Sn-Glycerol-3-phosphate is a Product of Starch Degradation in Isolated Chloroplasts from *Chlamydomonas reinhardii*

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Sn-glycerol-3-phosphate accumulates during starch catabolism by intact isolated chloroplasts from *Chlamydomonas reinhardii* in the dark. Further products detected mainly were 3-phosphoglyceric acid and triose phosphates with a ratio between 3-phosphoglyceric acid, sn-glycerol-3-phosphate and triose phosphates of 1.0:0.9:0.2. The production of sn-glycerol-3-phosphate by a plastidic glycerol-3-phosphate dehydrogenase provides a reasonable mechanism for regenerating reducing equivalents during continuous starch breakdown in the chloroplast of the alga *C. reinhardii*.

Introduction

The mobilization of starch in higher plant chloroplasts requires its degradation into transportable 3-phosphoglyceric acid (3-PGA) and triose phosphates (TP, [1]). For a persistent starch degradation in the dark reducing equivalents, generated either at the glyceraldehyde dehydrogenase (EC 1.2.1.12) or at glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44), had to be continuously reoxidized within the chloroplast. For balancing the reducing equivalents the occurrence of an electron linked reduction of O_2 (chlororespiration, [2]) or the activity of a plastidic malate/oxaloacetate shuttle [3] are discussed in higher plant chloroplasts. In this study we describe the detection of sn-glycerol-3-phosphate (Gly-3-P) as a regular main product of starch degradation in chloroplasts of the alga *Chlamydomonas reinhardii* and its importance in balancing the plastidic redox equilibrium.

Materials and Methods

C. reinhardii (11/32–90 from the Sammlung für Algenkulturen, Göttingen, FRG) was grown synchronuously (L/D, 12/12, with dilution to $A_{546} = 0.35$ at the beginning of the light phase) as described elsewhere [4]. Chloroplasts were prepared after digitonin lysis of autolysine [5] induced protoplasts from cells harvested after 5 h in the light following the method given in [4]. Purified chloroplasts (5 mg Chl/ml, 94 ± 5% SD intact as determined by ferrycyanide assay) were resuspended in 50 mM HEPES/KOH (pH 6.8) containing 120 mM mannitol, 5 mM KH_2PO_4, 1 mM $MgCl_2$ and 0.5% (w/v) defatted BSA and incubated at 22 °C in Warburg vessels (5 ml) in the dark. Aliquots (1 ml) were withdrawn at times indicated and immediately extracted for 15 min at 0 °C by addition of $HClO_4$ (0.3 M). Thereafter extracts were centrifuged (5 min at 13000 × *g*) neutralized by addition of 3 M K_2CO_3 and the supernatant (1.2 ml) used for metabolite assay, whereas starch content was estimated in the plastidic pellet [6]. Gly-3-P [7], Glc-1-P, Glc-6-P, Fru-6-P, Fru-1,6P_2, TP, 3-PGA [8] and Chlorophyll [9] were determined spectrophotometrically. Enzymes and substrates were purchased from Boehringer (Mannheim, FRG). All other chemicals (analytical grade) were from Merck (Darmstadt, FRG).

Abbreviations: Chl, Chlorophyll; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid; 3-PGA, 3-phosphoglyceric acid; TP, triose phosphates (dihydroxyacetone phosphate + glyceraldehyde-3-phosphate); Gly-3-P, sn-glycerol-3-phosphate.

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Results

Main products of starch degradation

Intact isolated chloroplasts from *C. reinhardii* degrade their assimilated starch in the dark with a rate of 0.36 μmol/mg Chl × h mainly to 3-PGA, Glyc-3-P and TP (Table I) in a ratio of 1.0:0.9:0.2, respectively. This corresponds to approximately 50% of the rate determined in intact cells of similar starch con-

Table I. Products of starch degradation in isolated chloroplasts from *C. reinhardii*. Chloroplasts (5 mg Chl/ml, 93% intact) were prepared and incubated as described under Materials and Methods. At 0 and 20 min aliquots (1 ml) were withdrawn and extracted with $HClO_4$ (0.27 M). After centrifugation (5 min, 13000 × *g*, Eppendorf 4114, Hamburg, FRG) the neutralized supernatant was used for metabolite assays.

