Journal of Cellular Biochemistry 20:369-380 (1982)

Influence of Growth Conditions on the Composition of the Plasma Membrane From Yeast and on Kinetic Properties of Two Membrane Functions

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The influence of different growth conditions on the phospholipid composition and on two membrane functions, the Mg-ATPase and the purine transport system, was investigated. Addition of cholinechloride to the growth medium led to a certain rise in the amount of phosphatidylcholine, whereas supplementation with ethanolamine resulted in a considerably higher portion of phosphatidylethanolamine. When yeast cells were cultured at lower temperatures we found more short-chain fatty acids with a higher content of monounsaturated chains as compared to higher growth temperatures. Addition of paraquat, a herbicide which enhances lipid peroxidation by free radicals, reduced the amount of unsaturated fatty acids without influencing their chain length.

The altered membrane composition had no influence on the basic mechanism of interaction between ATPase, MgATP, and free Mg^{2+} ions. However, several kinetic constants such as K_m, V_{max}, K_a, and especially K_i were influenced to some extent. Whereas the affinity of the purine transport system to its substrate was not significantly changed by the growth conditions, an effect on V_{max} could be seen. Lower growth temperatures clearly led to higher maximal uptake velocities. The presence of paraquat during growth resulted in a considerable decrease of V_{max}.

Key words: plasma membrane, yeast, phospholipids, fatty acids, Mg^{2+}-ATPase, purine transport system, growth conditions, paraquat

The physicochemical properties of cell membranes are mainly affected by their composition of phospholipids, fatty acid chains, and sterols. The chain length and the degree of unsaturation of the fatty acids especially determine membrane fluidity. Changes in membrane composition during several biological processes, such as development and aging, have been reported (for review see Grinna [1]).

Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid.

Received September 18, 1981; revised and accepted October 22, 1982.

Using microorganisms, changes in the composition of the membranes can be induced by altering the environmental conditions during growth [2–9]. The pattern usually observed is that a decrease of the growth temperature corresponds with an increase in the proportions of unsaturated and/or short-chain fatty acids [10–12]. Changes in the fatty acid composition are thought to be required to maintain membrane fluidity at a proper level, which is compatible with cellular growth and function [10,12,13].

Studies on procaryotic microorganisms like Acholeplasma laidlawii [3, 14–16], E coli [17] or Bacillus megaterium [18] have shown that environmental changes influence membrane composition considerably. However there is still some discussion concerning the respective effects on eucaryotic microorganisms. Hunter and Rose [9] found little change in either the fatty acid composition or the degree of unsaturation of the fatty acids from Saccharomyces cerevisiae following a change in incubation temperature. Okuyama et al [2], however, report observations that S cerevisiae cells produce considerably more C16 acids than C18 ones, shortly after a shift in growth temperature from 35°C to 10°C. In contrast Chavant et al [19], investigating four different fungi, found no correlation between cold-temperature acclimatization, increase in the fatty acid unsaturation, and an increased lipid fluidity.

Because of these differences in the results obtained from eucaryotic microorganisms, we investigated the influence of growth temperature and of supplementation of the medium with cholinechloride or ethanolamine as well as paraquat, which enhances lipid peroxidation [20], on the composition of the plasma membrane from yeast. Moreover we investigated the respective influence on the fatty acid composition of distinct phospholipids in the membrane.

There are few reports on the functional consequences of the observed structural changes of plasma membranes, although there are some papers concerning the action of lipids on the activity of membrane-bound enzymes [21–23, for review see 24] or transport systems [15]. Cunningham and Sinthusek [25], for example, found a linear relationship between the increase of negative charge of liposomes with increased activity of mitochondrial ATPase. Studies on interactions of the ATPase with phospholipids indicate that the stimulatory activity of the phosphoglycerides depends on the number of fatty acids per molecule, the degree of unsaturation in the fatty acids, and the ionic properties of the phospholipids [26].

These results suggest that phospholipids are not simply a matrix, in which the ATPase resides, but that they can influence directly the function of the enzyme complex by modulating its catalytic properties.

