ON THE ENERGY REQUIREMENTS OF FATTY ACID SYNTHESIS IN SPINACH CHLOROPLASTS IN THE LIGHT AND IN THE DARK

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1. Introduction

Intact photosynthesizing chloroplasts from spinach leaves synthesize fatty acids from acetate (reviewed in [1,2]) and other precursors [3]. The required ATP and reducing equivalents for this synthesis are delivered by photophosphorylation and photosynthetic generation of NADPH.

Here we show that in the light, exogenously added ATP at low concentration stimulates fatty acid synthesis, and at high concentration alters the distribution of synthesized acids within the plastidal lipids. Furthermore, we present evidence that in the dark, exogenously added ATP enables fatty acid synthesis only to a relatively low extent and that energy-rich compounds like dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, for which a translocator system exists in the chloroplast inner envelope membrane (reviewed in [4]) and phosphoenolpyruvate are even less effective.

2. Materials and methods

Spinach leaves (*Spinacia oleracea*) were chopped with a razor blade and homogenized with a knife homogenizer in 0.3 M sorbitol, 5 mM $MgCl_2$, 50 mM tricine/NaOH (pH 7.8). The homogenate was filtered through 3 layers of 40 μm mesh nylon cloth and the filtrate centrifuged for 1 min at 150 × *g* (Christ Minifuge). The supernatant was then centrifuged for 1 min at 1200 × *g*, the chloroplast pellet gently resuspended in the isolation medium and centrifuged again for 1 min at 1200 × *g*. The final chloroplast pellet contained $\geqslant$ 70% class I chloroplasts as judged by light and electron microscopy.

The incubation medium contained 0.3 M sorbitol, 100 mM tricine/NaOH (pH 7.8), 5 mM K_2HPO_4, 60 mM $NaHCO_3$, 1 mM $MgCl_2$, 1 mM dithioerythritol, 0.5 mM CoA, and [1-^{14}C]acetate (2 μCi, 34 nmol). Each assay contained chloroplasts measured as 200–500 μg chlorophyll in 0.5 ml total vol. Incubations in the dark additionally contained NADH (0.5 mM), NADPH (0.5 mM) and energy-rich compounds as indicated in table 1. K_2HPO_4 was omitted from these incubations. Assay mixtures were incubated at 25°C for 20 min on a shaker at 6000 lux (saturating light intensities for fatty acid synthesis [5]) or in the dark.

Lipid extraction, two-dimensional thin-layer chromatography and scintillation counting techniques were performed as in [6].

3. Results and discussion

3.1. *Fatty acid synthesis in the light*

The isolated chloroplasts incorporated [1-^{14}C]acetate into long-chain fatty acids at a sufficiently high rate of ~120 nmol .h^{-1} .mg chlorophyll^{-1} (equivalent to the 100% value at standard conditions in table 1) under the light conditions used. Most of the label was found to be associated with the free fatty acid fraction (table 1). Inhibitory and stimulating effects on chloroplast fatty acid synthesis in the light upon addition of ATP have been reported [5,7–9]. We observed a considerable increase in fatty acid formation, especially at a low ATP concentration, and an altered distribution of newly formed acids within the plastidal

Table 1
The incorporation of [1-^{14}C]acetate into fatty acids by isolated spinach chloroplasts in the light

Incubation conditions	^{14}C incorp. (%)	Distribution of ^{14}C-labelled fatty acids (Σ = 100%)				
		Acyl-CoA	PC	PG	FA	DG
Standard	100	–	1.0	0.5	82.8	15.7
+ATP (0.1 mM)	142	–	3.1	0.9	78.1	17.9
+ATP (0.2 mM)	219	4.6	2.0	1.0	70.9	21.5
+ATP (0.4 mM)	190	18.6	2.7	1.5	42.7	34.5
+ATP (0.8 mM)	139	33.2	4.8	2.1	21.7	38.2
+ATP (2.0 mM)	156	37.7	3.5	2.6	14.7	41.5
+ADP (0.2 mM)	153	1.3	2.8	1.0	74.8	20.1
+ADP (2.0 mM)	170	23.2	3.2	1.9	40.0	31.7

Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol; FA, free fatty acids; DG, diacylglycerol

lipids, especially at higher ATP concentrations (table 1). At high ATP concentration most of the label was recovered in the acyl-CoA and diacylglycerol fractions, i.e., in the pathway of galactolipid synthesis (galactolipids, however, were not labelled because only saturated and monoenoic acids were formed in vitro; discussed in [10]). Similar but less pronounced effects were obtained when ADP was added to the incubation mixtures (table 1).

