Lipophilic Monofunctional Aldehydes Inhibit Ferricyanide Reduction by Photosystem II of Spinach Chloroplasts

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Introduction

The Hill reaction, which measures photosystem II electron transport, was studied in 6% glutaraldehyde-fixed chloroplasts by Park (11), Hallier and Park (5) and Yoshida (17), in 0.5-1% glutaraldehyde-fixed chloroplasts by Oku *et al.* (10), and in 5% glutaraldehyde-fixed chloroplasts by Hardt and Kok (6, 7). In general it was found that even low concentrations of glutaraldehyde (<0.5%) inhibited photosystem I activity more severely than photosystem II activity, implying that photosystem I is more accessible in isolated chloroplasts.

The present study differs from previous studies in several aspects: (1) the effects of glutaraldehyde and other aldehydes was studies after a short incubation period (3 min.); and (2) both hydrophilic and lipophilic aldehydes were used. The purpose of this study was to find out if ferricyanide reduction by photosystem II could be inhibited selectively with aldehydes, as with thiols (15).

Materials and Methods

Chloroplasts were isolated from market spinach in 0.4M sucrose containing 0.05M NaCl (SN chloroplasts), as previously described (2). Likewise, chlorophyll was determined as in previous studies (2).

Electron transport in photosystem I and II was measured as O_2 evolution or uptake as previously described (2). Reaction mixtures for the various reactions are given in Table I. Tris-washed chloroplasts were prepared according to Yamashita and Butler (16).

All monofunctional aldehydes except formaldehyde were purchased from the Aldrich Chemical Company. Millimolar solutions were prepared fresh daily by adding the undiluted stock solutions to weighed bottles with a micropipette to obtain the desired weights of aldehydes to prepare solutions of desired concentrations. These solutions were protected from light by foil and kept on ice during use to minimize polymerization.

Glutaraldehyde, a bifunctional membrane cross-linking agent, was purchased from Sigma Chemical Company in individually sealed ampules.

Results and Discussion

It is well known (1, 2, 4, 5, 6, 7, 9, 10, 12, 17) that electron transport in spinach chloroplasts or algae is not affected by low concentrations (<0.5%) of glutaraldehyde or formaldehyde. Higher concentrations (>0.5-6%), however,

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TABLE I *The Effect of Monofunctional Aldehydes on Electron Transport in Spinach Chloroplasts.*

ALDEHYDE	Conc. (μM)	Stimulation or inhibition of electron transport rates (%) FeCN(+DBMIB)[a]	FeCN[b]	DMBQ(+DBMIB)[c]	PD_{ox}[d]	MV[e]
Paraformaldehyde	500	0	+ 4	+ 7	-	-14
Acetaldehyde	500	+ 3	-13	+ 7	-	- 4
Butyraldehyde	500	-20	+20	- 8	-10	-27
Benzaldehyde	500	-18	- 7	-21	+12	-50
Octylaldehyde	500	-30	+ 8	- 2	- 7	- 5

[a] $H_2O \rightarrow$ FeCN(+DBMIB) control rates varied from 90-120 μequiv./mg chl · hr; reaction mixture contained chloroplasts (50 μg chlorophyll), 25 mM Tris-Mes, pH 8, 0.5 mM FeCN, and 2 μM DBMIB.

[b] $H_2O \rightarrow$ FeCN control rates varied from 100-200 μequiv./mg chl · hr; reaction mixture contained chloroplasts as above, 25 mM Tris-Mes, pH 6, 250 μM FeCN and 2 mM NH_4Cl.

[c] $H_2O \rightarrow$ DMBQ(+DBMIB) control rates varied from 400-600 μequiv./mg chl · hr; reaction mixture contained chloroplasts as above, 25 mM Tris-Mes, pH 7, 2 mM NH_4Cl; 3 mM $MgCl_2$, 0.75 mM DMBQ and 2 μM DBMIB.

[d] $H_2O \rightarrow$ PD_{ox}(+DBMIB) control rates varied from 450-650 μequiv./mg chl · hr; reaction mixture contained chloroplasts and other reaction components as above except 0.5 mM PD and 0.5 mM FeCN in place of DMBQ.

[e] $H_2O \rightarrow$ MV control rates varied from 500-700 μequiv./mg chl · hr; reaction mixture contained chloroplasts as above, 25 mM Tris-mes, pH 7, 2 mM NH_4Cl, 3 mM $MgCl_2$, 0.5 mM azide and 0.5 mM MV.

