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SELECTIVE THIOL INHIBITION OF FERRICYANIDE REDUCTION IN PHOTOSYSTEM II OF SPINACH CHLOROPLASTS

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Summary

Selective inhibition of ferricyanide reduction in photosystem II by lipophilic thiols indicates a unique pathway of electron transport, which is not involved in reduction of class III acceptors or transfer of electrons to photosystem I. Both aromatic and aliphatic thiols induce the inhibition, but thiol binding reagents such as p-hydroxymercuribenzoate or N-ethylmaleimide do not inhibit. The inhibition can be observed using either dibromothymoquinone or bathophenanthroline to direct electrons away from photosystem I. No pretreatment of chloroplasts with thiols in the light was necessary to inhibit ferricyanide reduction by photosystem II or the O_2 evolution associated with ferricyanide reduction.

Introduction

The action of thiols on chloroplast electron transport has not been studied extensively. Kobayashi, Inoue and Shibata (1) showed that treatment of chloroplasts with p-nitrothiophenol in the light resulted in changes of chloroplast fluorescence and inhibition of PS II electron transport. In a later study (2) these authors showed that indophenol reduction was blocked on the oxidizing side of PS II after treatment with p-nitrothiophenol and other aromatic mercaptans in the light. Their purpose was to achieve membrane modification, light providing energy for acylthiol bond formation. In the present study, we tested various aromatic mercaptans and thiol reagents on several chloroplast partial reactions without preincubation or pretreatment in the light and found that ferricyanide reduction in PS II was inhibited selectively by various lipophilic thiol compounds. Preliminary results involving thiol inhibition indicate that a unique electron transport pathway operates in PS II, when forward electron flow toward PS I is inhibited by dibromothymoquinone

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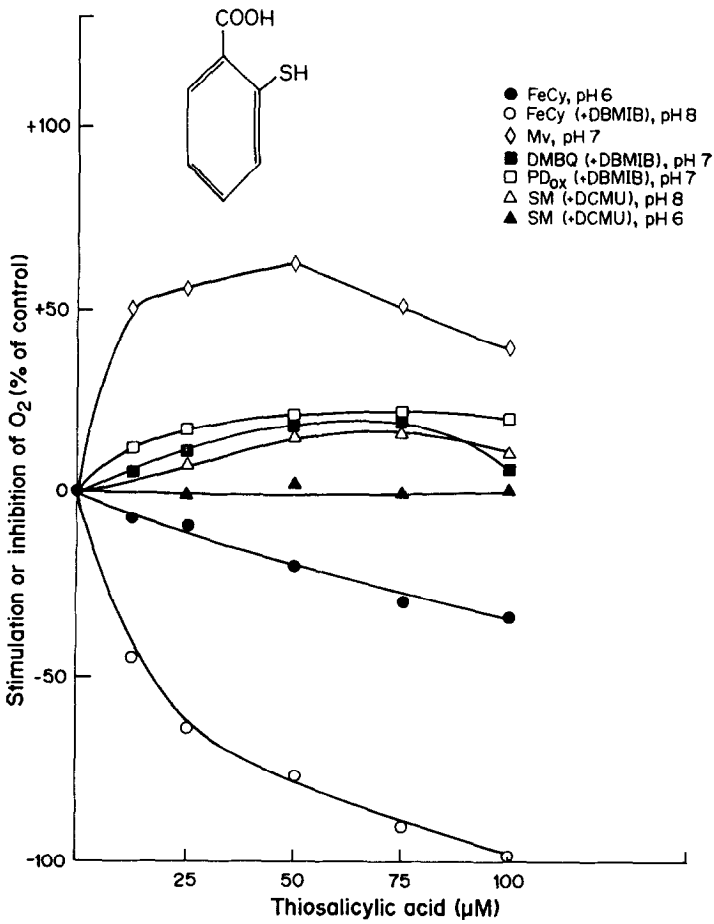

