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LIPID COMPOSITION AND LECTIN-MEDIATED AGGLUTINATION OF *CANDIDA KEFYR* CELLS GROWN IN MEDIA ENRICHED WITH CHOLINE OR ITS ANALOGUES

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Candida kefyr cells and their spheroplasts grown in media enriched with choline, *N,N'*-dimethylethanolamine (DMEA) or ethanolamine (EA) showed decreased concanavalin A (Con A) and ph tohemagglutinin (PHA) mediated agglutination while supplementation with *N*-monomethylethanolamine (MMEA) increased PHA-mediated agglutination. In all cases, the amount of phospholipid was increased and, consequently, a decrease in the free sterol:phospholipid ratio was observed except in the case of MMEA where this ratio remained almost unchanged. In the cells grown in media enriched with choline, DMEA or MMEA, but not in the EA-supplemented medium, the phosphatidylcholine to phosphatidylethanolamine ratio was increased. Saturation of fatty acids as well as their chain length decreased, which could lead to increased membrane fluidity. No breakpoint in Arrhenius plots of Mg^{2+}-ATPase of the choline-supplemented cells was observed. Cells grown in media enriched with EA and MMEA did not show any sharp break in Arrhenius plots of Mg^{2+}-ATPase.

Over the last decade, membrane biologists have been working to explain the correlation between lipid composition and the structure and function of particular membranes. Transport of various substances and osmotic fragility of yeast cells have been correlated with altered membrane lipid composition [1]. Schroeder [2] studied Con-A[1]-mediated haemadsorption of LM fibroblasts with altered lipid composition Yeast cells are very useful organisms whose lipid composition can be manipulated by simple means [3].

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[1] Abbreviations used: Con A, concanavalin A; PHA, phytohaemagglutinin; EA, ethanolamine; MMEA, *N*-monomethylethanolamine; DMEA, *N,N'*-dimethylethanolamine.

India. Con A, PHA and ATP disodium salt were purchased from CSIR correlated with lectin-mediated agglutination.

MATERIAL AND METHODS

N-Monomethylethanolamine[1], *N,N'*-dimethylethanolamine and phospholipid standards were purchased from Sigma Chemical Co., U.S.A. Choline, ethanolamine and silica gel H were from Sarabhai Chemicals, Baroda, India. Con A, PHA and ATP disodium salt were purchased from CSIR Centre for Biochemicals V. P. Chest Institute. New Delhi. All other chemicals of the purest grade available were purchased from local commercial sources. *Candida kefyr* and *Streptomyces* cultures were obtained from Microbiology Laboratory of the School of Life Sciences, Guru Nanak Dev University, Amritsar.

Growth of Candida kefyr. Yeast cells were grown in 100 ml Erlenmeyer flasks containing 20 ml of YEPG complete growth medium [4] composed of: 10.0 g glucose, 5.0 g peptone and 2.5 g yeast extract per litre. Choline or one of its analogues was added to the growth medium at a concentration of 20 mg/litre before autoclaving. The cells at the early logarithmic phase of growth were harvested by centrifugation at 10000 *g* for 15 min. The pellet obtained was washed with distilled water and then with phosphate saline buffer (0.02 M, pH 6.0, containing 0.6 M KCl).

Spheroplasts were prepared according to Villenueva & Gascon [5] using strepzyme prepared from *Streptomyces* species for digestion of the cell wall.

Agglutination assay. The assay for agglutination was performed according to the method of Gokhale & Mehta [6] with slight modifications: 0.5 ml of cell or spheroplast suspension (4.8×10^8 cells/ml) was mixed with an equal volume of lectin solution (200 μg/ml) and incubated at 30°C for 30 min. The percentage of agglutination was determined h emocytometrically by counting the number of free and clumped cells using the formula

$$100 - \left(100 \times \frac{Number\ of\ nonagglutinated\ cells}{Total\ number\ of\ cells} \right)$$

An aggregate of at least five cells was considered a clump.

Extraction and analysis of lipids. Lipids were extracted by the method of Folch *et al.* [7]. Phosphorus content was measured according to Ames [8] and the amount of phospholipids was calculated by multiplying the phosphorus content by 25. Individual phospholipids were separated by thin-layer chromatography. The slurry of silica gel H was prepared in 4% $(NH_4)_2SO_4$ solution. The solvent system for separation of individual phospholipids was chloroform/methanol/ammonia (28%) (14:11:2, by vol.). Various phospholipids were identified by comparing the R_F values with their standards as well as by specific spray reagents [9]. Free and esterified sterols in

the total lipid fraction were estimated according to the method of Sperry & Webb [10]. Fatty acid analysis was done by gas-liquid chromatography. Methyl esters were prepared by the method of Luddy *et al.* [11] and analyzed using NUCON Engineers gas chromatograph fitted with a flame ionisation detector, on a glass column containing 15% diethylene glycol succinate on 80 - 100 mesh chromosorb-W. The peaks were identified by comparison of their retention times with those of standard fatty acid methyl esters. Percentage of fatty acids was computed on the basis of peak area by an automatic data processor.

