Fatty-acyl Composition of the Lipids of *Saccharomyces cerevisiae* Grown Aerobically or Anaerobically in Media Containing Different Fatty Acids

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Saccharomyces cerevisiae grew at the same rate aerobically in a glucose/salts medium as anaerobically in a defined medium supplemented with ergosterol and either oleic, linoleic or α -linolenic acid. However, exponential growth extended for a longer time in aerobic cultures. Irrespective of the nature of the fatty acid provided, phosphatidylcholine from anaerobically grown organisms harvested from early mid-exponential phase cultures contained a higher proportion of residues chemically identical with that provided in the medium compared with the phospholipid from organisms harvested at later stages of batch growth. The fatty-acyl composition of phosphatidylethanolamine and of a fraction containing phosphatidylinositol and phosphatidylserine showed similar changes to those found in phosphatidylcholine. Similar trends were also detected in the fatty-acyl composition of triacylglycerols from organisms grown anaerobically in the presence of different fatty acids and harvested at different stages of growth. However, at all stages of growth there was a higher percentage incorporation of the exogenously provided acid into triacylglycerols. The fatty-acyl composition of individual classes of phospholipid and of triacylglycerols from aerobically grown organisms remained remarkably constant throughout growth in batch culture. There were, however, characteristic differences in the fatty-acyl composition of different classes of lipid.

INTRODUCTION

When strains of *Saccharomyces cerevisiae* are grown under strictly anaerobic conditions, nutritional requirements are induced for cell components or precursors whose synthesis requires molecular oxygen. Quantitatively, the two major nutritional requirements are a sterol (Andreasen & Stier, 1953) and an unsaturated fatty acid (Andreasen & Stier, 1954). Minor anaerobically induced nutritional requirements, such as nicotinic acid (Suomalainen *et al.*, 1965), are usually supplied by a low concentration of yeast extract. Requirements for both the sterol (Proudlock *et al.*, 1968) and the unsaturated fatty acid (Light *et al.*, 1962; Meyer *et al.*, 1963) are fairly non-specific, findings which have been exploited in studies on mitochondrial biogenesis (Jollow *et al.*, 1968) and, in this laboratory, in composition–function relationships in the plasma membrane of *S. cerevisiae* NCYC 366 (Alterthum & Rose, 1973; Hossack & Rose, 1976; Thomas *et al.*, 1978; Thomas & Rose, 1979). Apart from the report by Alterthum & Rose (1973) that total lipids from *S. cerevisiae* NCYC 366 grown anaerobically in medium supplemented with oleic, linoleic or α -linolenic acid, and harvested at a density of 0.22 to 0.24 mg dry wt ml⁻¹, contained, respectively, 65.1, 56.7 and 53.9% residues of the acid supplied, nothing has been reported on the extent to which

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exogenous fatty acids are incorporated into different types of lipid during anaerobic growth. The present paper reports on the extent to which the major phospholipids and triacylglycerols are enriched with the exogenously provided unsaturated fatty acid (oleic, linoleic or α -linolenic acid) at different stages of batch growth. Lipid compositions of anaerobically grown organisms are compared with those from aerobically grown cultures.

METHODS

Experimental cultures. *Saccharomyces cerevisiae* NCYC 366 was maintained on slopes of malt extract/yeast extract/glucose/mycological peptone/agar medium (Wickerham, 1951). The yeast was grown anaerobically as described by Alterthum & Rose (1973) with the medium (pH 4.5) supplemented with ergosterol (5 mg l^{-1}) and oleic, linoleic or α -linolenic acid (30 mg l^{-1}). Cultures were grown aerobically as described by Patching & Rose (1969) in 1 l batches of medium (pH 4.8) containing (per litre) 20 g glucose, 3 g $(\text{NH}_4)_2\text{SO}_4$, 3 g KH_2PO_4 , 10 g yeast extract (Oxoid), 5 g mycological peptone (Oxoid), 0.025 g CaCl_2 and 0.025 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Growth was followed by measuring turbidity at 600 nm. Turbidity values were related to dry weight equiv. which were determined by filtering suitable volumes of culture on to preweighed filters (Millipore; $0.80 \mu\text{m}$ pore size; 25 mm diam.), washing, and drying at 105°C for 24 h.

