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SULFHYDRYL REAGENTS INHIBIT ELECTRON TRANSPORT IN PHOTOSYSTEM II OF SPINACH CHLOROPLASTS

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A 120 min incubation period with sulfhydryl reagents, such as *p*-chloromercuribenzoic acid, shows greater than 50% loss of electron-transport activity in Photosystem (PS) II of spinach chloroplasts. Since *p*-chloromercuriphenylsulfonic acid, a nonpenetrating sulfhydryl reagent, and 4,4′-dithiopyridine, a bifunctional sulfhydryl reagent, show greater inhibition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea-insensitive silicomolybdate reduction than of dibromothymoquinone-insensitive indophenol reduction, it is postulated that two different sulfhydryl reagent-sensitive sites are involved in the PS II electron-transport chain of spinach chloroplasts.

Incubation of erythrocyte membranes with sulfhydryl reagents has aided in the elucidation of membrane topology, helping to distinguish extrinsic from intrinsic membrane proteins [1]. In chloroplasts, sulfhydryl reagents have mainly been used for the modification of CF_1, the coupling factor [2–10], or as energy-transfer inhibitors in photophosphorylation [11,12]. There are only scattered data on sulfhydryl reagent effect on electron transport [2,4,13], mainly because these reagents stimulate chloroplast electron-transport activities at low concentrations or show only a slight inhibition.

In the present study, we report inhibitions of up to 100% of electron-transport rates in PS II by incubating spinach chloroplasts with sulfhydryl reagents for 120 min. We describe two sulfhydryl sites in PS II, one of which is on the DCMU-insensitive silicomolybdate pathway, the other on the forward pathway toward PS I, located between the PS II reaction center, but before the dibromothymoquinone-inhibition site.

Sucrose/NaCl chloroplasts were prepared from market spinach in 0.4 M sucrose and 0.05 M NaCl as previously described [14]. Chlorophyll was determined according to the method of Arnon [15]. Oxygen evolution was measured with a Clark-type electrode, attached to a Yellow Springs oxygen monitor. Samples were illuminated with white light from a specially built light source, passed through a flask containing a saturated $CuSO_4$ solution (light intensity approx. $4 \cdot 10^5$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1}). Oxygen-evolution rates were recorded with a Sargent-Welch SRG recorder.

The procedure for obtaining sulfhydryl reagent inhibition of electron transport consisted of incubating spinach chloroplasts (1 mg Chl) with the desired concentration of thiol reagents in the dark at 4°C for a period of 120 min. Aliquots of chloroplasts (0.05 mg Chl) were removed every 10 min for assays. Controls consisted of an equal amount of chloroplasts to which either water or ethanol was added in the appropriate amounts.

Abbreviations: Mes, 4-morpholineethanesulfonic acid; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (dibromothymoquinone); DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; PCMB, *p*-chloromercuribenzoic acid; PCMBS, *p*-chloromercuriphenylsulfonic acid; PS, photosystem; Chl, chlorophyll.

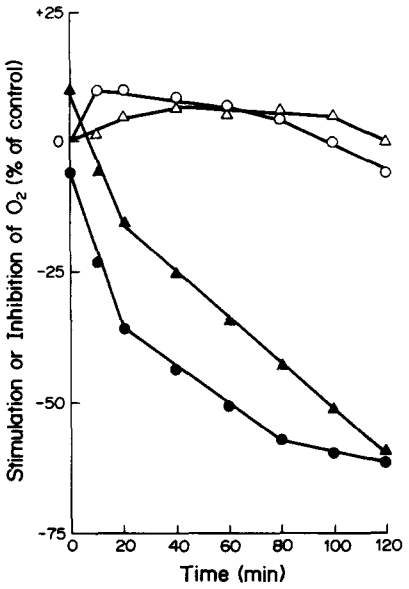


Fig. 1. The effect of PCMB on DCMU-insensitive silicomolybdate reduction in PS II of spinach chloroplasts. The control rate for the pH 6 reaction was 218 μequiv./mg Chl per h, at pH 8, 152. The reaction components for the pH 6 reaction included chloroplasts (0.05 mg Chl), 25 mM Tris-Mes, (pH 6), 5 μM DCMU, 5 mM NH_4Cl and 85 μM silicomolybdic acid. At pH 8 the reaction components were as above, except the pH of the buffer was 8 and the silicomolybdic acid concentration was 250 μM. pH 6: (○) control, (●) PCMB (100 μM). pH 8: (△) control, (▲) PCMB (200 μM).

