Effect of Growth Temperature on the Positional Distribution of Eicosapentaenoic Acid and *trans* Hexadecenoic Acid in the Phospholipids of a *Vibrio* Species of Bacterium

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ABSTRACT: Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) were isolated from a *Vibrio* species of bacterium, known to produce eicosapentaenoic acid (20:5n-3) and *trans*-hexadecenoic acid (16:1n-7), and subjected to phospholipase A_2 degradation to determine the positional distribution of component fatty acids. At the two growth temperatures studied (20 and 5°C), both 20:5n-3 and *trans* 16:1 n-7 were located mainly at position *sn*-2 in PE. Increases in the proportions of 20:5n-3 and *trans* 16:1n-7 in position *sn*-2 with decreasing growth temperature were balanced mainly by decreases in the level of *iso*-15:0. In PG, *trans* 16:1n-7 was located predominantly in position *sn*-1, although the difference between the two positions was not as great as in PE. Eicosapentaenoic acid was preferentially located in position *sn*-2 of PG, particularly at 5°C when it comprised 29.9% of the total fatty acids in this position. It is concluded that *trans* 16:1n-7/20:5n-3 is not a major molecular species of phospholipid in this species of *Vibrio* and that changes in the levels of molecular species of PE containing *iso*-15:0 may feature in thermal acclimation.

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It is well established that alterations in membrane lipid composition occur in poikilothermic organisms as an adaptation to changes in the temperature of their environment (1,2). An increase in the level of polyunsaturated fatty acids (PUFA) in phospholipids is an almost ubiquitous response in poikilothermic eukaryotes to a decrease in temperature. Bacteria do not, in general, contain PUFA (3). Temperature-induced adaptations in the fatty acid profile of bacteria that have been noted include increases in the proportions of monounsaturated fatty acids, shorter chain fatty acids and branched-chain fatty acids, as well as changes in the ratio of *anteiso*- to *iso* acids (4–6). Some strains of bacteria contain *trans*-monounsaturated fatty acids in addition to the more common *cis* isomers, and it has been shown that in the psychrophilic bacterium *Vibrio* sp. strain ABE-1, the relative proportion of *trans* 16:1n-7 decreases as a response to a decrease in environmental temperature (7,8).

We have recently reported the simultaneous occurrence of eicosapentaenoic acid (20:5n-3) and *trans* hexadecenoic acid (16:1n-7) in a *Vibrio* species of bacterium isolated from fish intestines and have shown that the level of both these fatty acids in the cellular phospholipids is higher at a growth temperature of 5 than 20°C (9). This increase in the level of *trans* 16:1 with decreasing growth temperature contrasts with the decrease observed with *Vibrio* ABE-1 species (7,8). In the latter species the *trans* fatty acid is known to be located mainly in position *sn*-2 of phosphatidylethanolamine (PE) (8). Although position *sn-2* of the phospholipids of most eukaryotes is characteristically enriched in PUFA (10,11), the C_{18} PUFA of photosynthetic cyanobacteria are known to be specifically located in the *sn*-1 position of phosphatidylglycerol (PG) (12). No information is available for the positional distribution of 20:5n-3 in the phospholipids of bacteria. We have previously postulated that the observed increase in both *trans* 16:1n-7 and 20:5n-3 in the *Vibrio* species with decreasing temperature results from the specific increase in a molecular species of phospholipid containing both these two fatty acids (9), a suggestion which implies that the fatty acids occupy different positions within the phospholipid. The study described here was undertaken to determine the positional distribution of 20:5n-3 and *trans* 16:1n-7 in the phospholipids of the *Vibrio* species of bacterium, which contains both these fatty acids. In addition to determining how the two fatty acids are distributed within the two constituent phospholipids of the *Vibrio* species, PE and PG (9), it was intended to establish whether specific changes in positional distribution occur in response to changes in growth temperature.