Lipid analyses. Organisms were harvested at the culture densities indicated, washed twice with water, and lipid was extracted by a modification of the Folch *et al.* (1957) procedure. Freshly washed organisms (2 g dry wt) were mixed with methanol (40 ml) and the suspension was shaken in a Braun homogenizer (B. Braun, Melsungen, West Germany) for 30 s at speed 2 ($4000 \text{ rev. min}^{-1}$) after addition of 60 g glass beads (Glas-perlen; B. Braun; 0.45 to 0.50 mm diam.). Chloroform was then added to the suspension to give the ratio 2:1 (v/v) chloroform/methanol, and the suspension was stirred on a flatbed stirrer for 2 h at room temperature (20 to 22°C). The suspension was then filtered through Whatman no. 44 filter paper, and the extraction procedure was repeated on the residue. The combined extracts were washed with 0.25 vol. 0.88% (w/v) KCl and the mixture was separated by centrifugation or left to separate overnight at 4°C . The lower phase was removed, taken to dryness on a rotary evaporator, and the residue was immediately dissolved in chloroform/methanol (2:1, v/v). Samples were stored under nitrogen gas at -20°C .

Phospholipids were separated by thin-layer chromatography on plates of silica gel H (0.5 mm thick) using a solvent system of chloroform/methanol/acetic acid/water (25:15:4:2, by vol.; Skipski *et al.*, 1964). Classes of phospholipid were located by exposing plates to iodine vapour, and the positions of spots were marked with a pin. Phospholipids were identified by reference to standards run simultaneously. Using these procedures, phosphatidylinositol and phosphatidylserine were not completely separated, and fatty-acyl compositions are quoted for the combined fraction. *Saccharomyces cerevisiae* NCYC 366 resembles other strains of this species in containing appreciably more phosphatidylinositol than phosphatidylserine (Ratcliffe *et al.*, 1973). The iodine was allowed to sublime at room temperature, phospholipid-containing spots were scraped off the plate, and fatty-acid methyl esters were prepared by refluxing the silica gel with methanolic HCl for 2 h. After cooling, an equal volume of water was added, and the methyl esters were extracted into hexane.

Neutral lipids were separated on plates (0.4 mm thick) of silica gel $\text{HF}_{366+254}$ using a solvent system of petroleum ether (b.p. 40 to 60°C)/diethyl ether/acetic acid (70:30:2, by vol.). Lipids were located by examining plates under ultraviolet radiation, and triacylglycerol spots were marked with a pin. Methyl esters of fatty-acyl residues in triacylglycerols were prepared as described for phospholipids.

Fatty-acid methyl esters were analysed by gas-liquid chromatography on a column (2 m) of 15% EGSS-Y supported on 100 to 120 mesh Gas-Chrom P at 175°C . The injection port was at 210°C , and the carrier gas (N_2) flow rate was 40 ml min^{-1} . Percentage fatty-acid compositions were calculated by multiplying the retention time by the peak height on the trace.

Chemicals. Ergosterol, oleic, linoleic and α -linolenic acids were obtained from Sigma, and phospholipids from Applied Science Laboratories, State College, Pa., U.S.A. Silica gels H and $\text{HF}_{366+254}$ were purchased from E. Merck. All other chemicals were reagent grade or of the highest purity available commercially.

RESULTS

Growth of anaerobic and aerobic cultures

The time-course of growth of anaerobic cultures of *S. cerevisiae* NCYC 366 in medium containing oleic, linoleic or α -linolenic acid was similar (Fig. 1). Yields at the end of exponential growth were 1.5 to 1.8 mg dry wt organisms ml^{-1} . Aerobic cultures grew at about

Fig. 1. Growth of *Saccharomyces cerevisiae* NCYC 366 aerobically (■), and anaerobically in medium containing oleic acid (○), linoleic acid (▲) or α -linolenic acid (◆). See Methods for details of media and conditions.

the same rate as anaerobic cultures, but exponential growth in aerobic cultures was more prolonged and, at the end of exponential growth, cultures contained 3.5 to 4.0 mg dry wt organisms ml^{-1} (Fig. 1).

