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Triacylglycerols as Fatty Acid Donors for Membrane Phospholipid Biosynthesis in Yeast

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The turnover of lipids was studied in the yeast, *Saccharomyces carlsbergensis* ATCC 9080, after prelabeling of the cells with [^{3}H] oleic acid and [^{14}C] palmitic acid. In inositol supplemented cells, a redistribution of fatty acids from triacylglycerols to phospholipids (mainly phosphatidylcholine and phosphatidylinositol) could be demonstrated. An increased transfer of fatty acids from triacylglycerols to phospholipids was observed when prelabeled cells were transferred to a growth medium containing cerulenin, which inhibits fatty acid synthesis and thus induces fatty acid deficiency in the growing cells. Inositol deficient cells contain increased levels of triacylglycerols, which are equally well utilized for phospholipid (mainly phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine) synthesis under conditions of fatty acid deficiency. The present results together with the previous finding that β-oxidation is practically absent in *Saccharomyces carlsbergensis* suggest that in this yeast triacylglycerols function as storage of fatty acids which can be mobilized for phospholipid biosynthesis.

(Keywords: Cerulenin; Fatty acid donor; Inositol deficiency; Phospholipid biosynthesis; Triacylglycerols; Yeast)

Triacylglycerine als Fettsäurespeicher für die Biosynthese von Membran-Phospholipiden in Hefe

Der Umsatz der Lipide von *Saccharomyces carlsbergensis* ATCC 9080 wurde nach Vormarkierung mit ^{3}H-Ölsäure und ^{14}C-Palmitinsäure untersucht. Inositversorgte Zellen zeigen eine Verschiebung der Fettsäuren von den Triacylglycerinen in die Phospholipide, im besonderen in Phosphatidylcholin und Phosphatidylinosit. Eine verstärkte Übertragung der Fettsäuren von Triacylglycerinen auf Phospholipide konnte festgestellt werden, wenn vormarkierte Zellen auf ein Nährmedium, welches Cerulenin enthielt, übertragen wurde. Cerulenin inhibiert die Fettsäuresynthese und ruft in wachsenden Zellen Fettsäuremangel hervor. Inositdefiziente Hefezellen, welche einen erhöhten Triacylglycerinspiegel aufweisen, verwenden diese Triacylglycerine unter Fettsäuremangelbedingungen ebenfalls für die Synthese von Phospholipiden, besonders von

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Phosphatidylcholin, Phosphatidyläthanolamin und Phosphatidylserin. Da aus früheren Arbeiten bekannt ist, daß in *Saccharomyces carlsbergensis* praktisch keine β-Oxidation existiert, können die Triacylglyerine in diesem Hefestamm als Speicher für Fettsäuren angesehen werden, welche zur Synthese von Phospholipiden dienen.

Introduction

In mammalian systems triacylglycerols are usually regarded as an energy source. *Paltauf* and *Johnston*[1,2] have shown, that in the yeast, *Saccharomyces carlsbergensis*, β-oxidation of fatty acids is practically not existent. The question then arose as to the role of triacylglycerols in this yeast. *Clausen* et al.[3] isolated lipid particles from baker's yeast and showed that they consist mainly of equal amounts of triacylglycerols and sterol esters. The authors concluded from the chemical composition of the lipid particles that they serve as a store not only for energy production but also for membrane synthesis. More direct proof for the function of microbial triacylglycerols as a storage form of precursors for the de novo synthesis of phospholipids was obtained by *Borowitz* and *Blum*[4] who studied the turnover of prelabeled endogenous triacylglycerols in the protozoan, *Tetrahymena pyriformis*.

It is the aim of the present study to investigate whether in yeast fatty acids from triacylglycerols can be utilized for the synthesis of phospholipids. Since enhanced mobilization of fatty acid depots can be expected under conditions of insufficient de novo fatty acid synthesis, the turnover of endogenous triacylglycerols was studied in the presence of cerulenin, an antibiotic that inhibits fatty acid synthesis in yeast[5–10]. The study was extended to inositol deficient cells of *Saccharomyces carlsbergensis* which accumulate abnormal quantities of triacylglycerol[1,2,11–14]. The question was whether this increased depot of triacylglycerols could be mobilized or whether it was metabolically inert.