Assay of Mg^{2+}-ATPase activity. Mg^{2+}-ATPase activity of microsomal fraction (crude plasma membrane) was determined according to the method of Lundborg *et al.* [12].

Statistical analysis. Statistical significance of the differences between mean values was evaluated using Student's *t* test.

RESULTS AND DISCUSSION

Data pertaining to lectin-mediated agglutination of yeast cells grown in the presence of choline or its analogue, and of their spheroplasts, are presented in Table 1. Supplementation with choline or any of its analogues: *N N'*-dimethylethanolamine, *N*-monomethylethanolamine or ethanolamine, decreased the percentage of Con A-mediated agglutination of the cells as well as of their spheroplasts. Cells grown in the MMEA-supplemented medium were agglutinated by Con A to the extent of 20% as compared with 80% of control cells. The cells cultured in the choline-, DMEA- or EA-supplemented medium showed 35, 30 and 45% Con A-induced agglutination respectively. Con A-mediated agglutination of cells grown in the choline or any of its analogues, enriched medium was smaller as compared with the agglutination of spheroplasts prepared from the respective cells. Contrary to these results is the twofold increase in Con A-mediated haemadsorption of LM cells grown in the presence of choline or its analogues [2]. The PHA-induced agglutination of yeast cells grown in the MMEA-enriched medium and of their spheroplasts was greater than of control cells. Cells grown in the choline- or EA-enriched medium showed no PHA-mediated agglutination at all.

To understand the mechanism by which choline or its analogues present in the growth media reduced the extent of agglutination of *C. kefyr* cells as well as of their spheroplasts, the lipid composition of yeast cells was studied (Table 2). The total lipid content of the cells grown in the EA-supplemented medium increased to 6.61%, as compared with 5.15% in nonsupplemented cells. Sterol content in total lipids decreased from 2.91% in normal cells to 2.25% and 1.98% in cells grown in choline and EA, respectively. Although the phospholipid content determined on dry weight

Table 1

Effect of choline and its analogues added to the growth medium on the lectin-mediated agglutination of C. kefyr cells and their spheroplasts

Concentration of each lectin used was 100 μg/ml. Values are means ± S.D. for five different sets of determinations. Statistical significance with respect to the control was in all cases $P < 0.01$.

Supplementation (20 mg/litre)	Agglutination (%)			
	Concanavalin A		Phytohaemagglutinin	
	Cells	Sphero-plasts	Cells	Sphero-plasts
Choline	35±3.0	45±4.6	0	0
N,N'-Dimethylethanolamine	30±2.5	40±3.5	10±1.0	15±1.8
N-Monomethylethanolamine	20±2.5	40±4.5	30±2.5	40±3.2
Ethanolamine	45±5.0	55±4.9	0	0
Control	80±6.2	95±9.0	15±1.6	25±2.2

Table 2

Effect of choline and its analogues on the lipid composition of C. kefyr cells

Values are means ± S.D. for five different sets of determinations

Supplementation (20 mg/litre)	Total lipids (% of dry weight)	Sterols (% of total lipids)	Phospholipids		Free sterol to phospholipid molar ratio
			% of dry weight	% of total lipids	
Choline	4.92±0.7	2.25±0.3**	0.36±0.02*	13.37±1.2*	0.19
N,N'-Dimethyl-ethanolamine	5.84±0.8	2.64±0.4	0.43±0.01*	13.57±1.4*	0.23
N-Monomethyl-ethanolamine	5.02±0.6	5.00±0.6*	0.71±0.02*	14.16±1.7*	0.35
Ethanolamine	6.61±0.5*	1.98±0.2*	0.43±0.02*	15.10±1.5*	0.15
Control	5.15±0.6	2.91±0.3	0.51±0.02	10.00±1.0	0.34

* $P < 0.01$;
** $P < 0.05$.

basis decreased when cells were grown in choline. DMEA or EA, it increased markedly when determined on total lipid basis Supplementation with MMEA increased the level of sterol and phospholipid significantly. These results are similar to those of Ratcliffe *et al.* [13] who reported that phospholipid content of *S. cerevisiae* increased when the growth medium was supplemented with choline of EA. The increase in phospholipid content was reported to be due to enhanced phosphatidylcholine and phosphatidylethanolamine synthesis. As a result of increased phospholipid content of yeast cells grown in the medium supplemented with choline or its analogue, the free sterol to phospholipid ratio decreased in the lipids of those cells. A similar decrease in the sterol to phospholipid ratio of *Neurospora crassa* cultured in the choline-supplemented medium has been reported by Johnston *et al.* [14]. Free sterol and phospholipids are almost exclusively located in the plasma membrane [15, 16] and their ratio is known to affect the physical state of the membrane [17].