Fig. 1. The Effect of Thiosalicylic Acid on Photosystem I and II Reactions in Isolated Spinach Chloroplasts. The reaction mixture contained chloroplasts (33 μg chl/ml), buffer (25 mM Tris-Mes, pH 7 or 8, 2 mM NH_4Cl as uncoupler where necessary, 3 mM $MgCl_2$, 250 or 500 μM FeCy, 2 μM DBMIB where needed, 0.5 mM MV, 0.75 mM DMBQ, 0.5 mM PD_{ox}, 5 μM DCMU where needed, and 0.2 mg SM/ml. The control rate (μequiv./mg chlor./hr) for $H_2O \rightarrow$ FeCy, pH 6 was 250; for $H_2O \rightarrow$ FeCy (+DBMIB), pH 8 - 150; for $H_2O \rightarrow$ MV - 450; for $H_2O \rightarrow$ DMBQ (+DBMIB), pH 7-500; for PD_{ox} (+DBMIB), pH 7-550; $H_2O \rightarrow$ SM (+DCMU), pH 6-82; for $H_2O \rightarrow$ SM (+DCMU), pH 8-115.

or by bathopnenanthroline. Thiol-sensitive ferricyanide reduction by PS II also occurs in KCN-treated chloroplasts. The site of thiol inhibition does not inhibit class III electron acceptors, indicating that a split pathway around PS II may be involved.

Materials and Methods

Sucrose-NaCl (SN) chloroplasts were isolated from market spinach in 0.4 M sucrose and 0.05 M NaCl as previously described (3). The stripped but

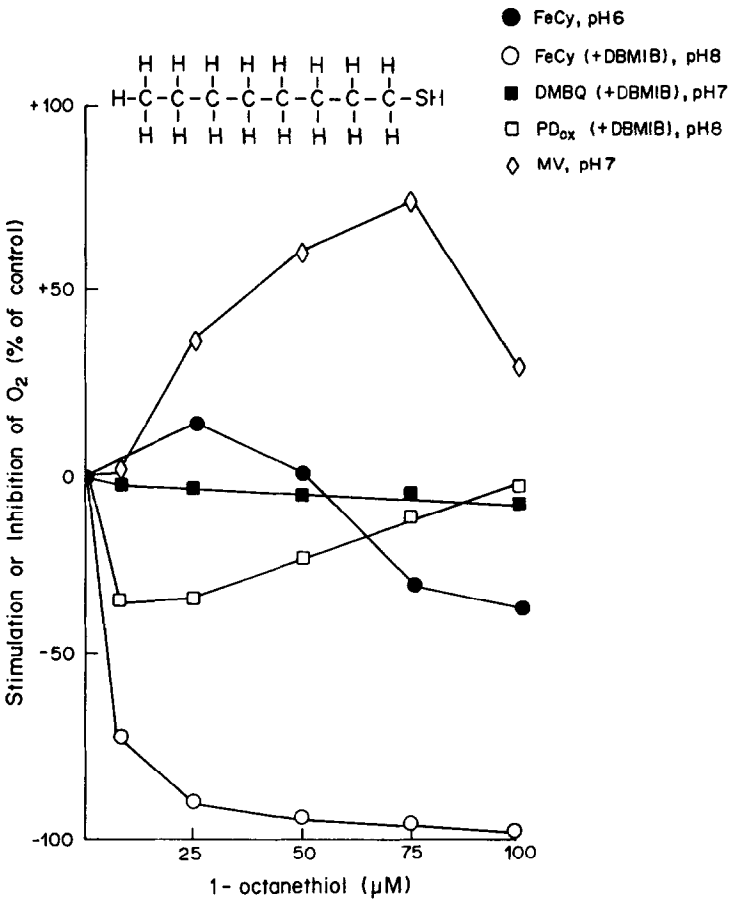

Fig. 2. The Effect of 1-Octanethiol on Photosystem I and II Reactions in Isolated Spinach Chloroplasts. Reaction conditions and control rates as in Fig. 1.

mainly unfragmented chloroplasts were suspended in SN for assays. Chlorophyll was determined according to Arnon (4). Ferricyanide-dependent O_2 evolution was measured with a Clark-type electrode at 24°C. Ferricyanide reduction was measured spectrophotometrically at 420 nm. Saturating white light (5×10^5 ergs/cm^2-sec^{-1}) was provided from a specially built lamp. Reaction mixtures are given in figure legends. Reaction rates were recorded with a Sargent-Welch Model SRG recorder.

Thiosalicylic acid and other thiols were purchased from the Aldrich Chemical Co.