MATERIALS AND METHODS

Chemicals. Inorganic chemicals were of AnalaR grade and were supplied by BDH (Lutterworth, Leics., United Kingdom). Solvents were of glass-distilled or high-performance

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Abbreviations. HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PUFA, polyunsaturated fatty acids; TLC, thin-layer chromatography; UV, ultraviolet.

liquid chromatography (HPLC) grade and were obtained from Rathburn Chemicals (Walkerburn, United Kingdom). Phospholipase A_2 (E.C. 3.1.1.4) prepared from *Naja naja* venom was purchased from Sigma Chemical Co. (Poole, Dorset, United Kingdom).

Growth of bacteria. The bacterium studied was the *Vibrio* species originally isolated from the gut of Arctic char *(Salvelinus alpinus* L.) (13). Cultures of the bacterium were grown under sterile conditions in defatted, freshwater medium in the dark at 20 and 5°C as described previously (9), and harvested during late log phase (24 h and 5 d for cultures at 20 and 5°C, respectively) by centrifugation at 7,000 × *g* for 10 min. Cultures were grown sequentially and did not arise from the same inoculating culture.

Phospholipid analysis. Lipid was extracted from pellets of bacterial cells by blending with chloroform/propan-2-ol (2:1, vol/vol) as described previously (9). All lipid extracts and fractions were maintained at −70°C under an atmosphere of oxygen-free nitrogen between analytical operations. PE and PG were isolated by thin-layer chromatography (TLC) of total lipid extracts on 20 × 20 cm glass plates precoated with silica gel G (E. Merck, Darmstadt, Germany) using chloroform/methanol/water (65:25:4, by vol) as developing solvent. Developed chromatograms were sprayed lightly with 0.01% (wt/vol) 2′,7′-dichlorofuorescein in methanol and viewed under ultraviolet (UV) light. Bands of adsorbent containing PE and PG were scraped separately from the plates and the phospholipids eluted from the adsorbent with chloroform/methanol/water (5:5:1, by vol). After removal of the solvent by evaporation and desiccation under vacuum, the phospholipids were redissolved in chloroform/methanol (2:1, vol/vol) and the purity of aliquots checked by high-performance TLC (HPTLC) on silica gel using chloroform/methanol/water (65:25:4, by vol) as developing solvent.

For the enzymatic hydrolysis of phospholipids, 2.5 mg PE or PG were dissolved in 0.75 mL diethyl ether, and 0.25 mL 0.1M Tris HCl, pH 7.5 containing 4 mM $CaCI_2$ and 20 units of phospholipase A_2, was added. The mixture was stirred vigorously at 30°C for 4 h. Lipids were then extracted from the assay with chloroform/methanol (2:1, vol/vol). After reduction of solvent volume by evaporation, lipid extracts were applied to HPTLC plates which were subjected to single-dimension, double development with methyl acetate/propan-2-ol/chloroform/methanol/0.25% KCl (25:25:25:10:9, by vol) followed by hexane/diethyl ether/glacial acetic acid (80:20:2, by vol) (14). Developed chromatograms were sprayed lightly with 0.01% (wt/vol) 2′,7′-dichlorofluorescein in methanol and viewed under UV light. Bands corresponding to lysoPE or lysoPG and free fatty acids were marked, scraped from the HPTLC plates into test tubes and subjected to acid-catalyzed transesterification with 1% sulfuric acid in methanol to produce the methyl ester derivatives of the constituent fatty acids (15). Aliquots of PE and PG were also subjected directly to acid-catalyzed transesterification. Fatty acid methyl esters were purified by HPTLC on silica gel plates using hexane/diethyl ether/glacial acetic acid (85:1 :1, by vol) as developing solvent and recovered from the adsorbent by elution with hexane/diethyl ether (1:1, vol/vol).

Analyses of fatty acid methyl esters were carried out by gas chromatography using a fused silica capillary column (25 m × 0.32 mm i.d. coated with BPX 70; S.G.E., Milton Keynes, United Kingdom). Sample application was by on-column injection, H_2 was used as carrier gas and the oven temperature was programmed to increase from 50 to 180°C during the course of an analysis. Separated methyl esters were identified by reference to authentic standards and by comparison with previously characterized samples of total fatty acids from the same species (9). The molar amounts of fatty acids were calculated by multiplying the area of each chromatographic peak by the appropriate arithmetic factor (15). The fatty acid compositions of the free fatty acid fraction and the lysophospholipid were considered to represent that of positions *sn-2* and *sn*-1, respectively, of the original intact phospholipid. The accuracy of the positional analysis was considered acceptable when the sum of the concentrations of a given fatty acid in positions *sn*-1 and *sn*-2, divided by two, was within 2% of the value for the fatty acid in the original phospholipid.

