RESTORATION OF CYCLIC PHOSPHORYLATION IN HEPTANE-EXTRACTED SPINACH CHLOROPLASTS BY α-TOCOPHEROL

T. BASZYŃSKI and A. TUKENDORF
Institute of Biology, Maria Curie-Skłodowska University, 20-033 Lublin, Poland

Received 14 June 1975

1. Introduction

Heptane extraction of lyophilized chloroplasts causes a loss of phosphorylation activity. Partial restoration of cyclic phosphorylation catalyzed by phenazine metosulphate was obtained when plastoquinone A or long-chain analogs down to plastoquinone A were added back to the extracted chloroplasts [1–3]. Addition of chloroplast lipids other than plastoquinone were not able to restore photophosphorylation by heptane-extracted chloroplasts.

Cyclic electron flow involves Photosystem I and seems to be concentrated in the stroma lamellae [4,5]. Photosystem I activity of heptane-extracted chloroplasts was recently restored by α-tocopherol [6] and partially by mono- and digalactosyl diglycerides [7]. As yet, α-tocopherol was uniformly lacking in the ability to replace plastoquinone in photophosphorylation [2].

In the present paper data is provided to show that α-tocopherol reconstitutes the cyclic phosphorylation of heptane-extracted chloroplasts.

2. Material and methods

In all experiments, chloroplasts were prepared from market spinach leaves as described by Sane et al. [5]. The pelleted chloroplasts were resuspended in a minimal volume of the isolation medium containing sucrose, necessary to protect phosphorylation from damage during lyophilization [8], and then lyophilized. Heptane extraction of freeze-dried chloroplasts and its reconstitution by α-tocopherol were carried out as described elsewhere [6]. Photophosphorylation catalyzed by phenazine metosulphate was measured according to the modified version of Avron [9]. Experimental details are described in the legends for the figure and tables. Inorganic phosphate analysis was measured by the method of Fiske and SubbaRow [10].

EDTA-treated chloroplasts were obtained according to McCarthy [11]. Preparation and assay of coupling factor 1 from chloroplasts (CF_1) were done as described by Lien and Racker [12]. Protein concentration was determined according to Lowry et al. [13] and chlorophyll according to Arnon [14].

3. Results and discussion

Heptane extraction of lyophilized chloroplasts shown in fig.1 (lower curve) causes progressive loss in photophosphorylation. After a 2-hr heptane extraction the activity of cyclic phosphorylation is stabilized on the level of 20–30% of its original rate and it does not change during 12 hr of extraction. Reconstitution of cyclic phosphorylation of heptane-extracted chloroplasts is shown by the upper curve of fig.1. A full recovery in activity was achieved by using α-tocopherol in a molar ratio to chlorophyll of about 10. Such molar concentration of α-tocopherol was also needed for recovery of Photosystem I activity of heptane-extracted chloroplasts [6]. The lack of restoration of phosphorylation reported by Krogmann and Oliviero [2] depended on a low molar ratio of α-tocopherol/chlorophyll, of about 2, used in their experiment. These amounts of α-tocopherol are not sufficient for reconstitution of photophosphorylation in extracted chloroplast membranes (see fig.1).

North-Holland Publishing Company – Amsterdam

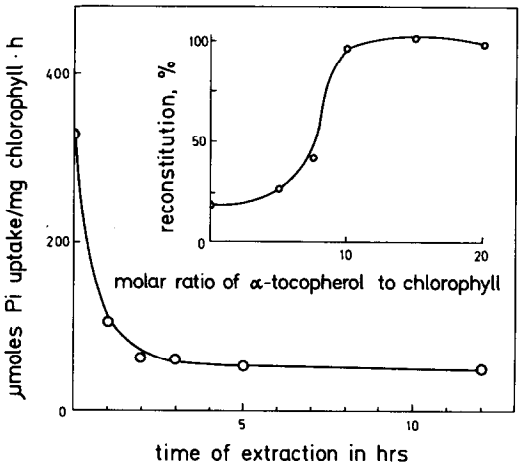

Fig.1. Cyclic phosphorylation of heptane-extracted and α-tocopherol reconstituted spinach chloroplasts. Lower curve represents the effect of extraction time on photophosphorylation; upper curve represents restoration of photophosphorylation in relation to α-tocopherol/chlorophyll molar ratio. The reaction mixture contained the following components, in μmoles: Tricine–NaOH buffer (pH 8.0) , 50; KCl, 50; $MgCl_2$, 10; Na_3PO_4, 10; ADP, 4; phenazine metosulphate, 0.15; sodium ascorbate, 20; chloroplast equivalent to 50 μg of chlorophyll in a final volume of 3 ml. Illumination for 5 min was provided by light at an incident intensity of 1.2×10^5 ergs·cm^{-2}·s^{-1}.

Table 1
Restoration of cyclic phosphorylation of EDTA-treated spinach chloroplasts

Chloroplasts Additions	μmoles Pi uptake/mg chlorophyll per hr
Lyophilized	293
EDTA-treated,	0
CF_1	271
α-tocopherol	69
α-tocopherol + CF_1	213
EDTA-treated and heptane-extracted,	0
CF_1	117
α-tocopherol	131
α-tocopherol + CF_1	264

The reaction mixture and assay conditions were described in the legend for fig.1. Where indicated α-tocopherol in a molar ratio to chlorophyll = 10 and CF_1 in concentration 50 μg protein per assay were added. EDTA-treated chloroplasts were lyophilized and next extracted with heptane for 12 hr.

