

INHIBITION OF PHOTOSYSTEM II ELECTRON TRANSPORT IN CHLOROPLASTS BY FATTY ACIDS AND RESTORATION OF ITS ACTIVITY BY Mn^{2+}

Paul-André SIEGENTHALER

*Laboratoire de Physiologie et Biochimie Végétales, Institut de Botanique,
Université de Neuchâtel, rue de Chantemerle 18, 2000 Neuchâtel, Switzerland*

Received 23 November 1973

1. Introduction

Fatty acids, especially C_{18} -unsaturated acids, represent a substantial portion of the thylakoid lipids [1] and play an important role in the maintenance of the structural and functional integrity of the chloroplast membrane [2, 3]. Interesting enough, free fatty acids, which accumulate in the membrane due to lipid hydrolysis under special conditions such as aging in vitro [4–7], seem to influence the structure and function of chloroplasts in a specific way. For instance, exogenous C_{18} -unsaturated fatty acids enhanced chloroplast swelling and inhibited light-induced shrinkage [7]. They also caused a sequential inhibition of photosystem II and photosystem I electron transports and the respective photosynthetic phosphorylations [8]. However, in spite of much evidence that fatty acids at appropriate concentrations are specific inhibitors of the photosystem II electron transport [8, 9], their precise inhibition site is still unknown. In order to localize this site, several specific inhibitors of photosystem II (DCMU and Tris-washing) and artificial electron donors (1,5-diphenylcarbazine and $MnCl_2$) were tested in various combinations, with or without fatty acids, for their action on the photoreduction of 2,6-dichlorophenol indophenol. The results indicate that the inhibition site of unsaturated fatty acids lies on the oxidizing side of photosystem II, probably between the hypothetical carriers Y_2 and Y_3 (see scheme, fig. 3).

An additional result of this investigation was the finding that Mn^{2+} interacts with some unknown electron carriers of the electron transport chain located between H_2O and the pigments complex II, allowing a shunt between Y_1 and Y_3 , bypassing thereby the fatty

acid block (see scheme, fig. 3). This again proves the requirement of Mn^{2+} in the photosystem II-mediated reactions [10] but it suggests that Mn^{2+} may have a function so far not postulated [11, 12].

2. Materials and methods

Chloroplasts were isolated from commercial spinach (*Spinacia oleracea* L.) as described elsewhere [13]. The resulting stock suspension contained 2 mg chlorophyll/ml, 100 mM Tris-HCl (pH 8) and 175 mM NaCl, and was kept at 0–4°C prior to use. Tris-washed chloroplasts were obtained according to Yamashita and Butler [14].

The electron transport for photosystem II (from H_2O to 2,6-dichlorophenol indophenol) was measured spectrophotometrically at 590 nm by the photoreduction of 2,6-dichlorophenol indophenol (DCPIP) as the oxidant, in the following reaction mixture: Tris-maleate (50 mM, pH 7), NaCl (35 mM), DCPIP (0.15 mM), ethanol (0.5%) and chloroplasts (20 μ g chlorophyll/ml). 1,5-diphenylcarbazine (DPC, 0.5 mM), $MnCl_2$ (5 mM), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 10 μ M), or fatty acids (various concentrations) were added to the basic reaction mixture, as indicated. The reaction was followed over a 1 min period at 20°C and at a light intensity of approximately $5 \cdot 10^5$ ergs. $cm^{-2} \cdot s^{-1}$.

DPC was dissolved in methanol, while fatty acids and DCMU were dissolved in ethanol.

