L-Methionine Sulfoximine as a New Electron Acceptor in Photosystem I of Spinach Chloroplasts

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Abbreviations: DCMU-3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB-2, 5-dibromo-3-methyl-6-isopropyl-p-benzoquinone or dibromothymoquinone; MSO-L-methionine sulfoximine; MV-methylviologen; PSI-photosystem I.

Introduction

Methylviologen, the best known electron acceptor in Photosystem I of spinach chloroplasts, or other viologen-type compounds presumably accept electrons from the reaction center complex or from a bound iron-sulfur protein associated with this complex (1). The purpose of this study was to look for other artificial electron acceptors in the methylviologen region and to find unique inhibitors, which would differentiate between these pathways. L-Methionine sulfoximine proved to be such an electron acceptor, capable of differentiating a site after the methylviologen site. Such a conclusion was reached on the basis of using 1-(2-thiazolylazo)-naphthol as a specific inhibitor for the new pathway.

Materials and Methods

Chloroplasts were isolated from market spinach in 0.4 M sucrose and 0.05 M NaCl as previously described (2). Chlorophyll was determined according to Arnon (3). Oxygen uptake was measured with a Clark-type oxygen electrode according to Barr and Crane (2). Reaction mixtures for assays are given in figure legends. Reaction rates were recorded with a Sargent-Welch SRG recorder.

Results and Discussion

L-methiomine sulfoximine gives oxygen uptake like methylviologen when used as an electron acceptor in PS I. As Table I shows, optimum reaction rates were obtained with a concentration between 50 and 100 μM at pH 7. The $H_2O \rightarrow$

Assay Conditions for the Photosystem I Reaction
H_2O → Methionine Sulfoximine (+ Na Azide)

Methionine Sulfoximine (μM)	Reaction Rate (μquiv. O_2/mg chl•hr)
I Optimum Concentration	
25	192
50	226
75	395
100	338
75 (minus Na azide)	68
75 (minus NH_4Cl)	90
II Optimum pH	
pH 6	297
pH 7	395
pH 8	221

Inhibition of the H_2O → Methionine Sulfoximine Reaction by Standard Inhibitors

Inhibitor	Conc.	Electron Transport H_2O → MV (+ azide), pH 7		H_2O → MSO (+ azide), pH 7	
		Rate[a]	Inhibition (%)	Rate[a]	Inhibition (%)
	(μM)				
None	—	538	0	310	0
DCMU	5.0	0	100	0	100
DBMIB	2.5	56	90	56	82
Bathophenanthroline	100	60	89	42	86
Polylysine (M.W. 30,000)	50 μg/ml	96	82	62	80

[a] μequiv. O_2mg chl•hr

methylviologen (+ azide) and the H_2O→ methionine sulfoximine (+ azide) reactions were inhibited to a similar extent by the standard inhibitors for sites between the 2 photosystems: DCMU for the B protein site (4), dibromothymoquinone for the plastoquinone pool (4), bathophenanthroline for the Rieske iron-sulfur protein located between the plastoquinone pool and cytochrome f (5), and polylysine for plastocyanin (6). These inhibitions range from 92-100% for both reactions (Table II). Ions, such as Ca^2+, Mg^2+, Sr^2+, or Ba^2+ stimulate both reactions (Fig. 1 and 2), although to a lesser degree on the H_2O→ methionine sulfoximine pathway.

