JOURNAL OF BACTERIOLOGY, Feb. 1982, p. 567-575
0021-9193/82/020567-09$02.00/0

Vol. 149, No. 2

Effects of Growth Temperature on Fatty Acid and Alk-1-Enyl Group Compositions of *Veillonella parvula* and *Megasphaera elsdenii* Phospholipids

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Received 3 June 1981/Accepted 17 September 1981

Veillonella parvula ATCC 10790, an anaerobic gram-negative coccus, contains diacyl and alk-1-enyl acyl (plasmalogen) forms of phosphatidylethanolamine and phosphatidylserine. We studied the effect of growth temperature on the lipid composition of this strain. There was a small increase in the phosphatidylethanolamine content but no change in the content of plasmalogens at the lower growth temperatures tested. The total acyl chains and the plasmalogen acyl chains contained between 73 and 80% mono-unsaturated fatty acids at all growth temperatures. The plasmalogen alk-1-enyl chains were significantly more unsaturated in cells grown at 30 and 25°C than in cells grown at 37°C. Differential scanning calorimetry of the hydrated phospholipids showed lower phase transition temperatures for the lipids from the cells grown at the lower temperatures. In *Megasphaera elsdenii* lipids, which are similar in composition to the lipids of *V. parvula*, the proportion of phosphatidylethanolamine also increased slightly at lower growth temperatures, with no significant change in the content of plasmalogens. *M. elsdenii* contained cyclopropane fatty acyl and alk-1-enyl chains in addition to the mono-unsaturated and saturated chains previously reported. As cells entered the stationary phase of growth at 30 and 42.5°C, there was a reciprocal increase in the proportion of cyclopropane acyl chains and decrease in the unsaturated moieties. The total proportion of cyclopropane and unsaturated acyl and alk-1-enyl chains was more than 65% at all growth temperatures studied, and there was no discernible increase in the sum of these moieties at the lower growth temperatures.

1-Alkenyl-2-acyl glycerolipids (plasmalogens) are major constituents of certain mammalian tissues, including heart, brain white matter, peripheral nerves, and skeletal muscle. These lipids are also found in avian tissues and in many invertebrates (15, 29, 30). The presence of plasmalogens in procaryotes appears to be confined to strict anaerobes, in which they often represent a substantial fraction of the total lipid (9, 17, 31). Studies on the role of plasmalogens in the maintenance of membrane fluidity have only begun to probe the adaptive functions of these compounds. The pioneer work of Roots and Johnston (25, 26) on the alkenyl ether lipid compositions of the neural tissues of goldfish acclimated to different temperatures suggested that the chain composition was regulated. Work in our laboratory has shown that both the acyl and alkenyl chains of the major plasmalogens of *Clostridium butyricum*, a strict anaerobe, undergo changes in composition as a function of growth temperature, as do the overall phospholipid composition and the plasmalogen contents of individual phospholipid classes (19, 20). These changes result in alterations in the phase behavior of the membrane phospholipids (10, 11). To extend this work to other anaerobes with different phospholipid, acyl, and alkenyl compositions, we performed a study on the effects of growth temperature on the lipids of *Veillonella parvula* and *Megasphaera elsdenii*. *V. parvula* is a small, gram-negative anaerobe which is a common constituent of the oral flora of humans. In this organism 45% of the total phospholipid is in the plasmalogen form of phosphatidylethanolamine, and 7.5% is in the plasmalogen form of phosphatidylserine (31). *M. elsdenii*, a large, gram-negative coccus which is found in the rumen, is also rich in the plasmalogen forms of phosphatidylethanolamine and phosphatidylserine, which together represent approximately 50% of the total phospholipid (32).

