EFFECTS OF MANGANESE ON THE FLUORESCENCE OF CHLOROPLASTS*

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In all schematic presentations of the electron transport system of chloroplasts, manganese is shown to act close to the site of water oxidation. Yet, the possibility has not been ruled out that manganese functions on the reducing side of the oxygen-evolving photosystem II. In the present communication evidence will be presented which supports the old concept of a participation of manganese in reactions close to the site of oxygen evolution. These results have emerged from comparative fluorescence studies on normal and manganese-deficient chloroplasts.

Three main parameters of the fluorescence of isolated chloroplasts were considered: (1) the level F_o reached immediately upon illumination. Its height depends, at least in part, on the structural organization of the chloroplasts (Homann, in press); (2) the level F_∞ attained after a few seconds by a slow fluorescence rise which is believed to reflect the reduction of a primary pool of electron acceptors Q (Duysens and Sweers, 1963); (3) the fluorescence yield F_{inh} in the presence of an inhibitor of oxygen evolution like DCMU. Since under the latter conditions no electrons from the acceptor pool are lost to photosystem I (Duysens and Sweers, 1963), F_{inh} is usually equal to or only slightly higher than F_∞.

Results and Discussion.

Chloroplast preparations which have low quantum efficiencies of oxygen evolution have a slow fluorescence rise and reduce their primary oxidants in

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the light incompletely. In such a case, F_∞ is relatively low and F_∞/F_{inh} remains well below unity. Chloroplasts which are unable to evolve oxygen should not display a fluorescence rise at all ($F_\infty = F_o$). Such chloroplasts can be obtained by heating a normal chloroplast suspension at 50° for 5 to 10 min. During this inactivating treatment all manganese is released from the chloroplast lamellae (Cheniae and Martin, 1966). After this treatment, the fluorescence level F_o was found to be higher than normal, while the level F_{inh} turned out to be much lower than in unheated control chloroplasts. The question arose whether it might be possible to prepare manganese-deficient chloroplasts while avoiding any structural damage which was considered to be responsible for the increased yield F_o. This could be achieved best by either washing the chloroplasts with 0.8 M Tris/HCl pH 8.0, as used by Yamashita and Butler (1968) to inactivate system II, or by heating chloroplasts in the intact leaf before isolation. These treatments released 65 to 85% of the bound manganese from the chloroplasts (Table I) and reduced the Hill activity by more than 90%. By heating the leaves at various temperatures, different levels of Mn-deficiency could be obtained. The fluorescence characteristics of such preparations are shown in Fig. 1. Although the untreated chloroplast fragments from the ^{54}Mn labeled plant used for this particular experiment were not of optimal quality (F_∞/F_{inh} considerably smaller than 1), we can deduce the following from our data.

Whereas the fluorescence level F_∞ decreases parallel with the Mn content, the fluorescence yield in the presence of DCMU (F_{inh}) does not change to the same extent. Moreover, the fluorescence kinetics in the presence of DCMU are not retarded to the extent one would expect, if the inactivated oxygen-evolving sites had to provide electrons from water for a reduction of the fluorescence quenching oxidants Q, even though Q may represent only a small part of the total pool (Kok et al., 1966). Joliot (1965) considers the fluorescence characteristics in the presence of DCMU a direct expression of the "activation reaction" of photosystem II. In the absence of DCMU, the activated factor EH

