Biochimica et Biophysica Acta, 347 (1974) 31–35

BBA 46727

EFFECT OF α-TOCOPHEROL ON RECONSTITUTION OF PHOTOSYSTEM I IN HEPTANE-EXTRACTED SPINACH CHLOROPLASTS

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(Received October 23rd, 1973)

SUMMARY

1. The effect of α-tocopherol, phytol and tocol on reconstitution of Photosystem I in heptane-extracted spinach chloroplasts was investigated.

2. α-Tocopherol completely restored Photosystem I activity. The maximum rate of methylviologen photoreduction in reconstituted chloroplasts occurred at an α-tocopherol:chlorophyll molar ratio of 8.

3. Phytol and tocol added to heptane-extracted chloroplasts partially stimulated Photosystem I activity.

4. The concentrations of lipids used for the reconstitution of Photosystem I suggested that α-tocopherol plays a structural role in restoration of thylakoid membranes.

INTRODUCTION

It has been known since 1957 that organic solvent extraction of chloroplasts damages the structure of the lamellae and decreases their photosynthetic activity [1]. A crude lipid extract is able to restore the Hill activity of extracted chloroplasts. Several authors [2–4] have shown plastoquinone A to be an active component of the lipid extract, which acts at a site close to Photosystem II. In 1972 Okayama and Butler [5] examined heptane-extracted chloroplasts and stated that β-carotene is essential for the primary photochemical activity of Photosystem II. When the Photosystem II reaction-center was restored and made functional, an additional requirement of plastoquinone A for the Hill reaction was demonstrated.

Photosystem I can also be inactivated during lipid extraction from chloroplast membranes [6–10] and then restored. Henninger and Crane [11], using heptane extraction of chloroplasts, found a loss of ascorbate–indophenol-dependent photoreduction of $NADP^+$, which was restored by plastoquinone C. The effect of extraction can also be reversed on addition of plastocyanin [12].

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3,4-dichlorophenyl-1,1-dimethylurea; TMPD, *N,N,N,N*-tetramethyl-*p*-phenylene diamine; Tricine, *N*-tris(hydroxymethyl)-methylglycine.

Brand et al. [13], measuring Photosystem I activity by transfer of electrons from *N,N,N,N*-tetramethyl-*p*-phenylene diamine (TMPD) through the chloroplast photosystem to methylviologen dye, found that a crude heptane extract added back to extracted chloroplasts partially restored the activity. Triglycerides containing unsaturated C_{18} fatty acids were also able to restore Photosystem I activity in heptane-extracted spinach chloroplasts. It seems, however, that triglycerides are not present in chloroplast membranes [14]. Several components of the heptane extract tested by Brand et al. [13] did not stimulate Photosystem I activity.

While the present work was in progress Milles [15] demonstrated an acid-bath phosphorylation in heptane-extracted chloroplasts.

The purpose of this paper is to examine whether other lipid components of chloroplast membrane are able to reconstitute Photosystem I in extracted chloroplasts. Initially α-tocopherol, which is present in chloroplast membranes and whose role is still uncertain, and later phytol and tocol, were examined.

MATERIALS AND METHODS

Spinach chloroplasts were isolated from market spinach leaves as described by Sane et al. [16]. Pelleted chloroplasts were washed twice with cold distilled water and lyophilized. Heptane extraction of lyophilized chloroplasts was done according to Brand et al. [13].

Reconstitution of extracted chloroplasts was attempted with solutions of α-tocopherol, phytol and tocol. Increasing amounts of these compounds, dissolved in iso-octane, were added to 25 mg samples of extracted chloroplasts and the iso-octane was evaporated under vacuum. The dried reconstituted chloroplasts, suspended in 3 ml of 0.01 M Tricine buffer (pH 7.4) with 0.1 M KCl and containing approx. 0.5 mg chlorophyll per ml were used for activity measurements.

Oxygen photoreduction assay

Photosystem I activity of the chloroplasts was measured as oxygen uptake with a Clark-type oxygen electrode at 24 °C using sodium ascorbate and TMPD as the electron donor and methylviologen as the electron acceptor. The reaction chamber was illuminated with red light at an incident intensity of $2.5 \cdot 10^5$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. Other experimental details are described in the figure legend.

NADP photoreduction assay

NADP photoreduction was measured by following the change in absorbance at 340 nm caused by illumination with red light of an intensity equal to $1.5 \cdot 10^5$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. Assay conditions and reagent concentrations are indicated in the legend to the table.

Ferredoxin and plastocyanin were prepared from spinach according to the procedure of Böger et al. [17].

Chlorophyll concentrations were determined by the method of Arnon [18].

All chemicals were reagent grade. α-Tocopherol and sodium ascorbate were purchased from Merck. Phytol and tocol were products of Koch-Light, and Tricine, methylviologen and NADP were purchased from Sigma. TMPD was obtained from Eastman and (3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) from K and K Laboratories.

