Effect of Plasma-membrane Phospholipid Unsaturation on Solute Transport into *Saccharomyces cerevisiae* NCYC 366

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A comparison was made of kinetics of solute accumulation by *Saccharomyces cerevisiae* NCYC 366 grown anaerobically under conditions that lead to enrichment of the plasma membrane with ergosterol and either oleyl or linoleyl residues. Values for K_T and $V_{\max}$ were identical for accumulation of L-asparagine, L-glutamine, $H_2PO_4^-$, Ca^{2+} and SO_4^{2-} , while for accumulation of D-glucose, the value differed slightly but not significantly. Values for K_T for accumulation of L-lysine, by both the low- and high-affinity systems, decreased when oleyl residues were replaced by linoleyl residues. Under these conditions, $V_{\max}$ values for the high-affinity system decreased while that for the low-affinity system increased. An Arrhenius plot for accumulation of lysine by the high-affinity system revealed a discontinuity when membranes were enriched in linoleyl residues. However, no discontinuity was evident on plots of lysine accumulation when membranes were enriched in oleyl residues. Similar plots for accumulation of L-asparagine, which was used as a control, showed that substitution of linoleyl for oleyl residues significantly raised the transition temperature, but had little effect on the activation energy at temperatures below the discontinuity. When palmitoleyl residues were incorporated into the yeast plasma membrane, the K_T value for L-lysine accumulation by the high-affinity system was hardly altered, although the $V_{\max}$ value was lowered, as compared with organisms with membranes enriched in oleyl residues. Replacement of oleyl by palmitoleyl residues lowered both the K_T and $V_{\max}$ values for accumulation of L-asparagine. A modified statistical method is described for calculating confidence limits for transition points on Arrhenius plots.

INTRODUCTION

The activities of several microbial plasma-membrane-bound proteins are influenced by lipids (Sanderman, 1978). The majority of these effects have been demonstrated in bacteria, and include interactions involving proteins that catalyse synthesis of wall components (Inouye, 1974; Gennis & Strominger, 1976; Hinckley *et al.*, 1972) and proteins involved in energy-generating metabolism (Cunningham & Hager, 1971; Peter & Ahlers, 1975; Salton & Schor, 1974; Aiyappa & Lampen, 1976; Bevers *et al.*, 1977). Reports on the effects of lipids on transport of molecules across the bacterial plasma membrane are less numerous. Beepe (1972) found that a mutant of *Bacillus subtilis* unable to synthesize phosphatidylethanolamine differed from the parent in its rate of uptake of several solutes, including eight amino acids, while Cox *et al.* (1975), using a fatty acid-requiring auxotroph of *Escherichia coli*, showed that the rate of transport of several amino acids by organisms enriched in *cis*-vaccenyl residues was slower than with organisms enriched in oleyl or palmitoleyl residues. Far fewer studies have been reported on the effect of plasma-membrane lipid composition on protein activity in eukaryotic micro-organisms (Sanderman, 1978). Phosphatidylcholine has, however, been implicated in L-lysine uptake by *Neurospora crassa*, since a phosphatidylcholine-deficient mutant had a decreased capacity for accumulating this amino acid (Sherr, 1969), while activity of one of the glucose-transporting

systems in another strain of this fungus decreased when it was grown under conditions of inositol deprivation, thereby suggesting a role for phosphatidylinositol in transport (Scarborough, 1973). A recent report indicated that the plasma-membrane ATPase in *Schizosaccharomyces pombe*, which is involved in formation of the proton gradient used in active uptake of solutes, requires phospholipids for activity (Dufour & Goffeau, 1980).

The present paper reports on the effect of the fatty-acyl composition of plasma-membrane phospholipids on accumulation of solutes by *Saccharomyces cerevisiae* NCYC 366. The study exploits a nutritional technique for changing the lipid composition of the plasma membrane, namely the anaerobically-induced requirement in *S. cerevisiae* for a sterol and an unsaturated fatty acid (Andreasen & Stier, 1953, 1954). Both of these nutritional requirements are fairly broad (Light *et al.*, 1962; Proudlock *et al.*, 1968) and this permits specific enrichments to be made in the sterol (Hossack & Rose, 1976) and fatty-acyl composition of plasma membranes (Thomas *et al.*, 1978) without altering the content and composition of other types of lipid in organisms (Rose, 1977).

