

β -Carotene as a factor in the reconstitution of cyclic phosphorylation in damaged chloroplast membranes

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(Received: March 28, 1980)

Abstract

Phenazine methosulphate mediated cyclic phosphorylation suppressed by heptane extraction or galactolipase treatment of spinach chloroplasts is restored by β -carotene, in 100% and 50%, respectively. Xanthophylls are not able to reconstitute this reaction. β -Carotene replaces galactolipids in reactivation of galactolipase treated chloroplasts, indicating a nonspecific effect of lipids in photosystem I dependent reactions.

Abbreviations: CF₁ — chloroplast coupling factor 1; EDTA — ethylenediaminetetracetic acid; PMS — phenazine methosulphate.

INTRODUCTION

Preliminary investigations on the role of carotenoids in the restoration of photosystem I activity and cyclic phosphorylation lost by heptane extraction of lyophilized chloroplasts show that β -carotene, as opposed to xanthophylls, was able to restore these reactions (Baszyński, Tukendorf, 1977). The present paper describes in more detail the effect of carotenoids, being components of chloroplast membranes, on PMS-mediated cyclic phosphorylation by examining their effect when added to lipid- or CF₁-depleted spinach chloroplasts.

MATERIAL AND METHODS

Chloroplasts were isolated from market spinach leaves (*Spinacia oleracea* L.) according to Sane et al. (1970). Pelleted chloroplasts were resuspended in a mixture of 0.4 M sucrose and 0.05 M NaCl and lyophilized by the method of Krogmann and Olivero (1962). The

procedure for heptane extraction has been described earlier (Baszyński, 1974).

The carotenoids necessary for reactivation of cyclic phosphorylation were extracted with acetone from spinach leaves and then purified by thin-layer chromatography on silica gel plates after Hager and Berenrath (1962).

Reconstitution was achieved by resuspending the extracted chloroplasts in heptane solution containing the quoted amounts of individual carotenoids. After evaporation of the solvent under vacuum, dry preparations resuspended in 0.02 M Tris buffer (pH 7.8) were used for activity measurements.

Cyclic phosphorylation catalyzed by phenazine methosulphate was measured according to the method of Avron (1960). The reaction mixture contained the following components, in μ moles: Tricine-NaOH buffer (pH 8.0) — 50; KCl — 50; $MgCl_2$ — 10; Na_3PO_4 — 10; ADP — 4; PMS — 0.15; sodium ascorbate — 20; chloroplasts equivalent to 50 μ g of chlorophyll; final volume 3 ml. Illumination was for 5 min, at an irradiance of $1.2 \times 10^4 \mu W \cdot cm^{-2}$ (Balzer K6 filter). Inorganic phosphorus was determined by the method of Fiske and Subbarow (1925).

CF_1 -deficient chloroplasts were prepared by the method of McCarty (1971), which involves washing the chloroplasts with 10 mM NaCl followed by treatment with 0.75 mM EDTA (pH 8.0). CF_1 was isolated from spinach chloroplasts and purified according to Younis et al. (1977).

The protein fraction containing galactolipase was obtained from fresh bean leaves and purified on Sephadex G-100 according to Anderson et al. (1974). Galactolipase was free of proteolytic activity when checked by the method of Nelson et al. (1961).

Protein content was estimated by the method of Schacterle and Pollack (1973). Chlorophyll was measured in 80% acetone extracts according to Arnon (1949).

RESULTS AND DISCUSSION

Fig. 1 shows the loss of cyclic phosphorylation capacity in lyophilized chloroplasts due to heptane extraction to about 20% of its original rate. Heptane extraction damages chloroplast membranes non-specifically and removes among other lipids nearly 90% of the β -carotene present in them. Full reconstitution of cyclic phosphorylation in these chloroplasts was achieved when β -carotene was readded to extracted membranes in a molar ratio to chlorophyll of about 0.5 (Table 1). Such molar concentration of β -carotene was also needed for the recovery of photosystem I activity in heptane-extracted chloroplasts (Baszyński, Tukendorf, 1977). Xanthophylls (lutein, violaxanthin and neo-

xanthin) restored its activity to a very small extent although Hawcroft and Friend (1975) were able to show the stimulative effect of violaxanthin and lutein on cyclic phosphorylation in isolated broad bean chloroplasts. Partial restoration of cyclic phosphorylation was shown earlier when plastoquinone A or long-chain analogs down to plastoquinone A (Krogmann, Olivero, 1962; Chang, Vedvick, 1968) and mono- and digalactosyldiacylglycerol or sulpholipid were added to extracted membranes (Krupa, Baszyński, 1977). From other components of chloroplast membranes only α -tocopherol, besides β -carotene, completely reconstitutes the cyclic phosphorylation (Baszyński, Tukendorf, 1975).