Statistical evaluation. Student's *t*-test was used for the statistical analysis of data, and the level of significance was defined as $P < 0.05$.

RESULTS

PE. The fatty acid composition of PE, the major phospholipid of the *Vibrio* (9), was significantly influenced by the temperature at which the bacteria were grown (Table 1). PE of bacteria grown at 5°C contained significantly higher levels of 20:5n-3 and *trans* 16:1n-7 and, conversely, lower levels of *iso*-14:0, *iso*-15:0, 17:0, 18:1n-9 and 18:1n-7 than that isolated from bacteria grown at 20°C. At both growth temperatures, more than half of the fatty acids esterified in position *sn*-1 of PE were saturated, whereas position *sn*-2 contained mainly monounsaturated fatty acids. The bulk of 20:5n-3 was always located in position *sn*-2, which also contained most of the *trans* 16:1n-7 present in PE.

Growth temperature influenced the fatty acid composition of both positions of PE. Bacteria grown at 5°C contained approximately threefold higher levels of both 20:5n-3 and *trans* 16:1n-7 in position *sn*-1 of PE than bacteria grown at 20°C, although these fatty acids together still accounted for only 2.7% of the fatty acids in this position at 5°C. In position *sn*-2, 20:5n-3 and *trans* 16:1n-7 comprised 9.6 and 17.7% of the fatty acids in this position at 5°C as compared to the lower corresponding values of 2.5 and 6.4% at 20°C. Conversely, the level of saturated fatty acids, particularly *iso*-15:0, in position *sn*-2 was lower at 5 than 20°C. The level of the major fatty acid in position *sn*-2, *cis* 16:1 n-7, was not significantly changed by growth temperature although its level in position *sn*-1 was higher at 5 than 20°C (13.8% vs. 7.7%).

PG. As with PE, the overall fatty acid composition of PG was influenced by growth temperature (Table 2). In particu-

TABLE 1
Effect of Growth Temperature on the Positional Distribution of Fatty Acids in Phosphatidylethanolamine of *Vibrio* sp.[a]