METHODS

Experimental cultures. *Saccharomyces cerevisiae* NCYC 366 was maintained on slopes of malt extract/yeast extract/glucose/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 an unsaturated fatty acid (30 mg l⁻¹) as indicated in the text. Growth was followed by measuring turbidity at 600 nm, and organisms were harvested from late-exponential phase cultures (0.24–0.26 mg dry wt ml⁻¹) by filtration through a membrane filter (0.45 µm pore size, 50 mm diam.; Oxoid). Control cultures lacking ergosterol were incubated with each batch of experimental cultures and when growth in the control exceeded 0.1 mg dry wt ml⁻¹, experimental cultures were discarded.

Measurement of rate of solute accumulation. Organisms which were to be used to measure the rate of solute accumulation were washed with PIPES buffer (20 mM) adjusted to pH 4.5 (pH 5.5 when uptake of Ca²⁺ was to be studied), and suspended at 15 mg dry wt ml⁻¹ in the same buffer in a screw-capped centrifuge tube (250 ml). The tube was stored in an ice-water mixture after the headspace had been flushed with oxygen-free nitrogen gas. The suspension (15 ml) used to measure the rate of solute accumulation consisted of PIPES buffer (pH 4.5 or 5.5) containing glucose (100 mM) (except when the rate of uptake of D-glucose was to be measured), 0.5 mg dry wt organisms ml⁻¹ and solute in the range 10⁻⁶–10⁻¹ M, including both radioactive and non-radioactive compounds. The concentrations of radioactive compounds in the solute solutions used were (µCi µmol⁻¹; 1 µCi = 37 kBq): L-[U-¹⁴C]arginine monohydrochloride, 0.05; L-[U-¹⁴C]asparagine, 3.3; ⁴⁵CaCl₂, 12.5; D-[6-³H]glucose, 0.75–750; L-[U-¹⁴C]glutamine, 3.3; L-[U-¹⁴C]lysine monohydrochloride, 0.013–1.67; KH₂³²PO₄, 2.0; and Na₂³⁵SO₄, 0.03–30. Where ranges are indicated, the tracer:carrier ratio was varied because of wide differences in the rate of accumulation at different solute concentrations. The suspension was contained in a round bottomed Quickfit flask (100 ml capacity) fitted with a glass-stoppered sampling port. When preparing the suspension, the flask was maintained in a water-bath at 30 °C except when otherwise stated. Its contents were stirred magnetically, and the inside of the flask continuously flushed with oxygen-free nitrogen gas. The experiment was started by adding solute (tracer and carrier) to the flask, after which portions (1 ml) were removed at predetermined times, rapidly filtered through membrane filters (0.45 µm pore size, 25 mm diam.; Millipore) and the filter and cells immediately washed with ice-cold PIPES buffer (pH 4.5 or 5.5) containing the solute at the concentration included in the cell suspension. Filters were then transferred to scintillation vials containing 7 ml Unisolve liquid scintillator no. 1 (Koch-Light). The radioactivity of the vial contents was measured in a Packard Tricarb liquid scintillation spectrometer (model 3385), and the values corrected for counting efficiency. Rates of accumulation were determined from plots of the time-course of accumulation for up to 195 s, although linear plots were obtained with low concentrations of solute for up to 30 min. Woolf–Hofstee plots (Hofstee, 1959) were used to calculate *K_T* and *V_{max}* values for uptake systems.