RESULTS

The activity of lyophilized and heptane-extracted chloroplasts was measured by oxygen uptake in reaction with methylviologen as electron acceptor and by NADP photoreduction.

Table I shows both activities under varying conditions. Heptane extraction of lyophilized chloroplast decreases the essential Photosystem I activity. Addition of saturating amounts of plastocyanin considerably protects electron transfer from TMPD to methylviologen as shown previously [13]. The restoration is much greater than that assayed by NADP photoreduction. The latter reaches only 1/3 of unextracted chloroplast activity.

TABLE I

RECONSTITUTION OF PHOTOSYSTEM I ACTIVITY OF HEPTANE-EXTRACTED SPINACH CHLOROPLASTS

The reaction mixture for measuring O_2 uptake contained the following components, in μmoles: Tricine–NaOH buffer (pH 8.0), 150; DCMU, 0.03; sodium ascorbate, 50; TMPD, 0.2; methylviologen 0.4; chloroplast equivalent to 15 μg of chlorophyll in a final volume of 3 ml. The reaction mixture for $NADP^+$ photoreduction measurements contained the following components, in μmoles: Tricine–NaOH buffer (pH 8.0), 150; DCMU, 0.03; sodium ascorbate, 50; TMPD, 0.2; $NADP^+$, 2.5; saturating amounts of ferrodoxin, chloroplasts containing 30 μg chlorophyll. In both assays, where indicated, saturating amount of plastocyanin was added. Addition of lipids to extracted chloroplasts were performed as described in methods.

Chloroplasts	Additions (μmoles/μmole chlorophyll)	TMPD → methylviologen (μmoles O_2 uptake/mg chlorophyll per h)		TMPD → $NADP^+$ (μmoles NADP reduced/mg chlorophyll per h)	
			plastocyanin		plastocyanin
Lyophilized	—	780	814	106	123
	10, α-tocopherol	760	820		
Heptane-extracted		138	504	9	35
	10, α-tocopherol	826	840	64	78
	10, phytol	427	765	18	45
	10, tocol	702	810	41	51

Fig. 1 shows the reconstitution of Photosystem I measured by oxygen uptake in relation to increasing amounts of lipids. Rate values are expressed as a percentage of the rate for lyophilized chloroplasts. As seen from the curve concerning α-tocopherol, a complete reconstitution of Photosystem I was attained when 8–10 times the amount of α-tocopherol (in comparison to chlorophyll present in chloroplast) was added to the extracted chloroplasts.

Taking into consideration the possibility that the tocopherol side chain may participate in reconstitution of Photosystem I, the influence of phytol was tested. Phytol stimulated electron flow in Photosystem I by about 60%.

Tocol, the unmethylated parent compound of α-tocopherol, gives an optimum rate of electron transport activity at a tocol:chlorophyll ratio of 10. This concentration of tocol reconstituted up to 90% of the original Photosystem I activity. Higher

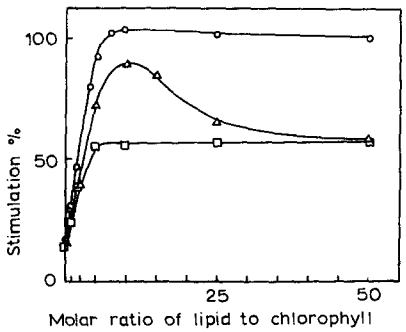

Fig. 1. The effect of α-tocopherol, phytol and tocol on reconstitution of Photosystem I in heptane-extracted spinach chloroplasts. The Photosystem I activity was assayed by the ascorbate+TMPD → methylviologen assay. The reaction conditions were identical to those described in Table I. Rate values are expressed as percentage of the rate of lyophilized chloroplasts activity. ○–○, α-tocopherol; □–□, phytol; △–△, tocol.

concentrations of tocol decrease the stimulating effect.

Taking into account the fact that, for all compounds tested, the optimal stimulation of Photosystem I activity is reached at a lipid:chlorophyll molar ratio of 10, the influence of saturated amounts of plastocyanin on the activity of chloroplasts reconstituted by the same amount of lipids was examined (Table I). Plastocyanin gives the least effect in increasing the activity of tocopherol-reconstituted membranes and maximum effect to membranes reconstituted with phytol.

In the presence of $NADP^+$ as the electron acceptor, an addition of plastocyanin also increases Photosystem I activity in reconstituted chloroplasts. The author, however, did not succeed in obtaining an activity equal to that of lyophilized chloroplasts.

DISCUSSION

The decrease in Photosystem I activity in heptane-extracted chloroplasts has been known for a number of years. Recent studies of Brand et al. [13] showed that the lipids of heptane extract restored the activity of System I.