Results and Discussion

Various chloroplast partial reactions including ferricyanide, oxidized p-phenylenediamine and 2,5-dimethylbenzoquinone reduction in presence of dibromothymoquinone (DBMIB) for PS II and $H_2O \rightarrow$ methylviologen for PS I and II

Table I. The Effect of Thiol Reagents on Photosystem II Reactions in Spinach Chloroplasts.

Reagent	Conc. (μM)	Inhibition or Stimulation of Electron Transport Reactions (% of control) FeCy[a]	DMBQ[b]	MV[c]
p-Chloromercuribenzoic acid	100	-26	+ 9	-33
n-Ethylmaleimide[d]	100	-42	0	-29
n-Phenylmaleimide	100	-26	+34	-14
Glutathione (reduced)	100	-50	+51	+24
Cysteine	100	-60	+13	+31
2-Mercaptoethanol	100	-37	- 7	-10
4-Nitrothiophenol	100	-100	-40	- 5
Butanethiol	100	-54	-70	+30

[a] Control rate of $H_2O \rightarrow$ FeCy (+DBMIB), pH 8 was 150 μequiv./mg chl · hr

[b] Control rate of $H_2O \rightarrow$ DBMQ (+DBMIB), pH 7 was 851 μequiv./mg chl · hr

[c] Control rate of $H_2O \rightarrow$ MV, pH 7 was 450 μequiv./mg chl · hr

[d] 10 min. treatment

overall electron transport were tested for thiol inhibition. It was found that thiosalicylic acid (Fig. 1), 1-octanethiol (Fig. 2) and other lipophilic thiols (Table I) selectively inhibited ferricyanide reduction in PS II, but mainly stimulated or only slightly inhibited the other reactions. Other thiol-reactive agents, such as p-chloromercuribenzoic acid or various maleimides (Table I) inhibited ferricyanide reduction in PS II less than 50%. Inhibition of PS II electron transport by lipophilic thiols is unique in several ways. (1) Relatively low concentrations of thiols (100 μM) give 100% inhibition, if ferricyanide reduction rates in PS II in presence of DBMIB at pH 8 are between 100-200 μequiv./mg chl · hr. It is also essential for complete thiol inhibition that ferricyanide reduction under the above conditions is unaffected by NH_4Cl or other uncouplers; otherwise ferricyanide is reduced at 2 different sites, one of which is stimulated by thiols. This is illustrated in Fig. 3. Here an increment of the total