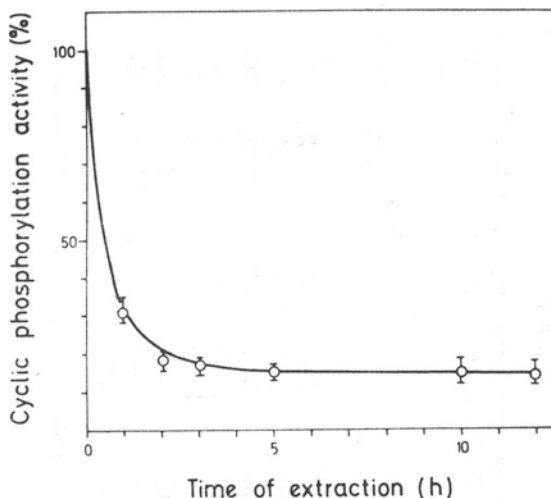


Fig. 1. The effect of heptane extraction on cyclic phosphorylation in lyophilized spinach chloroplasts

Rate values are expressed as a percentage of the lyophilized chloroplast activity (100%)

Contrary to the structural lipids, CF_1 is not removed from the thylakoid membranes by the heptane extraction (Baszyński, Tukendorf, 1975). The CF_1 therefore added to heptane-extracted and β -carotene-reconstituted chloroplasts did not influence cyclic phosphorylation of these membranes. In this case the decrease of cyclic phosphorylation activity is only due to structural lipids being removed from the chloroplast membranes. This fact supposes the view that damage of the membrane integrity affects the cyclic phosphorylation activity.

Treatment of lyophilized chloroplasts with EDTA solution liberates, as is known, the CF_1 required for phosphorylation and causes an uncoupling of phosphorylation from electron transport. The reconstitution of phosphorylation by recombining EDTA-treated chloroplasts with CF_1

Table 1

The effect of carotenoids on reactivation of cyclic phosphorylation in heptane-extracted and EDTA-treated spinach chloroplasts. Percentage in parentheses

Chloroplast treatment	Control		β -Carotene		Lutein		Violaxanthin		Neoxanthin	
	P _i uptake, μ mol/mg chl · h									
Lyophilized	79 \pm 8	(100)								
Heptane-extracted	21 \pm 2	(26)	87 \pm 9	(109)	30 \pm 2	(38)	24 \pm 2	(32)	24 \pm 2	(31)
+CF ₁	20 \pm 2	(25)	85 \pm 7	(107)	30 \pm 2	(38)	24 \pm 2	(30)	25 \pm 3	(31)
Heptane-extracted and EDTA-treated	0	(0)	52 \pm 7	(65)	9 \pm 2	(11)	2 \pm 2	(3)	6 \pm 2	(8)
+CF ₁	33 \pm 5	(42)	75 \pm 9	(94)	46 \pm 0	(58)	33 \pm 8	(42)	45 \pm 5	(57)
EDTA-treated	0	(0)	19 \pm 5	(24)	6 \pm 2	(7)	0	(0)	3 \pm 3	(3)
+CF ₁	77 \pm 11	(97)	95 \pm 12	(120)	76 \pm 7	(96)	69 \pm 11	(87)	70 \pm 7	(88)
EDTA-treated and heptane-extracted	0	(0)	41 \pm 6	(51)	9 \pm 0	(11)	4 \pm 1	(5)	5 \pm 2	(6)
+CF ₁	33 \pm 6	(42)	78 \pm 6	(98)	43 \pm 6	(55)	37 \pm 5	(46)	39 \pm 5	(49)

Molar ratios of β -carotene, lutein and violaxanthin, respectively to chlorophyll were 0.5; this ratio of neoxanthin to chlorophyll was 1.0. Where indicated 100 μ g CF₁ per 50 μ g chlorophyll was added.

was, in our experiments, nearly 90% of the original activity (Fig. 2). Incomplete reconstitution could be due to a wrong orientation by rebounding CF_1 molecules, which might prevent H^+ leakage without recovering catalytic activity (Livne, Racker, 1968; Schmid, Junge, 1975; Berzborn, Schröer, 1976).

