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Fatty Acid Positional Distribution in Phospholipids of a Psychrophilic Bacterium during Changes in Growth Temperature

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Abstract. The major phospholipids of the psychrophilic bacterium *Micrococcus cryophilus*, phosphatidylethanolamine and phosphatidylglycerol, have similar fatty acid compositions, comprising almost entirely palmitoleic and oleic acids. We show that there is a preference for the longer chains in the *sn*-1 position of both phosphatidylethanolamine and phosphatidylglycerol, both during isothermal growth and after temperature shifts, despite the fact that the overall phospholipid C18/C16 acyl chain ratio decreases with a lowering of growth temperature. Although it has been shown using model systems that the isomeric configuration *sn*-1-long, *sn*-2-short lowers lipid melting temperature, this paper reports the first clear-cut demonstration of such an isomeric preference in a natural system. We discuss how this acyl chain configuration contributes to membrane fluidity in *M. cryophilus*, in terms of adaptation to its psychrophilic habitat.

Besides the well-documented changes in unsaturation, bacteria may also adapt to changes in growth temperature by altering fatty acyl chain length [16]. A particularly striking example is the psychrophilic bacterium *Micrococcus cryophilus*, which alters four-fold the ratio of its two main fatty acids, palmitoleic and oleic, in response to growth temperature changes [14, 16]. Experiments with model systems have shown that the acyl chain positional distribution is also important in lipid fluidity, the *sn*-1-long, *sn*-2-short isomer having the lower melting temperature [5, 10]. We show clearly for the first time in a natural system that such an isomeric distribution contributes to membrane fluidity in *M. cryophilus*, both during isothermal growth and after temperature shifts. These results are discussed in terms of the adaptation of this organism to its psychrophilic habitat.

Materials and Methods

The growth of *Micrococcus cryophilus* ATCC 15174, the methods for acclimating cultures to a particular temperature, and carrying out temperature shift experiments are described in Sandercock and Russell [20]. Lipids were extracted and phospholipids isolated as detailed in Foot et al. [7]. Phospholipids were quantitated either by phosphate analysis [3] or by gas–liquid chromatographic analysis of the constituent fatty acids [19] using arachidic acid as the internal standard. The *sn*-1/*sn*-2 distribution of phospholipid acyl chains was determined by phospholipase digestion as described by Christie [4], using phospholipase A_2 from *Naja Naja* venom (Sigma [London] Chemical Co. Ltd., Poole, Dorset, England). Control experiments in which the rates of distearoyl-phosphatidylcholine and dipalmitoyl-phosphatidylcholine were compared showed that the phospholipase A_2 had a preference for C18 acyl chains over C16 acyl chains; during a 60-min incubation there was 1.59 ± 0.01 times more stearate released compared with palmitate. All values of the C18/C16 ratio presented in the paper have been corrected for this enzymatic preference. After separation by thin-layer chromatography, the lipid products were (trans)methylated using 2.5% (wt/vol) H_2SO_4 in dry methanol; the resulting fatty acid methyl esters were identified and quantitated by gas–liquid chromatography [19].

Results

The major phospholipids in *Micrococcus cryophilus* are phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin [15], and they do not vary with growth temperature (data not shown). At any one growth temperature, the C18/C16 acyl chain ratio of phosphatidylethanolamine was always greater than that of cardiolipin, which was approximately equal to that of phosphatidylglycerol (Table 1).

When the growth temperature was shifted up (0° to 20°C) or down (20° to 0°C), the phospholipid acyl chain length increased or decreased, respec-

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Table 1. The C18/C16 acyl chain ratio of phospholipids from *Micrococcus cryophilus* grown isothermally at various temperatures[a]

Bacterial growth temperature (°C)	Phospholipid	C18/C16 ratio of acyl chains		
		Combined	*sn*-1	*sn*-2
1	Total	1.17	1.77	0.86
	PE[b]	1.33	1.58	0.85
	PG	0.91	n.d.	n.d.
	CL	0.98	n.d.	n.d.
10	Total	1.80	2.79	1.07
	PE	2.94	4.15	1.54
	PG	1.31	2.43	1.03
	CL	1.38	n.d.	n.d.
20	Total	3.65	4.94	2.87
	PE	4.99	7.42	4.16
	PG	3.91	4.93	2.87
	CL	3.99	n.d.	n.d.

[a] Cultures were grown isothermally at the temperature indicated and the phospholipids isolated during late exponential phase. The individual phospholipid classes were isolated by thin-layer chromatography and their fatty acid compositions determined by gas–liquid chromatography. The acyl chain length ratio (C18/C16) is the sum of the areas of C18:0 + C18:1 peaks divided by the sum of the areas of the C16:0 + C16:1 peaks [20].