The fate following accumulation of one of the solutes examined, L-[U-¹⁴C]arginine, was followed by examining hot-water extracts of organisms and cell debris for ¹⁴C-containing compounds. Organisms were removed from suspensions as already described, and filters with organisms immersed in 10 ml water at 100 °C for 10 min. The suspension was then filtered through a membrane filter (0.45 µm pore size, 25 mm diam.; Millipore) and the radioactivity of the filtrate measured. The debris was then washed with arginine (200 µM), the filter with washed debris transferred to a scintillation vial and the radioactivity of the contents measured. Ninhydrin-positive compounds in the filtrate were separated by paper chromatography using the following solvent systems: ethanol/water/urea (80:20:0.5, v/v/w), butanol/pyridine/water (315:175:240, by vol.) and phenol/water/25% (v/v) aqueous ammonia (sp.gr. 0.88) (80:20:0.3, w/v/v). Chromatograms were dried, sprayed with ninhydrin (0.1%, w/v, in n-butanol saturated with water) and the location of spots marked. They were then exposed to photographic plates

(Kodak Industrex C) for four weeks. Filtrates from extracts of organisms sampled up to 30 min revealed only one radioactive spot, with an R_F value identical with that of arginine in all three solvents. Extracts from organisms incubated for 90 min contained a second radioactive compound which was not identified. Radioactivity was detectable in the debris from organisms incubated for 5 min, although this represented less than 3% of the total accumulated.

Lipid analysis. Organisms to be used for lipid analysis were washed twice with water on a membrane filter, and lipid was extracted by a modification of the procedures of Folch *et al.* (1957) and Watson & Rose (1980). Freshly washed organisms (1 g dry wt) were mixed with methanol (20 ml) and the suspension was shaken in a Braun homogenizer (B. Braun, Melsungen, F.R.G.) for three periods of 30 s at speed 2 (4000 r.p.m.) after addition of 30 g of glass beads (0.45–0.50 mm diam.; Glasperlen, B. Braun). Chloroform was then added to the suspension to give a ratio of 2:1 (v/v) chloroform/methanol, and the suspension stirred on a flatbed stirrer for 2 h at room temperature (20–24 °C). The suspension was then filtered through Whatman no. 44 filter paper, and the extraction procedure repeated on the residue. The combined extracts were washed with 0.25 vol. 0.88% (w/v) KCl, and the mixture left to separate overnight at 4 °C. The lower phase was removed, taken to dryness on a rotary evaporator, and the residue immediately dissolved in chloroform/methanol (2:1, v/v). Samples were stored under nitrogen gas at –20 °C. Phospholipids were separated from other lipid classes in extracts by TLC on plates of silica gel H (0.5 mm thick) using a solvent system of hexane/diethyl ether/acetic acid (70:30:2, by vol.). Lipid bands were located by exposing plates to iodine vapour, and were marked with pins. The phospholipid band was identified by reference to simultaneously run standards of phosphatidylcholine or phosphatidylethanolamine. Individual phospholipids were separated from extracts by TLC 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). Using this procedure, phosphatidylinositol and phosphatidylserine were not completely separated, and fatty-acyl compositions were determined 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). Phospholipid spots were located by exposing the plates to iodine vapour. The iodine was allowed to sublime at room temperature; spots of lipid classes or individual phospholipids were scraped off the plates, and fatty acid methyl esters were prepared by refluxing the silica gel with methanol containing 14% (w/v) BF_3 , for 10 min. After cooling, an equal volume of water was added, and the methyl esters were extracted into chloroform. The same procedure was used to prepare fatty acid methyl esters from neutral-lipid fractions containing sterol esters and di- and triacylglycerols. Fatty acid methyl esters were analysed by GLC on a column (2 m) of 15% EGSS-Y supported on 100 to 120 mesh Gas-Chrom P at 200 °C. The injection port was at 210 °C, and the carrier gas (nitrogen) flow rate was 60 ml min⁻¹. Percentage fatty acid compositions were calculated by multiplying the retention time by the peak height on the trace.