Our studies show that the major change in the composition of *V. parvula* phospholipids as a function of growth temperature is an increase in the total unsaturated alk-1-enyl chains at lower temperatures. This change leads to a decrease in the phase transition temperature, as observed by

differential scanning calorimetry. *M. elsdenii* phospholipids appear to change very little with respect to acyl and alk-1-enyl chain compositions as a function of growth temperature. In contrast to previous studies on this organism, we found that it contains substantial amounts of cyclopropane acyl and alkenyl chains.

MATERIALS AND METHODS

Materials. The solvents used for the preparation of lipids for gas chromatography were glass distilled (Burdick and Jackson Laboratories, Inc., Muskegon, Mich.). Silica Gel HR (E. Merck AG, Darmstadt, Germany) was slurried either in water or in 0.01 M Na_2CO_3 to produce Silica Gel HRB. The fatty acid methyl ester standards used were NIH-D and the branched-chain mixture BC-1 from Applied Science Laboratories, State College, Pa. and an odd-chain mixture (GLC-90) from Supelco, Inc., Bellefonte, Pa.

Bacteria and growth media. *V. parvula* ATCC 10790 was grown anaerobically in 1- or 2-liter flasks which were sparged with CO_2 before and after inoculation. The medium used has been described previously (31). Cultures were pregrown at the experimental temperatures during preparation of the final inocula. Cells were harvested after overnight growth or shortly after they had entered stationary phase in the case of the slow-growing, 25°C cultures. *M. elsdenii* ATCC 17752 was also grown in the medium described by Van Golde et al. (32). The periods of growth are described below. Cells were harvested by centrifugation at 6,000 × *g* for 15 min and were washed once in 0.05 M potassium phosphate buffer (pH 7.2) before being stored at −20°C.

Lipid analysis. Lipids were extracted and washed as described previously (8), and they were separated into neutral and polar fractions by chromatography on silicic acid (2). The phospholipids were separated by thin-layer chromatography on prerun Silica Gel HRB with a solvent mixture containing chloroform, methanol, acetone, acetic acid, and water (50:20:7.5:10:5, vol/vol) (solvent system A). Phospholipid analysis was performed as described previously (11). Total phospholipid acyl chains were obtained after saponification and methylation, and alkenyl chains were obtained by hydrolysis of the phospholipids in 90% acetic acid (19), followed by thin-layer chromatography on prerun Silica Gel HR with a solvent mixture containing hexane, chloroform, and methanol (135:60:5, vol /vol) (5, 6). The fatty acid methyl esters were purified further by preparative thin-layer chromatography on prerun Silica Gel HR with a solvent mixture containing petroleum ether, ether, and acetic acid (90:10:1, vol/vol). The gels were sprayed with a rhodamine B solution, and the esters were visualized under a UV light and eluted with ether. For analyses of the plasmalogen acyl chains, the lysophosphatide fractions were obtained by acetic acid hydrolysis of 4 to 5 mg of phospholipids (19), followed by chromatography on columns (0.8 by 3 cm) containing 0.5 g of Bio-Sil A (Bio-Rad Laboratories, Richmond, Calif.). The diacyl lipids were eluted with 8 ml of chloroform- methanol (3:1, vol/vol), and the lysophosphatides were eluted with 5 ml of methanol. Fatty acid methyl esters were hydrogenated under mild conditions in methanol over palladium on powdered charcoal. The catalyst was prereduced by bubbling with H_2 gas at atmospheric pressure for 15 min. Hydrogenation was accomplished by bubbling H_2 gas through the sample for 45 min, followed by a 30-min incubation of the sealed vessel at room temperature. The ester solution was filtered through Celite. The conditions used for opening cyclopropane rings have been described by Hofmann et al. (14). The fatty acids obtained by saponification of lipids were hydrogenated over Adams catalyst in glacial acetic acid under a slight positive pressure of hydrogen for 22 h. After filtration through Celite, the samples were lyophilized, and the methyl esters were prepared as described above. Lipid phosphorus was determined by the method of Bartlett (1). Vinyl ether was determined by the method of Gottfried and Rapport (12).