The total lipid contents of organisms grown anaerobically with any one of the fatty acids were similar to those reported by Alterthum & Rose (1973). Organisms from early-exponential phase aerobic cultures had a slightly higher lipid content similar to that reported by Hunter & Rose (1972).

Fatty-acyl compositions of lipids from anaerobically grown organisms

Phosphatidylcholine. Irrespective of the nature of the exogenously provided unsaturated fatty acid, phosphatidylcholine in organisms harvested from early mid-exponential phase cultures contained a higher proportion of residues chemically identical with those provided in the medium compared with the phospholipid from organisms harvested at later stages in the growth phase (Table 1). However, the extent of enrichment with the exogenously provided acid residues was considerably greater in organisms grown in the presence of oleic acid than in the presence of α -linolenic acid. Analyses of organisms grown in the presence of linoleic acid showed an intermediate pattern (Table 1). The decrease in the content of the exogenously provided C_{18} residues as growth proceeded was accompanied by an increased content of $\text{C}_{16:0}$ and of shorter chain ($\text{C}_{10:0}$, $\text{C}_{12:0}$ and $\text{C}_{14:0}$) residues.

Phosphatidylethanolamine. The fatty-acyl composition of phosphatidylethanolamine from organisms grown in the presence of different unsaturated fatty acids and harvested at various stages of growth was similar to that observed for phosphatidylcholine (Table 2). However, the changes in fatty-acyl composition of this phospholipid from organisms harvested at different stages of growth tended to be smaller than those observed with phosphatidylcholine.

Phosphatidylinositol and phosphatidylserine. The fatty-acyl composition of the fraction comprising phosphatidylinositol and phosphatidylserine showed changes in the content of the exogenously provided acid similar to those observed in phosphatidylcholine and phosphatidylethanolamine. However, the magnitude of the change in composition, as growth proceeded, was very much less marked than with the other two phospholipids, added to which the combined fraction contained a higher proportion of $\text{C}_{16:0}$ and $\text{C}_{18:0}$ residues, as reflected in the lower values for Δmol^{-1} (Table 3) compared with the other two phospholipids.

Triacylglycerols. Essentially similar trends to those reported for phospholipids were

Table 1. *Fatty-acyl composition of phosphatidylcholine from Saccharomyces cerevisiae NCYC 366 harvested at different stages of growth from anaerobic cultures supplemented with different fatty acids*

Values quoted are the averages of at least two separate experiments. Values for unsaturation ($\Delta \text{ mol}^{-1}$) were calculated as described by Kates & Hagen (1964). — indicates that the residue was not detectable.

Fatty-acid supplement	Culture density (mg dry wt ml ⁻¹)	Fatty-acyl composition (% of total)									Unsaturation (Δ mol ⁻¹)
		C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	
Oleic acid	0.42	—	—	0.7	5.0	0.4	1.4	92.7	—	—	0.93
	0.68	0.3	0.3	1.0	12.4	0.4	1.5	84.2	—	—	0.85
	1.12	0.3	1.4	3.3	16.3	0.7	2.1	76.0	—	—	0.77
Linoleic acid	0.42	—	—	1.9	20.8	0.4	4.7	0.4	71.8	—	1.44
	0.68	1.4	1.9	6.4	37.3	1.7	7.9	2.1	41.3	—	0.86
	1.12	4.1	4.3	9.8	38.7	1.8	6.9	—	33.9	—	0.70
α-Linolenic acid	0.42	0.2	0.3	2.0	24.6	0.4	7.7	0.3	0.2	64.8	1.95
	0.68	2.7	2.8	6.8	39.4	1.5	7.8	1.2	—	37.8	1.16
	1.12	4.6	4.6	10.2	41.4	2.4	8.5	1.0	—	27.5	0.86

Table 2. *Fatty-acyl composition of phosphatidylethanolamine from Saccharomyces cerevisiae NCYC 366 harvested at different stages of growth from anaerobic cultures supplemented with different fatty acids*

Values quoted are the averages of at least two separate experiments. Values for unsaturation ($\Delta \text{ mol}^{-1}$) were calculated as described by Kates & Hagen (1964). — indicates that the residue was not detectable.