Metabolite	0 min	20 min	C_1
	nmol/mg Chl		
Starch[a]	4300.0	4280.0	−720.0
Glc-1-P	1.4	1.4	0.0
Glc-6-P	6.6	6.4	−1.2
Fru-6-P	1.8	2.1	+4.8
Fru-1,6-P_2	1.3	5.8	−27.0
Tp	8.0	34.1	+78.3
Gly-3-P	13.0	118.0	+313.0
3-PGA	10.0	131.0	+363.0
CO_2	0.0	0.0	0.0
C $\frac{\text{degraded}}{\text{analyzed}}$			1.09

[a] Starch in glucose units.

tent [10]. From the starch carbon mobilized 96% is balanced in 3-PGA, Gly-3-P and TP analyzed, whereas only minor traces of hexose phosphates (*e.g.* Fru-1,6-P_2) are detected. No evolution of CO_2 has been observed within the given limits of detection (0.8 μmol/mg Chl × h).

Time course of Gly-3-P formation

The time course of starch degradation and of product formation by intact isolated chloroplasts from *C. reinhardii* is shown in Fig. 1a. 3-phosphoglyceric acid and Gly-3-P clearly accumulate in a ratio of 1:1, whereas DHAP transiently increases between 0 and 20 min of incubation but then declines to its original concentration. The increase of 3-PGA and Gly-3-P concentrations followed exactly the kinetics of starch degradation. At the end of incubation (30 min) nearly 80% of the chloroplasts were found intact (Phase contrast microscopy) and only few broken organelles were detectable. Significant starch degradation or product accumulation was not observed in control experiments with osmotically lysed chloroplasts (Fig. 1b).

Distribution of Gly-3-P between isolated chloroplasts and medium

The distribution of Gly-3-P between isolated chloroplasts and surrounding medium was proofed by silicon oil centrifugation of the incubated organelles (Table II). Most of 3-PGA, Gly-3-P and DHAP (99%, 96% and 94%, respectively) analyzed are exported from the chloroplast, probably by an active 3-PGA/phosphate translocator [11]. The ratio of 3-PGA, Gly-3-P and DHAP, as detected in the

