Variation in Chloroplast Lipid Content and its Correlation to Photosynthetic Activities

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During illumination infiltrated spinach leaf sections show after a short log a linearly increasing ^{14}C-incorporation into membrane lipids from $H^{14}CO_3^-$.

Isolated intact chloroplasts show this increase only for the neutral lipid fraction (NL). Glyco- and phospholipid accumulation declines in the light.

In chloroplast suspensions the increase in content and also in its ^{14}C-incorporation rate of the NL fraction is accompanied

1. by a proportional decrease of CO_2-fixation capacity,
2. by a decrease of the P/2 e-quotient.

From ^{14}C-incorporation kinetics, this increase of neutral lipids in plastid suspensions even after short illumination periods appears to be a consequence of an inhibited glycolipid — and phospholipid synthesis from diglycerides.

Introduction

The complex influence of products of enzymatic lipid hydrolysis [1–3] and transformations [4, 5] on photochemical activities in isolated chloroplasts has recently aroused considerable interest. From diurnal variations in the structure [6] and lipid composition [7] of chloroplast membranes a rapid "turnover" of lipids initially described by Mazliak [8] was suggested.

In isolated chloroplasts the lipid variations, expressed as MGDG/PL-ratio, show a linear correlation with the simultaneously measured P/2 e-quotient [9]. A similar relation between changes in the NL content and the photosynthetic capacity of chloroplast suspensions are the topic of this paper.

Methods

The techniques of isolation, illumination, lipid extraction, analysis and the estimation of photosynthetic activities of chloroplasts and particles has been described previously by Heise and Harnischfeger [9]. ^{14}C-incorporation into infiltrated leaf sections and their lipid extraction and analysis was according to Heise and Krapf [10]. CO_2-fixation of isolated intact chloroplasts was measured by O_2-production in a Clark electrode under saturating red light ($\lambda > 610$ nm) in a medium containing (μmol/ml): sorbitol 279; Hepes buffer (pH 7.6) 24.2; $MgCl_2$ 0.85; $MnCl_2$ 0.85; EDTA 1.69; KH_2PO_4 0.24; $NaHCO_3$ 4.83; 10 μl catalase and chloroplasts (40 μg Chl/ml).

After a preillumination period of 2 min, ^{14}C-incorporation was carried out using the same assay, containing in addition 11.7 μmol $H^{14}CO_3^-$/mg Chl (spec. activity 470 μCi). Chloroplast concentration was 0.1 mg Chl/ml.

After illumination for the periods indicated the chloroplast suspension was immediately extracted with $CHCl_3/CH_3OH$ according to Bligh and Dyer [11]. Separation and analysis of the lipid extract was as described by Heise and Jacobi [12].

Results and Discussion

In isolated intact chloroplasts the ^{14}C-incorporation into lipids declines as compared to that of infiltrated leaf sections (Fig. 1). After a short lag period, infiltrated leaf sections show a linearly increasing ^{14}C-uptake (Fig. 1 B). Isolated chloroplasts, in contrast, show an increase in ^{14}C content only for the neutral lipid fraction while the ^{14}C-incorporation rate into the remaining membrane lipids (galacto- and phospholipids) slows down

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Abbreviations: DGDG, digalactosyl diglyceride; MGDG, monogalactosyl diglyceride; (MGMG), possibly monogalactosyl monoglyceride as suggested from chromatographic behaviour as well as the stöchiometric composition of the lipid hydrolysate; NL, neutral lipids containing acyl glycerides and fatty acids; PL, total phospholipids.

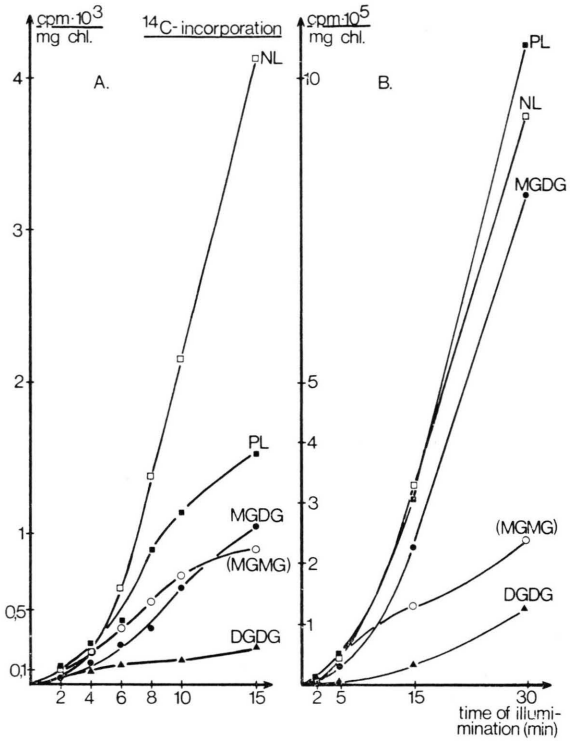

Fig. 1. ^{14}C-labelling kinetics in the lipids of isolated chloroplasts (A) and infiltrated leaf sections (B) of spinach in relation to the time of illumination. In order to overcome the lag in the rate of CO_2 assimilation, the leaf sections were freed of the lower epidermis, infiltrated with the incubation medium (absence of $H^{14}CO_3^-$) and preilluminated for 15 min. Isolated chloroplasts were preilluminated for 2 min. The data are mean values of four experiments.

more and more (Fig. 1 A) the longer the illumination period.

