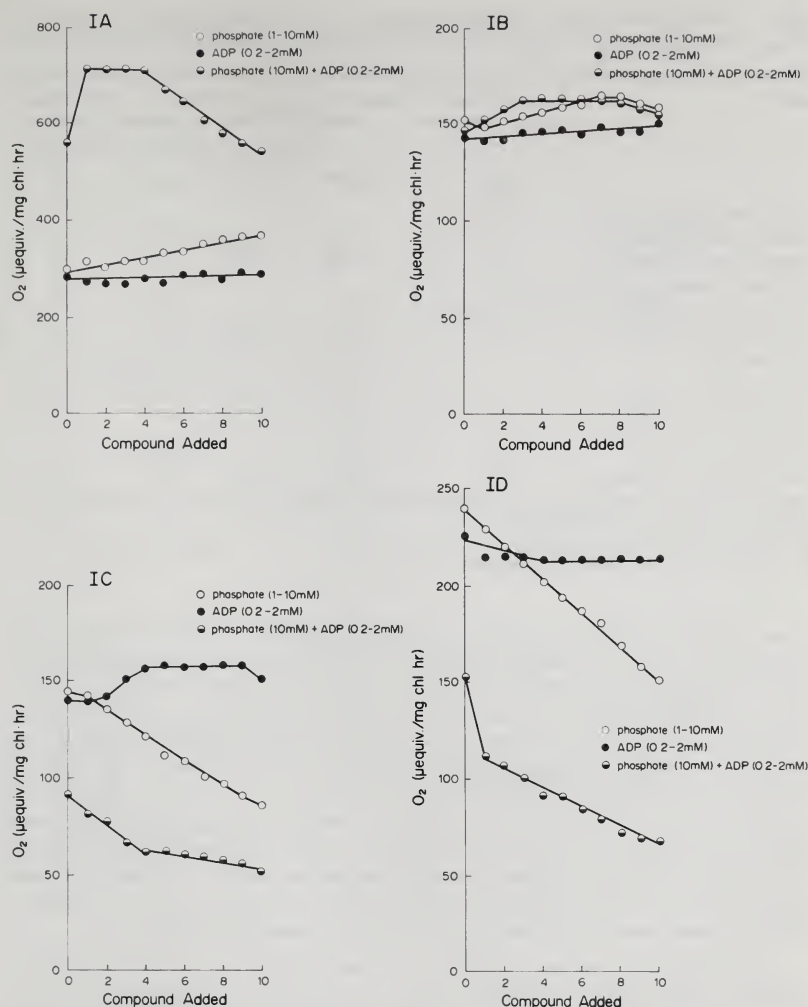


FIGURE 1A-D. *Stimulation or Inhibition of Electron Transport Reactions in Spinach Chloroplasts by Phosphate in a Buffered System.* 1-A. $H_2O \rightarrow MV$ (+ azide), pH 8; 1-B $H_2O \rightarrow SM$ (+ DCMU), pH 6; 1-C $H_2O \rightarrow SM$ (+ DCMU), pH 8, and 1-D $H_2O \rightarrow SM$, pH 8. The reaction mixture for 1-A contained 25 mM Tris-Mes, pH 8, 0.5 mM Na azide and 0.5 mM MV; for 1-B 0.25 mM Tris-Mes, pH 6, 5 μM DCMU, and 0.075 mM SM; for 1-C 25 mM Tris-Mes, pH 8, 5 μM DCMU, and 0.25 mM SM; for 1-D 0.25 mM Tris-Mes, pH 8, and 0.25 mM SM. Other compounds were added to these reaction mixtures as indicated.

in the $H_2O \rightarrow MV$ (+ azide) reaction and in the $H_2O \rightarrow SM$ (+ DCMU) reaction at pH 6 in PS II, while phosphate inhibited both the DCMU-sensitive and the DCMU-insensitive silicomolybdate reduction at pH 8. This inhibition of electron transport by phosphate at pH 8 is not associated with uncoupling, because it is not eliminated in uncoupled chloroplasts (Table 1). This effect can also be considered

TABLE I. *Stimulation of Silicomolybdc Acid Reduction By Phosphate Anions In Spinach Chloroplasts In Absence of Tris-Mes Buffer and NH₄Cl and Decreased Stimulation In Buffered, Uncoupled Chloroplasts*