Assay of asparaginase activity. The activity of the cell wall enzyme asparaginase II (Dunlop *et al.*, 1978; L-asparagine amidohydrolase, EC 3.5.1.1), was assayed by following the appearance of L-aspartate in a buffered suspension of organisms containing L-asparagine. Washed organisms were suspended at a density of 15 mg dry wt ml⁻¹ in PIPES buffer (pH 4.5) in a Quickfit flask (100 ml capacity) and the suspension sparged with oxygen-free nitrogen gas as described for measurement of the rate of solute accumulation. The assay was started by adding L-asparagine to a final concentration of 1 mM. Portions of suspension were removed at intervals and rapidly filtered through membrane filters (0.45 µm pore size, 25 mm diam.; Oxoid). The concentration of L-aspartate in filtrates was determined spectrophotometrically by coupling with L-glutamate-oxaloacetate transaminase and L-malate dehydrogenase as described by Dunlop & Roon (1975).

Statistical analysis. Slopes of lines on Arrhenius plots, and points of intersection of these lines, were calculated using the method of Hinkley (1969). Confidence limits for transition points were calculated using a corrected version of Hinkley's method.

Chemicals. All chemicals used were of Analar grade or of the highest purity available commercially. Ergosterol, palmitoleic acid (Δ^9 -*cis*-hexadecenoic acid), oleic acid (Δ^9 -*cis*-octadecenoic acid), linoleic acid ($\Delta^{9,12}$ -*cis,cis*-octadecadienoic acid), cetoleic acid (Δ^{11} -*cis*-eicosaenoic acid) and PIPES were purchased from Sigma. Radioactively labelled compounds were obtained from Amersham.

RESULTS

Effect of storage time on solute-accumulating ability of organisms

The viability of the suspension of organisms maintained in an ice–water mixture prior to use in solute accumulation measurements, as judged by plate counts using the medium of Wickerham (1951), did not change over a period of 5 h. The ability of organisms enriched in oleyl residues to accumulate L-arginine declined only slowly during storage in an ice–water mixture, but after about 1 h the arginine-accumulating ability of organisms enriched in linoleyl residues

Fig. 1. Effect of storage time on the ability of *S. cerevisiae* NCYC 366 enriched in oleyl (●) or linoleyl (○) residues to accumulate L-arginine. Organisms were stored in an ice-water mixture in PIPES buffer (pH 4.5) under nitrogen gas. Cell suspensions used for uptake measurements contained 100 μ M-L-arginine. Each value represents the average of at least three independent measurements.

declined rapidly (Fig. 1). Comparable observations on the ability of organisms to accumulate other solutes were not made, although it was established that the ability of organisms enriched in either oleyl or linoleyl residues to accumulate any of the other solutes investigated hardly declined over the first hour of storage. All determinations were therefore made on organisms that had been stored in an ice-water mixture for not more than 1 h, and usually for considerably less.

Solute accumulation by organisms enriched in oleyl or linoleyl residues

Keenan & Rose (1979) reported that the K_T and V_{max} values for accumulation of L-arginine in *S. cerevisiae* NCYC 366, by both high- and low-affinity systems, differed in organisms enriched in oleyl as compared with linoleyl residues. A survey was therefore made of the effect of fatty-acyl enrichment on the kinetics of accumulation of a representative range of other solutes. Values for K_T and V_{max} for five of these solutes, namely L-asparagine, L-glutamine, $H_2PO_4^-$, Ca^{2+} and SO_4^{2-} , were identical, irrespective of the nature of the fatty-acyl enrichment. Kinetic values for accumulation of phosphate, calcium and sulphate ions were, moreover, similar to those already reported for these transport systems in strains of *S. cerevisiae* grown aerobically (Borst-Pauwels & Jager, 1969; Fuhrmann & Rothstein, 1968; Breton & Surdin-Kerjan, 1977). Values for K_T and V_{max} for accumulation of L-asparagine and L-glutamine, which have not previously been reported for *S. cerevisiae*, were: L-asparagine, K_T 4×10^{-4} M, V_{max} 14 nmol (mg dry wt) $^{-1}$ min $^{-1}$; and L-glutamine, K_T 3.3×10^{-4} M, V_{max} 15 nmol (mg dry wt) $^{-1}$ min $^{-1}$. Organisms were shown to lack cell wall asparaginase II activity reported in other strains of *S. cerevisiae* by Dunlop & Roon (1975). Values for K_T for accumulation of D-glucose by organisms enriched in oleyl or linoleyl residues were virtually identical with those already reported for *S. cerevisiae* grown aerobically (Koty, 1967), although the V_{max} values differed slightly, but not significantly [232 nmol (mg dry wt) $^{-1}$ min $^{-1}$ for organisms enriched in oleyl residues, and 266 nmol (mg dry wt) $^{-1}$ min $^{-1}$ for those enriched in linoleyl residues]. Accumulation of L-lysine, like that of L-arginine (Keenan & Rose, 1979), was effected by both high- and low-affinity systems (Fig. 2). The equations derived by Neal (1972) were used to obtain separate K_T and V_{max} values for each of the uptake systems, with the assumption that both systems are described by Michaelis-Menten kinetics. Both kinetic values differed when organisms enriched in either oleyl or linoleyl residues accumulated L-lysine by either the high-affinity or low-affinity system.