inhibit PS II and PS I activity. Hardt and Kok (7) have shown that preincubation with glutaraldehyde for at least 30 min. destroys plastocyanin. It is also assumed that the methylviologen site in PS I can be inhibited, since it is located on the outside of the thylakoid membrane. A number of studies (10, 17) demonstrated inhibition of the Hill reaction, but the inhibition site in PS II was not pinpointed with precision. In this study we chose to test a number of aldehydes with a progressively longer sidechain (1-12 carbons) on various PS II reactions to see if lipophilicity or bifunctionality produced more inhibition. The results varied, depending on the reaction tested, as shown in Table I, but aldehydes with 1-8 carbons gave no significant inhibition except benzaldehyde, which inhibited the $H_2O \rightarrow$ MV reaction up to 50%. Glutaraldehyde, a bifunctional reagent, which polymerizes rapidly to polymers of unknown molecular weight (8) can cross-link membrane components (13). As seen in (Fig. 1), the greatest effect was on PS II ferricyanide and p-phenylene diamine reduction in presence of DBMIB to block electron flow to PS I. It was not a very effective PS I inhibitor in the 3 min. incubation period employed in this study, since the $H_2O \rightarrow$ MV reaction was only inhibited 35-50%, at most. Dodecylaldehyde (Fig. 2), a lipophilic monofunctional reagent, gave better inhibition of methylviologen reduction in PS I and ferricyanide reduction in PS II, (70-85%) inhibition. Diphenylacetaldehyde, a lipophilic short chain monofunctional aldehyde (Fig. 3), stimulated methylviologen reduction in PS I, but selectively inhibited ferricyanide reduction by PS II in presence of DBMIB. A similar selective inhibition of ferricyanide reduction in PS II has been observed by us with lipophilic thiols, such as octane thiol (15).

Proof that the lipophilic aldehydes act selectively on electron transport, but not on water oxidation in PS II, is provided in Table II, using Tris-washed

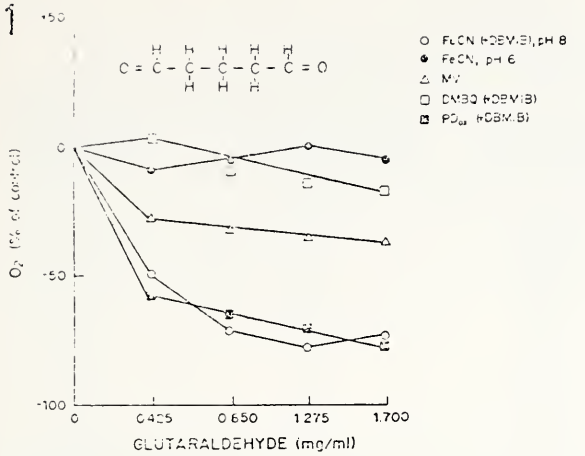

FIGURE 1. *The Effect of Glutaraldehyde on Electron Transport in Spinach Chloroplasts. Reaction mixtures for the various reactions as in Table I. Control rates were as follows: 1020 μequiv./mg chl · hr for $H_2O \rightarrow MV$; 176 for $H_2O \rightarrow FeCN(+DBMIB)$; 182 for $H_2O \rightarrow FeCN$; 955 for $H_2O \rightarrow PD_{ox}(+DBMIB)$ and 1072 for $H_2O \rightarrow DMBQ(+DBMIB)$. + indicates stimulation of rate, — inhibition.*

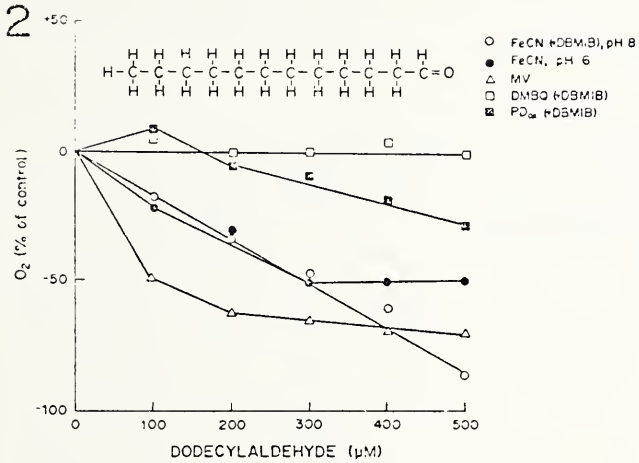

FIGURE 2. *The Effect of Dodecylaldehyde on Electron Transport in Spinach Chloroplasts. Reaction mixtures and control rates as in (Fig. 1). + indicates stimulation, — inhibition of rate.*

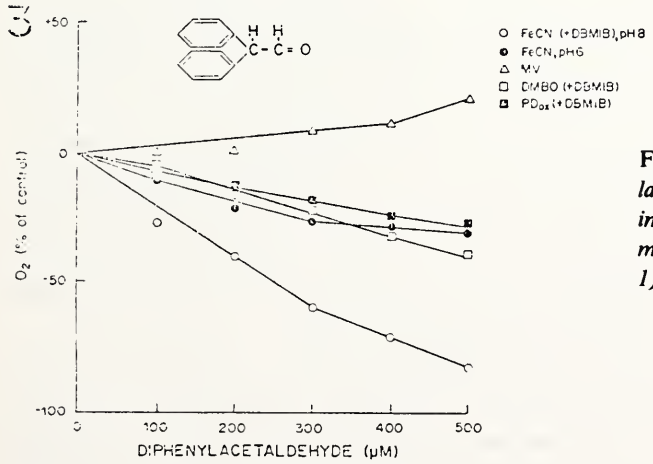

FIGURE 3. *The Effect of Diphenylacetaldehyde on Electron Transport in Spinach Chloroplasts. Reaction mixtures and control rates as in (Fig. 1). + indicates stimulation, — inhibition of rate.*