In addition to changes in total lipid content, changes in phospholipid composition of the base-supplemented cells were also studied (Table 3). Supplementation with choline increased the phosphatidylcholine content from 29.36 to 43.24% of total phospholipids while the contents of phosphatidylethanolamine and phosphatidylinositol remained unaffected. However, phosphatidylserine content decreased from 22.00 to 8.52%. It should be noted that an increase in the level of phosphatidylcholine in *S. cerevisiae* on addition of choline into its growth medium has been reported earlier [11, 18, 19]. The unchanged phosphatidylethanolamine level (Table 3) indicate that probably phosphatidylcholine is not synthesized by methylation of phosphatidylethanolamine. Moreover, in yeast, methylation of phosphatidylethanolamine to phosphatidylcholine has been shown to be repressed in the presence of choline [20]. Therefore, the increase in phosphatidylcholine content may be a consequence of base exchange reactions in phospholipids. The level of phosphatidylethanolamine increased significantly when EA was added to the growth medium. Similar observations have been reported for *S. cerevisiae* [11, 19] and *Microsporum gypseum* [18]. The higher content of phosphatidylethanolamine might be the result of the base exchange reaction. DMEA supplementation increased phosphatidylcholine content from 29.36 to 38.68% of total phospholipids. The amount of phosphatidylethanolamine remained unchanged while the content of phosphatidylserine decreased. Upon supplementation of the growth medium with MMEA the content of phosphatidylcholine and phosphatidylinositol in *C. kefyr* increased from 29.36 to 34.61% and from 17.47 to 23.00%, respectively. The amount of phosphatidylethanolamine and phosphatidylserine decreased from 29.11 to 22.23% and from 22.00 to 16.53%, respectively. As a result of the relative increase in phosphatidylcholine levels of *C. kefyr* grown in the choline- or its analogue-enriched media, the phosphatidyl-

Table 3

Effect of choline and its analogues added to the growth medium on the content of individual phospholipids in C. kefyr cells

PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; LPL, lysophospholipids. Trace amounts of cardiolipin were also detected. Results are means ± S.D. for five different sets of experiments

Supplementation (20 mg/litre)	Phospholipids (% of total)					PC:PE ratio
	PE	PC	PI	PS	LPL	
Choline	29.73±3.2	43.24±4.0*	18.48±1.6	8.52±0.9*	0.80±0.07**	1.45
N,N'-Dimethylethanolamine	30.15±4.0	38.68±3.4*	15.07±1.5	15.10±1.3*	1.00±0.01**	1.30
N-Monomethylethanolamine	22.23±2.3*	34.61±3.5**	23.00±1.9*	16.53±1.5*	3.63±0.04**	1.56
Ethanolamine	40.55±4.4*	32.97±2.9	9.18±1.0*	15.18±1.0*	1.02±0.08**	0.81
Control	29.11±3.0	29.35±3.6	17.43±1.4	22.00±2.0	2.00±0.16	1.00

* $P < 0.01$;
** $P < 0.05$.

choline to phosphatidylethanolamine ratio increased, except in the EA-supplemented cells where this ratio decreased. The contents of zwitterionic phospholipids, particularly phosphatidylcholine and phosphatidylethanolamine, have been shown to alter drastically the binding of Con A to liposomes containing glycolipids [21].

Changes in fatty acids composition of the phospholipid fraction of *C. kefyr* cultured in media supplemented with choline and its analogues were also studied (Table 4). The major fatty acids were 16:0, 16:1, 18:1, 18:2 and 18:3, while 12:0, 14:0, 18:0 and 22 1 were present in small amounts. As iudged from the decrease in the saturated to unsaturated fatty acid ratio, the overall unsaturation of fatty acids increased upon addition of choline or its analogues to the growth medium. The lower chain to higher chain fatty acid ratio also decreased. Both these factors show that choline and its analogues produce an increase in the membrane fluidity.