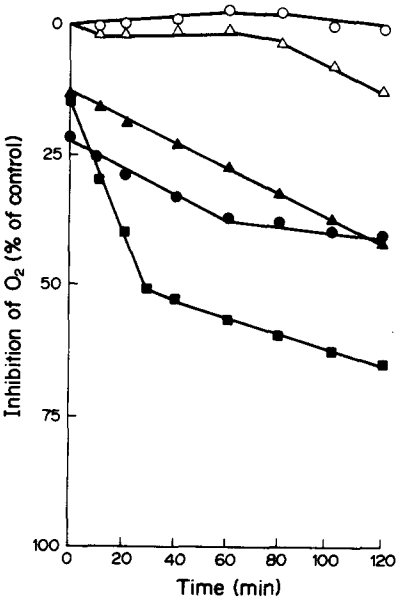


Fig. 2. The effect of PCMB on DBMIB-insensitive indophenol reduction in PS II of spinach chloroplasts. The control rate at pH 6 was 552 μequiv./mg Chl per h, at pH 8, 356. The reaction components for the pH 6 reaction included chloroplasts (0.05 mg Chl), 25 mM Tris-Mes, (pH 6), 5 mM NH_4Cl, 2 μM DBMIB and 200 μM DCIP. At pH 8 the reaction components were as above, except the pH of the buffer was 8 and the DCIP concentration was 100 μM. pH 6: (○) control, (●) PCMB (100 μM), (■) PCMB (200 μM). pH 8: (△) control, (▲) PCMB (100 μM).

Controls were also monitored every 10 min for comparison, but timing was started 5 min ahead of assaying the treated samples.

Tris-washed chloroplasts were prepared according to the procedure of Yamashita and Butler [16].

PCMB, PCMBS, *N*-ethylmaleimide and 4,4′-dithiopyridine were obtained from Sigma, *N*-butylmaleimide from ICN Pharmaceuticals, Inc., and phenylmaleimide from Aldrich Chemical Co.

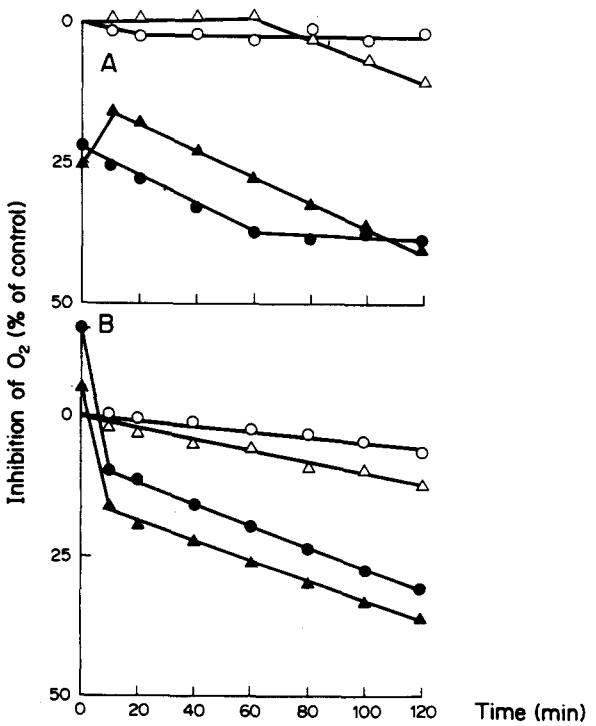
In early chloroplast studies with sulfhydryl reagents, it was discovered by Arnon and associates [17,18] and later by Jagendorf and Avron [19] that PCMB inhibits photophosphorylation and CO_2 fixation better than electron transport. Subsequently, Izawa and Good [11,12] showed that PCMB was an energy-transfer inhibitor. Since then, *N*-ethylmaleimide and other sulfhydryl reagents have been shown to inhibit various subunits of the chloroplast coupling factor, CF_1 [2–10]. Recently, Underwood and Gould [13] showed that sulfhydryl-modifying agents caused H^+ efflux through the chloroplast ATP synthase (CF_0-CF_1). With the exception of a few scattered pieces of data [2,4,13], the present paper is the first concentrated effort, which shows that long-term incubation of chloroplasts with sulfhydryl reagents inhibits electron transport in spinach chloroplasts. As shown in Figs. 1 and 2, the DCMU-insensitive silicomolybdate reduction and dibromothymoquinone-insensitive indophenol reduction at pH 6 is inhibited more than 50% by PCMB. At pH 8, there is less than 50% inhibition of indophenol reduction, but inhibition on the DCMU-insensitive silicomolybdate pathway by PCMB reaches more than 50%. This shows that two separate sulfhydryl sites must be involved in the PS II electron-transport chain. Data in Table I confirm this assumption, because a nonpenetrating sulf-

