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THE RELATIONSHIPS BETWEEN GROWTH TEMPERATURE, FATTY ACID COMPOSITION AND THE PHYSICAL STATE AND FLUIDITY OF MEMBRANE LIPIDS IN *YERSINIA ENTEROCOLITICA*

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Summary

The relationship between membrane lipid composition and membrane lipid phase transitions was investigated in *Yersinia enterocolitica* cells grown at 5, 22 and 37°C. The total phospholipid concentrations were 9.4, 7.3 and 6.3% of the cell dry weight for cells grown at 5, 22 and 37°C, respectively. The relative concentrations of the three major phospholipids, phosphatidylethanolamine (73—76%), phosphatidylglycerol (9—11%) and cardiolipin (11—13%) were essentially the same at all three growth temperatures. The ratios of unsaturated to saturated fatty acids were 2.2, 1.1 and 0.4 for cells grown at 5, 22 and 37°C, respectively. This change in the fatty acid composition in response to temperature changes is similar to the patterns reported for other organisms. Reversible thermotropic phase transitions were detected by calorimetric analysis in both pure lipid preparations and membrane preparations. The mid-points of the thermotropic phase transitions were at —13, —9 and 1°C for membranes from cells grown at 5, 22 and 37°C, respectively. The phase transitions of the membranes from cells grown at the three different temperatures occurred below the lowest growth temperature (5°C). The alternations in the fatty acid composition in *Y. enterocolitica* did not, therefore, appear to be required to adjust membrane fluidity but might rather be required for some other membrane function.

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Introduction

It has been previously shown that the physical state and fluidity of the membrane lipids of an organism are directly related to the fatty acid composition which in turn is regulated by the growth temperature [1—4]. The pattern usually observed is that as the growth temperature is increased, a greater proportion of long-chain saturated fatty acids is incorporated into the membrane lipid with a corresponding decrease in the proportions of unsaturated, branched chain and/or saturated short chain fatty acids [3,5—9]. Changes in the fatty acid composition are thought to be required to maintain the membrane lipids at the proper degree of fluidity which is compatible with cellular growth and function [2,3,8—11].

Membrane fluidity can be measured by differential scanning calorimetry [11—13]. Using this technique, it has been shown that membranes undergo a reversible thermotropic gel-to-liquid-crystalline phase transition similar to that shown by synthetic phospholipid bilayer preparations. This phase transition arises from a cooperative melting of the hydrocarbon chains in the interior of the lipid bilayer. Since the lipid phase transition is dependent on the fatty acid acyl groups of the membrane lipids, changes in the fatty acid composition would result in shifting the temperature range of the transition. This shift is assumed to be required to minimize the effect of temperature changes on the fluidity and physical state of the membrane lipid. *Yersinia enterocolitica* is an enteric organism with an unusually broad growth temperature range. The strain used in this study will grow at 4°C and, if it is first acclimated by growth at 37°C, as high as 45°C. In this paper, we report that although the ratios of unsaturated to saturated fatty acids changes in a typical pattern, this did not appear to be required to change the phase transition temperature for growth at lower temperatures.

Methods and Materials

Organism and growth conditions. *Y. enterocolitica* strain TN 559582 was grown in Trypticase Soy Broth (BBL Microbiology Systems, Cockeysville, MD) at 5, 22 and 37°C. Cells were harvested at mid-log phase ($2 \cdot 10^9$ cells/ml) by centrifugation and washed twice with buffer containing 0.1 mM EDTA and 10 mM Tris-HCl (pH 7.8).

Membrane preparations. Cell membranes were prepared exactly as described by Kaback [14] and stored under N_2 at —20°C until used. Examination of the membrane preparations by phase-contrast microscopy showed no whole cells or unlysed protoplasts.

Lipid extraction and analysis. The lipids were extracted by a slight modification [15] of the procedure of Bligh and Dyer [16]. The phospholipids were separated by two-dimensional chromatography on Whatman SG-81 silica gel-loaded paper with the following solvents. Solvent A, chloroform/methanol/diisobutylketone/acetic acid/water (23 : 10 : 45 : 25 : 4, v/v); solvent B, chloroform/methanol/diisobutylketone/pyridine/0.5 M NH_4Cl (pH 9.9) (60 : 35 : 50 : 70 : 12, v/v). Solvent A was used in the first dimension and solvent B in the second dimension. The phospholipids were located with a

molybdate spray for lipid phosphorus. Spots were cut from the chromatogram, digested with 70% perchloric acid and then analyzed for phosphorus as described previously [15].