As the heptane extract effects Photosystem I activity, it is evident that the components of the extract should be responsible for this reconstitution. Several compounds known to be present in chloroplasts such as plastoquinone, vitamin K_1, α-tocopherolquinone, β-carotene, galacto-, sulfo- and phospholipids fractionated by thin-layer chromatography and then tested for activity gave no significant stimulation [13].

In our studies attention was drawn to α-tocopherol and we succeeded in showing its presence in heptane extract. When added to extracted chloroplasts in an amount similar to that found in the heptane extract, it did not show a significant effect on Photosystem I activity. Therefore, in further extraction and reconstitution experiments much higher concentrations of α-tocopherol were used which produced a 100% reactivation of System I. α-Tocopherol added to the suspension of fresh or lyophilized chloroplasts at the concentration reconstituting Photosystem I activity (i.e., for α-tocopherol:chlorophyll molar ratio of 10) of extracted chloroplasts does not cause an increase in the rate of methylviologen reduction.

Since the prenyl side chain may be the active part of the α-tocopherol molecule, the effect of phytol was tested. A much smaller effectiveness of phytol suggest the chromane ring also exerts an effect. This is confirmed by a higher activity of tocol in comparison with phytol.

Lack of α-tocopherol influence on Photosystem I activity in fresh chloroplasts and a high concentration of this lipid in relation to chlorophyll restoring oxygen uptake to extracted chloroplasts give evidence of structural rather than redox role of this lipid. Such an assumption was put forward by Brand et al. [13] and Milles [15] in regard to triglycerides. It is also supported by the fact that α-tocopherol maintains the integrity of chloroplasts as an antioxidant [20].

Recently it was also shown that unsaturated C_{18} fatty acids are effective in stimulating swelling of fresh chloroplasts and electron flow in Photosystem I at a similar molar ratio of fatty acids to chlorophyll [20, 21].

It seems that the lipids studied play a structural role in restoration of the thylakoid membranes. This supposition also accounts for the fact that re-isolation of reconstituted chloroplasts does not significantly change the α-tocopherol:chlorophyll molar ratio. Our results, however, are not a sufficient evidence for the structural role of α-tocopherol in chloroplast because in nature the α-tocopherol concentration in chloroplast membranes is much lower than used in the above experiments. Further investigations will be continued along this line.

ACKNOWLEDGEMENTS

The author is greatly indebted to Dr F. L. Crane for the critical remarks and for helpful discussion.

REFERENCES

1 Lynch, V. H. and French, C. S. (1957) Arch. Biochem. Biophys. 70, 382–391
2 Bishop, N. I. (1959) Proc. Natl. Acad. Sci. U.S. 45, 1696–1702
3 Trebst, A. (1963) in Photosynthesis Mechanism of Green Plants (Kok, B. and Jagendorf, A. T., eds), p. 174, National Academy of Sciences-National Research Council, Washington D.C.
4 Wood, P. M., Bhagavan, H. N. and Crane, F. L. (1966) Plant Physiol. 41, 633–640
5 Okayama, S. and Butler, W. L. (1972) Plant Physiol. 49, 769–774
6 Krogmann, D. W. (1961) Biochem. Biophys. Res. Commun. 4, 275–277
7 Krogmann, D. W. and Oliviero, E. (1962) J. Biol. Chem. 237, 3292–3295
8 Chang, S. B. and Vedvick, T. S. (1968) Plant Physiol. 43, 1661–1665
9 Henninger, M. D. and Crane, F. L. (1963) Biochemistry 2, 1168–1171
10 Henninger, M. D., Dilley, R. A. and Crane, F. L. (1963) Biochem. Biophys. Res. Commun. 10, 237–242
11 Henninger, M. D. and Crane, F. L. (1967) J. Biol. Chem. 242, 1155–1159
12 Elstner, E., Pistorius, E., Böger, P. and Trebst, A. (1968) Planta 79, 146–161
13 Brand, J., Krogmann, D. W. and Crane, F. L. (1971) Plant Physiol. 47, 135–138
14 Nichols, B. W. and James, A. T. (1968) in Progress in Phytochemistry (Reinhold, L. and Liwschitz, Y., eds) pp. 1–48, Interscience Publishers, London
15 Miles, C. D. (1972) Plant Physiol. 50, 591–593
16 Sane, P. V., Goodchild, D. J. and Park, R. B. (1970) Biochim. Biophys. Acta 216, 162–178
17 Böger, P., Plack, C. C. and San Pietro, A. (1966) Arch. Biochem. Biophys. 115, 35–43
18 Arnon, D. I. (1949) Plant Physiol. 24, 1–15
19 Green, J., Diplock, A. T., Bunyan, J., Mc Hale, D. and Muthy, I. R. (1967) Br. J. Nutr. 21, 69–101
20 Siegenthaler, P.-A. (1972) Biochim. Biophys. Acta 275, 182–191
21 Siegenthaler, P.-A. (1973) Biochim. Biophys. Acta 305, 153–162