Materials and Methods

Growth Conditions

Saccharomyces carlsbergensis ATCC 9080 was grown on a synthetic medium containing 2% of glucose[11]. Inositol supplemented media contained 100 mg myo-inositol in 1 liter; no inositol was present in inositol deficient media. For aerobic growth cells were shaken on a New Brunswick Scientific incubator with 250 rpm at 30 °C.

Turnover Studies with Radioactively Labeled Fatty Acids

Cells were grown aerobically in the presence of [^{3}H]oleic acid (10^7 cpm; specific radioactivity 2.2 mCi per mmole) and [^{14}C] palmitic acid (10^7 cpm, specific radioactivity 55.2 mCi per mmole) for 18 hours. After this period the individual lipids had identical specific radioactivities. Cells were harvested by

centrifugation and washed once with ethanol-water (1:10, *v/v*) and twice with water to remove extracellular labeled fatty acids. Aliquots of the cells were transferred to media containing 10 mg/l cerulenin (supplied by Senn Chemicals, Dielsdorf, Switzerland). Control media contained in addition to cerulenin 500 mg oleic acid plus 500 mg palmitic acid per liter. The amount of fatty acids added to the control media was sufficient for an optimum reversion of the growth inhibiting effect of cerulenin.

From the culture media samples were taken at the times indicated and cells were separated by centrifugation after the addition of non-radioactive cells (about 100 mg dry weight per sample); cells were washed with ethanol-water (1:10, *v/v*) and with water. Turnover studies with unlabeled lipids were carried out in the same way, but without the addition of labeled fatty acids to the inoculum.

Lipid Extractions

Cells were disintegrated for 4 min in a Braun-Melsungen homogenizer in the presence of glass beads (diameter 0.25–0.30 mm) under CO_2-cooling. Lipids were extracted with chloroform/methanol (2:1) by the method of *Folch* et al.[15]. Aliquots were used for lipid analyses.

Lipid Analyses

Total acylglycerols were quantitated enzymatically using the test combination "Neutral Lipids" from Boehringer, Mannheim.

Total lipid phosphorus was determined by the method of *Bartlett*[16]. Thin-layer chromatography of neutral lipids and phospholipids was carried out as described by *Daum* et al.[11].

Radioactivity was measured by liquid scintillation counting on a Beckman LS 200. For measuring radioactivity in lipid extracts a scintillation mixture containing 8 g Butyl-*PBD* (Beckman) in 1 liter toluene was used. Individual lipids from thin layer plates were counted in a scintillation mixture consisting of 6 g Butyl-*PBD* (Beckman), 50 ml water, 100 ml BBS-3 solubilizer (Beckman) and 850 ml toluene.

Cell dry weights were measured after filtering aliquots of cultures through membrane filters, pore size 0.45 μm (Sartorius) and drying at 95 °C for 24 hours.

Cell growth was followed by reading O.D. at 546 nm. The proportionality between O.D. and dry weight of cells had previously[11] been determined.

Results

Figures 1*a* and 1*b* show the data obtained after the transfer of inositol supplemented (1*a*) and deficient (1*b*) cells of *Sacch. carlsbergensis*, prelabeled with [^{3}H] oleic acid and [^{14}C] palmitic acid, to growth media containing cerulenin or cerulenin plus unlabeled fatty acids, respectively. After growth for 4 h in the media containing only cerulenin, a marked decrease of ^{3}H and ^{14}C radioactivity of triacylglycerols in both inositol supplemented and inositol deficient cells was observed, which is accompanied by a proportionate increase of the ^{3}H and ^{14}C radioactivities in total phospholipids. In the control experiments where cells were grown in the presence of cerulenin plus

exogenous fatty acids, changes in the radioactivities of triacylglycerol and phospholipids were also observed. The decrease of triacylglycerol radioactivity in inositol supplemented cells grown in the presence of cerulenin plus fatty acids was not as pronounced as compared to cells