Most of these structure-function investigations have been performed using artificial liposomes. To study the functional enzymes in their natural lipid environment we altered the composition of the yeast plasma membrane by growth under different environmental conditions. As functional consequence of changes in the membrane structure, we not only determined the enzyme activity, but also examined the influence of the membrane alterations on the kinetic constants and on the mechanism of substrate binding and splitting at the active site.

As examples of membrane functions we chose the plasma membrane ATPase and the purine transport system, since a profound kinetic characterization of both functions has been performed previously [27,28] and since we observed an influence of membrane fluidity on it [29,30].

MATERIALS AND METHODS

Reagents

ATP was a product of Boehringer (Mannheim, Germany). N-Tris(hydroxymethyl)-methylglycine (Tricin), and the phospholipids as well as the fatty acids used for reference in the chromatographic procedures were obtained from Serva (Heidelberg, Germany). Paraquat (methyl viologen) and $MgCl_2$ were supplied by Sigma (München, Germany). 8-^{14}C-labeled hypoxanthine (54 Ci/mol) was purchased from NEN (Dreieich, Germany), and the unlabeled hypoxanthine was obtained from Pharma Waldhof (Düsseldorf, Germany). The TLC-plates (silica gel 60, thickness 0.25 mm) and all other reagents were products of Merck (Darmstadt, Germany).

Growth Conditions

Saccharomyces cerevisiae (strain R XII, a kind gift of Dr Kotyk, Prague) was grown under aerobic conditions, either in a medium containing 2% glucose, 1% Difco yeast extract, and 5% Merck-peptone or in a synthetic medium containing per liter: 20 g glucose, 20 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 500 mg $MgSO_4 \cdot 7\ H_2O$, 100 mg NaCl, 100 mg $CaCl_2 \cdot 2\ H_2O$, 500 μg H_3BO_3, 470 μg $MnSO_4 \cdot 4\ H_2O$, 400 μg $ZnSO_4 \cdot 7\ H_2O$, 200 μg $FeCl_3 \cdot 6\ H_2O$, 120 μg $(NH_4)_2Mo_7O_{24} \cdot 4\ H_2O$, 100 μg KJ, 40 μg $CuSO_4 \cdot 5\ H_2O$, 10 mg nicotinic acid, 10 mg meso-inositol, 1 mg Ca-pantothenate, 500 μg thiamin-HCl, 500 μg pyridoxine-HCl, 20 μg biotin.

The synthetic medium was supplemented either with 1 mM cholinechloride or ethanolamine. Growth temperature was 10°C, 30°C, or 40°C. The cells were normally harvested in the logarithmic phase ($3–5 \times 10^7$ cells/ml); in some cases, however, in the stationary phase.

To test the influence of paraquat, 5 mM of this herbicide was added for the last two generations (2.5 hr) of growth.

Plasma membranes were prepared by osmotic shock and differential centrifugation after converting the cells into spheroplasts as has been described earlier [27]. The procedure up to the osmotic shock treatment was performed at the same temperature at which the cells were grown.

Measurement of ATPase Activity and Kinetic Analysis

The ATPase activity was determined by continuously recording the amount of inorganic phosphate released as described earlier [31–33]. The assay contained varying amounts of ATP and $MgCl_2$, 50 mM Tricin, pH 7.5, 80 mM KCl, and 10–40 μg protein in a total volume of 5 ml. The concentrations of substrate (MgATP) and free Mg^{2+} ions were calculated from the total concentrations of ATP and $MgCl_2$ using dissociation constants of $K_{MgATP}=0.215$ mM at 30°C or 0.245 mM at 20°C [29].

The derivation of the reaction mechanism and of the rate equation was performed on the basis of a rapid equilibrium reaction as described earlier [27,31]. The kinetic constants were calculated from the reciprocal plots, by means of the direct linear plot [34] or from a computer program using the least-squares fit to the Michaelis-Menten equation [35] assuming a constant relative error [27].

All values used for kinetic characterization are averages of at least five individual experiments performed in duplicate. The constants were obtained from the extrapolation of the variable constants from the primary plots against the second

parameter. Since additionally the individual preparations showed some differences, a relative large error of 40% for each kinetic constant must be taken into account.