^{14}C-incorporation into the neutral lipid fraction of isolated chloroplasts seems to be correlated to their photosynthetic activity. Fig. 2 shows that chloroplasts with a relatively high CO_2-fixation rate (40 μmol/mg Chl·h) incorporate approximately 35% of the label into the neutral lipids (Fig. 2 A). A reduction of the fixation rate to 23 μmol/mg Chl·h is followed by an increase of ^{14}C-incorporation into neutral lipids to approximately 50% (Fig. 2 B). The ratio of the initial slopes of the time courses are inversely related to the ratio of the initial fixation rates. Thus, it appears that the high neutral lipid pool may be due to reduced CO_2 assimilation capacity. The different behaviour of leaf sections and whole chloroplasts is puzzling since in both systems the envelope supposed to be the main site of membrane lipid biosynthesis [13 – 15] in this organelle is still present.

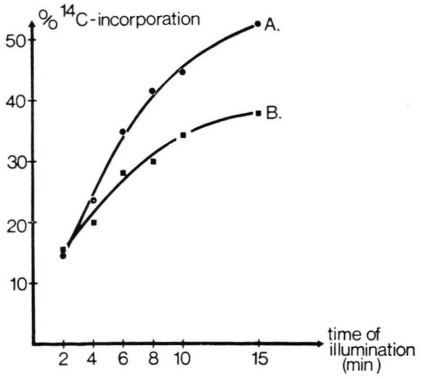

Fig. 2. Relative ^{14}C-incorporation in the neutral lipid fraction of isolated chloroplasts which differing O_2-production capacity (A: 23,4 μmol O_2/mg·Chl·h; B: 40 μmol O_2/mg Chl·h) in relation to the time of illumination. ^{14}C-incorporation into the total lipid fraction was taken as 100%.

The interpretation of these findings is difficult due to the fact, that a correlation with electron transport and photophosphorylation capacity is only possible after an osmotic shock of the plastids, while changes in lipid composition of intact chloroplasts can be directly compared with their CO_2-fixation capacity.

Therefore the relative proportion of NL in the total lipid pool of chloroplasts (Fig. 3 A) and chloroplast particles prepared by osmotic shock (Fig. 3 B) was varied by illuminating a dense suspension (1 mg Chl/ml) in the absence of an electron acceptor. Electron transport rates and photophosphorylation were measured immediately afterwards in

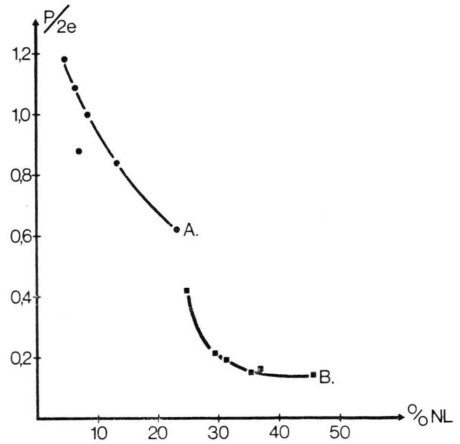

Fig. 3. Correlation between the relative NL proportion in the lipid content of chloroplasts (A) and chloroplast particles (B), varied by illumination of a dense (1 mg Chl/ml) suspension, and the P/2 e-ratio determined in a subsequent measurement. The data are based on four experiments.

Table I. Correlation between light induced lipid changes in intact (a) and broken (b) chloroplasts and their photosynthetic activity.

Time of illumination [min]	Relative proportion (%) of neutral lipids (NL)*		Photosynthetic activities					
			1		2		3	
	a	b	a	b	a	b	a	b
0	7	25	136	209	60	44	0.88	0.42
2	5	30	131	240	77	25	1.18	0.21
4	6	31	132	248	72	24	1.09	0.19
6	8	37	136	264	68	21	1.00	0.16
8	13	35	157	258	66	20	0.84	0.15
10	23	46	200	273	62	19	0.62	0.14

1, e^--Transport (ADP+Pi) [μmol FeCy red/mg Chl×h].
2, Photophosphorylation [μmol ATP/mg Chl×h].
3, P/2 e-quotient.

All values are averages of at least 4 independent experiments
* The total lipid content was taken as 100%.

a subsequent illumination period (3 min) under hypotonic conditions.

The result shows that with an increase of the NL pool the P/2 e-ratio decreases from a value of 1.2 to 0.6 in intact and from 0.4 to approximately 0.2 in broken chloroplasts (Table I). This indicates that in intact chloroplasts the decline in the P/2 e-ratio appears to be mainly due to an increasing electron transport rate only, whereas in broken chloroplasts uncoupling effects superimpose themselves as well. Thus, an inhibition of photosynthetic phosphorylation by products of lipid breakdown, found in other laboratories [1 – 3] can be discussed.

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