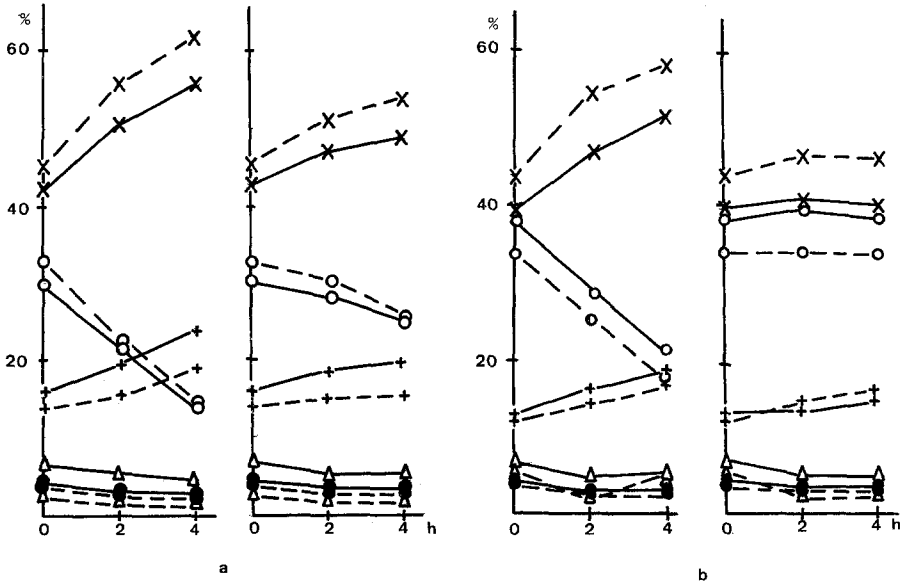

Fig. 1. Lipid turnover after transfer of prelabeled yeast cells to cerulenin-containing media; cells of *Saccharomyces carlsbergensis* were grown in the presence of [^{3}H] oleic acid and [^{14}C] palmitic acid. After 18 h cells were collected and transferred to media containing 10 mg cerulenin/l or 10 mg cerulenin plus exogenous fatty acids. Redistribution of lipid radioactivity in cells transferred to cerulenin containing media is shown on the left side and values from cells transferred to media containing cerulenin plus fatty acids on the right side of the Figures; *a* results from inositol supplemented; *b* results from inositol deficient cells; values are expressed as percent of total lipid radioactivity. × phospholipids, ○ triacylglycerols, ● diacylglycerols, Δ fatty acids, + sterolesters, ——— ^{3}H-oleic acid, — — — ^{14}C-palmitic acid

not supplemented with exogenous fatty acids. In inositol deficient cells, on the other hand, the control culture with added exogenous fatty acids showed a slight increase of triacylglycerol radioactivity, mainly at the expense of free acids (Fig. 1*b*).

The main acceptors for oleic and palmitic acid derived from triacylglycerols are phosphatidylcholine and phosphatidylinositol in inositol supplemented cells (Tab. 1*a*) and phosphatidylcholine, phos-

Table 1. *Changes in the distribution of* [^{3}H] *oleic acid and* [^{14}C] *palmitic acid in phospholipids after transfer of prelabeled cells to media containing cerulenin**

a: Inositol supplemented cells	0	2	4 hours	0	2	4 hours
		^{3}H-oleic acid			^{14}C-palmitic acid	
Cardiolipin	8.5	8.2 (8.9)	9.6 (9.8)	4.5	4.6 (4.8)	5.4 (5.4)
Phosphatidylethanolamine	8.6	9.6 (9.5)	9.6 (9.6)	11.3	13.0 (13.0)	12.8 (14.7)
Phosphatidylcholine	12.0	14.2 (13.1)	17.5 (15.2)	19.0	23.2 (21.2)	26.2 (27.8)
Phosphatidylinositol	10.8	15.3 (12.4)	14.2 (11.0)	6.9	11.3 (8.4)	10.9 (7.5)
Phosphatidylserine	1.1	1.3 (1.1)	1.2 (1.0)	0.8	1.2 (1.0)	1.2 (1.1)
Phosphatidic acid	1.4	1.9 (1.3)	2.8 (1.6)	1.9	2.4 (1.8)	3.5 (2.2)
Lysophosphoglycerides	0.2	0.1 (0.2)	0.4 (0.3)	1.0	1.1 (0.8)	1.1 (1.1)
b: Inositol deficient cells	0	2	4 hours	0	2	4 hours
		^{3}H-oleic acid			^{14}C-palmitic acid	
Cardiolipin	8.3	8.2 (8.7)	8.8 (9.5)	4.0	4.2 (4.2)	4.6 (4.7)
Phosphatidylethanolamine	8.2	10.6 (8.8)	11.4 (7.9)	9.9	13.2 (11.4)	14.7 (11.3)
Phosphatidylcholine	14.4	18.2 (15.4)	19.2 (15.4)	22.2	27.6 (23.6)	27.8 (23.9)
Phosphatidylinositol	2.3	3.1 (2.3)	3.2 (1.7)	1.8	2.6 (1.8)	2.5 (1.3)
Phosphatidylserine	2.5	3.8 (2.7)	4.2 (2.0)	2.1	3.4 (2.7)	3.8 (2.4)
Phosphatidic acid	1.6	2.0 (1.1)	2.6 (1.1)	1.9	2.1 (1.4)	2.7 (1.5)
Lysophosphoglycerides	1.5	1.5 (1.3)	2.0 (1.3)	2.0	1.5 (2.3)	2.3 (1.5)