Uptake of Purine Bases

For the uptake experiments the yeast cells were pretreated for maximum activity as already described [28]. The measurements were performed with 8-^{14}C-hypoxanthine as substrate for reasons discussed earlier [28]. The incubation temperature was 15°C or 35°C. The constants K_m and V_{max} were obtained by both the direct linear plot [34] and from a computer program using the least-squares fit to the Michaelis-Menten equation [35]. All values used for kinetic characterization are averages of at least four individual experiments. The uptake experiments with cells grown in the presence of paraquat have been performed in the absence of paraquat during the glucose-pretreatment and the incubation. Previous experiments had shown no significant differences of the obtained kinetic constants if paraquat was present or absent during the measurement.

Determination of Phospholipid Composition

The plasma membranes were freeze-dried and the phospholipids were extracted with chloroform/methanol=2/1 (vol/vol). The determination of the individual phospholipids was performed by quantitative thin-layer chromatography according to the method of Peter and Wolf [36] modified by Weinmann [37].

The single phospholipid-spots were scraped off the TLC-plates, resuspended, and the content of inorganic phosphate was determined using the method of Bartlett [38] modified by Sokoloff et al [39]. From these data the composition was calculated in mole-percent. To obtain the total amount of phosphate in the plasma membranes, the phosphate-determination was performed in parallel to the one just described immediately after extraction, without separation on TLC.

Analysis of Fatty Acids

For the determination of the fatty acids two distinct procedures were employed. The first consists of direct transesterification of the extract of freeze-dried plasma membranes, the second consists of separating the phospholipids quantitatively on TLC, scraping off every spot, and then transesterificating the redissolved phospholipids. The method of Weinmann [37] was modified, in so far as no saponification was made, because the obtained results were qualitatively equal. For the gaschromatographic analysis a Packard model 433 was used. The temperature program was performed on 1/4 in × 7-ft glass columns packed with Silar 10C on Gaschrom Q (100–120 mesh) as supplied by W.G.A. (Griesheim, Germany). Injectors and flame ionization detectors were at 250°C; the oven heated up from 150°C with an initial delay time of 2 min to 220°C with a rising rate of 4°C/min, holding the final temperature for 10 min. The gas flows were 18 ml/min H_2, 40 ml/min N_2, and 250 ml/min O_2.

The percentage of single fatty acids was obtained by integrating the respective peak area.

Determination of Protein Concentration

Protein was determined by the method of Schaffner and Weissmann [40] using bovine serum albumin as standard.

RESULTS

The effects of different growth conditions on membrane composition were studied parallel to the effects on two membrane functions, the Mg-ATPase and the purine transport system.

Influence of Growth Conditions on Membrane Composition

Figure 1 shows the phospholipid composition of the plasma membranes from yeast grown at different conditions as well as the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE). It can be seen that the membranes are composed mainly of phosphatidylcholine followed by phosphatidylinositol (PI)-phosphatidylserine (PS) and phosphatidylethanolamine. Lysocompounds could not be detected and phosphatidic acid (PA) was present only in small amounts.

Comparing the plasma membranes obtained from different growth conditions, one recognizes that the relative amount of PC is independent of growth temperature, but is increased by the addition of cholinechloride. In contrast the amount of PE is significantly less if cholinechloride is added during growth. Supplementation of the medium with ethanolamine had no significant effect on the phospholipid composition of the plasma membranes. The amount of PI/PS is enhanced to a certain extent when the growth temperature is raised.

Figure 2 shows the composition of the fatty acyl chains of the total plasma membrane phospholipids from cells grown under standard conditions and of the individual phospholipids.

