BBA 43242

Activation by manganese of photochemical oxygen evolution and $NADP^+$ photoreduction in chloroplasts

The chloroplasts washed with 0.8 M Tris–HCl buffer (pH 8.0) (Tris-washed chloroplasts) are known to exhibit no Hill reaction activity with water as electron donor[1,2]. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU)-sensitive $NADP^+$ photoreduction[1] with an artificial electron donor such as ascorbate *plus* *p*-phenylenediamine and DCMU-sensitive photobleaching of carotenoids[3] in Tris-washed chloroplasts have been interpreted as indicating that the treatment with Tris buffer blocks the photosynthetic electron transport on the oxidizing side (the water side) of System II at a site between water and an electron carrier designated as *Y* (see the scheme in ref. 3 or 4). The oxidized form, Y^+, which accumulates in Tris-washed chloroplasts[3] or in chloroplasts with carbonyl cyanide *m*-chlorophenylhydrazone[4] on illumination and is consumed by ascorbate *plus* phenylenediamine causes the carotenoid bleaching. On the other hand, it was demonstrated[3] that Mn in chloroplasts is removed by the Tris treatment and that 2,6-dichlorophenolindophenol (DCIP) photoreduction once lowered by Tris treatment is greatly restored by addition of Mn^{2+}. The restoration of DCIP photoreduction by Mn was accompanied by suppression of carotenoid bleaching. It was postulated[3] from these results that Mn, like ascorbate *plus* phenylenediamine, donates electrons to Y^+. This supposition is consistent with the effects of added Mn on the fluorescence yield of Mn-depleted chloroplasts[3,5]. In the present study, $NADP^+$ photoreduction and photochemical O_2 evolution were measured with Tris-washed chloroplasts in the presence or absence of added Mn in order to see whether the electron transport from water to $NADP^+$ is supported by Mn.

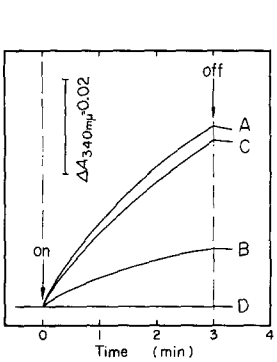
Tris-washed chloroplasts and normal (control) chloroplasts without the treatment with Tris buffer were prepared from spinach leaves as described previously[1,3]. These samples were suspended in 50 mM Tris–HCl buffer (pH 7.8) containing 0.4 M sucrose and 10 mM NaCl and were subjected to the measurements of $NADP^+$ reduction and O_2 evolution. $NADP^+$ reduction in the chloroplast suspension illuminated with blue light (4000 lux) obtained with a combination of glass filters with maximum transmission around 480 mμ was estimated from the light-induced absorbance increase at 340 mμ of the suspension measured with a Shimadzu Multipurpose recording spectrophotometer model MPS-50 (temperature, 20 $\pm$ 2°). Ferredoxin and ferredoxin–$NADP^+$ reductase, which are requisites for $NADP^+$ photoreduction in isolated chloroplasts, were prepared from spinach leaves according to the methods of SAN PIETRO[6] and KEISTER AND SAN PIETRO[7], respectively. O_2 evolution was measured with a Clark-type O_2 electrode as described previously[4]. In the experiment, chloroplasts in a semi-closed lucite vessel were illuminated with white light ($1 \cdot 10^5$ lux) at 17°.

Curves A and B in Fig. 1 show the time-courses of $NADP^+$ photoreduction in normal and Tris-washed chloroplasts, respectively. The rate of reduction obtained for normal chloroplasts, which was estimated from the absorbance increase at 340 mμ during 3 min of illumination with blue light, was 8.8 μmoles $NADP^+$ per mg chloro-

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol.

phyll per h (Curve A). The rate obtained for Tris-washed chloroplasts was lower (2.9 μmoles $NADP^+$ per mg chlorophyll per h, see Curve B), being in agreement with previous observations[1,2]. The $NADP^+$ photoreduction once lowered by treatment with Tris buffer was greatly activated by addition of Mn^{2+}. The rate of reduction obtained for the Tris-washed chloroplasts supplied with 0.1 mM $MnCl_2$ was 8.0 μmoles $NADP^+$ per mg chlorophyll per h (Curve C in Fig. 1), which is as high as 91 % of the rate for normal chloroplasts. As described previously[3], addition of 0.1 mM $MnCl_2$ to Tris-washed chloroplasts caused a similar degree of activation in DCIP photoreduction. It should be noted that the reduction of $NADP^+$ in the presence of Mn proceeds as long as 3 min in the light (Curve C in Fig. 1), whereas DCIP reduction in the presence of Mn proceeds only for 20 sec in the light[3]. The $NADP^+$ photoreduction restored by Mn was inhibited completely by 10 μM DCMU (Curve D in Fig. 1).