	5°C			20°C		
Fatty acids	Overall composition	Position *sn*-1	Position *sn*-2	Overall composition	Position *sn*-1	Position *sn*-2
i-13:0	0.7 ± 0.5	0.5 ± 0.5	5.0 ± 4.3	1.7 ± 1.0	0.3 ± 0.1	4.9 ± 07
13:0	0.7 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.8 ± 0.2	0.1 ± 0.0	0.3 ± 0.1
i-14:0	0.1 ± 0.1[f]	0.1 ± 0.1	0.3 ± 0.2[f]	0.4 ± 0.1	0.1 ± 0.1	0.8 ± 0.1
14:0	2.1 ± 0.7	3.2 ± 2.3	0.8 ± 0.1[f]	1.5 ± 0.3	2.1 ± 0.1	1.3 ± 0.2
14:1n-5	—	—	0.1 ± 0.1	—	—	0.1 ± 0.1
i-15:0	11.1 ± 1.5[f]	18.3 ± 4.4	7.6 ± 1.5[f]	17.1 ± 2.3	21.9 ± 1.0	18.6 ± 0.9
a-15:0	0.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.3
15:0	4.7 ± 0.8	8.8 ± 2.4	0.7 ± 0.2	4 3 ± 0 3	8.0 ± 0.5	0.8 ± 0.1
15:1n-6	2.0 ± 0.5[d]	1.3 ± 0.3[f]	2.2 ± 0.6[c]	1.3 ± 0.1	0.4 ± 0.1	1.9 ± 0.5
16:0	14.2 ± 1.0	26.4 ± 2.5	2.8 ± 1.1	15.6 ± 1.3	28.7 ± 1.8	2.1 ± 1.1
16:1n-9	1.6 ± 0.3	2.0 ± 0.3	1.7 ± 0.4[f]	1.6 ± 0.6	2.0 ± 0.1	2.5 ± 0.2
cis 16:1n-7	26.3 ± 2.4	13.8 ± 2.3[f]	39.7 ± 3.6	25.6 ± 1.6	7.7 ± 0.4	40.7 ± 1.0
trans 16:1n-7	9.9 ± 1.8[f]	2.1 ± 0.4[f]	17.7 ± 3.1[f]	2.2 ± 1.5	0.6 ± 0.1	6.4 ± 1.4
17:0	1.1 ± 0.3[f]	1.8 ± 0.4[f]	0.7 ± 0.7	2.4 ± 0.2	4.1 ± 0.4	—
17:1n-8	8.6 ± 1.4	7.8 ± 1.7	6.9 ± 1.3[f]	9.8 ± 1.1	6.5 ± 0.5	10.6 ± 0.9
17:1n-6	1.2 ± 0.2	1.7 ± 0.5	0.2 ± 0.1	1.0 ± 0.1	1.4 ± 0.2	0.2 ± 0.0
18:0	2.1 ± 3.3	0.7 ± 0.3[f]	0.9 ± 0.3	1.2 ± 0.2	1.4 ± 0.2	0.7 ± 0.3
18: In-9	1.8 ± 0.4[f]	1.7 ± 0.6[d]	1.7 ± 0 4[b]	4.0 ± 0.5	2.9 ± 0.4	4.2 ± 2.6
18:1n-7	4.6 ± 0 7[b]	7 7 ± 2.4	0.6 ± 0.2[e]	5 3 ± 0 5	7.9 ± 0.6	1.0 ± 0.1
20:5n-3	7.0 ± 1.0[f]	0.6 ± 0.1[f]	9.6 ± 2.3[f]	1.8 ± 0.2	0.2 ± 0.1	2.5 ± 0.2
Total sat.	37.0 ± 2.6[f]	60.3 ± 7.5	19.5 ± 6.0[f]	45.4 ± 2.6	67.1 ± 1.6	30.0 ± 1.4
Total mono.	56.0 ± 1.7[f]	38.1 ± 6.9[f]	70.8 ± 3.8	50.8 ± 2.3	29.4 ± 1.7	67.5 ± 1.6
Total PUFA	7.0 ± 1.0[f]	0.6 ± 0.1[f]	9.6 ± 2.3[f]	1.8 ± 0.2	0.2 ± 0.1	2.5 ± 0.3
Unidentified	—	1.0 ± 0.2	0.1 ± 0.1	2.0 ± 0.1	3.3 ± 0.2	—

[a]Values are mol% and are means ± SD of six cultures. Values at 5°C with superscripts are significantly different from the corresponding 20°C value; [b]$P < 0.05$; [c]$P < 0.02$; [d]$P < 0.01$; [e]$P < 0.002$; [f]$P < 0.001$. *i-, iso; a-, anteiso;* sat., saturated fatty acids; mono.. monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

lar, the level of monounsaturated and polyunsaturated fatty acids were lower and higher, respectively, at 5 than 20°C. The level of saturated fatty acids, around 28% of total PG fatty acids, was not significantly affected by growth temperature.

Regardless of growth temperature, position *sn*-1 of PG was characterized by a high content of saturated fatty acids, particularly 16:0, and the monounsaturated fatty acids *cis* 16:1n-7 and 18:1n-7. Position *sn*-2 contained less saturated fatty acids than position *sn*-1, but had a higher content of PUFA, in the form of 20:5n-3, and was richer in *cis* 16:1n-7. More *trans* 16:1n-7 was located in position *sn*-2 than in position *sn*-1.