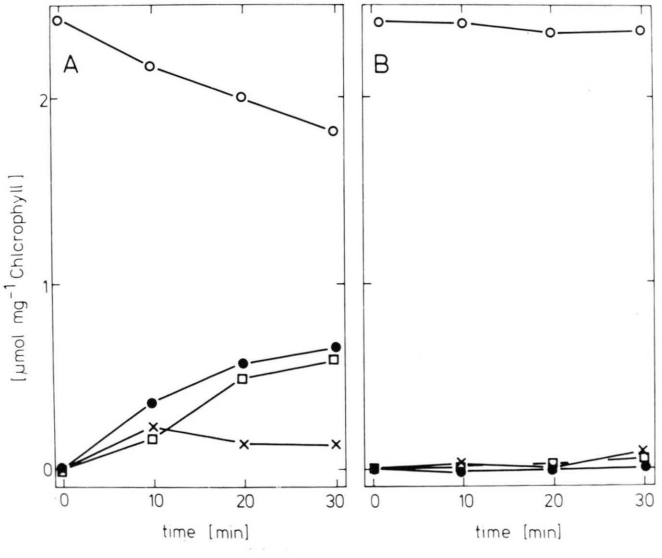

Fig. 1. Time course of starch degradation in isolated chloroplasts from *C. reinhardii*. Chloroplasts (5 ml, 10 mg Chl, 93% intact) were centrifuged (2 min, 2500 × *g*) and resuspended in 5 ml incubation medium (50 mM HEPES/KOH (pH 6.8) containing 120 mM mannitol, 5 mM KH_2PO_4, 1 mM $MgCl_2$, 0,5% BSA). Incubation was performed in Warburg vessels (5 ml) at 22 °C in the dark (Fig. 1a). For osmotic lysis in control experiments (Fig. 1b) chloroplasts were incubated in 4 ml of 5 mM HEPES/KOH (pH 6.8). After 5 min at 4 °C all organelles were broken. Then 1.0 ml of 5-fold concentrated incubation buffer were added and chloroplasts incubated as in Fig. 1a. Aliquots were withdrawn and starch (○), 3-PGA (●), Gly-3-P (□), and DHAP (×) determined as described under Materials and Methods.

Table II. Distribution of plastidic starch degradation between the stroma and the suspending medium. Chloroplasts prepared as described under Materials and Methods were incubated at 22 °C in the dark. At 0 min and after 30 min aliquots (0.5 ml, 1 mg Chl, 96% intact) were centrifuged (1 min, 13000 × *g* Eppendorf 4114, Hamburg, FRG) through a mixture of 1-bromodecane/silicone oil (AR 150 Wacker Chemie, München, FRG; 1.3:1, v/v) into 0.1 ml 0.5 M $HClO_4$. Immediately after centrifugation, the supernatant was treated with 0.1 ml of 0.5 M $HClO_4$. After phase separation the extracts were centrifuged again (5 min, 13000 × *g*) and the neutralized supernatants used for spectrophotometrical metabolite assays.

Metabolite		Stroma	Medium
		nmol/mg Chl (%)	
Gly-3-P	0 min	4.6 (39)	7.3 (61)
	30 min	5.3 (4)	126.6 (96)
DHAP	0 min	3.1 (50)	3.1 (50)
	30 min	4.2 (6)	63.5 (94)
3-PGA	0 min	2.3 (52)	2.1 (48)
	30 min	1.3 (1)	131.0 (99)
Glc-6-P	0 min	2.4 (96)	0.1 (4)
	30 min	3.5 (95)	0.2 (5)

surrounding medium, was 1.0:1.0:0.5, respectively. Other stroma metabolites (*e.g.* Glc-6-P) were not exported outside the chloroplasts.

Discussion

In contrast to higher plant chloroplasts [1] 3-PGA and Gly-3-P are detected as main products of starch degradation in chloroplasts of the green alga *C. reinhardii* (Table I, Fig. 1). Both were exported outside the chloroplast (Table II) thus representing the main transportable metabolites via the envelope of this alga. Synthesis of Gly-3-P was observed by the activity of a plastidic Gly-3-P dehydrogenase (EC 1.1.1.8) in Dunnaliella [12]. The demonstration and further chracterization of this enzyme even from chloroplasts of *C. reinhardii* [13] let suggest a tight coupling of Gly-3-P dehydrogenase to plastidic starch catabolism. Thus reducing equivalents, which were produced at least at the plastidic glyceraldehydephosphate dehydrogenase could be partially reoxidized by reducing DHAP to Gly-3-P. The failure of any CO_2 evolution (Table I) makes likely starch catabolism via plastidic glycolysis. In this case one molecule DHAP has to be reduced to Glyc-3-P for each molecule 3-PGA generated for maintaining the plastidic redoxbalance.

Thus continuous starch degradation in *C. reinhardii* can be performed without a concomitant activity of chlororespiration [2]. A contribution of a plastidic malate/oxaloacetate shuttle as known from higher plant chloroplasts [3] is not obligate during starch catabolism in *C. reinhardii* due to the export of Gly-3-P. However exclusion of a malate/oxaloacetate shuttle and the importance of a plastidic Gly-3-P exportation has to be elucidated in further experiments with intact cells.

Acknowledgements

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