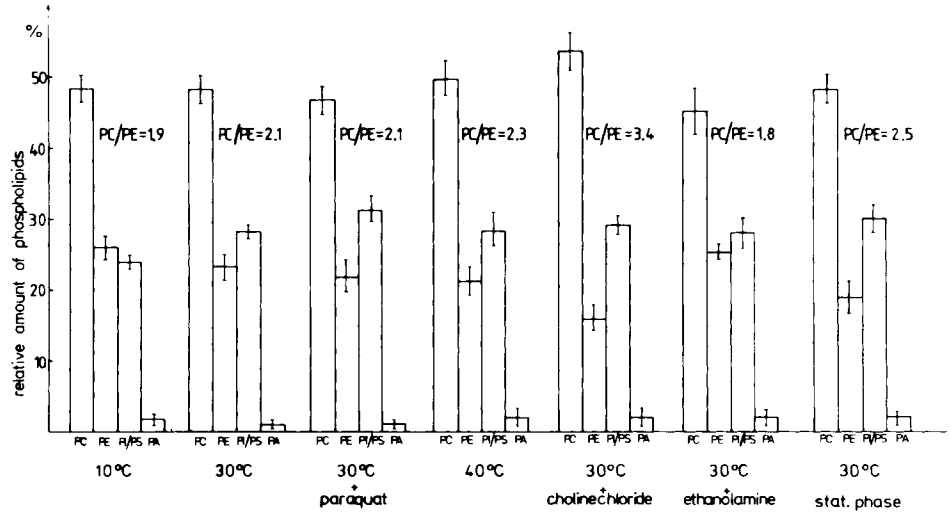

Fig. 1. Phospholipid composition of plasma membranes from cells grown under different conditions. The composition is expressed as percentage of total lipid phosphorus. The values are means ± SD of at least six individual membrane preparations. The ratio of PC/PE is given for every growth condition. PC = phosphatidylcholine, PE = phosphatidylethanolamine, PA = phosphatidic acid, PI/PS = sum of phosphatidylinositol and phosphatidylserine, mainly PI [43]. It was not possible to separate PI from PS with the one-dimensional TLC used.

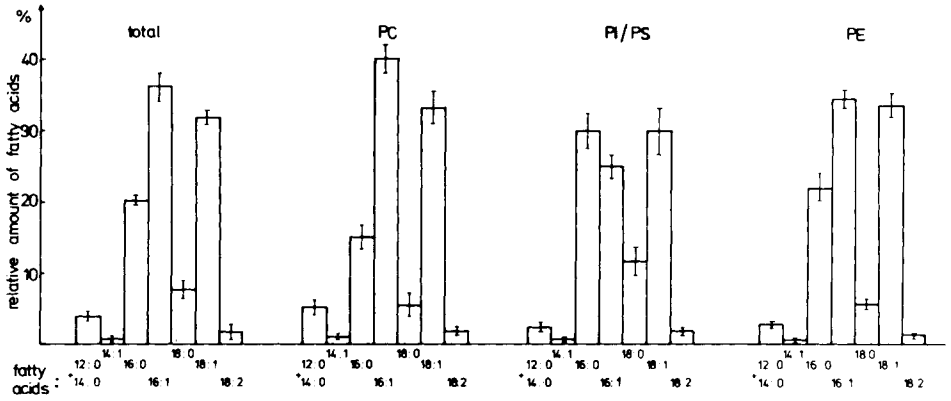

Fig. 2. Fatty acid composition of single phospholipids and of the extract of the plasma membrane obtained from cells grown at 30°C. Values are means±SD in mole% of at least ten individual preparations. Each determination was performed twice, one directly from the lipid extract (total), the other after separation of the phospholipids on TLC as described in methods. The numbers before the colon represent the carbon atoms of the fatty acids, the numbers after the colon indicate their double bonds.

It can be seen that the main fatty acids are the monounsaturated ones. We observed the following order: 16:1 > 18:1 > 16:0 > 18:0, as well as minor parts of 14:0, 18:2, 14:1, and 12:0. Interestingly, the individual phospholipids possess a rather distinct spectrum of fatty acyl chains.

Among the fatty acids found in PC there is a greater percentage of small (C12, C14) and especially unsaturated ones. On the other hand PI/PS possess larger amounts of saturated fatty acids and smaller amounts of unsaturated and short-chain ones. In PE a fatty acid spectrum is found which represents more or less that of the total membrane.

As the individual phospholipids possess a distinct fatty acid composition (Fig. 2) and as the spectrum of the phospholipids depends on growth conditions (Fig. 1) we expected some effect on the fatty acyl chains as well.