These two observations can be interpreted in consideration of the properties of the intact chloroplast. The permeability of the inner chloroplast envelope membrane to adenine nucleotides is small in proportion to normal rates of ATP formation and utilization [4,11]. Thus, the added nucleotides certainly do not stimulate fatty acid synthesis directly, but rather indirectly by increasing the internal nucleotide pool to a certain level which then is accessible for photophosphorylation. This stimulation of fatty acid synthesis by external ATP may be more or less pronounced according to the adenine nucleotide pools in the stroma of the isolated chloroplasts.

The site of acyl-CoA and diacylglycerol synthesis has been shown to be the inner membrane of the chloroplast envelope [12,13]. The results presented above indicate that this reaction in vitro requires a relatively high ATP concentration (and CoA concentration which is present in the incubation mixture) to proceed and, perhaps, to overcome the acyl-CoA hydrolase activity which sometimes seems to be very high in envelope membranes [14]. Similar observations have been made using daffodil chromoplasts [6]. At which membrane surface (inner or outer) ATP is utilized in this reaction is not exactly known, although the inner surface would be the more reasonable to assume. This view is sustained by the similar effects of ADP which should be postulated first to be converted to ATP by the plastidal adenylate kinase.

3.2. *Fatty acid synthesis in the dark*

The active synthetic phase of chloroplasts is under light conditions, although ATP consumption by chloroplast metabolism in the dark is certainly not negligible [11]. In view of the dependence on external energy supply, the dark situation of chloroplasts may resemble in a sense the normal situation of other non-green plastids. The adenylate translocator is relatively ineffective in chloroplast envelopes [15]. Thus, it has been postulated that the dihydroxyacetone phosphate/3-phosphoglycerate shuttle (mediated by the phosphate translocator) operating in the light for indirect transport of NADH and ATP into the cytoplasm may be reversed in the dark in order to supply the chloroplast with ATP [11]. Here we have tested ATP, ADP, dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and phosphoenolpyruvate (shown [16] to be also transported by the phosphate translocator) for their ability to sustain

Table 2
The incorporation of [1-^{14}C]acetate into fatty acids by isolated spinach chloroplasts in the dark

Incubation conditions	^{14}C incorp. (%)	Distribution of ^{14}C-labelled fatty acids (Σ = 100%)			
		Acyl-CoA	PL	FA	DG
Standard (light)	100	–	1.5	82.8	15.7
Standard (dark)	–				
+ATP (2 mM)	25	20.6	0.6	27.6	51.2
+ADP (2 mM)	13	–	–	38.2	61.8
+DHAP (2 mM)	5	–	–	60.4	39.6
+GAP (2 mM)	7	–	–	60.3	39.7
+PEP (2 mM)	7	–	–	62.5	37.5

Abbreviations: PL, phospholipids; FA, free fatty acids; DG, diacylglycerol; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde phosphate; PEP, phosphoenolpyruvate

P_i was omitted from the dark incubations because of its competition with the other phosphates for transport

fatty acid synthesis in isolated chloroplasts in the dark (table 2). In the presence of 2 mM ATP, 25% of the synthetic rate in the light was measured. With decreasing ATP concentrations the rate of the synthesis also decreased. No synthesis was observed when ATP was omitted from the incubation medium. Interestingly, relatively high proportions of acyl-CoA and diacylglycerol were found under these conditions as were also observed in the light in the presence of high amounts of ATP (tables 1,2). ADP was also tested, but proved less effective. Even less effective as energy donors were dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, and phosphoenolpyruvate under the conditions used (table 2) which might be unexpected from the present knowledge of the properties of the chloroplast envelope and which cannot be explained at the moment. In parallel experiments it was shown that the phosphate translocator was effectively operating in these chloroplasts [16].

A generally reduced rate of fatty acid synthesis in the dark is certainly caused by the limited availability of energy within the plastids due to limited transport and, perhaps, competing energy-requiring reactions. This may also be deduced from the finding that disrupted spinach chloroplasts retained the ability to form fatty acids in the presence of ATP in a pattern similar to that of intact chloroplasts [17]. A limiting reservoir of reducing equivalents should also be considered because pyridine nucleotides are not permitted to penetrate the envelope, although it has been claimed that the chloroplast NADP system may be kept reduced to some extent in the dark by, e.g., the oxidative pentose phosphate pathway [11].

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