[b] n.d., not determined; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin.

tively (Figs. 1 and 2). There was a lag period of approximately one generation time before the acyl chain ratio changed in the shift-up experiments, although the shift-up and shift-down experiments were reciprocal in the sense that they reached equivalent C18/C16 ratios within the same overall period of four generation times (Figs. 1 and 2).

There was consistently a greater proportion of oleoyl acyl chains in the *sn*-1 position than in the *sn*-2 position of the phospholipid from bacteria grown at one particular temperature (Table 1). This preference was seen in both major phospholipids, phosphatidylethanolamine and phosphatidylglycerol (Table 1). Furthermore, the preference for oleoyl acyl chains in the *sn*-1 position was maintained after a temperature shift-down, even though the overall fatty acid composition changed from an excess of oleate at 20°C to a near equivalence of oleate and palmitoleate at 0°C (Fig. 3). The same was true for a shift-up (data not shown).

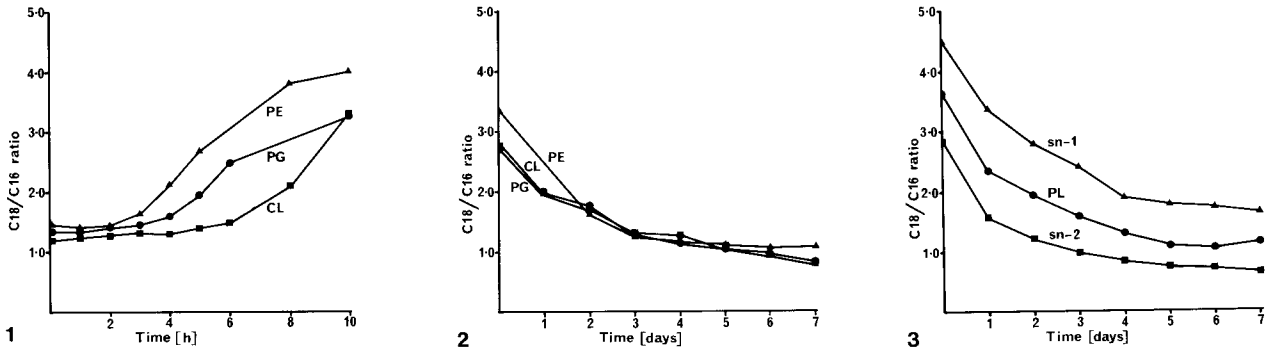
Discussion

The lack of change in phospholipid head-group composition with growth temperature indicates that the enzymes of phospholipid synthesis in *Micrococcus cryophilus* do not have differential temperature sensitivities. In contrast there was a difference in the acyl chain composition of phosphatidylethanolamine on the one hand and phosphatidylglycerol and cardiolipin on the other at all growth temperatures; this could originate in the acyl chain specificity of phosphatidylserine synthetase and/or phosphatidylglycerol-phosphate synthetase. It cannot arise from selective acyl chain turnover, because there is none during isothermal growth in *M. cryophilus* (L. McGibbon, unpublished results). In view of this fact, it was expected that phosphatidylglycerol and cardiolipin would have the same acyl composition, because cardiolipin is derived directly from phosphatidylglycerol [13].

Although the magnitude of the changes in the C18/C16 ratio in the shift-up and shift-down experiments were reciprocal in that they reached equivalent C18/C16 ratios, there was a clear difference in their time-courses. The reason for the lag in the shift-up but not the shift-down experiments is unclear. The acyl chain length change in *M. cryophilus* is brought about by a membrane-bound elongation/retroconversion enzyme system that is temperature sensitive [20]. Experiments using antibiotics to inhibit protein synthesis, to test whether enzyme induction is involved, are presently inconclusive (L. McGibbon, unpublished results). Alternatively the effects of a shift-down may be more "stressful" in that membrane fluidity decreases and activates the adaptation mechanism involving retroconversion of C18 to C16 acyl chains [18], whereas a shift-up produces a possibly less serious increase in fluidity.

The preferred *sn*-1/*sn*-2 distribution is assumed to arise from the activity of the acyltransferases acting on glycerol-3-phosphate, although this has not been tested directly in *M. cryophilus*. The growth temperature-dependent changes in the C18/C16 ratio of the fatty acid synthesized were reflected in the C18/C16 ratios of both *sn*-1 and *sn*-2 positions of the phospholipid. But during a temperature shift there was no growth temperature-dependent change in the *sn*-1/*sn*-2 specificity of the acyltransferases, so that the difference in the C18/C16 ratios of the two positions was maintained.