In Table I, the chain length distribution (C18/C16) and the degree of unsaturation of the total amount of fatty acids in the plasma membrane are summarized. The data are obtained from plasma membranes of cells grown under various conditions. It can be seen that with increasing growth temperature the saturation of the fatty acids increases. Addition of paraquat during the last two cell divisions leads to similar results as obtained with cells grown at 40°C. The growth phase had only a small effect on the results obtained. A comparison of the degree of unsaturation of short (C16) and long (C18) chain fatty acids indicates that an increase in temperature from 10°C to 30°C results mainly in a decrease of the number of double bonds in the short acyl chains, whereas at higher temperatures both chains are hydrated to a similar extent. The presence of paraquat leads mainly to a decrease of unsaturation of the C18 chains. The chain length distribution is influenced only slightly by different growth conditions.

Furthermore in Table 1 data obtained from individual phospholipids are given in relation to growth conditions. One can see that under most conditions PI/PS possess a higher amount of C18 fatty acids, which are most saturated, compared with those

TABLE I. Fatty Acid Composition of Whole Plasma Membranes (WPM) and of Their Individual Phospholipids*

Growth conditions	Phospholipid	Chain length distribution C18 (%) / C16 (%)	Degree of unsaturation C16:1 / C16:0	Degree of unsaturation C18:1+18:2 / C18:0	Total
	PC	0.5	4.4	5.5	4.6
10°C	PI/PS	0.8	0.5	2.6	1.3
	PE	0.6	2.2	6.5	3.7
WPM		0.6	2.7	4.8	3.4
	PC	0.7	2.6	6.3	3.7
30°C	PI/PS	0.8	0.8	2.2	1.3
	PE	0.7	1.5	6.0	3.2
WPM		0.7	1.8	5.0	2.9
30°C	PC	0.7	3.0	6.0	4.0
stationary	PI/PS	1.1	0.8	3.5	1.6
phase	PE	0.7	1.8	9.0	3.5
WPM		0.8	2.0	5.6	3.1
30°C	PC	0.6	2.0	4.4	2.8
+5 mM	PI/PS	0.8	0.8	2.1	1.4
paraquat	PE	1.1	2.1	3.4	2.6
WPM		0.75	1.6	3.4	2.2
	PC	0.6	1.7	4.6	2.6
40°C	PI/PS	0.8	0.8	2.9	1.6
	PE	0.8	1.7	3.6	2.1
WPM		0.65	1.4	3.7	2.1
30°C	PC	0.6	2.4	5.7	3.5
+ 1 mM	PI/PS	1.0	0.6	2.7	1.6
choline-chloride	PE	0.7	1.8	7.0	3.8
WPM		0.7	1.7	4.9	2.9
30°C	PC	0.7	3.5	5.6	4.1
+ 1 mM	PI/PS	1.0	0.5	2.5	1.4
ethanol-amine	PE	0.5	1.3	4.2	2.1
WPM		0.7	1.9	4.0	2.6

*Lipid extracts of whole cells were directly used for fatty acid analysis, individual phospholipids were determined after separation on TLC. Chain length distribution was obtained by dividing the sum of stearic, oleic, and linoleic acids by the sum of palmitic and palmitoleic acids. The degree of unsaturation of the fatty acids is shown separately for the C16 and C18 acids. The ratio of the sum of all unsaturated fatty acids divided by the sum of all saturated ones is listed in the last column (total). Values are averages of at least six determinations, no value differing from any other by more than 15%.

from PC and PE. These data are almost independent of growth conditions. On the other hand fatty acids of phosphatidylcholine usually show the highest degree of unsaturation.

These results are similar to those given in Figure 2 for cells grown at 30°C. The influence of growth temperature on the C18 and C16 chains, respectively, in the individual phospholipids do not differ much from the values found for the total plasma membranes. Generally the degree of unsaturation is greater in C18 than in C16 as

seen in Figure 2 and Table I. Obviously, cells grown in the presence of cholinechloride possess a relative high amount of unsaturated acyl chains in C18 compared with the unsaturation at C16. In this case the relation of the degrees of unsaturation is even higher than cells grown at 10°C.