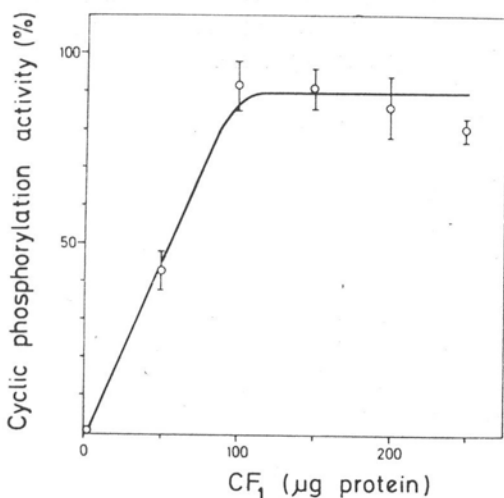


Fig. 2. The effect of the chloroplast coupling factor (CF_1) on cyclic phosphorylation in EDTA-extracted spinach chloroplasts

In order to check the participation of both CF_1 and carotenoids in the restoration of cyclic phosphorylation, in other experiments the spinach chloroplasts were treated with EDTA and extracted with heptane. Such treatment of chloroplasts caused, of course, full loss of cyclic phosphorylation. CF_1 and β -carotene, separately added to those chloroplasts, recovered the activity only partially. Both factors added together restored cyclic phosphorylation to over 90% of the initial rate. Recovery of cyclic phosphorylation was independent of the order of removal of components from chloroplast membranes. The effect of xanthophylls on the reconstitution of the above reaction in the chloroplasts mentioned was very small.

In our previous investigations we have shown that the photosystem I activity of heptane-extracted chloroplasts can be unspecifically restored by the addition of endogenous components of chloroplast membranes (Baszyński, 1974; Krupa, Baszyński, 1975, 1977) as well as exogenous lipids (Krupa, Baszyński, 1978).

The photosystem I activity and cyclic phosphorylation, we believe, to be located in the same regions of chloroplast membranes. Thus the

non-specific action of lipids in the restoration of cyclic phosphorylation is also taken into consideration. To resolve this problem we have used, for the reactivation of cyclic phosphorylation, chloroplasts incubated with galactolipase. Galactolipase treatment, as seen from Fig. 3 affects the photosystem I-dependent cyclic phosphorylation catalyzed by PMS. In contrast to heptane extraction, galactolipase affects only or mainly the galactolipids in chloroplasts. Thus if other lipids (in our case carotenoids) are effective in the restoration, this suggests that the restoration effect is really non-specific.

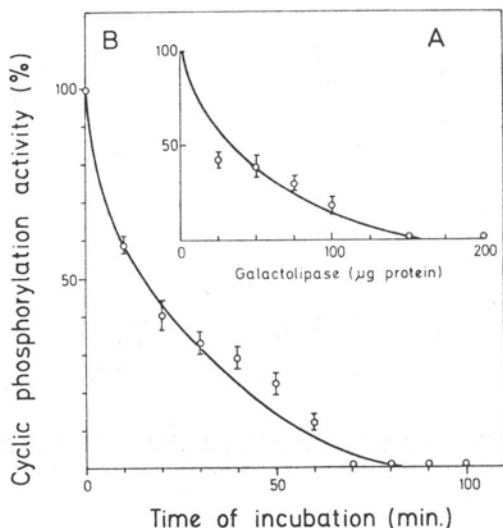


Fig. 3. The effect of galactolipase on cyclic phosphorylation in lyophilized spinach chloroplasts: (A) in relation to galactolipase content (incubation time 30 min); (B) in relation to incubation time

The galactolipase content in (B) was 50 µg protein per 300 µg chlorophyll. Rate values are expressed as a percentage of the rate of lyophilized chloroplast activity (100%)

From the results pointed out in Table 2 one might speculate that the decrease of cyclic phosphorylation in chloroplast membranes after galactolipase treatment is related to a degradation of galactolipids. Assuming that CF_1 is bound to thylakoid membranes with some galactolipid (Livne, Racker, 1969; Kannangara et al., 1970; Schopf et al., 1975), galactolipase treatment could release CF_1 from the membrane. However, this is not certain, because CF_1 added to galactolipase treated chloroplasts does not reconstitute cyclic phosphorylation.