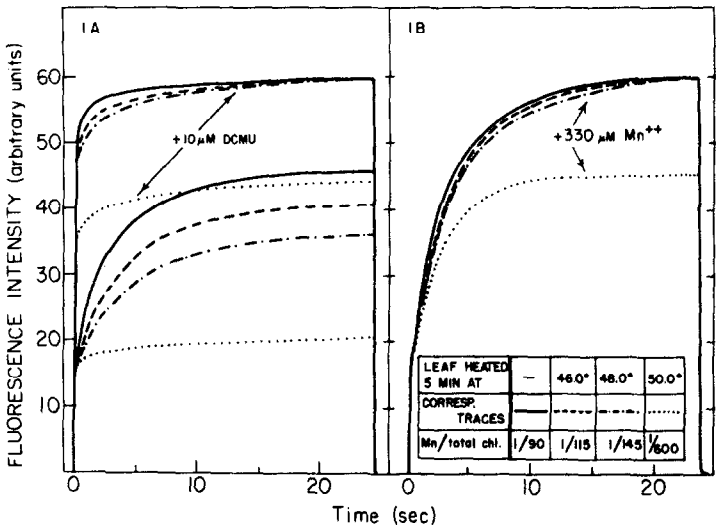

Fig. 1a - Effect of the manganese content of chloroplasts on their fluorescence in the presence and absence of DCMU. The green tobacco NC 95 was grown in water culture with 2.5 μM Mn (labeled with ^{54}Mn). Chloroplasts were prepared from leaves heated in a water bath for 5 min (see insert of Fig. 1b). Exciting beam for the fluorescence (isolated at λ 684 nm): 7.5 n einst sec^{-1} cm^{-2} λ_{max} 570 nm. T = 17°; 5.5 μM chlorophyll.

Fig. 1b - Effect of added manganous ions on the fluorescence rise. Chloroplast material and experimental conditions as in Fig. 1a. (A similar regeneration of the fluorescence rise was observed with 1 mM hydroxylamine or with 33 μM p-phenylenediamine + 330 μM ascorbate.)

(which corresponds to QH of Duysens and Sweers, 1963), loses its electron more rapidly to system I than it regains another one from an inactive oxygen-evolving system. Hence, a high level of fluorescence is made possibly only by blocking the electron flow between EH and system I with DCMU. Figure 1a shows that a slight manganese deficiency strongly inhibits the electron flow from water, but not the activation reaction. Only the highest degree of manganese deficiency is accompanied by some decrease of F_{inh}. While it cannot be excluded that in this case effects not related to the release of chloroplast-bound manganese have occurred, it may also be that the lowered level F_{inh} indicates a requirement for some manganese of the activation reaction as recently suggested by Cheniae and Martin (1968). A fluorescence yield equal to F_{inh} can be reached in the absence of DCMU only if the oxygen-evolving

system is intact, or if electrons are supplied by an artificial electron donor, e.g., a mixture of p-phenylenediamine and ascorbate (Yamashita and Butler, 1968). We found that a fluorescence rise in our manganese-deficient chloroplasts was regenerated not only by these agents but also by manganous ions or hydroxylamine. Hydroxylamine has long been known to be an inhibitor of oxygen evolution. Its action, however, has been shown to be different from that of DCMU (Bertsch et al., 1963), and can now be explained by its oxidation by an intermediate of photosystem II. Manganous ions, on the other hand, have been considered as reductants for $HO_2\cdot$ radicals formed by the reduction of oxygen on the reducing side of system I (Homann, 1965), as well as for intermediates on the oxidizing side of system II (Habermann et al., 1968). The latter action would resemble the role attributed to the normal bound manganese of the oxygen-evolving reaction complex. With our inactivated chloroplast the manganese-induced fluorescence rise disappears upon washing with buffer.

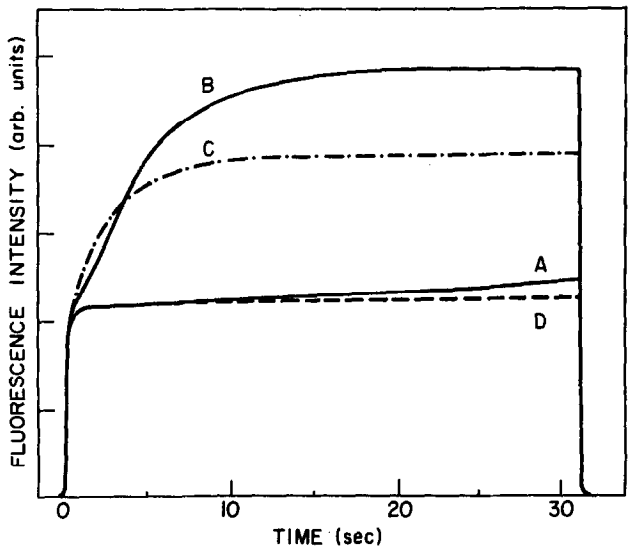

Fig. 2 - Effect of ferricyanide on the Mn^{++} induced fluorescence rise of chloroplasts from leaves of Cassia obtusifolia, heated at 50° for 5 min. Experimental conditions as in Fig. 1. A: no addition; B: example of a fluorescence induction curve obtained in the presence of 330 μM $MnCl_2$ after illumination with far-red light (λ_{max} 717 nm); traces C and D were obtained after addition of 30 or 300 μM ferricyanide respectively to the suspension which also contained 330 μM $MnCl_2$.