Influence on Kinetic Properties of the Plasma Membrane ATPase

The question arises whether the observed compositional changes are accompanied by functional alterations. Of all functions of the membrane usually only enzyme activity is investigated. However, as the activity is the function of several kinetic constants, an investigation of, for example, growth conditions on these constants and on the mechanism of the reaction between enzyme, substrate, and effector yields more insight into protein-lipid interactions.

As an example of a membrane function, we chose the Mg^{2+}-ATPase, since this enzyme is an integral part of the plasma membrane. We varied MgATP at several constant concentrations of free Mg^{2+} ions, within a concentration range, in which these ions lead to an activation, as well as within a range in which they inhibit the ATPase. The measurements were performed at 20°C and also at 30°C since a transition of the plasma membrane from the gel to the liquid-crystalline phase occurs between these two temperatures. This transition influences the kinetic properties of the ATPase [29].

We found that the growth conditions had no influence on the reaction mechanism described recently [29]. A random activation mechanism and a pseudocompetitive inhibition was observed in all preparations (Fig. 3).

Moreover, several kinetic constants of these reactions are not altered significantly (Table II), although some of these constants are changed by the growth conditions. Most significant are the effects on K_i respectively K_i'. Cells grown at

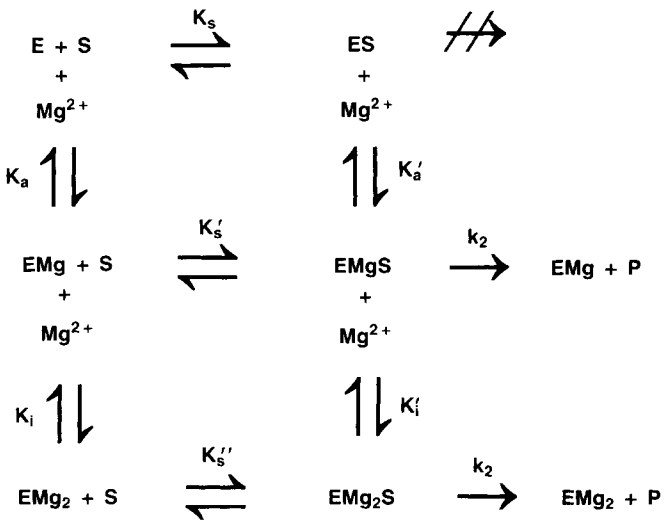

Figure 3.

TABLE II. Influence of Growth Conditions on Kinetic Constants of the Plasma Membrane ATPase From Yeast*

	Kinetic constants											
	$K_a = K_a'$(mM)		$K_s = K_s'$(mM)		K_s''(mM)		K_i(mM)		K_i'(mM)		V_{max}(U/mg)	
Growth conditions	30°C	20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C	20°C
10°C	0.06	0.09	0.04	0.05	0.54	0.45	0.2	0.5	2.5	4.9	2.1	1.0
30°C	0.04	0.06	0.07	0.06	0.50	0.49	0.4	0.9	2.8	7.0	2.3	1.1
40°C	0.03	0.04	0.06	0.05	0.50	0.52	0.6	1.0	5.4	9.8	3.4	1.7
30°C stationary	0.04	0.08	0.05	0.06	0.45	0.47	0.8	1.4	6.9	10.4	1.2	0.6
30°C + ethanolamine	0.04	0.04	0.04	0.05	0.50	0.52	0.3	0.7	3.6	6.9	1.4	0.6
30°C + cholinechloride	0.03	0.05	0.10	0.07	0.59	0.51	0.3	1.0	1.8	5.5	1.5	0.7
30°C + paraquat	0.02	0.04	0.05	0.04	0.47	0.36	>1	>1	>10	>10	1.3	0.6

*The kinetic constants of activation and inhibition by Mg^{2+} ions are derived according to Figure 3. The values are obtained from reciprocal and secondary plots of initial rate data. They are averages of at least five individual experiments performed in duplicate. The relative error is maximal 40%.