TABLE I

INHIBITION OF ELECTRON TRANSPORT IN PS II OF SPINACH CHLOROPLASTS BY VARIOUS SULFHYDRYL REAGENTS AFTER 120-min INCUBATIONS

Electron transport values expressed as μequiv. O_2/mg Chl per h.

Sulfhydryl reagent	Concentration (mM)	Electron transport			
		$H_2O \rightarrow$ silicomolybdate (+DCMU), pH 6		$H_2O \rightarrow$ indophenol (+DBMIB), pH 6	
		Rate	Inhibition (%)	Rate	Inhibition (%)
N-Ethylmaleimide					
control	–	211	–	260	–
treated	0.5	136	35	224	14
Phenylmaleimide					
control	–	291	–	282	–
treated	0.5	73	75	143	48
Butylmaleimide					
control	–	253	–	343	–
treated	0.5	101	60	248	28
PCMBS					
control	–	208	–	318	–
treated	0.5	106	51	239	25
4,4'-Dithiopyridine					
control	–	209	–	349	–
treated	0.4	115	45	332	5



hydryl reagent, PCMBS, inhibits the DCMU-insensitive silicomolybdate pathway up to 50%, but less than 25% inhibition is seen on the dibromothymoquinone-insensitive indophenol reduction. Dithiopyridine (Table I), a bifunctional sulfhydryl reagent, also shows more inhibition of the DCMU-insensitive silicomolybdate reduction than indophenol reduction in PS II. The inhibitions given by maleimides (Table I) are also stronger on the DCMU-insensitive silicomolybdate pathway, which may represent the cycling of

Fig. 3. A comparison of PCMB inhibition of DBMIB-insensitive indophenol reduction in PS II of spinach chloroplasts (A) and inhibition of the diphenylcarbazide-to-indophenol pathway in Tris-treated chloroplasts (B). The control rate in A at pH 6 was 480 μequiv./mg Chl per h, at pH 8, 301; in B the control rate at pH 6 was 186 and at pH 8, 105. The reaction components were as in Fig. 2; the concentration of diphenylcarbazide in Tris-treated chloroplasts was 0.5 mM. In both A and B the chloroplasts were initially incubated with 100 μM PCMB as indicated. pH 6: (○) control, (●) PCMB (100 μM). pH 8: (△) control, (▲) PCMB (100 μM).

electrons around PS II, than on the forward pathway represented by the dibromothymoquinone-insensitive indophenol reduction. That the sulfhydryl reagent inhibitions seen here are not affecting water oxidation is illustrated in Fig. 3, in which a similar extent of inhibition is seen on the diphenylcarbazide donor pathway in Tris-treated chloroplasts as on indophenol reduction in PS II.

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