Effect of temperature on accumulation of L-lysine and L-asparagine by organisms enriched in oleyl or linoleyl residues

Further insight into the effect of fatty-acyl unsaturation on L-lysine accumulation was sought by examining the effect of temperature on accumulation by the high-affinity system. For comparison, an examination was made of the effect of temperature on accumulation of L-

Fig. 2. Woolf-Hofstee plots for accumulation of L-lysine by *S. cerevisiae* NCYC 366 enriched in oleyl residues (a) or linoleyl residues (b). Rates of accumulation were determined as described in Methods. Each value represents the average of at least three independent observations. The lines represent the relationship between points showing the regression coefficient nearest unity. The maximum variation on any one velocity value was $\pm 15\%$. Values for K_T (M) for the high-affinity system were 6.5×10^{-6} for organisms enriched in oleyl residues, and 0.25×10^{-6} for organisms enriched in linoleyl residues. Corresponding values for the low-affinity system were 2.8×10^{-4} and 1.1×10^{-4} , respectively. Values for $V_{\max}$ [nmol (mg dry wt) $^{-1}$ min $^{-1}$] for the high-affinity system were 4.2 (organisms enriched in oleyl residues) and 0.5 (organisms enriched in linoleyl residues). Corresponding values for the low-affinity system were 1.9 and 3.1, respectively.

Fig. 3. Arrhenius plots for accumulation of L-lysine (a) and L-asparagine (b) by *S. cerevisiae* NCYC 366 enriched in oleyl residues (○) or linoleyl residues (●). Cell suspensions contained $10 \mu\text{M}$ -amino acid. Each value represents the average of at least two independent measurements. Rates of accumulation were determined as described in Methods. Slopes of lines and transition temperatures were calculated using a corrected version of the method of Hinkley (1969).

asparagine, K_T and $V_{\max}$ values for which were not affected by the change in plasma-membrane fatty-acyl unsaturation, Hinkley's method for statistical analysis of these types of data (Hinkley, 1969) assumes that there is a linear response in each of two regimes, with a change of slope at the point γ . To the left of γ the slope is β_0 , to the right, β_1 . Application of Hinkley's algorithm (Hinkley, 1969) for finding the least-squares fit of the model for the data yielded point estimates $\hat{\beta}_0$, $\hat{\beta}_1$, and $\hat{\gamma}$ of the parameters in the model. The procedure is complicated, and a FORTRAN program was written to implement it, incorporating also the statistical modifications described below.

Application of the test of Hinkley (1969) for the existence of a transition point showed that, with data for uptake of L-lysine by organisms enriched in oleyl residues, there was no significant change in slope of the Arrhenius plot (Fig. 3). The activation energy was calculated from the

Table 1. Values for transition temperatures and activation energies derived from Arrhenius plots for accumulation of L-lysine and L-asparagine by *S. cerevisiae* NCYC 366 enriched in oleyl or linoleyl residues

Amino acid*	Fatty-acyl enrichment	Transition temperature (°C)†	Activation energy (kJ mol ⁻¹)‡	
			Below transition	Above transition
L-Lysine	Oleyl	—	113 ± 10	113 ± 10
	Linoleyl	20.5 (19.8, 21.2)	295 ± 21	-46 ± 24
L-Asparagine	Oleyl	19.0 (17.7, 20.2)	285 ± 40	52 ± 26
	Linoleyl	23.2 (21.1, 30)	237 ± 44	-32 ± 64

* Rates of amino acid uptake were measured in suspensions containing the amino acid at 10 µM.