* Cells of *Saccharomyces carlsbergensis* (inositol supplemented and inositol deficient) were grown in the presence of [^{14}C] palmitic acid and [^{3}H] oleic acid for 18 h and then transferred to media containing cerulenin (10 mg/l). ^{3}H and ^{14}C radioactivities present in individual lipids were determined at the times indicated. Values are expressed as percent of total lipid radioactivity. In brackets are the data obtained from cells transferred to media containing cerulenin plus exogenous fatty acids. The values are the mean of 3 independent experiments; deviation was less than 4%.

phatidylethanolamine and phosphatidylserine in inositol deficient cells (Tab. 1*b*). The preferred incorporation of oleic acid into cardiolipin and phosphatidylinositol and of palmitic acid into phosphatidylethanol-

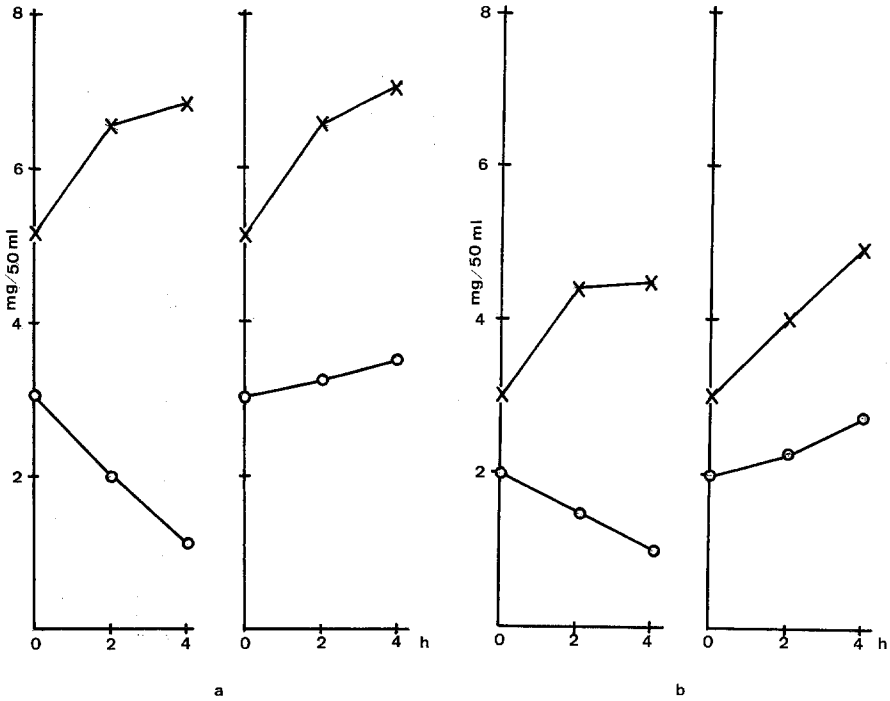

Fig. 2. Changes in the lipid composition of yeast cells after transfer to media containing cerulenin; cells were treated as described in the legend to Fig. 1 except for the radioactive labeling of fatty acids. At the times indicated lipids were extracted and analysed. Values are expressed as mg of the respective lipid class per aliquot (50 ml) of culture medium. On the left side are the curves obtained from cultures containing only cerulenin and on the right side those obtained from cultures containing cerulenin plus fatty acids; *a* results from inositol supplement; *b* results from inositol deficient cells; × phospholipids, ○ acylglycerols

amine and phosphatidylcholine during the period of prelabeling reflects the fatty acid composition of the respective phospholipids.