The usual configuration of phospholipids in bacteria is *sn*-1-saturated, *sn*-2-unsaturated, particularly in Gram-negatives [8]; since the commonest saturated and unsaturated fatty acids in Gram-negatives are palmitic and *cis*-vaccenic, respectively, this means, therefore, that the preferred phospholipid configuration is *sn*-1-short, *sn*-2-long. Thus *M. cryophilus*, which is a Gram-negative organism



Figs. 1–3. (1) The change in acyl chain C18/C16 ratio of phospholipids in *Micrococcus cryophilus* after a change in growth temperature from 0° to 20°C. A culture grown at 0°C was shifted up to 20°C and at intervals the acyl chain length ratio of phospholipids determined as described in Table 1. Abbreviations: see Table 1.

(2) The change in acyl chain C18/C16 ratio of phospholipids in *Micrococcus cryophilus* after a change in growth temperature from 20° to 0°C. A culture grown at 20°C was shifted down to 0°C and at intervals the acyl chain length ratio of phospholipids determined as described in Table 1. Abbreviations: see Table 1.

(3) The change in acyl chain C18/C16 ratio of the *sn*-1 and *sn*-2 positions of the phospholipids in *Micrococcus cryophilus* after a change in growth temperature from 20° to 0°C. A culture grown at 20°C was shifted down to 0°C and at intervals the phospholipids extracted. Phospholipid samples were digested with phospholipase A_2 [4] and the unesterified fatty acid separated from lysophospholipid and remaining phospholipid by thin-layer chromatography. Fatty acid compositions were determined and acyl chain length ratios expressed as described in Table 1. Abbreviation: PL, combined *sn*-1 + *sn*-2 acyl chain ratio of total phospholipids.

despite its classification [15], does not conform to this general pattern, although relatively few species have been investigated in this detail.

Bacteria require a fluid membrane for growth, although a very wide range of fatty acid compositions may be tolerated, even to the extent of having a proportion of gel phase lipid [11]. It has been assumed that the large percentage of unsaturated fatty acids and the acyl chain length changes in *M. cryophilus* are necessary to maintain a fluid membrane at low growth temperatures [7, 14]. The liquid-crystalline to gel phase transition temperature of extracted lipids, detected by differential scanning calorimetry, occurs at sub-zero temperatures (L. McGibbon and N. J. Russell, unpublished results). The preference for the longer, oleoyl acyl chains in the *sn*-1 position may be a further adaptation to ensure the maintenance of liquid-crystalline lipids. Studies with model systems show that the difference in melting temperatures between pairs of positional isomers differing in length by 2 carbon atoms can be considerable; for example, it is 6.7° for *sn*-1-16:1, *sn*-2-18:1-phosphatidylcholine and *sn*-1-18:1, *sn*-2-16:1-phosphatidylcholine [5]. The reason for this difference is believed to be due to the fact that the *sn*-1 acyl chain of a phospholipid penetrates 1.5 alkyl carbon atoms further into the bilayer than does the *sn*-2 acyl chain [21]; thus an *sn*-1-long, *sn*-2-short phospholipid exaggerates this difference, disrupting bilayer packing and thereby lowering melting temperature. A difference of 6.7° could contribute significantly to membrane fluidity in *M. cryophilus*, because this represents 25% of the growth temperature range. Although the positional isomers of phospholipids in some organisms have been determined [12] and have been implicated in temperature adaptation in *Tetrahymena* [6], this is the first example where such changes can be correlated clearly with known effects in model systems.

Besides a depression of lipid melting point, there may be another reason for the isomeric distribution in *M. cryophilus*. Experiments with *Escherichia coli* auxotrophs show that when the fatty acid composition is dominated by a single *cis*-unsaturated species, strong lipid–lipid interactions arise, leading to less fluid domains [1] and the inactivation of some membrane-bound enzymes [2]. The simple fatty acid composition of *M. cryophilus* could lead to the synthesis of large amounts of dipalmitoleoyl- or dioleoyl-phospholipids, with the subsequent possible deleterious effects on lipid bilayer structure and membrane function. In fact, at least half of the total phospholipid from cells grown at 20°C must be dioleoyl-phospholipid, when oleate represents 70%–80% of the total fatty acids. A mechanism that favored acylation of the *sn*-1 position with oleate would keep the proportion of dioleoyl-phospholipid to a minimum. As the growth temperature is lowered, the phospholipid C18/C16 acyl chain ratio falls to approximately unity, so that theoretically it would be possible for there to be no dipalmitoleoyl- or dioleoyl-phospholipid. This is not the case (Table

1), but there is still the same preference for oleoyl chains in the *sn*-1 position. Thus, by maintaining this preference throughout its growth temperature range, *M. cryophilus* ensures that the levels of phospholipids having a fatty acid composition that would reduce membrane fluidity do not reach damaging proportions.

Such a mechanism may be necessary in *M. cryophilus* because of its simple fatty acid composition (and subsequent lack of "alternative" fatty acid combinations in phospholipids). Several other psychrophilic bacteria have more diverse fatty acid compositions, often with shorter-chain fatty acids [9], which would give the necessary disruption to acyl chain packing and thereby preserve membrane fluidity.

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