Fatty-acid supplement	Culture density (mg dry wt ml ⁻¹)	Fatty-acyl composition (% of total)									Unsaturation (Δ mol ⁻¹)
		C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	
Oleic acid	0.42	—	—	1.1	11.0	1.3	0.3	86.5	—	—	0.88
	0.68	—	0.4	2.1	18.9	1.2	1.0	76.4	—	—	0.78
	1.12	0.2	0.2	3.5	20.9	1.7	1.4	72.1	—	—	0.74
Linoleic acid	0.42	0.6	0.6	2.6	26.9	0.9	3.8	0.5	64.3	—	1.30
	0.68	0.9	1.2	3.1	34.2	1.5	6.5	0.5	51.9	—	1.06
	1.12	1.5	2.1	5.6	37.1	2.7	7.6	2.0	41.5	—	0.88
α-Linolenic acid	0.42	—	—	1.8	28.8	1.5	3.9	1.0	0.3	62.4	1.90
	0.68	3.9	3.0	4.2	33.9	1.0	6.4	1.0	—	46.6	1.42
	1.12	3.6	3.8	6.3	40.8	2.3	7.1	1.9	—	34.4	1.08

Table 3. *Fatty-acyl composition of phosphatidylinositol plus phosphatidylserine from Saccharomyces cerevisiae NCYC 366 harvested at different stages of growth from anaerobic cultures supplemented with different fatty acids*

Values quoted are the averages of at least two separate experiments. Values for unsaturation (Δ mol⁻¹) were calculated as described by Kates & Hagen (1964). — indicates that the residue was not detectable.

Fatty-acid supplement	Culture density (mg dry wt ml ⁻¹)	Fatty-acyl composition (% of total)										Unsaturation (Δ mol ⁻¹)
		C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}		
Oleic acid	0.42	—	—	1.8	27.3	0.6	8.3	62.1	—	—	0.63	
	0.68	0.2	0.5	2.6	33.6	0.6	7.1	55.6	—	—	0.56	
	1.12	1.4	1.3	2.7	36.7	0.6	7.3	50.2	—	—	0.51	
Linoleic acid	0.42	0.8	1.1	1.6	36.0	0.3	12.2	0.5	47.7	—	0.96	
	0.68	5.2	5.2	4.9	38.1	0.5	11.5	0.4	34.2	—	0.69	
	1.12	5.2	4.9	5.6	47.2	1.1	11.9	0.8	27.2	—	0.56	
α -Linolenic acid	0.42	0.9	1.1	2.4	35.9	0.5	14.3	0.7	—	44.4	1.34	
	0.68	3.9	5.5	4.2	40.8	0.8	14.6	0.8	—	29.4	0.90	
	1.12	4.6	4.3	5.0	45.0	1.8	16.3	1.6	—	21.6	0.68	

Table 4. *Fatty-acyl composition of triacylglycerols from Saccharomyces cerevisiae NCYC 366 harvested at different stages of growth from anaerobic cultures supplemented with different fatty acids*

Values quoted are the averages of at least two separate experiments. Values for unsaturation (Δ mol⁻¹) were calculated as described by Kates & Hagen (1964). — indicates that the residue was not detectable.

Fatty-acid supplement	Culture density (mg dry wt ml ⁻¹)	Fatty-acyl composition (% of total)									Unsaturation (Δ mol ⁻¹)
		C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	
Oleic acid	0.42	—	—	1.1	4.0	0.6	2.0	92.2	—	—	0.93
	1.12	0.3	0.3	2.3	10.5	0.8	3.7	82.3	—	—	0.83
Linoleic acid	0.42	0.4	0.4	2.0	13.3	0.8	5.1	1.2	77.0	—	1.56
	1.12	2.3	2.9	5.2	23.5	2.1	6.8	1.4	56.1	—	1.16
α -Linolenic acid	0.42	—	0.2	1.7	12.5	1.1	5.2	1.5	0.4	77.5	2.36
	1.12	2.3	2.8	5.6	28.7	1.4	8.2	3.4	0.8	47.0	1.47

Table 5. *Fatty-acyl composition of phospholipids and triacylglycerols from Saccharomyces cerevisiae NCYC 366 grown aerobically and harvested at different stages of growth*

Values quoted are the averages of two separate experiments. Values for unsaturation (Δmol^{-1}) were calculated as described by Kates & Hagen (1964).