† Transition temperatures were calculated using the corrected version of the method of Hinkley (1971). Values in parentheses are asymmetrical 95% confidence limits.

‡ Activation energies are quoted ± two standard errors of the mean.

slope of the line, and confidence limits determined by standard linear regression theory (Table 1). Analysis of the other three Arrhenius plots revealed that each showed a significant change in slope. The method of Hinkley (1969) was then used to calculate point estimates $\hat{\gamma}$ and $\hat{\beta}_1$ and approximate 95% confidence limits for the activation energy β_1 . Hinkley's procedure for finding a confidence interval for the transition point was examined and found to be applicable to data for L-lysine accumulation by organisms enriched in linoleyl residues and L-asparagine accumulation by organisms enriched in oleyl residues.

However, data on L-asparagine accumulation by linoleyl-enriched organisms exposed the need for a modification of the method of Hinkley (1969, 1971), as follows. Suppose that the distinct abscissae of the data are $u_1 < u_2 < \dots < u_r$ (with $r \leq n$). Hinkley (1969, 1971) suggested erroneously that, if the estimated change point be less than u_2 , then the lower confidence limit should be set as $-\infty$, and further that this would be associated with a lack of significance in the test for the existence of a change point. The error is revealed by the data under consideration; the existence of a change point near u_1 is perfectly possible, leading to the phenomenon that Hinkley (1971) ruled out. To arrive at the correct procedure, let $S(\gamma_0)$, for any γ_0 , be the residual sum of squares about the least squares fit with change point γ_0 . Then $S(\gamma_0) = S(u_2)$ for $u_1 < \gamma_0 \leq u_2$, and hence if Hinkley's confidence intervals include u_2 they must also include the interval (u_1, u_2) . However, in contrast to the remarks of Hinkley (1969), points $\gamma_0 \leq u_1$ should not be included in the confidence interval if there is known to be a significant change of slope since, on the basis of the data, the situations $\gamma_0 \leq u_1$ and no change of slope are indistinguishable. Therefore, in the case where u_2 is acceptable as a transition point by the criterion of Hinkley (1969), but there is a significant change of slope, we set the lower confidence limit to u_1 . Rigorously the limit should be $u_1^\dagger$ since u_1 itself is not an acceptable value for the transition point.

For data on L-asparagine accumulation by organisms enriched in either fatty-acyl residue, the part of the function $S(\gamma)$ on the appropriate side of the least squares estimate γ was found to be approximately quadratic with curvature corresponding to the asymptotic formulae of Hinkley (1969). Accordingly, manipulations involving his estimated standard errors were applicable. These lead to a lower 95% confidence limit of 2.5 °C for the increase in transition temperature for L-asparagine accumulation by linoleyl-enriched organisms relative to oleyl-enriched organisms.

Accumulation of L-lysine by organisms enriched in palmitoleyl residues

An attempt was made to examine the effect of fatty-acyl chain length of membrane phospholipids on accumulation of L-lysine, by the high-affinity system, using organisms grown in the presence of ergosterol and palmitoleic (C_{16:1}) or cetoleic (C_{20:1}) acid, and to compare the results with those for organisms grown in the presence of oleic acid. The phospholipid fraction from

Table 2. Fatty-acyl composition of phospholipids and neutral lipids from *S. cerevisiae* NCYC 366 grown anaerobically in the presence of ergosterol and palmitoleic or oleic acid

Fatty acid supplement	Lipid*	Contents of residues (percentage of total)†					
		C _{12:0}	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}
Palmitoleic acid	PC	2.3	2.1	33.8	47.3	14.4	ND
	PE	2.8	2.7	38.7	50.7	5.1	ND
	PS + PI	5.9	2.4	37.1	28.3	26.2	ND
	NL	1.5	3.0	15.5	70.5	9.4	ND
Oleic acid	PC	1.0	6.4	25.2	ND	14.3	53.2
	PE	0.3	11.5	29.3	ND	11.7	48.0
	PS + PI	1.2	10.4	32.1	1.3	12.9	41.9
	NL	0.1	2.2	12.3	ND	5.9	79.6

ND, Not detectable.

* Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; NL, neutral lipids.

† The values quoted are the averages of two independent analyses.

Fig. 4. Woolf-Hofstee plot for accumulation of L-lysine by *S. cerevisiae* NCYC 366 enriched in palmitoleyl residues. Rates of accumulation were determined as described in Methods. The line represents the relationship between points with the regression coefficient nearest unity.

organisms grown in the presence of palmitoleic acid was enriched with residues of this fatty acid to a slightly greater extent (60.1% of total) than phospholipids from organisms grown in the presence of oleic acid, the value for which (49.7%) was somewhat lower than that previously reported (62.3%) for *S. cerevisiae* NCYC 366 by Thomas *et al.* (1978). However, the phospholipid fraction from organisms grown in the presence of cetoleic acid was enriched with cetoleyl residues to the extent of only 21.5%, a low value which precluded these organisms being used to study the effect of fatty-acyl chain length on the kinetics of amino acid accumulation. In organisms grown in the presence of palmitoleic or oleic acid, phosphatidylcholine and phosphatidylethanolamine, two lipids which account for the bulk of the phospholipids in *S. cerevisiae* NCYC 366 (Hunter & Rose, 1972), were enriched to approximately 50% of the total with residues of the fatty acid supplied in the medium (Table 2). Neutral lipids from organisms grown in the presence of either palmitoleic or oleic acid were also extensively enriched in residues of the fatty acid exogenously supplied (Table 2). Organisms grew at approximately the same rate in media containing either palmitoleic or oleic acid, although the duration of the lag phase of growth was somewhat extended in cultures supplemented with the former acid. From a Woolf-Hofstee plot for accumulation of L-lysine by the high-affinity system in organisms enriched in palmitoleyl residues (Fig. 4), K_T and V_{max} values were 0.5×10^{-5} M and 1.6 nmol (mg dry wt) $^{-1}$ min $^{-1}$, respectively.

DISCUSSION

A major finding from this study was that the kinetics of solute transport, as judged from K_T and $V_{\max}$ values, were altered in only two of the eight systems studied, when organisms enriched in oleyl residues were compared with those enriched in linoleyl residues. There are two possible explanations for identical or very similar K_T and $V_{\max}$ values for accumulation of solutes by organisms with plasma membranes enriched in oleyl rather than linoleyl residues. The proteins that make up these transport systems may be totally unaffected by the degree of unsaturation of C_{18} residues on phospholipids that surround them in the membrane. Alternatively, one or more of the proteins in the transport system may specifically attract phospholipid molecules that contain neither oleyl nor linoleyl residues. If so, the fatty-acyl residues on these phospholipids must be present in organisms enriched in either oleyl or linoleyl residues. The only major fatty-acyl residue common to phospholipids from organisms grown anaerobically in the presence of oleic or linoleic acid is $C_{16:0}$, which accounts for 25–30% of the total residues (Thomas *et al.*, 1978). The very similar activation energies for accumulation of L-asparagine by organisms enriched in either oleyl or linoleyl residues indicated that the transport systems operate with equal efficiency in both types of organism, being largely unaffected by the change in fatty-acyl enrichment. The small but significant increase in transition temperature when oleyl residues were replaced by linoleyl residues suggests that, in organisms enriched in the doubly unsaturated residue, one or more of the proteins in the asparagine transport system are surrounded by phospholipids with a higher melting point compared with organisms enriched in oleyl residues. This can be explained not by assuming that the transport system has a higher affinity for C_{16} residues in membranes enriched in linoleyl residues, but rather that it has a lower affinity for $C_{18:2}$ than for $C_{18:1}$ residues, thereby increasing the chance of proteins in the uptake system being surrounded by C_{16} residues.

