ISOLATION OF *sn*-GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE FROM ACHLOROPLASTIC *EUGLENA*

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1. Introduction

The acylation of *sn*-glycerol-3-phosphate is a key reaction in the metabolic pathways leading to the biosynthesis of glycerol-based phospholipids, glycolipids, and sulfolipids. In *Euglena gracilis* variety *bacillaris*, *sn*-glycerol-3-phosphate acyltransferase activity is associated with chloroplast, microsomal, and mitochondrial membrane [1]. The chloroplast enzyme has been solubilized, purified, and characterized [2]. Parallel studies of the enzymes solubilized from all three membrane sources will contribute to knowledge of the mechanisms which control membrane lipid composition and the sites of lipid biogenesis within a cell. To this end, membrane fractions devoid of cross contamination have been obtained from W10BSML, an achloroplastic mutant of the wild type *Euglena* [3,4].

2. Materials and methods

Cells were grown in darkness in 1 l cultures [5]. Harvested cells were stored at −50°C. Extracts of cell particulate matter were prepared by sonifying the cells suspended (5 ml/g cells) in 0.01 M TES buffer, (pH 7.0), 0.4 M in sucrose for organelle marker enzymes or 0.01 M potassium phosphate (pH 7.0), 0.4 M in sucrose, and 0.001 M in dithiothreitol for acyltransferase studies. The broken cell suspension was centrifuged twice at 480 × *g* for 10 min to remove unbroken cells and nuclear debris. The supernatant particulate mixture was separated into fractions enriched in various organelles by ultracentrifugation in a discontinuous sucrose density gradient described [1]. Fractions (1 ml) were collected from the bottom of the tubes and assayed for protein [6], organelle-specific enzyme activities, and *sn*-glycerol-3-phosphate acyltransferase activity.

For solubilization of *sn*-glycerol-3-phosphate acyltransferase, the pooled fractions of enriched membranes were combined with an equal volume of 0.01 M potassium phosphate buffer (pH 7.0), 0.001 M in dithiothreitol, and 2×10^{-4}% in Triton X-100. Following a 1 s burst with ultrasound, the mixture was centrifuged at 100 000 × *g* for 2 h. The supernatant solutions were collected, and the pellets were resuspended in 0.01 M potassium phosphate buffer (pH 7.0), 0.001 M in dithiothreitol. All procedures were done at 4°C unless otherwise specified.

Assays for *sn*-glycerol-3-phosphate acyltransferase were done at 35°C and contained in 0.2 ml: potassium phosphate, 10 mM (pH 7.75); dithiothreitol, 1 mM; bovine serum albumin, 60 μg; palmitoyl CoA, 25 mM; *sn*-[^{14}C]glycerol-3-phosphate, 1 mM (1 μCi/μM); and protein, 25–50 μg. After 12 min, the assay tubes were placed on ice and 50 μl 2 N HCl and 750 μl chloroform–methanol (1:2) were added. Following thorough mixing, 250 μl 2 M KCl in 0.2 M H_3PO_4 and 250 μl chloroform were added and mixed vigorously. Centrifugation in a clinical centrifuge separated the organic and aqueous phases. The aqueous layer was removed. A 100 μl aliquot of the chloroform layer was measured with a Hamilton syringe into a 5 ml Econovial (New England Nuclear).

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The chloroform was evaporated under a stream of N_2, and the residue was dissolved in 5 ml toluene containing 15.1 g/gal. of 2,5-diphenyloxazole. Radioactivity was measured in a Packard Model 3320 liquid scintillation counter at 93% efficiency.

The following enzyme activities associated with specific membranes were assayed: succinate dehydrogenase, mitochondria [7]; β-hydroxypyruvate reductase, peroxisomes [8,9]; 5′-ribonucleotide phosphohydrolase, plasma membrane [10]; glucose-6-phosphatase, microsomes [11,12]; and acid phosphatase, lysosomes [12]. Inorganic phosphate produced by the action of 5′-phosphohydrolase, glucose-6-phosphatase, and acid phosphatase was measured by the method in [13].

The products of the *sn*-glycerol-3-phosphate acyltransferase reaction were identified by thin-layer chromatography on Bakerflex silica gel plates using two different development systems, lipid standards and techniques described in [1].