Lipid fraction	Culture density (mg dry wt ml ⁻¹)	Fatty-acyl composition (% of total)							Unsaturation (Δmol^{-1})
		C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	
Phosphatidylcholine	0.63	0.2	0.5	0.6	4.1	73.1	0.7	20.9	0.94
	1.55	0.2	0.2	0.5	5.0	72.5	0.6	21.0	0.94
	2.35	0.2	0.7	0.8	5.4	72.0	0.7	19.9	0.92
	3.00	0.2	1.1	1.1	5.8	68.4	1.2	22.2	0.91
Phosphatidylethanolamine	0.63	0.1	0.1	0.9	17.7	43.0	0.8	37.4	0.80
	1.55	0.2	0.1	0.7	16.4	45.7	1.4	35.8	0.82
	2.35	0.2	0.2	0.8	15.6	45.1	1.0	37.0	0.82
	3.00	0.2	0.2	0.3	11.6	50.3	1.2	36.4	0.87
Phosphatidylinositol + Phosphatidylserine	0.63	0.6	0.5	0.9	30.9	25.8	5.4	37.3	0.63
	1.55	1.1	0.9	1.2	31.9	24.8	5.2	34.9	0.60
	2.35	1.0	0.8	1.1	32.8	25.3	4.9	34.1	0.59
	3.00	0.3	0.6	1.0	25.6	33.1	4.1	35.4	0.69
Triacylglycerols	0.63	1.7	1.8	2.6	20.2	42.1	6.6	25.0	0.67
	1.55	2.0	2.1	2.8	21.2	41.1	5.9	24.9	0.66
	2.35	2.7	1.4	2.7	19.8	40.3	6.0	27.1	0.67
	3.00	1.1	1.8	1.8	19.9	48.2	4.0	23.2	0.71

observed in the fatty-acyl composition of triacylglycerols from organisms harvested at different stages of growth. However, there was a higher percentage incorporation of the exogenously provided acid at all stages of growth (Table 4).

Fatty-acyl composition of lipids from aerobically grown organisms

Compared with lipids from anaerobically grown organisms, the composition of phospholipids and triacylglycerols from aerobically grown organisms remained remarkably constant throughout growth in batch culture (Table 5). There was, however, a marked variation in the fatty-acyl composition of the different lipids. Phosphatidylcholine remained highly unsaturated, with palmitoleyl and oleyl residues predominant. These two residues also predominated in phosphatidylethanolamine, but in lower proportions, as indicated by the overall lower Δmol^{-1} value for this phospholipid. The fraction comprising phosphatidyl-inositol and phosphatidylserine contained a much higher proportion of saturated residues, particularly $\text{C}_{16:0}$, compared with the other two phospholipids. Triacylglycerols had a similar overall Δmol^{-1} value to the mixture of phospholipids in the combined fraction, but differed in their contents of $\text{C}_{16:1}$ and $\text{C}_{18:1}$ residues.