Reaction	Phosphate conc. for maximum stimulation	Stimulation by phosphate in unbuffered chloroplasts		Decreased stimulation by phosphate in buffered, uncoupled chloroplasts	
		(mM)	rate ^{1,2]} % ^{3]}	rate ^{1,2]} % ^{3]}	
H ₂ O - SM (+ DCMU), pH 6					
reaction rate (- buffer)	10	45	-	192	-
reaction rate (+ phosphate)	10	124	+ 176 ^{3]}	192	0
H ₂ O - SM (+ DCMU), pH 8					
reaction rate (- buffer)	5	62	-	107	-
reaction rate (+ phosphate)	5	118	+ 90	85	- 21
H ₂ O - SM, pH 8					
reaction rate (- buffer)	5	51	-	136	-
reaction rate (+ phosphate)	5	146	+ 186	79	- 42
H ₂ O - MV (+ azide), pH 8					
reaction rate (- buffer)	10	203	-	919	-
reaction rate (+ phosphate)	10	519	+ 156	896	- 2

1] Reaction rate equals $\mu\text{equiv./mg chl} \cdot \text{hr}$

2] The composition of reaction mixtures is given in figure legends

3] + indicates % stimulation over control rates.

an anti-uncoupling effect. Its origin is unknown at present. Arsenate (Figures 2A-C) showed a comparable slight stimulation of the various reactions and an inhibition of silicomolybdate reduction at pH 8. A slight stimulation in place of inhibition in the overall silicomolybdate pathway (Figure 2-D) by arsenate also occurred. Arsenate had previously been shown to stimulate electron transport through uncoupling (8). Sulfate (Figures 3A-D) was not a good stimulator of electron transport in this study, which shows that the phosphate and arsenate effects were not simple ionic strength effects, compared to phosphate or arsenate. Sulfate was shown by Pick and Avron to act as an energy transfer inhibitor (10). Stimulation by phosphate in all the reactions studied was greatest, when no other buffer was present in the reaction mixtures. Under these conditions (Table 1) electron transport rates were stimulated from 90-186% in absence of ADP.

Stimulation of electron transport by anions appeared to be influenced by uncoupling. When NH₄Cl was present (Table 1), stimulation was abolished, implying that phosphate, arsenate, and sulfate could stimulate electron transport by acting as uncouplers themselves. This is not surprising, since arsenate has been used as an uncoupler in mitochondria.

The stimulation of electron transport by phosphate in the DCMU-insensitive silicomolybdate pathway at pH 6 is consistent with Berg and Izawa's (5) interpretation, that this short segment of the electron transport chain represents a PS II energy conservation site with a P/e_2 of 0.2.

In summary, this study shows that anions can stimulate overall electron transport and the DCMU-insensitive silicomolybdate reduction at pH 6, while inhibiting all silicomolybdate pathways at pH 8. The order of effectiveness of anions is as follows: phosphate > arsenate > sulfate. The mechanism of this stimulation may be related to an uncoupling-type reaction, involving the coupling factor, although lesser effects by anions, such as alteration of local surface charges, cannot be excluded as contributing factors of the stimulation observed.