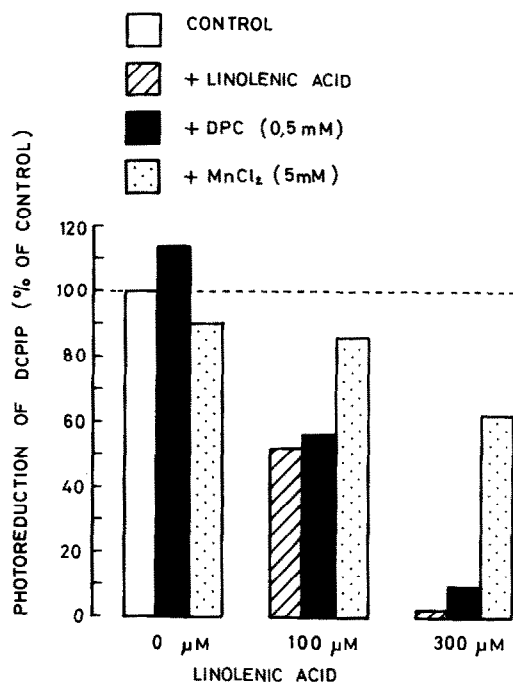


Fig. 1. Ability of DPC and MnCl_2 to restore the photosystem II activity which was inhibited by various concentrations of linolenic acid. The value 100% corresponded to $\Delta E_{590} = 1.720/\text{min}/20 \mu\text{g chlorophyll/ml}$.

3. Results

Fig. 1 shows the effect of increasing linolenic acid concentrations on the electron transport activity of photosystem II, and the ability of DPC and MnCl_2 to restore this activity. DPC [15] and MnCl_2 [11, 12] have been shown to be artificial electron donors to the oxidizing side of photosystem II. The addition of 100 μM linolenic acid caused a 50% inhibition of the activity which was restored up to 85% in the presence of MnCl_2 , but not in the presence of DPC. Higher concentrations of linolenic acid (200–300 μM) obliterated completely the electron flow rate which was restored up to 60% with MnCl_2 . DPC had only a slight restoring effect. MgCl_2 could not replace MnCl_2 (not shown).

These first observations suggested that the inhibition block caused by linolenic acid in the electron transport could be located after the entry point of DPC. Moreover, MnCl_2 might either feed electrons in the system after the fatty acid block or bypass it.

In order to test these two possibilities, the photo-

Table 1

Influence of Tris-washing, linolenic acid and DCMU on the photoreduction of DCPIP in chloroplasts and ability of DPC and MnCl_2 to restore the activity.

Conditions	Photoreduction of DCPIP (% of control)	
	Controls +	300 μM Linolenic acid
Untreated chloroplasts*	100**	2
+ DPC	107	10
Tris-washed chloroplasts	10	5
Tris-washed + DPC	65	9
Tris-washed + DPC + DCMU	3	2
Tris-washed + Mn^{2+}	5	2
Tris-washed + Mn^{2+} + DPC	30	6

* The basic reaction is described in Materials and methods. Where indicated, the concentrations of DPC, MnCl_2 and DCMU were 0.5 mM, 5 mM and 10 μM , respectively.

** The value of 100% corresponded to $\Delta E_{590} = 1.365/\text{min}/20 \mu\text{g chlorophyll/ml}$.

reduction of DCPIP by Tris-washed chloroplasts was studied. As previously reported, washing chloroplasts with 0.8 M Tris (pH 8.0) caused an inhibition of the Hill reaction by blocking electron transport between water and photosystem II [14]. The results reported in table 1 (column 1) confirmed that such a treatment inhibited photosystem II activity and that DPC restored the electron flow to an extent of 65%. MnCl_2 did not have such a restoring effect indicating that it did not behave as an electron donor similar to DPC. A combination of DPC and MnCl_2 was not as effective as DPC alone.

The addition of 300 μM linolenic acid (table 1, column 2) strongly inhibited electron flow in the presence and absence of DPC. Likewise, the activity of Tris-treated chloroplasts which was restored by DPC, again was inhibited by the fatty acid. In the presence of linolenic acid, neither the addition of MnCl_2 alone nor a combination of MnCl_2 and DPC was able to restore the activity of Tris-washed chloroplasts. Taken together, these results might indicate that Mn^{2+} establishes a shunt between Y_1 and Y_3 (see fig. 3), i.e. over the point of entry of DPC (Y_2) and the fatty acid block.