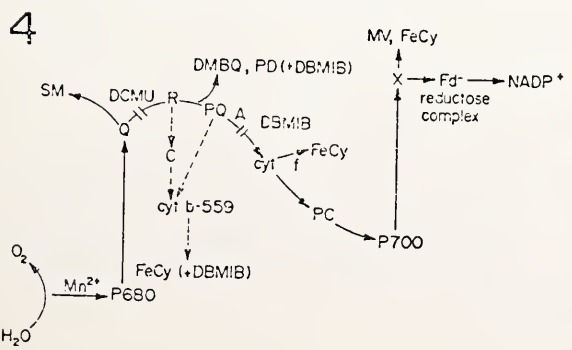

FIGURE 4. *Electron Transport Scheme in Spinach Chloroplasts, Showing Possible Involvement of Cytochrome b-559 in Ferricyanide Reduction by Photosystem II.*

TABLE II *Stimulation or Inhibition of the Diphenylcarbazide → DCPIP Pathway by Various Aldehydes in Tris-Treated Spinach Chloroplasts*

Aldehyde	Conc. (μM)	Electron Transport Rate (μequiv/mg chl · hr)	Stimulation or Inhibition (%)
Control Tris-washed Chloroplasts	-	72	0
Paraformaldehyde	500	76	+ 6
Acetaldehyde	500	76	+ 6
Butyraldehyde	500	75	+ 4
Benzaldehyde	500	60	- 17
Octylaldehyde	500	60	- 17
Glutaraldehyde	1.7 mg/ml	104	+44
Diphenylacetaldehyde	500	36	- 50
Dodecylaldehyde	500	24	- 66

[1]Reaction mixture contained in 3 ml total volume: chloroplasts (50 μg chlorophyll), 25 mM Tris-Mes, pH 7, 2 μM NH_4Cl, 3 μM $MgCl_2$, 0.5 mM DPC and 0.5 mM DCPIP.

chloroplasts, in which water does not serve as electron donor any longer, but is substituted by diphenylcarbazide. As can be seen in this table, the effect of glutaraldehyde is different from the effect of the 2 more lipophilic aldehydes, diphenylacetaldehyde and dodecylaldehyde. Since glutaraldehyde stimulates the DPC → DCIP reaction, which involves only PS II, the inhibition observed with glutaraldehyde on the H_2O → MV pathway (Fig. 1) must involve sites in PS I, or an effect on water oxidation itself. Since PS I is located on the outside of the thylakoid membrane, cross-linking of outer membrane proteins by glutaraldehyde to give inhibition of electron transport in PS I is not unreasonable (13). Diphenylacetaldehyde and dodecylaldehyde, on the other hand, clearly inhibit electron transport in PS II. This indicates the monofunctional lipophilic aldehydes can penetrate the thylakoid membrane to reach sites located on the inner half of the thylakoid membrane, where PS II, including water oxidation, is located. Another possibility is that only that portion of PS II exposed to the outside, as shown by DABS labeling studies (3) is the segment affected. Since benzaldehyde and octylaldehyde only give a slight inhibition of PS II electron transport (17% on the DPC → DCIP pathway, Table II), compared to (>50%) inhibition by diphenylacetaldehyde and dodecylaldehyde, a more lipophilic compound than a straight-8-carbon chain skeleton is necessary to inhibit PS II electron transport.

The mode of action of the monofunctional lipophilic aldehydes in causing inhibition of PS II electron transport is unknown, but the possibility of interaction between iron-sulfer centers and these aldehydes exists, as was shown in mitochondria by Salerno and Ohnishi (14). Further studies are necessary to prove this point in chloroplast PS II.

Abbreviations Used

DBMIB — dibromothymoquinone; DCPIP — 2,6-dichlorophenol indophenol; DCMU — 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMBQ — 2,5-dimethylbenzoquinone; DPC — diphenylcarbazide; FeCN — potassium ferricyanide; MV — methylviologen; PD — p-phenylene-diamine; PS I — photosystem I; PS II — photosystem II.

Summary

Various mono- and bifunctional aldehydes with chainlength from 1-12 carbon atoms affect electron transport in spinach chloroplasts. Glutaraldehyde, a bifunctional reagent, affected photosystem I activity, but lipophilic dodecylaldehyde inhibited both photosystems. Diphenylacetaldehyde, another lipophilic compound, inhibited specifically photosystem II activity, assayed by ferricyanide reduction in presence of dibromothymoquinone to block electron flow toward photosytem I. The localization of the two photosystems in the thylakoid membranes is discussed in view of their accessibility to aldehydes.

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