A higher level of 20:5n-3 was present in the *sn*-1 position of PG from the *Vibrio* grown at 5°C (5.6%) than in that of the same phospholipid of bacteria grown at 20°C (1.2%). The level of *trans* 16:1n-7 in position *sn*-1 was also higher at 5 than 20°C (4.2 vs. 1.6%). The fatty acid profile of position *sn*-2 was markedly influenced by growth temperature. In PG of bacteria grown at 5°C, *cis* 16:1n-7 accounted for 24.8% of the fatty acids in position *sn*-2, and this proportion increased to 43.6% at 20°C. In contrast, the content of 20:5n-3 decreased from 29.9% at 5°C to 8.2% at 20°C. The level of 17:1n-8 in position *sn*-2 also increased significantly with increasing growth temperature, and the overall content of monounsaturated fatty acids in this position was substantially higher at 20 than at 5°C. The level of *trans* 16:1n-7 in position *sn*-2 was not significantly influenced by growth temperature. Overall, the temperature-related changes in fatty acid composition of PG were more obvious in position *sn*-2 than in position *sn*-1.

DISCUSSION

It is well established that position *sn*-1 of bacterial phospholipids contains predominantly saturated fatty acids, whereas the *sn*-2 position is enriched in monounsaturated fatty acids (3,5). The data obtained in the present study confirm this pattern and further establish the unsaturated nature of position *sn*-2 by demonstrating that 20:5n-3 is located mainly in this position in both PE and PG in a *Vibrio* species of bacterium containing this PUFA. This is in keeping with the positional distribution patterns observed in eukaryotic phospholipids, where position *sn*-2 generally contains the highest proportion of PUFA (10,11), but is in contrast to the situation in cyanobacteria where C_{18} PUFA are concentrated in position *sn*-1 (12). It is likely that the preferential location of PUFA in the *sn*-2 position of bacterial phospholipids extends to 22:6n-3 in those species of bacteria which can produce this PUFA (16), although this remains to be established.

The finding that most of the *trans* 16:1n-7 in PE of the species of bacterium analyzed here is esterified to the *sn*-2 position of the glycerol moiety is in agreement with a previous

TABLE 2
Effect of Growth Temperature on Positional Distribution of Fatty Acids in Phosphatidylglycerol of *Vibrio* sp.[a]

	5°C			20°C		
Fatty acids	Overall composition	Position *sn*-1	Position *sn*-2	Overall composition	Position *sn*-1	Position *sn*-2
i-13:0	—	—	—	—	0.1 ± 0.1	1.2 ± 0.8
13:0	0.8 ± 0.4	0.4 ± 0.4	0.4 ± 0.4	0.3 ± 0 3	—	—
i-14:0	—	—	—	—	—	0.1 ± 0.1
14:0	1.5 ± 0.4[d]	0.9± 1.2	0.6 ± 0.3	0.5 ± 0.3	0.8 ± 0.4	0 6 ± 0 2
14:1n-5	—	—	—	—	—	0.1 ± 0.1
i-15:0	3.9 ± 0.5	4.2 ± 4.5	1.3 ± 0.7[f]	4.6 ± 1.1	5.7 ± 2.8	4.2 ± 0.5
a-15:0	—	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	—
15:0	3.2 ± 0.5	3.3 ± 2.7	0.8 ± 0.1[e]	3.0 ± 0.3	4.0 ± 1.0	0.5 ± 0.1
15:1n-6	1.4 ± 0.1	0.3 ± 0 4[c]	0.8 ± 0.5	1.9 ± 1.1	0.4 ± 0.0	1.3 ± 0.2
16:0	14.8 ± 1.3	23.5 ± 1.3	10.7 ± 3.3[d]	15.0 ± 1.1	25.3 ± 1.3	3.6 ± 1.3
16:1n-9	1.7 ± 0.2	1.5 ± 0.4	1.3 ± 0.1[f]	1.2 ± 0.6	1.6 ± 0.1	2.7 ± 0.1
cis 16:1n-7	25.2 ± 1.0[f]	11.7 ± 4.0	24.8 ± 5.5[f]	33.3 ± 3.0	13.3 ± 1.4	43.6 ± 4.1
trans 16:1n-7	7.0 ± 1.7[f]	4.2 ± 1.2[d]	8.0 ± 3.3	1.7 ± 0.5	1.6 ± 0.6	5.8 ± 1.3
17:0	1.0 ± 0.2[f]	2.3 ± 0.6[f]	0.5 ± 0.2[e]	2.2 ± 0.2	5.1 ± 1.0	0.2 ± 0.1
17:1n-8	7.7 ± 1.6[f]	9.2 ± 1.2	7.5 ± 1.1[f]	14.0 ± 1.4	10.4 ± 1.0	16.4 ± 1.5
17:1n-6	1.2 ± 0.1	1.6 ± 0.3	0.2 ± 0.1	1.1 ± 0.1	1.6 ± 0.2	0.2 ± 0.1
18:0	2.8 ± 0.7	8.2 ± 6.1	5.2 ± 1.8[d]	1.8 ± 0.9	3.7 ± 0.7	1.6 ± 0.7
18:1n-9	4.0 ± 0.9[d]	6.5 ± 3.3	5.9 ± 1.1[d]	7.2 ± 1.4	6.4 ± 0.7	8.0 ± 0.2
18:1n-7	5 4 ± 0.4[d]	15.7 ± 5.6	1.9 ± 0.4[e]	6.6 ± 0.6	13.9 ± 1.2	1.3 ± 0.1
20:5n-3	17.7 ± 1.3[f]	5.6 ± 1.6[f]	29.9 ± 6.4[f]	3.7 ± 2.2	1.2 ± 0.7	8.2 ± 1.0
Total sat.	28.0 ± 1.6	42.9 ± 4.3	19.7 ± 4.1[e]	27.5 ± 0.6	45.1 ± 5.1	12.4 ± 3.1
Total mono.	53.6 ± 1.2[f]	50.7 ± 4.0	50.4 ± 8.7[f]	67.0 ± 2.9	49.1 ± 4.1	79.4 ± 4.1
Total PUFA	17.7 ± 1.3[f]	5.6 ± 1.6[f]	29.8 ± 7.0[f]	3.7 ± 2.2	1.2 ± 0.7	8.2 ± 1.0
Unidentified	0.7 ± 0.2	0.8 ± 0.3	0.1 ± 0.1	1.8 ± 0.1	4.8 ± 0.4	—