40°C or those harvested in the stationary phase lead to considerably higher dissociation constants compared with the results obtained with plasma membranes from yeast grown at lower temperatures. The presence of paraquat during the last two cell divisions reduces the affinity of the enzyme for inhibitory Mg^{2+} ions even more, so that up to a concentration of 10 mM only a slight inhibition could be observed.

From Table II it can be seen that the other alterations of kinetic constants with different growth conditions are of less significance. K_a of cells grown at 10°C are the highest, K_s of cells grown in the presence of ethanolamine and V_{max} of cells grown at higher temperatures seem to be greater than these constants at lower temperatures.

When the experiments were performed at 20°C we obtained in essence the same dependencies on the growth conditions as at 30°C; however, K_a and especially K_i as well as K_i' are greater and V_{max} smaller at 20°C as has been discussed recently [29]. No significant temperature influence could be detected on constants representing substrate binding.

Influence of Growth Conditions on the Purine Transport System

As a second well-defined membrane function [28], the purine transport system was used to test the influence of growth conditions on the plasma membrane. As is shown in Table III, the affinity constants vary between the two incubation temperatures of 15°C and 35°C, indicating a temperature dependency which was found to be linear over the whole range of temperatures examined (0°–40°C) [41]. The growth conditions have only slight effects on K_m.

Cells grown at 10°C show nearly twice the maximal uptake velocity V_{max} at both incubation temperatures as compared to control cultures grown at 30°C. This effect seems to be greater at 15°C than at 35°C. The cells grown in the presence of paraquat demonstrate a significant reduction of V_{max}, indicating an immobilization of the transport system as a consequence of the higher content of saturated fatty acids. The other growth conditions have only slight effects.

TABLE III. Kinetic Constants of the Purine Transport System With Hypoxanthine as Substrate*

Growth conditions	$K_m(\mu M)$		$V_{max}\left(\frac{10^{-18}\ mol}{cell \times min}\right)$	
	15°C	35°C	15°C	35°C
30°C standard	0.38 ± 0.04	2.3 ± 0.4	7.4 ± 1.1	100 ± 15
30°C + paraquat	0.71 ± 0.26	3.0 ± 0.6	0.5 ± 0.2	11 ± 2
30°C + cholinechloride	0.47 ± 0.1	3.2 ± 0.3	9.1 ± 3.5	203 ± 50
30°C + ethanolamine	0.65 ± 0.05	3.3 ± 0.2	10.5 ± 1.2	126 ± 14
10°C	0.62 ± 0.1	4.1 ± 0.6	19.0 ± 3.0	191 ± 11
40°C	0.44 ± 0.07	2.8 ± 0.5	5.6 ± 1.2	107 ± 11

*Incubation temperature was 15°C or 35°C. Values are means ± SD of at least four individual measurements. They were obtained from reciprocal plots and from a computer program using the least-squares fit to the Michaelis-Menten equation.

DISCUSSION

In contrast to other microorganisms such as Acholeplasma laidlawii [3,14] the composition of plasma membranes from Saccharomyces cerevisiae changes less with growth conditions.

However, Figure 1 demonstrates that the hydrophilic head groups of the phospholipids can be influenced for the most part by growth in the presence of an excess of ethanolamine and especially of cholinechloride. Hossack et al [7] describe an influence through the same supplementation of the growth medium on the ratio of PC/PE which is somewhat greater than those values reported in Figure 1. But the phospholipid composition we found corresponds largely with data of Hunter and Rose [9] with the exception that the amount of phosphatidylcholine is larger in our preparations. The differences between our results and those of Hunter and Rose [9] may be explained by the fact that they investigated whole cells, whereas we studied isolated plasma membranes. This assumption is supported by investigations of Hossack et al [7] reporting significant differences between phospholipid analysis of whole cells and membranes.

The growth temperature has only a minor effect on phospholipid composition resulting in an enhancement of phosphatidylinositol-phosphatidylserine at higher growth temperatures and a decrease in phosphatidylethanolamine. However, the growth temperature influences the degree of unsaturation considerably. Table I demonstrates an increase in unsaturated fatty acids when cells were grown at 10°C. Paraquat, a herbicide, whose presence in several organisms is reported to lead to an increased concentration of free radicals giving rise to lipid peroxidation [20] has, as expected, no influence on chain length but reduces the content of double bonds.