Gas chromatography. Fatty acid methyl esters and aldehydes were chromatographed on a Perkin-Elmer model 990 instrument equipped with a hydrogen flame ionization detector. Columns (1.83 m by 3.2 mm) of 10% DEGS-SP and 3% SE-30 (both on 80/100 Supelcoport; Supelco) were operated at 170 and 215°C, respectively. The proportion of each component was determined by multiplying the peak height by the retention time (10, 18).

Differential scanning calorimetry. Samples (2.5 to 3.5 mg) of the phospholipids were dried under nitrogen in aluminum pans, and 8 to 9 mg of water was added. After the pans were sealed and heated at 50°C for 1 h, calorimetric measurements were carried out with a Perkin-Elmer model DSC-2 calorimeter. Heating and cooling were at 5°C/min.

^{1}H nuclear magnetic resonance. Spectra were recorded with a Brucker model WH360/180 spectrometer that was operated at 360 MHz. Lipids were dissolved in $CDCl_3$, and 32 to 1,000 scans were accumulated.

RESULTS

Effect of growth temperature on the phospholipid composition of *V. parvula*. Table 1 shows the phospholipid compositions of *V. parvula* cells grown at three different temperatures. As Van Golde et al. found (31), the major components were serine and ethanolamine-containing phosphoglycerides. In addition to these compounds, in cells grown at 37 and 30°C there was an unknown phospholipid at R_f 0.77 after thin-layer chromatography in solvent system A; this compound constituted about 8% of the total phospholipid. At 25°C the unknown phospholipid was not present in detectable amounts, and the proportion of ethanolamine phosphoglycerides increased from 58 to 70% of the total. The plasmalogen content of the total phospholipids did not vary significantly over the temperature range studied.

Effect of growth temperature on the acyl and alk-1-enyl chains of *V. parvula*. Table 2 and Fig. 1 show the results of a gas chromatographic analysis of the acyl and alk-1-enyl chains of *V. parvula*. The acyl moieties contained a high proportion of mono-unsaturated chains at all of the temperatures studied, and the increase in total acyl chain unsaturation at 25°C was not

TABLE 1. Phospholipid compositions of *V. parvula* cells grown at different temperatures

Temp (°C)	% of total lipid phosphorus in:			% Plasmalogen[a]
	Phosphatidylethanolamine	Phosphatidylserine	Unknown phospholipid	
25	70.3 ± 7.0[b]	29.7 ± 7.0		39.4 ± 1.6
30	58.5 ± 2.2	33.3 ± 0.4	8.2 ± 2.5	37.6 ± 1.4
37	58.3 ± 0.4	33.3 ± 0.7	8.4 ± 1.1	36.0 ± 4.1

[a] Ratio of alk-1-enyl bonds to phosphorus in total phospholipids × 100.
[b] Mean ± standard error of the mean.

statistically significant (*t* test). The increases in the content of mono-unsaturated alkenyl chains (Table 2) at 30 versus 37°C and at 25 versus 37°C were quite marked and highly significant ($P < 0.01$). This was accomplished largely through a major increase in the 17:1 moiety and a decrease in 15:0 alk-1-enyl chains. The acyl chains of the ethanolamine plasmalogen were analyzed separately. As Verkley et al. found (33), these chains had a high proportion of the 17:1 moiety at 37°C, and the total mono-unsaturated content was 76%. The degree of unsaturation did not change at the lower growth temperatures.