[a]Values are mol% and are means ± SD of six cultures. Values at 5°C with superscripts are significantly different from the corresponding 20° value; [b]$P < 0.05$: [c]$P < 0.02$; [d]$P < 0.01$; [e]$P < 0.002$; [f]$P < 0.001$. *i-, iso; a-, anteiso;* sat., saturated fatty acids; mono., monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

study of the ABE-1 strain of *Vibrio,* which also contains this *trans*-monounsaturated fatty acid but no PUFA (8). In the species examined here, both *trans* 16:1n-7 and 20:5n-3 are located predominantly in the same position of PE with the level of the *trans* fatty acid exceeding that of the PUFA. It is notable that although the proportions of both fatty acids increased in position *sn*-2 with a reduction in growth temperature, the ratio of *trans* 16:1n-7 to 20:5n-3 was lower at 5°C (1.8) than at 20°C (2.6). This demonstrates that the magnitude of increase in 20:5n-3 in response to decreases in growth temperature is greater than that observed in the *trans* fatty acid.

The increase in the level of 20:5n-3 observed in bacterial phospholipids in response to a decreased growth temperature is in keeping with the frequently reported increases in the degree of unsaturation of phospholipids which occur during the adaptation of poikilothermic animals and plants to a reduced environmental temperature (1,2). However, in marked contrast to the situation observed here, the level of *trans* 16:1n-7 has been reported to be lower at 5°C than at 20°C in the *Vibrio* sp. strain ABE-1 (7,8). This difference in response between the two species of *Vibrio* may be related to differences in the other fatty acids present in PE. In comparison with the species of *Vibrio* examined here, *Vibrio* strain ABE-1 contains a more restricted pattern of fatty acids and, within its PE, changes in *trans* 16:1n-7 are reciprocated almost exclusively by changes in the level of *cis* 16:1n-7 (7,8). In contrast, the *Vibrio* in this study contains a wide range of fatty acids, and the levels of several fatty acids are influenced by growth temperature. In particular, it is notable that the increase in *trans* 16:1n-7 (11 mol%) observed when the bacteria were grown at 5°C compared to 20°C was matched by an almost equivalent decrease in the level of *iso* 15:0. Although the melting point and phase transition temperature of *iso*-15:0 are lower than those of saturated C_{14}–C_{18} straight chain fatty acids, the melting point of *iso* 15:0 (51.7°C) (5) is still higher than that of *trans* 16:1n-7 (32–33°C) (17). Consequently, it may be expected that an increase in the proportion of the latter fatty acid with a lowering of growth temperature would assist in maintaining membrane fluidity.