3. Results and discussion

The separation of the enriched zones of the different organelles throughout the gradient (fig.1) shows that acyltransferase activity in the achloroplastic mutant is associated with mitochondrial and microsomal fractions. The plasma membrane fraction and peroxisomes are largely excluded from the microsomal and mitochondrial membranes. The lysosomes occur throughout both microsomal- and mitochondrial-enriched fractions. The highest acid phosphatase specific activity occurs, however, between the two acyltransferase-bearing membranes.

In order to obtain mitochondrial membrane practically free of microsomes, a narrow pooling of the mitochondria containing fractions was made from 2–9 ml of the gradient. This pool contained 57% of the succinate dehydrogenase activity, 2% of the glucose-6-phosphatase activity, 25% of the *sn*-glycerol-3-phosphate acyltransferase activity, and 31% of the protein.

Microsomes were pooled from 20–30 ml of the gradient. This pool contained 34% of the glucose-6-phosphatase activity, 12% of the succinate dehydrogenase, 57% of the acyltransferase activity, and 26% of the protein. Flotation of the pooled microsomes over

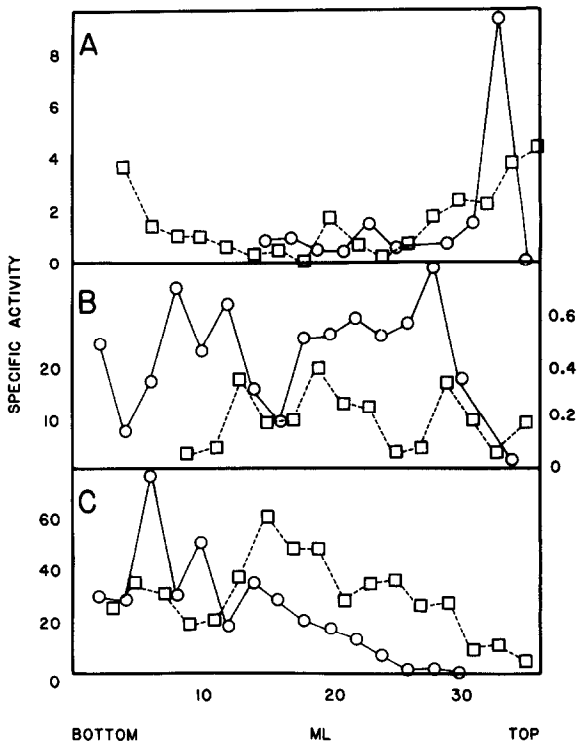

Fig.1. *sn*-glycerol-3-phosphate acyltransferase activity in organelles of achloroplastic *Euglena*. The specific activity for each enzyme is nmol product $.min^{-1}$ $.mg\ protein^{-1}$. Enzyme assay procedures are referenced or given in the text. Details of the preparation and processing of the discontinuous sucrose density gradient are in the text. Particulate extracts from ~1 g cells were applied to each gradient. (A) Squares, β-hydroxypyruvate reductase; circles, 5′-ribonucleotide phosphohydrolase. (B) Squares, glucose-6-phosphatase; circles, *sn*-glycerol-3-phosphate acyltransferase (right side coordinate). (C) Squares, acid phosphatase; circles, succinate dehydrogenase.

75% sucrose reduced contamination by mitochondrial membranes to 1.8% of the total succinate dehydrogenase activity and spec. act. 0.86 nmol/min/mg protein compared to 77 nmol/min/mg protein for the gradient peak fraction of this activity. On this basis, the final microsomal enriched sampling was estimated to be ~1% contaminated with mitochondrial membrane. This was a minimal contribution to the acyltransferase activity, and the microsomes were judged sufficiently pure for study of the enzyme.