It should be noted that the designation 18:1 is based on the report of Verkley et al. (33) concerning the acyl and alk-1-enyl chains of *V. parvula* phospholipids and on the close agreement of 18:1 standards after gas chromatography on DEGS. However, we had to consider the possibility that one or more of the presumed unsaturated chains could be a cyclopropane compound when we found in subsequent work with *M. elsdenii* that cyclopropane acyl and alkenyl chains were present (see below). To check the designations, we hydrogenated the fatty acid methyl esters in methanol with a palladium catalyst. The major 17:1 acyl chain peak decreased from 60.5% of the total to 2.9%, and the smaller 15:1 and 16:1 peaks disappeared; however, the 18:1 peak increased slightly, from 11 to 14% of the total. After hydrogenation of the fatty acids over the Adams catalyst in glacial acetic acid for 26 h, the 18:1 peak decreased from 11.9 to 4.8% of the total, and the 16:1 and 17:1 peaks were quantitatively converted to the respective saturated compounds. Proton magnetic resonance studies of the total phospholipids revealed signals that were characteristic of cyclopropane rings (see below). We suggest that much of the 18:1 fraction was most likely 18:cyc, but further chemical study of the isolated fatty acid and biosynthetic studies will be needed to verify this designation.

Phase transitions in *V. parvula* phospholipids. In previous studies, Verkley et al. (33) showed by freeze-fracture electron microscopy that the membrane lipids of *V. parvula* undergo phase separation between 23 and 0°C. Differential

TABLE 2. Acyl and alk-1-enyl chain compositions of phospholipids from *V. parvula* cells grown at different temperatures[a]

Moiety	Composition of acyl chains at:			Composition of alk-1-enyl chains at:		
	25°C	30°C	37°C	25°C	30°C	37°C
13:0[b]	7.23 ± 1.43	7.48 ± 1.41	7.30 ± 0.96	<1	<1	<1
15:0	7.12 ± 1.15	11.3 ± 0.66	12.2 ± 0.32	18.2 ± 1.2	26.7 ± 2.9	30.9 ± 1.1
15:1	1.87 ± 0.20	2.78 ± 0.21	2.93 ± 0.34	1.33 ± 1.33	2.75 ± 0.44	3.65 ± 1.23
16:0	1.00 ± 0.16	1.22 ± 0.04	1.22 ± 0.11	4.81 ± 0.20	5.07 ± 0.27	5.07 ± 0.35
16:1	3.97 ± 0.37	4.30 ± 0.06	4.15 ± 0.13	6.95 ± 0.75	6.87 ± 0.42	5.50 ± 0.15
17:0	4.50 ± 0.71	4.95 ± 0.63	4.78 ± 0.27	15.4 ± 3.8	15.7 ± 2.9	16.7 ± 2.1
17:1	61.3 ± 2.5	55.4 ± 1.9	49.2 ± 0.67	43.3 ± 1.1	36.9 ± 0.7	32.4 ± 0.67
18:1[c]	9.83 ± 0.64	9.60 ± 1.32	15.9 ± 1.2	5.28 ± 0.73	3.17 ± 0.43	2.82 ± 0.40
19:1	1.76 ± 0.33	1.03 ± 0.09	1.37 ± 0.22	1.08 ± 0.42	<1	<1
Total saturated	19.6 ± 3.2	26.3 ± 3.0	25.8 ± 1.5	39.0 ± 2.8	47.8 ± 2.3	52.6 ± 1.7
Total unsaturated	80.0 ± 3.4[d]	73.4 ± 3.2	74.0 ± 1.6	57.6 ± 0.53[e]	49.6 ± 1.2[e]	44.8 ± 0.2[e]

[a] Cultures were grown at the different temperatures as described in the text. Each set of data represents the compositions of three cultures grown at the same temperature, and each sample was analyzed by gas chromatography twice, except for the sample of alk-1-enyl chains from cells grown at 25°C, which were obtained from two cultures. The values represent means ± standard errors of percentages, by weight.
[b] Chains are designated by the number of carbons in the chain, followed by the number of double bonds.
[c] This fraction may have contained a mixture of the 18:1 moiety and a C_{18} cyclopropane chain (see text).
[d] The difference between the total unsaturated acyl chain values at 25 and 37°C was not significant ($P > 0.2$).
[e] The difference between the total unsaturated alk-1-enyl chain values at 25 and 37°C was significant ($P < 0.01$), as was the difference between the values 30 and 37°C ($P < 0.01$).