The observed positional distribution patterns refute our previous suggestion that a *trans* 16:1n-7/20:5n-3 molecular species of phospholipid is involved in temperature adaptation of membranes in this species of bacterium (9). Although the relative amounts of individual molecular species of PE which occurred at the two growth temperatures were not measured directly, it can be deduced from the positional distribution of fatty acids that the principal molecular species of PE at 20°C probably contained 16:0 or *iso*-15:0 in the *sn*-1 position coupled with *iso*-15:0 or *cis* 16:1 n-7 at position *sn*-2. At 5°C, higher proportions of molecular species containing *cis* 16:1n-7 at position *sn*-1 and *trans* 16:1n-7 or 20:5n-3 at position *sn*-2 would have been present. Thus, a *trans*

16:1n-7/20:5n-3 molecular species, if present, would only have been a minor molecular species at both temperatures.

The positional distribution of *trans* fatty acids in PG of bacteria has not been previously reported. Although the present results demonstrate that position *sn*-2 of PG contained more *trans* 16:1n-7 than position *sn*-1, as in PE, the difference between the two positions was not so great as observed with PE. At the same time, the position *sn*-2 of PG was much richer in 20:5n-3 than the same position of PE, and the proportion of 20:5n-3 always exceeded that of *trans* 16:1n-7 regardless of temperature. Because PE and PG both arise from CDP-diacylglycerol in Gram negative bacteria, similarities in fatty acid composition might be expected. However, PG is known to be a highly active substrate in the lipid metabolism of bacteria and has more potential than PE for changing its fatty acid composition (3).

The positional data suggest that the biggest temperature-induced changes in molecular species composition of PG involve those containing 20:5n-3 rather than *trans* 16:1n-7. Thus, at 20°C molecular species of PG containing 16:0 or a *cis* monounsaturated fatty acid coupled with *cis* 16:1n-7 at position *sn*-2 are major molecular species, while a decrease in growth temperature results in an increase in molecular species with 20:5n-3 at position *sn*-2. At the same time, however, small amounts of di-20:5n-3 molecular species may also be formed. Analysis by HPLC of the molecular species of PE and PG is of obvious interest in elucidating thermal adaptation effects in this *Vibrio* species but presents a challenge on account of the constituent levels of odd-chain and branched fatty acids.

In *Vibrio* sp. strain ABE-1, the increase in *trans* 16:1n-7 observed during adaptation to growth at a high temperature is matched closely by the decrease in *cis* 16:1n-7, and the conversion of *cis* to *trans* isomers by an isomerase has been demonstrated when the bacterium adapts to growth at higher temperature in the presence of inhibitors of *de novo* fatty acid synthesis (7,8). However, it is notable that, in the *Vibrio* species studied here, the levels of *cis* and *trans* 16:1n-7 show no inverse relationship in either PE or PG during temperature adaptation, and it remains to be established whether the bacterium can adopt the isomerization of preexisting *cis* 16:1n-7 to its *trans* isomer as a strategy for adaptation to growth at low temperature.

It has been recently shown that, in the phospholipids of some species of lower fungi, n-3 PUFA are located predominantly in position *sn*-1, whereas n-6 PUFA mainly occupy position *sn*-2 (18). This specific positional distribution has been attributed to the Δ15 desaturase having a specificity for the acyl group in position *sn*-1. Although the preferential esterification of 20:5n-3 in position *sn*-2 of *Vibrio* suggests that such a positional specificity of desaturases does not operate in this bacterium, very little is known of the mechanisms of PUFA biosynthesis in bacteria. It remains to be established whether intact phospholipids, as in fungi, plants and protozoa, or acyl thioesters as in animals, serve as substrates for desaturase enzymes in bacteria.

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