DISCUSSION

Yeast phospholipids are located almost exclusively in membranes. Changes in the degree of unsaturation of phospholipids will therefore alter the fluidity of the membrane, that is the extent to which phospholipid fatty-acyl chains are mobile in the bilayer. Changes in unsaturation could also affect the activity of membrane-bound enzymes and proteins concerned with transport processes, since individual proteins may have requirements for individual phospholipid molecules with specific unsaturated fatty-acyl residues, as already reported for certain bacterial membrane-bound enzymes (Finnerty, 1979). Lipids of *S. cerevisiae* NCYC 366 grown anaerobically in the presence of oleic acid had a fluidity (as judged by the Δmol^{-1} value) which was reasonably constant throughout growth in batch culture. Unsaturation values for phosphatidylcholine and phosphatidylethanolamine in these organisms were similar to those reported for these phospholipids from aerobically grown organisms and are typical for phospholipids from yeasts grown in the range 25 to 30 °C (Kates & Baxter, 1962; Hunter & Rose, 1972; Erwin, 1973). On the other hand, comparable values for unsaturation in phospholipids from organisms grown in the presence of linoleic or α -linolenic acid were only observed with lipids from late-exponential phase organisms. Exceptionally high unsaturation values were detected in phospholipids from organisms grown in the presence of multiply unsaturated acids and harvested earlier during exponential growth. Such high values for lipid unsaturation have generally only been recorded for psychrophilic yeasts (Kates & Baxter, 1962; Arthur & Watson, 1976; Watson *et al.*, 1978) when grown at suboptimum temperatures, when the high degree of lipid unsaturation is likely to be essential to maintain membranes in a functional state.

It might be inferred from these findings that, in organisms grown in the presence of linoleic or α -linolenic acid, membrane fluidity during the early- and mid-exponential growth phases might be so high as to render membranes unstable. The comparable rates of growth and final yields of organisms observed with anaerobic cultures containing each of the three different unsaturated fatty acids indicate that membrane instability is not sufficient to affect basic growth parameters. There is, however, evidence for instability in plasma membranes in *S. cerevisiae* NCYC 366 containing multiply unsaturated fatty-acyl residues. Sphaeroplasts prepared from cells enriched in either linoleic or α -linolenic acid residues leaked protein faster than sphaeroplasts with membranes enriched in oleyl residues (Diana M. Belk & A. H. Rose, unpublished observations). Furthermore, α -mannosyltransferase activities of plasma-membrane preparations were more variable when the membranes were enriched in linoleyl rather than oleyl residues, which might be explained by a greater loss of

components (protein and/or phospholipid) from the more fluid membranes (L. Julia Douglas, unpublished observations). Finally, M. H. J. Keenan & A. H. Rose (unpublished observations) detected a greater rate of decay of certain transport systems in *S. cerevisiae* NCYC 366 with membranes enriched in linoleyl rather than oleyl residues, which again might be explained by loss of proteins from the more fluid membranes.

There are several possible ways in which *S. cerevisiae* growing anaerobically in the presence of multiply unsaturated fatty acids might attempt to minimize fluidity in cellular membranes. One strategem would be to incorporate, preferentially, exogenously provided multiply unsaturated fatty acids into neutral lipids (e.g. triacylglycerols) which are not membrane components. Significantly, triacylglycerols from anaerobically grown organisms were more unsaturated than the phospholipids from these organisms. A second possibility is that decreased fluidity could arise as a result of an increased synthesis of shorter chain fatty-acyl residues, which was observed in organisms grown in the presence of multiply unsaturated fatty acids. Yet another mechanism whereby membrane fluidity could be balanced is through synthesis of phosphatidylinositol and phosphatidylserine, with a higher proportion of saturated fatty-acyl residues, strategically located in the membrane thereby maintaining a degree of rigidity in that domain.

Finally, our data on differences in the fatty-acyl composition of phospholipids from anaerobically and aerobically grown organisms could indicate operation of different biosynthetic pathways in organisms grown under these two conditions. Two pathways for synthesis of phosphatidylcholine are known to operate in *S. cerevisiae* (Steiner & Lester, 1972; Waechter & Lester, 1973), namely the methylation pathway in which phosphatidylcholine arises by methylation of phosphatidylethanolamine, and the cytidine nucleotide pathway in which free choline or ethanolamine is incorporated into phospholipids. The dissimilarity of the fatty-acyl composition of phospholipids from aerobically grown organisms could be indicative of a biosynthetic pathway in which specific phosphatidic acid species are used for synthesis of particular phospholipid classes. On the other hand, under anaerobic conditions with lipid supplements, it might appear that phosphatidylcholine and phosphatidylethanolamine are synthesized from a common pool of phosphatidic acid.

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