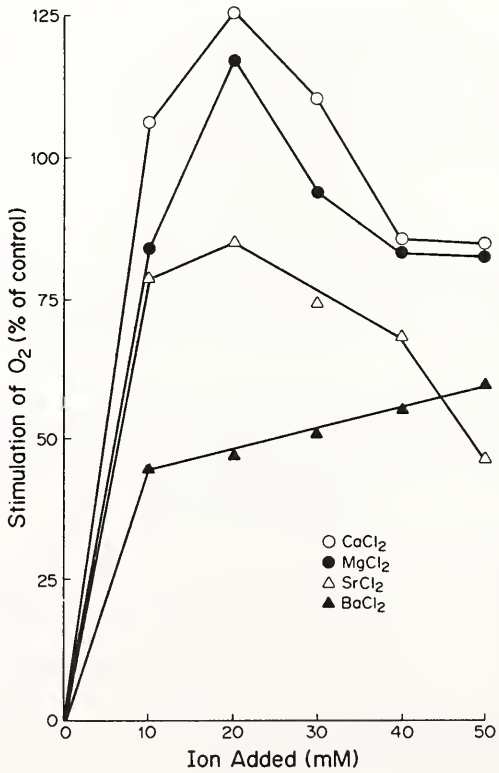

FIGURE 1. *Stimulation of Electron Transport in Spinach Chloroplasts by Ions. The reaction tested was H_2O→ methylviologen (+azide), pH 7. The reaction mixture contained chloroplasts (0.05 mg. chlorphyll), 25 mM Tris-Mes, pH 7, 5mM NH_4Cl, 0.5 mM Na azide, and 0.5 mM MV. The control rate was 530 uequivalents O_2/mg chl•hr.*

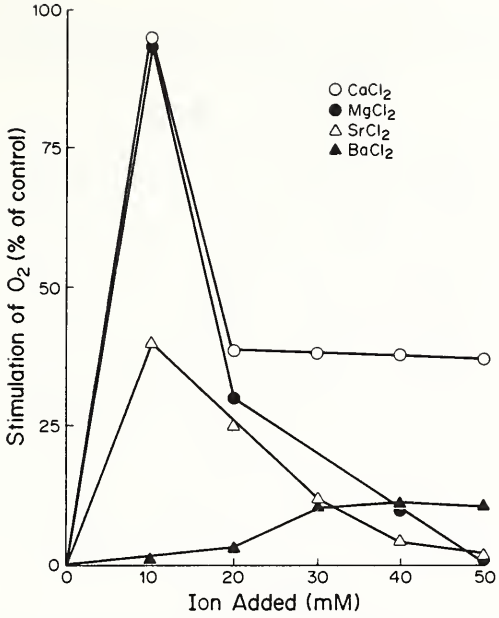

FIGURE 2. *Stimulation of Electron Transport in Spinach Chloroplasts by Ions. The reaction tested was $H_2O \rightarrow$ methionine sulfoximine (+azide), pH 7. The reaction mixture contained chloroplasts (0.05 mg chlorophyll), 25 mM Tris-Mes, pH 7, 5mM NH_4Cl, 0.5 mM Na azide, and 75 μM MSO. The control rate was 281 μquiv. O_2/mg chl•hr.*

The data presented so far allow no differentiation between the two pathways. Only the use of such an iron chelator as 1-(2-thiazolylazo)-naphthol (Fig.3) or incubation with sodium selenite (Fig. 4) uncovers differences. As Fig. 3 shows, the iron chelator inhibits the $H_2O \rightarrow$ methionine sulfoximine pathway more than 80%,