The effect of DCMU on the MnCl_2 -restored activity is illustrated in fig. 2. First, it can be seen that the inhibition of electron transport in photosystem II caused

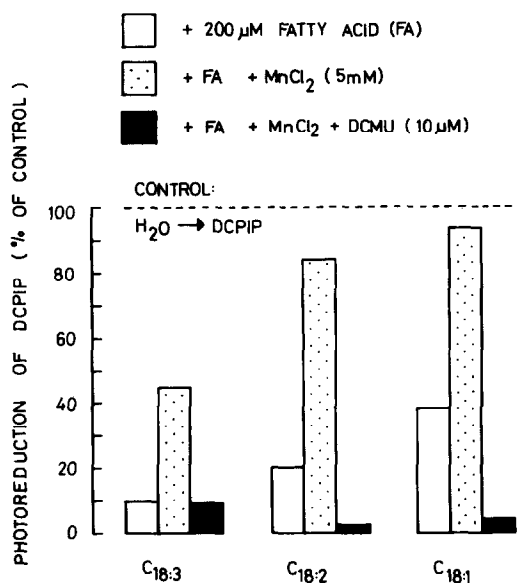


Fig. 2. Restoration by MnCl_2 of the photoreduction of DCPIP inhibited by three C_{18} -unsaturated fatty acids and subsequent inhibition by DCMU. The value 100% corresponded to $\Delta E_{590} = 1.720/\text{min}/20 \mu\text{g}$ chlorophyll per ml.

by $200 \mu\text{M}$ of C_{18} -fatty acids depended on the degree of saturation of the molecule; for instance, linolenic acid ($\text{C}_{18:3}$) had a more pronounced inhibitory effect than linoleic ($\text{C}_{18:2}$) and oleic ($\text{C}_{18:1}$) acids. The restoration of the electron flow rate by MnCl_2 also depended on the degree of saturation of the molecule. MnCl_2 was less effective in restoring the activity in the presence of linolenic acid than in the presence of the other acids. Fig. 2 also shows that in the presence of all three fatty acids, the MnCl_2 -restored activities were strongly inhibited by DCMU. This suggested that the inhibition and restoration events take place at a site prior to the DCMU block.

4. Discussion

The present findings confirm that fatty acids are inhibitors of the photosystem II electron transport as reported earlier [8,9]. Furthermore, we are now in a position to propose that their inhibition site is located on the oxidizing side of photosystem II, most probably between the hypothetical carriers Y_2 and Y_3 , as shown in fig. 3. In this scheme, electrons from water

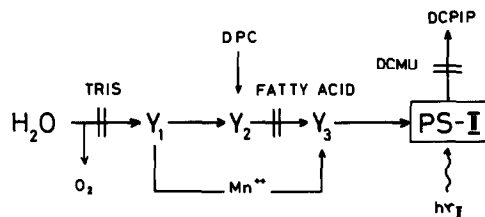


Fig. 3. Schematic representation of the action sites of C_{18} -unsaturated fatty acids, Tris-washing, 1,5-diphenylcarbazine (DPC), MnCl_2 and DCMU in the photosystem II electron transport in chloroplasts.

are transported through three hypothetical carriers (Y_1 , Y_2 , Y_3) to the photosystem II pigments, and boosted through photosystem II to DCPIP. Our results suggest that C_{18} -unsaturated fatty acids and Tris-washing inhibit the electron flow between Y_2 and Y_3 and between H_2O and Y_1 , respectively, and that DPC feeds electrons in the chain through the Y_2 -carrier. DCMU, a specific inhibitor on the reducing side of photosystem II, of course inhibited all the electron flow pathways.