Heptane extraction decreases cyclic phosphorylation due to partial removing of lipids from chloroplast membranes. Some protein factors which affected cyclic phosphorylation are also likely to be removed. Elstner et al. [15] have reported that heptane extraction removes some amounts of plasticyanin from chloroplast membranes. Plastocyanin, however, has no effect on phenazine metosulphate-dependent phosphorylation [16].

The protein factor required for photophosphorylation is CF_1 located in the outer surface of thylakoid membrane [17]. The heptane-extract did not contain CF_1, but it was found to remain in the extracted chloroplasts.

Restoration of cyclic phosphorylation of CF_1 depleted chloroplasts is shown in table 1. Treatment of lyophilized chloroplasts with a dilute EDTA solution removes CF_1 and causes an uncoupling of photophosphorylation from electron transport. Addition of CF_1 to EDTA-treated chloroplasts reinstates cyclic phosphorylation, as found by Vambutas and Racker [18]. The degree of reconstitution with α-tocopherol was not higher than 25%. Both factors used together partially decreased the phosphorylation rate in comparison with CF_1 reconstituted chloroplasts. α-Tocopherol and CF_1 partially restored the activity of EDTA-treated chloroplasts extracted with heptane. When added together, they restored nearly 90% of the original phosphorylation rate. The results of this experiment suggest that full restoration of cyclic phosphorylation with CF_1 is possible only in the undamaged structure of the lipid part of the membrane. Partial removal of lipids with heptane decreases CF_1 effectiveness. Introduction of α-tocopherol in place of the extracted lipids makes full restoration of cyclic phosphorylation with CF_1 possible.

Support for this suggestion can be found in another experiment (table 2). Cyclic phosphorylation of heptane-extracted chloroplasts is not influenced by CF_1 whereas α-tocopherol gave full recovery of its activity (see also fig.1). EDTA-treatment of heptane-extracted chloroplasts caused full loss of cyclic phosphorylation. CF_1 and α-tocopherol, separately added to these chloroplasts, recover the activity to no more than 30–40%. Both factors added together restored 66% of the original activity. The low phosphorylation rate of the reconstituted chloroplasts was due to the

Table 2
Restoration of cyclic phosphorylation of heptane-extracted spinach chloroplasts

Chloroplasts Additions	μmoles Pi uptake/mg chlorophyll per hr
Lyophilized	176
Heptane-extracted,	73
CF_1	94
α-tocopherol	178
α-tocopherol + CF_1	157
Heptane-extracted and EDTA-treated,	0
CF_1	56
α-tocopherol	75
α-tocopherol + CF_1	117

The reaction mixture and assay conditions were described in the legend for fig.1. 12-hr heptane-extracted chloroplasts were treated with EDTA according to McCarthy [11]. Concentrations of CF_1 and α-tocopherol added as in table 1.

lyophilization of the heptane-extracted chloroplasts after the EDTA-treatment.

It appears that α-tocopherol serves as a binding agent between CF_1 and the chloroplast membrane. Livne and Racker [19] had found earlier that other components of chloroplast membranes such as sulpho-, galacto- and phospholipids conferred heat stability on CF_1. It is possible that α-tocopherol is not a specific lipid component which reacts with CF_1 in the chloroplast membranes. Recently Miles [20] has found that exogenous triglycerides gave partial restoration of acid-bath phosphorylation. It is not excluded that some endo- and exogenous lipids are also able to reconstitute cyclic phosphorylation.

Acknowledgements

This work was supported by the Committee of Biochemistry and Biophysics of the Polish Academy of Sciences.

References

[1] Krogmann, D. W. (1961) Biochem. Biophys. Res. Communs 4, 275–277.
[2] Krogmann, D. W. and Oliviero, E. (1962) J. Biol. Chem. 237, 3292–3295.
[3] Whatley, F. R. and Harton, A. A. (1963) Acta Chem. Scand. 17, S140–143.
[4] Anderson, J. M. and Boardmann, N. K. (1966) Biochim. Biophys. Acta 112, 403–421.
[5] Sane, P. V., Goodchild, D. J. and Park, R. B. (1970) Biochim. Biophys. Acta 216, 162–178.
[6] Baszyński, T. (1974) Biochim. Biophys. Acta 347, 31–35.
[7] Krupa, Z. and Baszyński, T. (1974) Materiały XII Zjazdu Polskiego Towarzystwa Biochemicznego, Warszawa, p. 52.
[8] Hauska, G. A. and Sane, P. V. (1972) Z. Naturforsch. 27b, 938–942.
[9] Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272.
[10] Fiske, C. H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375.
[11] McCarthy, R. E. (1971) Methods in Enzymology Vol. XXIII A pp. 251–253, (A. San Pietro ed) Academic Press, New York and London.
[12] Lien, S. and Racker, E. (1971) Methods in Enzymology Vol. XXIII A pp. 547–555 (A. San Pietro ed) Academic Press, New York and London.
[13] Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[14] Arnon, D. I. (1949) Plant Physiol. 24, 1–15.
[15] Elstner, E., Pistorius, E., Böger, P. and Trebst, A. (1968) Planta 79, 146–161.
[16] Anderson, M. M. and McCarthy, R. E. (1969) Biochim. Biophys. Acta 189, 193–206.
[17] Kannangara, C. G., Van Wyk, D. and Menke, W. (1970) Z. Naturforsch, 25b, 613–618.
[18] Vambutas, V. K. and Racker, E. (1965) J. Biol. Chem. 240, 2660–2667.
[19] Livne, A. and Racker, E. (1969) J. Biol. Chem. 244, 1332–1338.
[20] Miles, C. D. (1972) Plant Physiol. 50, 591–593.