Fatty acid methyl esters were obtained by transesterification with H_2SO_4 in anhydrous methanol as described by Read and McElhaney [17]. The methyl esters were analyzed by gas-liquid chromatography on a 6 ft × 1/8 inch stainless-steel column coated with 3% SE-30 in a Varian Aerograph Gas-Liquid Chromatograph 1800 series equipped with a flame-ionization detector. The relative area percentages were determined by a Hewlett Packard 3380 A Integrator. The fatty acid methyl esters were identified by comparing their retention times to those of known standards and their identities confirmed by coinjections of standards and unknown samples.

Differential scanning calorimetry (DSC). Total phospholipids were dried under vacuum and the redissolved in one to two drops of benzene. The benzene was then removed under a stream of N_2. The dried lipids were resuspended in water or in 50% ethylene glycol in water and dispersed by sonication (Branson Model W-350). The lipid vesicles were sedimented by centrifugation at 176 000 × *g* (Beckman Ls-65B) at 4°C for 3 h. The pellets were removed and placed into pre-weighted DSC sample pans and weighed using a Perkin-Elmer Autobalance Model AD-2. Water or 50% ethylene glycol in water was added to the pellets and the pans were sealed using a Perkin-Elmer sealing press.

Calorimetric analyses were carried out using a Dupont 990 Thermal Analyser, equipped with a DSC cell. Thermograms were obtained at a scan rate of 5° per min and a full-scale sensitivity of 1 mcal · s^{-1}. Samples scanned above freezing were suspended in distilled water or 0.1 M potassium phosphate buffer, pH 6.6, against a reference pan containing the same suspending solution. Samples scanned below the freezing-point of water were suspended in 50% ethylene glycol in water and 50% ethylene glycol was used in the reference pans.

Results and Discussion

Phospholipid composition

The major phospholipid groups of *Y. enterocolitica* were phosphatidylethanolamine, phosphatidylglycerol and cardiolipin. Small amounts of lysophosphatidylethanolamine and lysophosphatidylglycerol were also present (Table I). If EDTA was not included in the buffer used to wash the cells there was a marked increase in the amount of lysophosphatidylethanolamine and a corresponding decrease in the amount of phosphatidylethanolamine recovered in the lipid extract (data not shown). This suggested that at least a portion of the lysophospholipids was formed during the extraction procedure. The total amount of phospholipid per g dry weight of cells was higher at the lower growth temperatures. The relative concentrations of the different phospholipids, however, were essentially the same at all three growth temperatures (Table I). The adjustment of *Y. enterocolitica* to growth at different temperatures involved a change in the total lipid concentration but did not appear to require a change in the relative phospholipid concentrations.

TABLE I

PHOSPHOLIPIDS OF *YERSINIA ENTEROCOLITICA*

All cultures were harvested at a cell density between 0.7 and 0.9 mg dry wt. of cells per ml of culture (mid log-phase of growth). The cells were washed immediately with 10 mM Tris-HCl buffer (pH 7.8) containing 0.1 mM EDTA. Total lipid expressed as percentage of cell dry weight. Phospholipid composition expressed as percentage of total lipid phosphorus. PG, phosphatidylglycerol; CL, cardiolipin; PE, phosphatidylethanolamine; LPG, lysophosphatidylglycerol; LPE, lysophosphatidylethanolamine. All values represent the average ±S.D. of four determinations.

Growth temperature (°C)	Total lipid (%)	Percentage phosholipid composition			
		PG	CL	PE	LPG + LPE
5	9.4 ± 2.3	10.6 ± 0.6	13.2 ± 1.4	72.7 ± 6.4	3.4 ± 0.8
22	7.3 ± 0.4	9.0 ± 0.3	13.0 ± 0.9	72.9 ± 1.7	5.2 ± 3.4
37	6.3 ± 0.6	11.6 ± 0.6	11.4 ± 0.1	76.4 ± 0.9	1.0 ± 0.7

Fatty acid composition

The fatty acid compositions of cells grown at 5, 22 and 37°C are listed in Table II. The major fatty acids found in this organism were C16:1, C16:0, C17:0 and C18:1. No branched or cyclopropane side-chain fatty acids were detected. The following changes in the fatty acid composition were observed. At 5°C, the unsaturated fatty acids (C16:1 and C18:1) predominated; whereas at 22°C, there was a decline in these fatty acids coupled with an increase in the fatty acids from C12:0 to C15:0. The major fatty acids in the cells grown at 37°C were the saturated fatty acids C16:0 and C17:0. The changes in the ratio of unsaturated to saturated fatty acids in response to temperature changes were similar to the pattern which have been reported for other organisms [2,3,18].