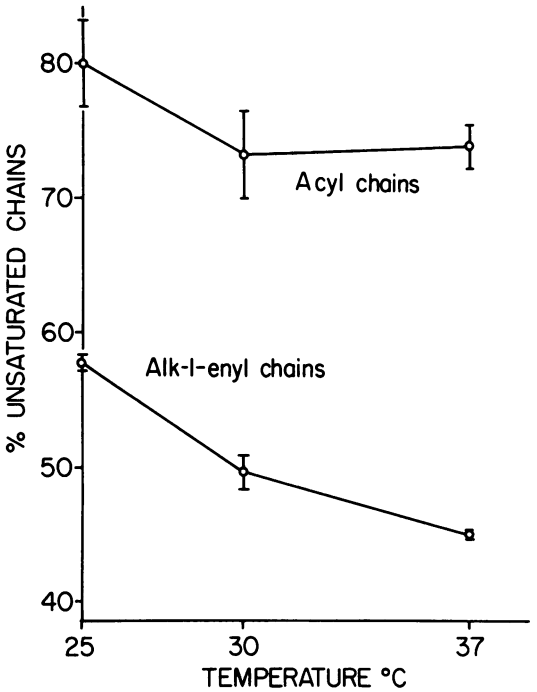

FIG. 1. Effect of growth temperature on the total acyl and alk-1-enyl chain compositions of *V. parvula* phospholipids. Any cyclopropane chains were included among the unsaturated chains.

scanning calorimetry of the total lipids showed a phase transition between 5 and 20°C upon heating. We examined the phase behavior of the phospholipids from cells grown at three temperatures (Fig. 2 and Table 3). The beginning of melting was difficult to observe; however, the temperature at which heat was absorbed most rapidly (T_{max}) and the end of melting were easily seen, and the curves were similar to the curve of Verkley et al. (33). We found that both T_{max} and the temperature at which melting ended decreased in the phospholipids from cells grown at 30 and 25°C compared with cells grown at 37°C. The total decrease in T_{max} was 5.7°C. When calorimetric cooling scans were done, the onset of the transition to the gel state occurred at 11.6°C in the phospholipids from cells grown at 37°C and at 6.8°C in the phospholipids from cells grown at 25°C. T_{max} values were 9.1 and 5.3°C for the phospholipids from cells grown at 37 and 25°C, respectively. The phospholipids from cells grown at 30°C gave values approximately halfway between those for cells grown at 37 and 25°C.

Effect of growth temperature on the phospholipid composition of *M. elsdenii*. As in *V. parvula*, the proportion of phosphatidylethanolamine in *M. elsdenii* increased slightly at the lower growth temperatures, and there was no significant change in the total plasmalogen content (Table 4).

Analysis of acyl and alk-1-enyl chains of *M. elsdenii*. The acyl and alk-1-enyl moieties of *M. elsdenii* phospholipids were analyzed by Verkley et al. (33). In agreement with the results of these authors for cells harvested in the late exponential phase of growth, we found that the 16:0 and 18:1 moieties were the major acyl and alk-1-enyl moieties; there were lesser amounts of the 14:0, 15:0, 16:1, and 18:0 moieties (Table 5). In contrast to the results of Verkley et al., we found that the major acyl and alk-1-enyl moieties designated 17:1 and *iso*-20:0 were C_{17} and C_{19} cyclopropane chains, respectively. Our designations were based on the following three lines of evidence. (i) Although the gas chromatographic retention times of the methyl esters of the acyl chains on the polar DEGS phase could be interpreted as either 17:1 or 17:cyc and the retention time of *iso*-20:0 was close to that of 19:cyc, the retention time of the longer-chain component on an SE-30 column was in close agreement with the value of a 19:cyc methyl ester and not with