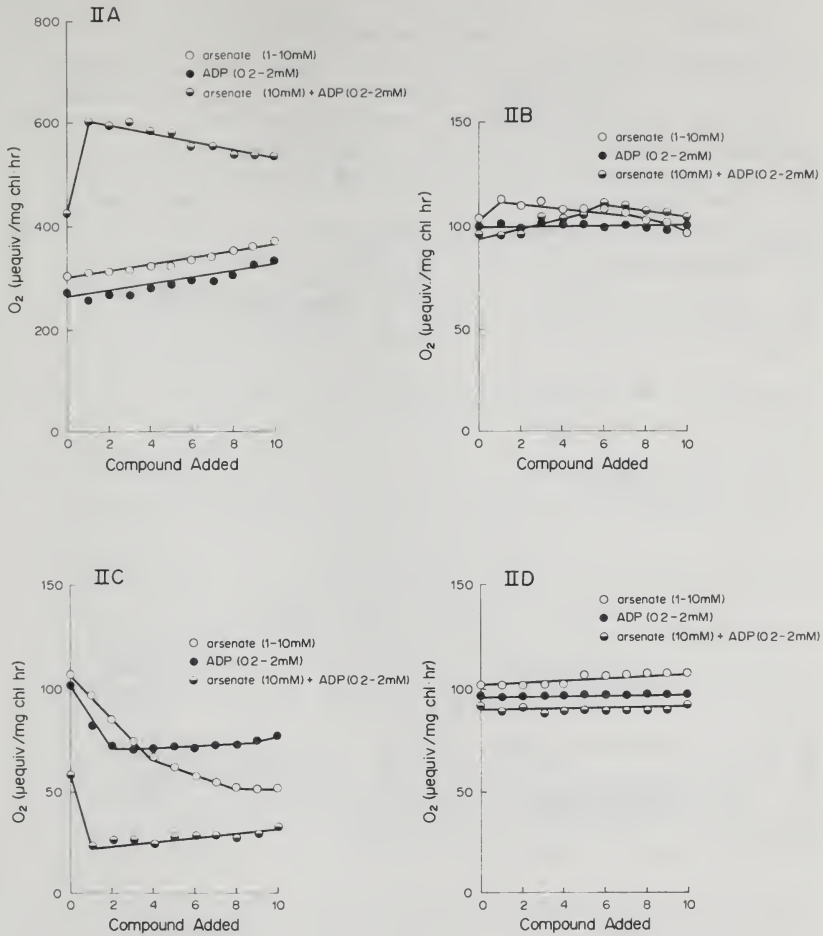


FIGURE 2A-D. Stimulation or Inhibition of Electron Transport Reactions in Spinach Chloroplasts by Arsenate in a Buffered System. 2-A $H_2O \rightarrow MV$ (+ azide), pH 8; 2-B $H_2O \rightarrow SM$ (+ DCMU), pH 6; 2-C $H_2O \rightarrow SM$ (+ DCMU), pH 8; 2-D $H_2O \rightarrow SM$, pH 8. Reaction mixtures as in Fig. 1A-D.

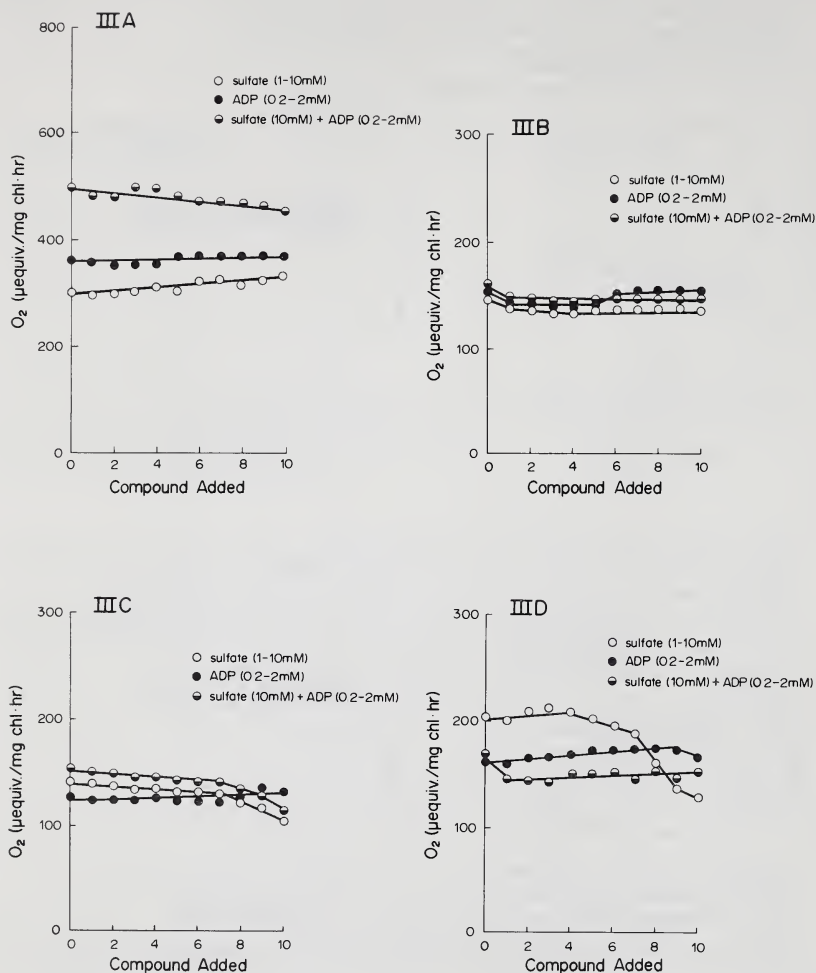


FIGURE 3A-D. Inhibition of Electron Transport Reactions in Spinach Chloroplasts by Sulfate in a Buffered System. 3-A $H_2O \rightarrow MV$ (+ azide), pH 8; 3-B $H_2O \rightarrow SM$ (+ DCMU), pH 6; 3-C $H_2O \rightarrow SM$ (+ DCMU), pH 8; 3-D $H_2O \rightarrow SM$, pH 8. Reaction mixtures as in Fig. 1A-D.

Acknowledgements

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