TABLE II

FATTY ACID COMPOSITION OF THE TOTAL MEMBRANE LIPID

The analysis of fatty acid methyl esters was carried out using a Varian Aerograph gas-liquid chromatograph series 1800 equipped with a flame-ionization detector at programmed temperature rates from 90 to 240°C and using a stainless-steel column, coated with 3% SE-30. USFA/SFA, the ratio of the sum of unsaturated fatty acids divided by the sum of saturated fatty acids. The number before the colon represents the number of carbon atoms, the number after the colon represents the number of double bonds. Values represent the average ±S.D. of four determinations. tr, trace.

Fatty acid methyl esters	Temperature of growth (°C)		
	5	22	37
Unidentified	tr	tr	tr
12 : 0	2.2 ± 0.1	6.9 ± 1.4	3.9 ± 0.3
13 : 0	1.7 ± 0.1	4.3 ± 0.9	2.4 ± 0.1
14 : 0	2.2 ± 0.5	4.7 ± 0.9	4.2 ± 0.4
15 : 0	0.9 ± 0.3	2.7 ± 0.6	2.0 ± 0.3
16 : 1	43.3 ± 0.7	29.8 ± 3.6	15.2 ± 0.3
16 : 0	21.1 ± 0.5	21.4 ± 2.6	34.7 ± 2.1
17 : 0	1.8 ± 0.2	4.4 ± 1.1	24.0 ± 0.7
18 : 1	25.9 ± 0.3	21.5 ± 0.1	11.2 ± 0.9
19 : 0	0.8 ± 0.6	4.4 ± 1.5	2.2 ± 0.4
USFA/SFA	2.2	1.1	0.4

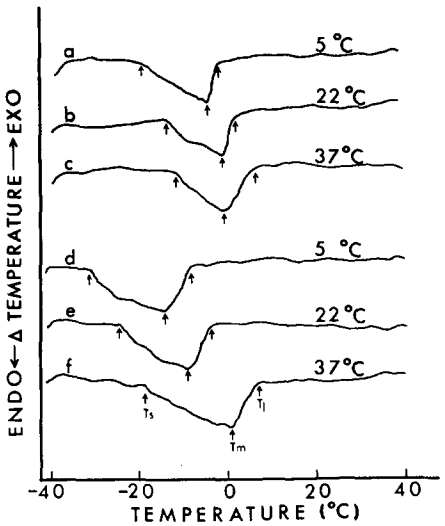

Fig. 1. Temperature-base thermograms of lipid preparations (a—c) and membrane preparations (d—f) of *Yersinia enterocolitica* grown at 5°C (a, d), 22°C (b, e) and 37°C (c, f). The lower boundary (T_s), transition midpoint (T_m) and upper boundary (T_l) of the phase transitions are denoted by the arrows. Second scans of the same preparations were essentially the same except that the entire transitions were shifted by 1—2°C to lower temperatures. The mass of the samples scanned ranged from 2.7 to 6.9 mg. All samples scanned were suspended in a 50% ethylene glycol/water mixture. Scans were conducted at a heating rate of 5°C/min and full-scale sensitivity of 1 mcal · s^{-1}.

It has been suggested that such changes are required to maintain the proper degree of membrane fluidity at different temperatures [3,19].

Lipid phase transitions

Representative reversible endotherms obtained by DSC scans of extracted lipid and membrane preparations are shown in Fig. 1. These reversible endotherms were interpreted as lipid phase transitions which have been demonstrated by a number of workers to represent the cooperative melting of the fatty acid acyl chains within lipid bilayers [2,13,20,21].

The transitions detected with the lipid preparations (Fig. 1a—c) occurred over a narrower range than the corresponding membrane preparations (Fig. 1d—f). The upper and lower boundaries of the transitions are listed in Table III.

TABLE III

LOWER AND UPPER PHASE TRANSITION BOUNDARIES

Values are from the scans shown in Fig. 1. Scans of four preparations showed a variation of less than 2°C in the transition boundaries.