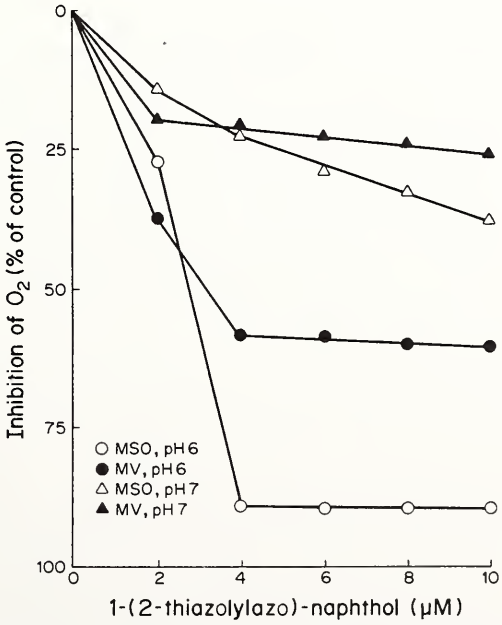

FIGURE 3. *The Inhibition of Electron Transport in Spinach Chlorophasts by 1-(2-Thiazolylazo)-Naphthol. The control rate for $H_2O \rightarrow MV$ (+azide), pH 7 was 575 μequiv. O_2/mg chl•hr, for $H_2O \rightarrow MSO$ (+azide), pH 7, it was 293. The reaction mixtures were as in Figures 1 and 2.*

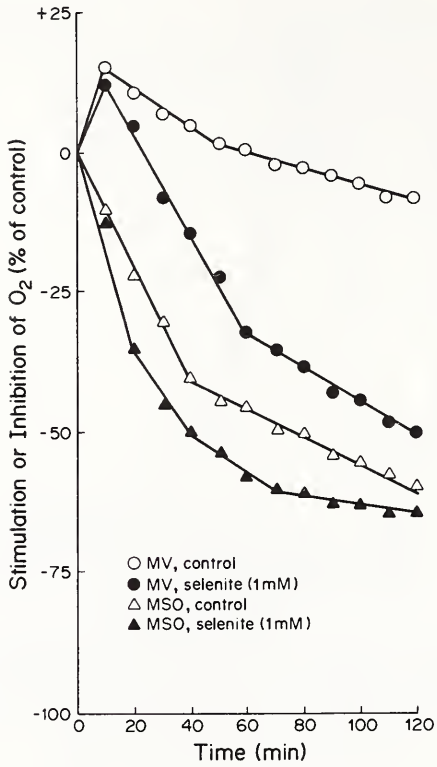

FIGURE 4. *The Inhibition of Electron Transport in Spinach Chloroplasts by Sodium Selenite. The control rate for $H_2O \rightarrow MV$ (+azide), pH 7 was 496 μequiv. O_2mg chl•hr, for $H_2O \rightarrow MSO$ (+azide), pH 7 it was 338. The reaction mixture were as Figures 1 and 2.*

but the $H_2 \rightarrow$ methylviologen pathway over 50%. This indicates that the new electron acceptor methionine sulfoximine is able to reach a site past where methylviologen accepts electrons in PS I. This new site may be a bound iron-sulfur protein, several of which have been detected by studies of PS I reaction center particles by flash-induced absorption changes (7,8) or by EPR spectroscopy (9). With the idea in mind that selenium ions can replace sulfur in iron-sulfur protein cores (10), a 120-min. incubation of chloroplasts with sodium selenite was undertaken. As Fig. 4 shows, the $H_2O \rightarrow$ methylviologen reaction was inhibited about 50% in two hours, but the $H_2O \rightarrow$ methionine sulfoximine pathway was inhibited only about 5% compared to its sucrose-NaCl control. However, since the methionine sulfoximine pathway and its control lost about 60% activity, it is possible that the acceptor site reached by methionine sulfoximine is an iron-sulfur site exposed to the outside of the membrane, which has lost its labile sulfur during incubation, while exposed to atmospheric oxygen. A total of 3-4 bound iron-sulfur centers are known to exist in PS I (11). It is also known that the sulfur in iron-sulfur clusters is labile, particularly when exposed to atmospheric oxygen (12). The fact that selenite was able to inhibit the $H_2 \rightarrow$ methylviologen pathway 50%, implies that its iron-sulfur was originally less exposed to the outside than the presumed iron-sulfur on the $H_2O \rightarrow$ methionine sulfoximine pathway.

In conclusion, it can be seen that L-methionine sulfoximine can act as an electron acceptor in PS I and that it may accept electrons at a different point that methylviologen. This site is more sensitive to loss of activity in contrast to the selenium-inhibited iron-sulfur site found on the methylviologen pathway.

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