The function of Mn^{2+} in relation to the fatty acid inhibition was found to be interesting (fig. 3). Although the absolute requirement of Mn^{2+} for a functional photosystem II is well documented [10], it is by no means certain whether Mn^{2+} participates directly as a carrier in the electron transfer reactions [10] and/or is an electron donor to some other carrier prior to photosystem II pigments [11,12]. Moreover, neither the exact position of Mn^{2+} as an electron carrier nor the entry point of Mn^{2+} as an electron donor are well defined. Izawa [11] showed that in EDTA- and heat-treated chloroplasts, Mn^{2+} can be an efficient electron donor for photosystem II. Since Mn^{2+} failed to reactivate NADP^+ photoreduction in Tris-treated chloroplasts, Ben-Hayyim and Avron [12] concluded that the Mn^{2+} site of action not only precedes photosystem II but is rather close to the O_2 evolution step itself. Our results are in agreement with this latter conclusion since Tris-treated chloroplasts, either with or without added fatty acids, failed to show DCPIP photoreduction activity in the presence of Mn^{2+} (see table 1). However, when H_2O was the electron donor, Mn^{2+} was able to restore the electron flow activity in the presence of fatty acids, as measured by DCPIP reduction (fig. 1) and O_2 evolution (results to be published). This suggested a third role for Mn^{2+} . The ion, func-

tioning as a catalyst, might establish a shunt between Y_1 and Y_3 bypassing Y_2 and the fatty acid block (fig. 3).

Finally, we would like to point out that compared to classical inhibitors of photosystem II, such as DCMU, *o*-phenanthroline, Tris-washing, etc., we may consider fatty acids as physiological inhibitors. The study of their action allows us not only to probe the various components involved in the photosystem II electron flow, but to gain some insight into the functional role of the fatty acids themselves in the complex machinery of the electron transport chain. Such a study and the role that Mn^{2+} plays in the photosystem II electron flow is currently under investigation in our laboratory.

Acknowledgements

Financial support by the Swiss National Science Foundation (project no. 3.566.71) is gratefully acknowledged. I would like to thank Mrs. Jarmilà Horakova for her excellent technical assistance and Prof. E. Stutz and Dr. E. Crouse for correcting the manuscript.

References

- [1] Allen, C.F., Good, P., Davis, H.F. and Fowler, S.D. (1964) *Biochem. Biophys. Res. Commun.* 15, 424.
- [2] Costes, C., Bazier, R. and Lechevallier, D. (1972) *Physiol. Vég.* 10, 291.
- [3] Benson, A.A. (1971) in: *Structure and Function of Chloroplasts* (Gibbs, M., ed), p. 129, Springer-Verlag, Berlin, Heidelberg, New York.
- [4] McCarthy, R.E. and Jagendorf, A.T. (1965) *Plant Physiol.* 40, 725.
- [5] Constantopoulos, G. and Kenyon, C.N. (1968) *Plant Physiol.* 43, 531.
- [6] Wintermans, J.F.G.M., Helmsing, P.J., Polman, B.J.J., Van Gisbergen, J. and Collard, J. (1966) *Biochim. Biophys. Acta* 189, 95.
- [7] Siegenthaler, P.A. (1972) *Biochim. Biophys. Acta* 275, 182.
- [8] Siegenthaler, P.A. (1973) *Biochim. Biophys. Acta* 305, 153.
- [9] Cohen, W.S., Nathanson, B., White, J.E. and Brody, M. (1969) *Arch. Biochem. Biophys.* 135, 21.
- [10] Cheniae, G.M. (1970) *Ann. Rev. Plant Physiol.* 21, 467.
- [11] Izawa, S. (1970) *Biochim. Biophys. Acta* 197, 328.
- [12] Ben-Hayyim, G. and Avron, M. (1970) *Biochim. Biophys. Acta* 205, 86.
- [13] Siegenthaler, P.A. (1969) *Plant Cell Physiol.* 10, 801.
- [14] Yamashita, T. and Butler, W.L. (1968) *Plant Physiol.* 43, 1978.
- [15] Vernon, L.P. and Shaw, E.R. (1969) *Biochem. Biophys. Res. Commun.* 36, 878.