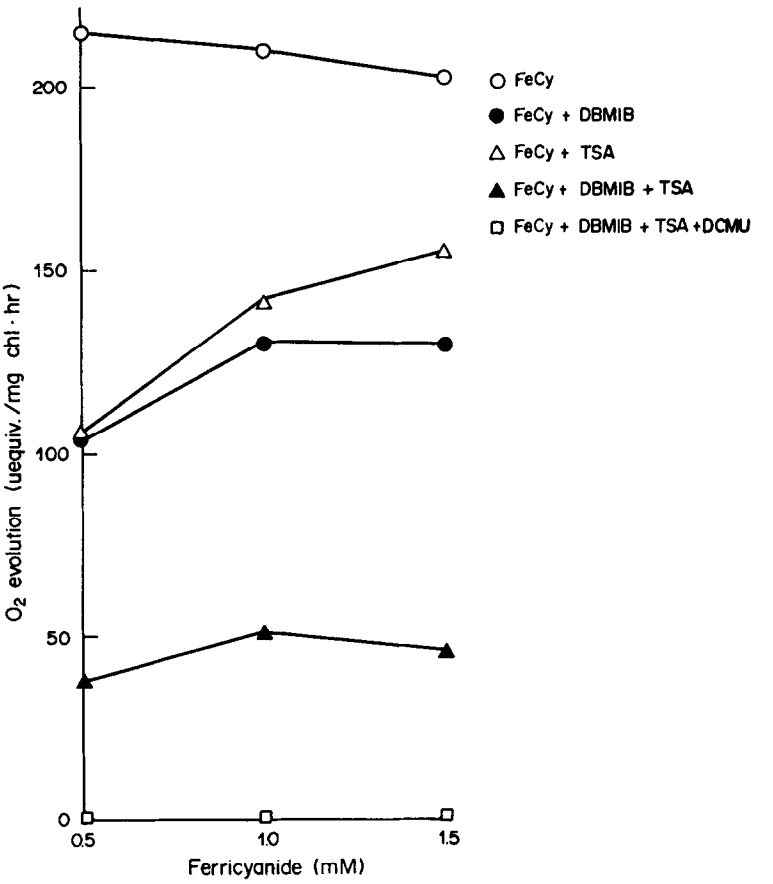

Fig. 3. Inhibition of O_2 Evolution Associated with Ferricyanide Reduction in Spinach Chloroplasts by Thiosalicylic Acid and Other Inhibitors. Reaction mixtures and control rates as in Fig. 1, except all reactions run at pH 7 with 2 μM DBMIB and 100 μM thiosalicylic acid present.

ferricyanide reduction rate at pH 7 is insensitive to DBMIB. This indicates the PS II site, which can be inhibited by thiols. Without DBMIB, less inhibition by thiols is seen, because electrons then preferentially use the forward pathway to PS I, which is stimulated by thiols. (2) As shown in Fig. 3, and as explained above, thiol inhibition of ferricyanide reduction in PS II requires the presence of an inhibitor of forward electron transport, such as 2 μM DBMIB or 100 μM bathophenanthroline. KCN-treated chloroplasts (5) can also be substituted in studies of thiol inhibition in PS II. (3) Both aromatic and aliphatic thiols inhibit ferricyanide reduction in PS II within a 3-minute preincubation period.

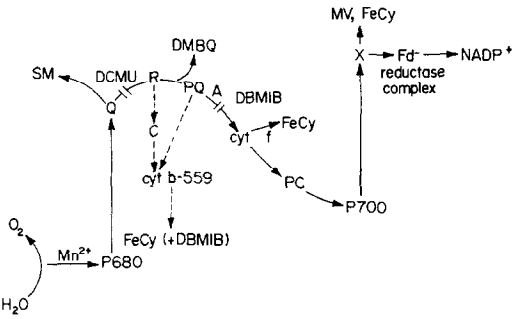

Fig. 4. A Modified Z-Scheme of Electron Transport in Spinach Chloroplasts, Showing a Split Pathway in PS II, which May Involve Cytochrome b-559.

The only requirement for inhibition appears to be the necessity for a lipophilic compound, which can penetrate chloroplast membranes easily, since octane thiol (Fig. 2) gives better inhibition than butanethiol (Table I), for example. (4) The thiols used in this study show a differential effect on ferricyanide reduction in presence of DBMIB and regular class III acceptor sites, such as dimethylbenzoquinone or p-phenelenediamine reduction in presence of DBMIB, thereby providing evidence for a new pathway in PS II. This split pathway could originate from R, the hypothetical acceptor located between the DCMU inhibition site and the plastoquinone pool (6,7) or go directly through plastoquinone A itself (Fig. 4) in a pathway around PS II involving cytochrome b-559, as described by Whitmarsh and Cramer (8).

The mode of inhibition by thiols on ferricyanide reduction in presence of DBMIB in PS II is unknown. However, the effect studied here appears to involve a site different from Kobayashi, Inoue and Shibata's (1,2) site, which required pretreatment of chloroplast membranes in the light with p-nitrothiophenol or other aromatic thio compounds. Aliphatic thiols were ineffective in their studies. Since Kobayashki et al. (1,2) detected changes in chloroplast fluorescence after their treatment with aromatic mercaptans, they localized their site of inhibition on the oxidizing side of PS II. They interpreted the thiophenol treatment as modification of the carboxyl groups of some PS II proteins, because thiophenol is known to combine by a covalent bond with a carboxyl side chain in a protein

to form an acylthiol bond. We would like to consider additional alternatives for the mode of action of thiols in PS II. Since thiol reagents, such as PCMB or n-ethylamaleimide (Table I) are not as effective as thiosalicylic acid or octanethiol in causing inhibition on the ferricyanide pathway in PS II, disulfide bond formation with SH groups on a chloroplast protein can be excluded from further consideration. Studies are underway in our laboratory to test the possibility of thioquinone formation between lipophilic thiols and plastoquinone A, the effect of thiols on non-heme iron proteins, hemes, such as found in cytochrome b-559 in PS II, or the reaction between copper proteins and lipophilic thiols in PS II.

Acknowledgements

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