The changes to the K_T and $V_{\max}$ values for transport of L-lysine, by both high- and low-affinity systems, caused by replacing oleyl by linoleyl residues, suggest that, in the two types of organism, different lipid molecules surround the proteins that go to make up the transport system. With the high-affinity system in oleyl-enriched organisms, the absence of a discontinuity between 5 °C and 30 °C suggests that over this temperature range there was no change in the state of the lipid molecules that surround proteins in the transport system. However, with organisms enriched in linoleyl residues, there was a change from a negative to a positive slope over the same temperature range. The change to a positive slope indicates a decreasing rate of L-lysine transport with increasing temperature. Assuming that the properties of the proteins that constitute the high-affinity lysine uptake system are not changed when oleyl residues in plasma-membrane phospholipids are replaced by linoleyl residues, it must be concluded that the instability in the uptake system in linoleyl-enriched organisms is attributable to the change in phospholipid composition. Implicit in this view is that the lipid annulus surrounding the proteins is made up of phospholipids containing a high proportion of the fatty-acyl residues that constituted the enrichment. Since the $V_{\max}$ value for the high-affinity system was greater in oleyl- or palmitoleyl-enriched organisms, while the activation energy was much lower in oleyl- as compared with linoleyl-enriched organisms, it would seem that the presence of increased fatty-acyl mobility, and therefore less tight packing in the interior of the plasma membrane, retards the rate at which lysine is moved across the membrane. On the other hand, it is clear that the nature of the fatty-acyl enrichment has a more specific effect on the affinity of the high-affinity transport system for lysine, the affinity being lower in linoleyl-enriched and higher in palmitoleyl-enriched organisms, compared with those enriched in oleyl residues.

Any attempt to explain further the changes in K_T and $V_{\max}$ values for uptake of lysine by the high-affinity system, following switches in the fatty-acyl unsaturation, are complicated by three further considerations. The first of these is that lysine, when taken up by *S. cerevisiae*, is accumulated in vacuoles (Wiemken & Dürr, 1974). It follows that, to understand the reason for changes in the kinetic values, studies will need to be made on lysine accumulation by isolated vacuoles from organisms enriched in each of the two types of residue. The second complication arises from the likelihood that the lysine uptake system is made up of several proteins, with the possibility that only certain of these proteins are affected by the fatty-acyl composition of

surrounding phospholipids. Evidence for the possible existence of this complexity in the lysine uptake system comes from studies on uptake by *S. cerevisiae* of the closely related amino acid arginine, where periplasmically-located proteins with a high affinity for arginine as well as membrane-bound transport proteins (Opekarova *et al.*, 1975) are involved, together with an ATPase that generates a proton gradient (Eddy, 1982). It would seem unlikely that the ATPase activity is influenced by the fatty-acyl composition of the plasma membrane, since, if one assumes that there is not a multiplicity of specific ATPases, this would have been reflected in an effect on K_T and V_{max} values for transport of asparagine and glutamine. Finally, sterols might affect the kinetics of transport systems. Enriching the plasma membrane with specific fatty-acyl residues may cause changes in the location of sterol molecules and of proteins in transport systems, thereby causing a change in K_T and V_{max} values.

Little has been reported on the stability of membrane-bound solute-transport systems in micro-organisms. The decline in arginine-transporting ability in organisms enriched in linoleyl residues might be attributable to the high fluidity in the plasma membrane in these organisms, which in turn could lead to a loss of membrane-bound proteins. Some evidence for this phenomenon comes from the finding that spheroplasts from organisms enriched in linoleyl residues leaked protein faster than spheroplasts with membranes enriched in oleyl residues (D. M. Belk & A. H. Rose, unpublished observations). Also supporting this explanation is the finding that α -mannosyl-transferase activities of plasma membrane preparations were more variable when the membranes were enriched in linoleyl rather than oleyl residues (L. J. Douglas, unpublished observations). It is clear, nevertheless, that plasma-membrane instability in organisms enriched in linoleyl residues has minimal physiological effects, since organisms enriched with linoleyl or oleyl residues grew at the same rate.

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