The physical state of the cellular membranes from *C. kefyr* cells grown in base analogue-supplemented media was compared with that of the membranes from normal cells by studying Arrhenius kinetics of microsomal Mg^{2+}-ATPase (Fig. 1) [17]. Microsomal Mg^{2+}-ATPase from yeast cells grown in nonsupplemented medium showed a break in its Arrhenius plots at 17°C while yeast cells grown in the choline-supplemented medium did

Table 4

Effect of choline and its analogues on the fatty acid profile of C. kefyr phospholipids

S, saturated fatty acid; US, unsaturated fatty acid; L, lower chain fatty acids up to C_{16}; H, higher chain fatty acid C_{18}; –, not present. Values are means ± S.D. for five different sets of determinations

Supplementation (20 mg/litre)	Fatty acids (%)									S:US ratio	L:H ratio
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	22:1		
Choline	1.35 ±0.12	1.61 ±0.13*	20.14 ±2.1	8.03 ±1.0	2.13 ±0.21*	32.14 ±3.4	20.29 ±2.1	10.36 ±1.0**	3.09 ±0.3	0.34	0.45
N,N'-Dimethylethanolamine	1.21 ±0.10**	2.72 ±0.23*	16.77 ±2.0**	9.00 ±0.9	3.69 ±0.33	31.73 ±2.9	17.78 ±2.0	15.14 ±1.6*	1.36 ±0.2	0.32	0.42
N-Monomethylethanolamine	1.00 ±0.09*	1.00 ±0.08*	12.66 ±1.3*	10.19 ±1.0**	2.00 ±0.22*	37.41 ±3.6	27.15 ±2.6*	7.52 ±0.9	1.00 ±0.1	0.22	0.36
Ethanolamine	2.50 ±0.20*	1.99 ±0.20*	14.46 ±1.5*	7.73 ±0.8	1.63 ±0.15*	33.81 ±3.5	17.99 ±2.0	17.99 ±1.8*	1.42 ±0.13	0.24	0.34
Control	1.45 ±0.12	3.57 ±0.34	21.42 ±2.1	8.13 ±0.9	3.78 ±0.36	34.75 ±4.0	17.94 ±1.9	8.32 ±0.9	–	0.44	0.52

* $P < 0.01$;
** $P < 0.05$.

not show any break. Probably, the membranes of choline-supplemented cells were more fluid and the phase transition temperature (T_t) might have fallen below 10°C. In the case of cells grown in the DMEA-enriched medium, the breakpoint in Arrhenius plot decreased to 12°C as compared

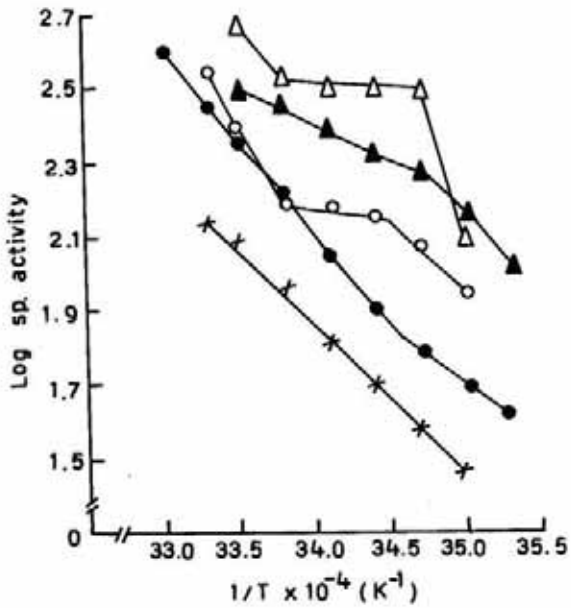

Fig. 1. Arrhenius plots of microsomal Mg^{2+}-ATPase of *Candida kefyr*. Specific activity: nmol P_i released/30 min per μg protein. Symbols: ▲, Choline; ○, ethanolamine; △, *N*-monomethylethanolamine; ●, *N,N'*-dimethylethanolamine; ×, control.

to 17°C for the control. This indicates that T_t of these membrane lipids was lowered. The Arrhenius plots in the case of MMEA and EA did not show any sharp phase transition point but the transition occurred over a wide range of temperature from 12° to 22.5°C and 15° to 22°C, respectively. The changes in physical state of yeast membranes as indicated by Arrhenius kinetics might be responsible for the observed change in cell surface phenomenon, i.e. lectin-mediated agglutination.

Alterations in membrane lipid composition of *C. kefyr* caused by supplementation of the growth medium with choline or its analogues might be responsible for changes in the lectin-mediated agglutination of these cells. Greater PHA-mediated agglutination of cells/spheroplasts cultured in MMEA might be due to exposure of more PHA receptors on the cell surface. However, membrane fluidity is not the only factor affecting lectin-mediated

agglutination. If it were so, then it should have affected lectin (PHA and Con A) mediated agglutination in a similar manner.

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