The complete deactivation of cyclic phosphorylation after a 70 min treatment of chloroplasts with galactolipase indicates that this reaction

Table 2

Reactivation of cyclic phosphorylation with β -carotene in galactolipase-treated spinach chloroplasts. Percentage in parantheses

Chloroplast treatment	μ moles P_i uptake mg chl/h
Lyophilized	59 \pm 5 (100)
Galactolipase-treated	0 (0)
+CF ₁	0 (0)
+ β -carotene	31 \pm 6 (53)
+CF ₁ + β -carotene	30 \pm 5 (50)

Lyophilized spinach chloroplasts were incubated with galactolipase (50 μ g protein per 300 μ g chlorophyll) in Tris buffer (pH 7.8) for 70 min at temp. 24°C. After incubation pelleted chloroplasts were washed twice with 0.05 M NaCl and then lyophilized. Galactolipase-treated chloroplasts were reactivated with β -carotene in a molar ratio to chlorophyll of 0.5. Where indicated 100 μ g of CF₁ per 50 μ g chlorophyll were added.

depends on the degradation of galactolipids and subsequent membrane disintegration.

Cyclic phosphorylation activity was found to be more dependent on intact chloroplast structure than photosystem I activity. We have shown earlier (Krupa, Baszyński, 1975) that galactolipase treatment inhibited photosystem I activity only up to 30% of control chloroplasts, but the present data indicate a complete deactivation of cyclic phosphorylation.

β -Carotene, in a constant ratio to chlorophyll of 0.5, added to galactolipid-depleted chloroplasts reactivates cyclic phosphorylation to a degree of 50%. CF₁ added with β -carotene have no influence on the enhancement of reconstitution. Significantly lower reactivation rates of enzyme-treated chloroplasts in comparison with heptane-extracted ones probably depends on the different action of the agents examined on thylakoid membranes. Polar organic solvents remove intact lipid particles, but galactolipase removes one or two acyl residues only.

According to our previous investigations and the present data (Baszyński, 1974; Baszyński, Tukendorf, 1975, 1977; Krupa, Baszyński, 1975, 1977, 1978; Tukendorf et al., 1977), it can be assumed that for cyclic phosphorylation, as for photosystem I activity, the integrity of chloroplast membranes is required. The recombination of CF₁ and thylakoid membranes in reconstitution experiments shows that the addition of chloroplast lipids enhances the incorporation of CF₁ into the membranes (Harnischfeger, Schopf, 1977). Lipids could probably play some role in the binding of CF₁ to thylakoid membranes or in the conformation of CF₁ particles.

The replacement of galactolipids by β -carotene in the partial reactivation of cyclic phosphorylation indicates a non-specific activity of lipids in photosystem I-dependent reactions.

Acknowledgments

These investigations were supported by the Committee of Biochemistry and Biophysics of the Polish Academy of Sciences.

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β -Karoten jako czynnik reaktywujący fosforylację cykliczną w uszkodzonych błonach chloroplastowych

Streszczenie

Badano wpływ karotenoidów chloroplastowych na przywracanie zdolności do fosforylacji cyklicznej, katalizowanej przez PMS, w chloroplastach szpinaku, które utraciły tę zdolność na skutek ekstrakcji heptanem. β -Karoten dodany do ekstrahowanych chloroplastów, w stosunku molarnym do chlorofilu = 0,5, całkowicie reaktywował fosforylację cykliczną. Ksantofile (luteina, wiolaksantyna i neoksantyna) nie wykazywały zdolności do reaktywacji. β -Karoten reaktywował także fosforylację cykliczną utraconą na skutek inkubacji chloroplastów z galaktolipazą, rozkładającą galaktolipidy błony chloroplastowej. Zdolność β -karotenu do zastępowania galaktolipidów w reaktywacji wskazuje na, stwierdzone wcześniej, niespecyficzne działanie lipidów w reakcjach związanych z I układem fotosyntezy.