Quantitative analysis of the changes in lipid composition after transfer of inositol supplemented and inositol deficient cells into media containing cerulenin or cerulenin plus fatty acids, respectively, are

shown in Fig. 2*a* and 2*b*. The decrease in the triacylglycerol content in cells grown in the presence of cerulenin correlates very well with the decrease observed in triacylglycerol radioactivity under these conditions (Figs. 1*a* and 1*b*). In cells supplemented with exogenous fatty acids, the amount of triacylglycerols per aliquot of culture medium

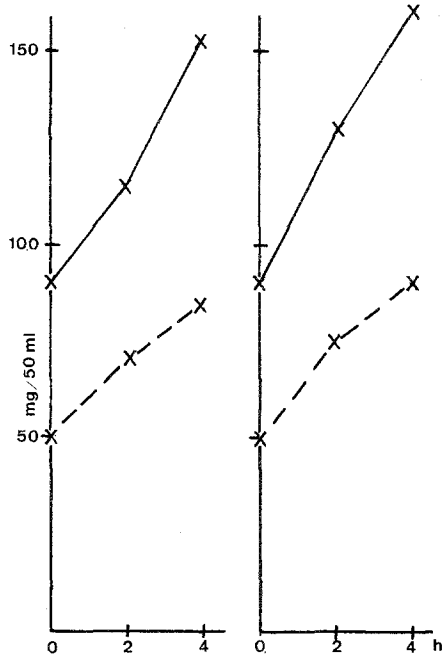

Fig. 3. Growth of *Saccharomyces carlsbergensis* in media containing cerulenin; growth of the cells (inositol supplemented and inositol deficient) in culture media containing 10 mg/l cerulenin (left side) or 10 mg cerulenin plus 1 g per liter fatty acids (equal amounts of oleic and palmitic acid) (right side) was followed by measuring the dry weight of cells in 50 ml culture medium; ×——× inositol supplemented, ×— — —× inositol deficient

increases. The increase of phospholipids in cultures grown on cerulenin alone and on cerulenin plus exogenous fatty acids, respectively, reached almost identical values.

Discussion

Fatty acid deficiency was induced in growing cells of *Saccharomyces carlsbergensis* by transfer of normally grown cells to a medium containing cerulenin, which totally blocks fatty acid synthesis and leads with

time to a cessation of cell growth unless the cultures are supplied with exogenous fatty acids. During the time of the experiments described here (4 h) cell growth continues in the absence of exogenous fatty acids at rates which are only slightly reduced as compared to cultures supplemented with exogenous fatty acids (Fig. 3). Data obtained with inositol supplemented cells prelabeled with ^{14}C palmitic acid and ^{3}H oleic acid (Fig. 1*a*) show that during the time of growth under fatty acid deficiency the endogenous pools of triacylglycerols are utilized for the synthesis of phospholipids. In control experiments with exogenously added fatty acids a transfer of labeled fatty acids from triacylglycerols to phospholipids is also observed although it is not as pronounced as under fatty acid deficiency.

In addition to the experiments with prelabeled lipids changes in the total quantities of triacylglycerols and phospholipids under the described conditions of fatty acid deficiency were analysed in order to preclude the possibility that prelabeling of triacylglycerols with ^{14}C palmitic acid and ^{3}H oleic acid had not been uniform and that different pools of triacylglycerols existed with different specific radioactivities. A comparison of data in Fig. 1*a* and 1*b* with data in Figs. 2*a* and 2*b* clearly shows that the observed changes in the radioactivities of triacylglycerols and phospholipids correlate very well with quantitative changes in these lipid classes induced by fatty acid deficiency. It is noteworthy that fatty acids from ergosterol esters are not utilized for phospholipid synthesis (Figs. 1*a* and 1*b*). Rather the reverse is observed, i.e. fatty acids from triacylglycerols are utilized for the formation of ergosterol esters under fatty acid deficiency.

The results described in this communication together with the previous finding[1,2] that fatty acids are not utilized as an energy source in *Saccharomyces carlsbergensis* provide good evidence that triacylglycerols in this yeast are a depot for fatty acids that can be utilized for membrane phospholipid synthesis. This effect is especially pronounced under conditions of limited fatty acid synthesis. It could also be shown that increased depots of triacylglycerols accumulated under inositol deficiency are utilized as normal triacylglycerol stores in fatty acid deficient cells. This means that the triacylglycerols in inositol deficient cells are not metabolically inert. The slight increase in triacylglycerol radioactivity in inositol deficient cells grown in the presence of cerulenin and exogenous fatty acids as compared to the decrease observed with inositol supplemented cells under otherwise identical conditions sustain our assumption that triacylglycerol accumulation in inositol deficient cells is not caused by an excessive fatty acid synthesis, but by an as yet not understood regulatory effect of inositol deficiency on glycerolipid synthesis and degradation.

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