Tris-washed chloroplasts show a decreased activity of photochemical O_2 evolution with $NADP^+$ as electron acceptor. The rate of evolution for normal chloroplasts and that for Tris-washed chloroplasts, which were estimated from the amount of O_2 evolved during 5 min of illumination with white light, were 13.2 and 0.95 μmoles O_2 per mg chlorophyll per h, respectively (Curves A and B in Fig. 2). Addition of 0.1 mM $MnCl_2$ to Tris-washed chloroplasts caused a further decrease in the rate of evolution. This is probably due to the fact that the photochemical O_2 uptake observable in the absence of $NADP^+$ is accelerated by Mn^{2+} at such a high concentration (see also ref. 8). However, an accelerating effect of Mn on O_2 evolution was demonstrated for the Tris-washed chloroplasts which were incubated with 0.3 mM $MnCl_2$ for 10 min in the dark and then resuspended in 50 mM Tris–HCl buffer (pH 7.8) containing



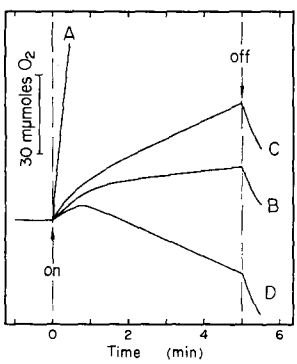


Fig. 1. The time-courses of the light-induced absorbance change at 340 mμ in the presence of 0.33 mM $NADP^+$ *plus* 1 μM ferredoxin *plus* a saturating amount of ferredoxin–$NADP^+$ reductase. Curve A, normal chloroplasts; Curve B, Tris-washed chloroplasts; Curve C, Tris-washed chloroplasts with 0.1 mM $MnCl_2$; Curve D, Tris-washed chloroplasts with 0.1 mM $MnCl_2$ *plus* 10 μM DCMU. The chloroplast suspensions contained 17.5 μg of chlorophylls per ml. Blue light (4000 lux) was turned on or off as indicated by arrows. Absorbance increase (upward change) indicates $NADP^+$ photoreduction.

Fig. 2. The time-courses of the light-induced O_2 evolution in the presence of 0.33 mM $NADP^+$ *plus* 1 μM ferredoxin *plus* a saturating amount of ferredoxin–$NADP^+$ reductase. Curve A, normal chloroplasts; Curve B, Tris-washed chloroplasts; Curve C, Tris-washed chloroplasts pretreated with 0.3 mM $MnCl_2$; Curve D, Mn-treated chloroplasts in the presence of 50 μM DCMU. The chloroplast suspensions (vol., 3 ml) contained 262 μg of chlorophylls. White light ($1 \cdot 10^5$ lux) was turned on or off as indicated by arrows. Upward change in the figure corresponds to evolution of O_2.

0.4 M sucrose and 10 mM NaCl after centrifugation. In fact, the chloroplasts pretreated with Mn by such a procedure showed an enhanced O_2 evolution when illuminated in the presence of $NADP^+$ (Curve C in Fig. 2). The rate of evolution obtained for the Mn-treated chloroplasts was 2.0 μmoles O_2 per mg chlorophyll per h (Curve C), which is 2.1 times higher than the rate for Tris-washed chloroplasts. O_2 evolution in Mn-treated chloroplasts was strongly inhibited by 50 μM DCMU (Curve D in Fig. 2). It should be noted that the pretreatment of Tris-washed chloroplasts by the above procedure increased the $NADP^+$ photoreduction in blue light by 43 %.

The accelerating effect of Mn on O_2 evolution was also observed with 3 mM *p*-benzoquinone as electron acceptor in place of $NADP^+$. In the presence of quinone, the rate of evolution in μmoles O_2 per mg chlorophyll per h was 144 for normal chloroplasts, 26 for Tris-washed chloroplasts and 40 for the Tris-washed chloroplasts pretreated with 0.3 mM $MnCl_2$.

As fully discussed previously[3], Mn in chloroplasts functions as a donor of electron to Y^+. The observed acceleration of $NADP^+$ photoreduction by Mn is consistent with this proposition. The acceleration of O_2 evolution by Mn indicates that the electron transport from water to Y is mediated by Mn. In conclusion, electrons from water migrate in photosynthesis to $NADP^+$ through Mn.

Tokugawa Institute for Biological Research,
Mejiromachi, Tokyo, and
Tokyo Institute of Technology,
Meguroku, Tokyo (Japan)

KATSUJI YAMASHITA
MICHIO ITOH
KAZUO SHIBATA

1 T. YAMASHITA AND W. L. BUTLER, in K. SHIBATA, A. TAKAMIYA, A. T. JAGENDORF AND R. C. FULLER, *Comparative Biochemistry and Biophysics of Photosynthesis*, University Park Press, State College, Pa. and Tokyo University Press, Tokyo, 1968, p. 179.
2 T. YAMASHITA AND T. HORIO, *Plant Cell Physiol.*, 9 (1968) 267.
3 M. ITOH, K. YAMASHITA, T. NISHI, K. KONISHI AND K. SHIBATA, *Biochim. Biophys. Acta*, 180 (1969) 509.
4 K. YAMASHITA, K. KONISHI, M. ITOH AND K. SHIBATA, *Biochim. Biophys. Acta*, 172 (1969) 511.
5 P. H. HOMANN, *Biochem. Biophys. Res. Commun.*, 33 (1968) 229.
6 A. SAN PIETRO, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 6, Academic Press, London, 1963, p. 439.
7 D. L. KEISTER AND A. SAN PIETRO, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 6, Academic Press, London, 1963, p. 434.
8 A. H. MEHLER, *Arch. Biochem. Biophys.*, 34 (1951) 339.

Received June 23rd, 1969