Since the native manganese is bound tightly to the chloroplast lamellae and cannot be removed in aqueous solutions even by chelators, it appears that our finding does not represent a true reconstitution of the oxygen-evolving system. Such a "reactivation" has only been achieved with living algae. Further studies should answer the question how close added manganous ions get to their natural binding site in our illuminated, manganese-depleted chloroplasts.

In agreement with our expectations, the fluorescence level F_{inh} marks the limit of the fluorescence yield F_{∞} which can be reached in the presence of any type of electron donor described above. In order to allow a comparison with Fig. 1a, representative examples are shown in Fig. 1b using the same ^{54}Mn labeled chloroplast preparations as in Fig. 1a, with 330 μM Mn^{++} added as electron donor. It is noteworthy that the condition F_{∞} (e-donor) = F_{inh} (no donor) holds true for all other types of chloroplasts studied, including the structurally disorganized chloroplasts from Mn-deficient plants and the partitionless chloroplasts of the variegated tobacco NC 95, both of which have F_o/F_{inh} ratios close to unity (Homann, in press). We also verified that the induced fluorescence rise is comparable to the fluorescence induction of normal

TABLE I - MANGANESE CONTENT OF NORMAL AND INACTIVATED TOBACCO CHLOROPLASTS

Plant	NC 95 (green)			Su/su (aurea)	
Treatment	none	leaf heated (50°)	0.8 M Tris/HCl (pH 8.0)	none	leaf heated (50°)
Mn per total chl	1/93	1/500	1/330	1/45	1/450
	1/100	1/680	1/360		
	1/90	1/600	-		

Mn-deficient plants in water culture supplied with 2.5 μM Mn labeled with ^{54}Mn (3 to 6 x 10^5 cpm/μatom Mn) 4 to 8 weeks before harvest of the leaves.

chloroplasts in all respects: for example, it can be restored by a far-red illumination or prolonged darkness and its level F_{∞} is suppressed by oxidants like ferricyanide (Figure 2).

The observations presented in this communication clearly support the old concept that manganese functions close to or at the site of water oxidation. They also show that the fluorescence level of isolated chloroplasts in the presence of DCMU (F_{inh}) is a true measure for the number of functional trapping centers of the oxygen-evolving photosystem II.

References

Bertsch, W. F., Davidson, J. B., and Azzi. J. R. In "Photosynthetic Mechanisms in Green Plants", Publ. 1145 N. A. S. -N. R. C., p. 701, Washington (1963).

Cheniae, G. H., and Martin, I. Brookhaven Symp. in Biology 19: 406 (1966).

Cheniae, G. H., and Martin, I. Biochem. Biophys. Acta 153: 819 (1968); Plant Physiol. 43: S-12 (1968).

Duysens, L. N. M., and Sweers, H. E. In "Studies on Microalgae and Photosynthetic Bacteria", p. 353, University of Tokyo Press (1963).

Habermann, H. M., Handel, M. A., and McKellar, P. Photochem. Photobiol. 7: 211 (1968).

Homann, P. H. Biochemistry 4: 1902 (1965).

Homann, P. H. Biochim. Biophys. Acta, in press.

Joliot, P. Biochim. Biophys. Acta 102: 135 (1965).

Kok, B., Malkin, S., Owens, O., and Forbush, B. Brookhaven Symp. in Biology 19: 446 (1966).

Yamashita, T., and Butler, W. L. In "Comparative Biochemistry and Biophysics of Photosynthesis", K. Shibata et al., editors, p. 179, University of Tokyo Press (1968).