Exposure of the isolated mitochondrial and microsomal membranes to dilute Triton X-100 resulted in the removal of large amounts of acyltransferase activity

Table 1
Solubilization of *sn*-glycerol-3-phosphate acyltransferase from organelles

Enzyme source	mg protein		Acyltransferase activity[a]		Specific activity[b]	
	Mitochondria	Microsomes	Mitochondria	Microsomes	Mitochondria	Microsomes
Untreated membranes	25.8	46.8	4.5	33.8	0.17	0.72
Triton X-100 supernatant solution	3.4	21.6	8.1	40.1	2.38	1.86
Triton X-100-treated membrane pellet	22.0	19.4	0.7	8.3	0.03	0.43
Total recovery from Triton X-100 treatment	25.4	41.0	8.8	48.4	–	–

[a] A unit of activity is 1 nmol acyl-*sn*-glycerol-3-phosphate/min
[b] Specific activity is units enzyme activity/mg protein

from the respective membranes (table 1). Increases in the amounts of activity over that present in the untreated membranes were always observed. This may be due to assay geometry in that solubilized acyltransferase is more accessible to substrate than the membrane-bound enzyme. With mitochondria, 92% of the enzyme activity is found in the 100 000 × *g* 2 h supernatant solution after exposure to Triton X-100, for microsomes, 83%. A 14- and 3-fold increase in specific activity upon solubilization was seen for the acyltransferases from mitochondria and microsomes, respectively. No acyltransferase activity was detected in the 100 000 × *g* 2 h supernatant solutions from either membrane source when Triton X-100 was omitted.

Both mono and diacyl products were formed consistently by all sources of acyltransferase. Data from a typical experiment are summarized in table 2. Although for both microsomal and mitochondrial sources of enzyme, the monoacyl compounds comprised the bulk of the product, the diacyl product was always formed in small amounts. It is not known at present whether separate acyltransferases are responsible for the formation of mono and diacyl compounds in this system.

The use of an achloroplastic mutant has permitted separation of microsomal and mitochondrial acyltransferase activities and eliminated the problem of contamination from the same enzyme present in chloroplasts of wild-type cells. It is expected that further study will enable comparison of these isofunctional enzymes from the same cell.

Table 2
Products formed by the acyltransferases before and after membrane exposure to Triton X-100 (% of total product)[a]

	Untreated membrane		Triton X-100 supernatant solution		Triton X-100-treated pellet	
	Mitochondria	Microsomes	Mitochondria	Microsomes	Mitochondria	Microsomes
Lysophosphatidate	56	91	80	85	82	70
Phosphatidate	20	2	6	3	10	6
Monopalmitin	18	5	11	8	6	19
Dipalmitin	7	2	3	4	2	6

[a] Separation was by thin-layer chromatography on silica gel developed with diisobutylketone–acetic acid–water (40:25:5)

The total amount of product (nmol) produced in each assay analyzed was from left to right: 0.74, 0.57, 0.41, 0.56, and 0.57

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References

[1] Boehler, B. A. and Ernst-Fonberg, M. L. (1976) Arch. Biochem. Biophys. 175, 229–235.
[2] Tenbarge, K., Boehler, B. A. and Ernst-Fonberg, M. L. (1978) Fed. Proc. FASEB 37, 1494.
[3] Schiff, J. A. and Zeldin, M. H. (1968) J. Cell Physiol. suppl. 1. 72, 103–128.
[4] Ernst-Fonberg, M. L., Dubinskas, F. and Jonak, Z. L. (1974) Arch. Biochem. Biophys. 165, 646–644.
[5] Ernst-Fonberg, M. L. (1973) Biochemistry 12, 2449–2455.
[6] Ross, E. and Schatz, G. (1973) Anal. Biochem. 54, 304–562.
[7] Ells, H. A. (1959) Arch. Biochem. Biophys. 85, 561–562.
[8] Collins, N. and Merrett, M. J. (1975) Biochem. J. 148, 321–328.
[9] Tolbert, N., Yamazaki, R. and Deser, A. (1970) J. Biol. Chem. 245, 5129–5136.
[10] Wattiaux, R. and Wattiaux-DeConick, S. (1969) Biochim. Biophys. Acta 183, 118–128.
[11] Nordlie, R. C. and Arion, W. J. (1966) in: Methods in Enzymology (Kaplan, N. and Coldwick, S. P. eds) vol. 9, pp. 615–625, Academic Press, New York.
[12] Leighton, F., Poole, B., Beaufay, H., Baudhin, P., Coffey, J., Fowler, S. and DeDuve, C. (1968) J. Cell. Biol. 37, 482–513.
[13] Ames, B. N. (1966) in: Methods in Enzymology (Neufeld, E. and Ginsburg, V. eds) vol. 8, pp. 115–118, Academic Press, New York.