From Figure 2 and Table I it can be seen that the individual phospholipids possess a distinct fatty acid pattern. Thus an effect on phospholipid composition due to growth conditions should also result in alterations of the acyl chains. Table I demonstrates that cells grown in the presence of cholinechloride compared with those grown in the presence of ethanolamine not only exhibit a higher phosphatidylcholine level, but also a higher degree of unsaturation due to the general observation that phosphatidylcholine carries more unsaturated fatty acids. However, there is an addi-

tional effect found exclusively in the fatty acids. Again from Table I it can be seen that an enhancement of growth temperature or the addition of paraquat lead to an increase in the degree of saturation with no response seen in the polar head groups of the phospholipids. Moreover, the change in the number of double bonds at different growth temperatures is not equally distributed among the different acyl chains and individual phospholipids. The results of the analysis of fatty acid composition are similar to those reported by Wilson and McLeod [42] for yeast cells. Furthermore, the degree of unsaturation of C16 as well as C18 acyl chains corresponds well with data given by Hunter and Rose [9], who, however, only observed a slight influence of growth temperature on their results.

The protein content of the plasma membranes did not vary significantly with different growth conditions. However, since we did not determine the protein pattern, we cannot give information regarding possible alterations of protein composition in response to the growth conditions applied.

As the growth conditions applied resulted only in limited compositional changes, one should not expect great effects on membrane functions. However, the microenvironment of the membrane proteins may be different from the total composition of the membrane. Our examinations on kinetic properties of the Mg-ATPase (Table II) show that the affinity of the enzyme for the activator Mg^{2+} is enhanced when the yeast cells were grown at higher temperatures. The growth temperature has an even more significant effect on K_i and K_i', respectively, whereby higher temperatures lead to a reduced affinity of Mg^{2+} ions at the inhibitory site of the ATPase. These results on activation and inhibition lead to a broader Mg^{2+} optimum, suggesting that higher growth temperatures may be advantageous for the cells. However, a regulation of enzyme activity by varying Mg^{2+} concentrations is reduced, which may be, on the other hand, disadvantageous.

These effects of growth temperatures on ATPase function correspond with the decrease in double bonds of the fatty acids (Table I). This is supported by the results from paraquat-treated cells, showing a reduced amount of unsaturation and, on the other hand, smaller K_a and larger K_i values. The effects of growth temperature on inhibition of the ATPase by Mg^{2+} ions correspond with the predicted effect of membrane fluidity on the same constants, which has been discussed recently [29] and which can also be seen from Table II. Lower temperatures of measurement, ie, decreased fluidity, have the same effect as higher growth temperature or addition of paraquat, which decrease the amount of double bonds and thus reduce fluidity.

Therefore we assume that the effector sites of the ATPase are influenced by the hydrophobic surrounding. A similar effect on the substrate-binding site (K_s and K_s'' values of Table II) cannot be recognized. Possibly this binding site is located mainly in the hydrophilic moiety of the polar head groups of the phospholipids and is influenced by their composition. The differences in K_s between ATPase from cells grown in the presence of cholinechloride or ethanolamine suggest such an explanation.

Interestingly, the effects of growth conditions respectively membrane composition on another function of the same membrane—the purine transport system—show some comparable results, eg, lower V_{max} of cells grown in the presence of paraquat. In both cases the Arrhenius plots of log V_{max} versus 1/T show inflection points at about 24°C [30]. At the same temperature calorimetric measurements indicate structural alterations of the plasma membrane [29]. Therefore an influence of the membrane structure on the functions investigated seems probable.

However, as the effects observed are not very large, greater alterations in membrane composition by using auxotrophic mutants or inhibitors of fatty acid synthesis are necessary to obtain more significant effects.

ACKNOWLEDGMENTS

We thank Mrs Eva Nehls for technical assistance. This work was supported by a research grant of the Deutsche Forschungsgemeinschaft.

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