Growth temperature (°C)	Membranes		Lipids	
	Lower (T_s)	Upper (T_l)	Lower (T_s)	Upper (T_l)
5	—29	—8	—18	0
22	—24	—4	—12	4
37	—18	8	—10	8

The midpoints of the transitions for the lipid preparations extracted from cells grown at 5, 22 and 37°C were at −3, 1 and 1°C, respectively. The midpoints of the transitions for the membrane preparations were at −13, −9 and 1°C for membranes from cells grown at 5, 22 and 37°C, respectively. DSC scans of four different preparations showed a variation of less than 2°C in the transition boundaries. Similar scans of lipid preparations obtained from *Escherichia coli* grown at 37°C showed transitions between 18 and 38°C with a T_m values of 30°C.

The midpoints (T_m) of the transitions in the cell membrane preparations (−13 and −9°C) from the cells grown at 5 and 22°C were lower than the midpoints of the corresponding lipid preparations (−3 and 1°C). The midpoints were the same (1°C), however, in the membrane and lipid preparations from the cells grown at 37°C. In studies with model systems, lipid-protein interactions have been shown to lower T_m and broaden the lipid phase transition [3,11,20–23]. In line with this view, our results suggested that the hydrophobic lipid-protein interactions in *Y. enterocolitica* grown at 5 and 22°C were more pronounced (or involved a greater percentage of the total membrane lipid) than in the cells grown at 37°C. This observation was unexpected considering the fact that cells grown at the lower temperatures contained more total lipid than cells grown at 37°C (Table I). Estimates of the amount of the membrane lipid which is involved in the phase transition (and therefore in the bilayer arrangement) in other organisms range from 70 to 90% of the total membrane lipid [2,20,21,23,24]. It will be of interest to determine if the lipid protein interactions in *Y. enterocolitica* are altered either by changes in the membrane lipid or protein in response to changes in the growth temperature.

Changes in the fatty acid composition have been shown to shift the temperature at which the phase transition occurs so that growth is observed at a temperature that coincides with or is higher than the temperature of the midpoint (T_m) of the transition [1,2,3,25–27]. Membranes from *Y. enterocolitica* grown at 37 and 22°C had transition temperatures well below 22 and 5°C, respectively (Fig. 1). Therefore, a change in fatty acid composition would not appear to be required to maintain the fluid state when cells were shifted from 37 to 22°C or from 22 to 5°C. This would seem to suggest that the changes observed in the fatty acids of membrane lipids of *Y. enterocolitica* in response to a change in the growth temperature may have a different role than maintaining the liquid-crystalline state.

The response of *Y. enterocolitica* to shifts in the growth temperature may give some clue as to the purpose of the fatty acid changes. When cultures were grown at 37°C and then shifted to 45°C, good growth was observed. Cultures grown at 22°C, however, failed to grow when shifted directly to 45°C. In contrast, growth was observed at 5°C regardless of the previous growth temperature. When cells were shifted from a higher to a lower temperature, they were able to make the necessary adjustments for growth at the lower temperature. On the other hand, cells grown at 22°C apparently could not survive long enough after the shift to 45°C to make the adjustments necessary for growth at 45°C. McElhaney and his coworkers [17,28] have shown that membrane stability and membrane permeability are markedly influenced by the fatty acid composition. A lack of stability or excessive permeability at 45°C, of the cells

grown at 22°C, might account for their failure to survive when shifted directly to 45°C. Membrane transport activity and the activity of a number of membrane associated enzymes have also been shown to be influenced by the fatty acid composition [6,10,13,17,27—33].

In general, organisms grow at a temperature that lies within the range of the transition or at a temperature that coincides with its upper end, but the phase transition may be detected at a considerably lower temperature than the growth temperature [3,13,21,27,33]. The upper boundary of the phase transition T_1) does not define the maximum growth temperature; however, growth ceases at a point well above the lower boundary (T_s) of the phase transition [3]. Cronan and his coworkers [19,25] have noted that the upper boundaries of the membrane lipid phase transitions of *E. coli* grown at 25 and 37°C were lower than the growth temperature by more than 10°C. They have suggested that this might be an environmental adaptation which would protect cells from sudden decreases in temperature. *Y. enterocolitica* is widely distributed in nature and grows well at temperatures below 5°C. The low phase transition temperatures of *Y. enterocolitica* might represent an adaptation to growth at low temperatures.

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