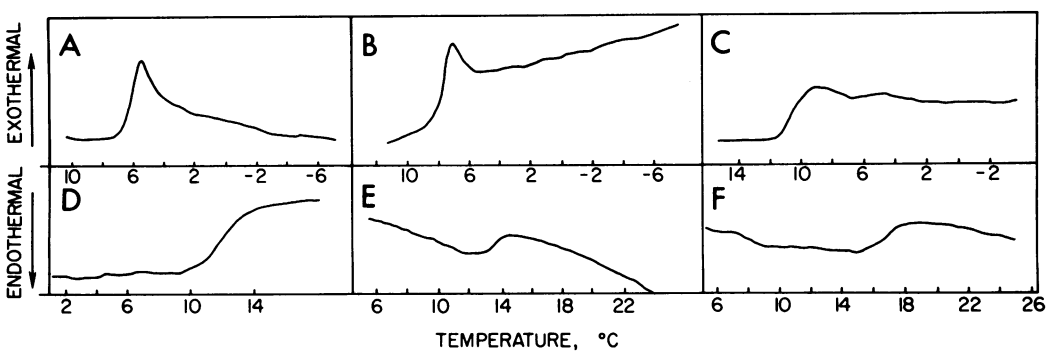

FIG. 2. Calorimetric scans of hydrated phospholipids from *V. parvula*. (A through C) Cooling scans. (D through F) Heating scans. The phospholipids were from cells grown at 27°C (A and D), 30°C (B and E), and 37°C (C and F).

TABLE 3. Phase behavior of *V. parvula* phospholipids

Growth temp (°C)	Phase transition temp on heating (°C)[a]		
	T_s	T_{max}	T_f
37	7.3 ± 0.05	14.7 ± 0.2	17.7 ± 0.5
30		12.4 ± 0.1	14.8 ± 0.2
25		9.0 ± 0.55	13.6 ± 0.2

[a] T_s, Temperature at which melting began; T_f, temperature at which melting ended.

the value of *iso*-20:0 (Table 5). (ii) When the acyl chains were reduced with hydrogen and a palladium catalyst in methanol for 40 min and this was followed by gas chromatography, we observed that the 16:1 and 18:1 acyl chains were reduced, but that the 17:cyc and 19:cyc chains were not. After reduction with hydrogen and a platinum catalyst in acetic acid for 22 h, the 17:cyc and 19:cyc chains, as well as the 16:1 and 18:1 acyl chains, were no longer visible upon gas chromatography. These results were consistent with the expected behavior of cyclopropane fatty acids (3). (iii) When the total phospholipids, the fatty acid methyl esters, or the alk-1-enyl chains were examined by ^1H nuclear magnetic resonance, signals were observed at $\delta = 0.572$ to 0.641 (multiplet) and at δ −0.327 to −0.340 (doublet) relative to tetramethysilane. These signals are highly characteristic of 1,2-dialkyl cyclopropanes (3, 7).

Compositions of the acyl and alk-1-enyl chains of *M. elsdenii* as a function of growth phase and growth temperature. Further evidence for the presence of 17:cyc and 19:cyc chains was obtained in studies on the acyl chain compositions during the growth of *M. elsdenii* at 30 and 42.5°C. As cells grew in batch cultures at these two temperatures, there was a reciprocal increase in 19:cyc and decrease in 18:1 (Fig. 3). At 42.5°C the proportion of 18:1 fell to 7.5% of the total acyl chains, and the proportion of 19:cyc increased to 39% of the total. These values were comparable to those previously reported for 18:1 and *iso*-20:0 acyl chains at the late exponential phase of growth (33). Increases in 17:cyc concomitant with decreases in 16:1 were also observed during growth at 30 and 42.5°C, but these changes were not as marked as those observed with the longer-chain pair. A similar result was obtained with the alk-1-enyl chains of strain B159, which was obtained from M. P. Bryant (University of Illinois, Urbana) and was the source of strain ATCC 17752. At 42.5°C, exponential-phase cells had 27% 18:1 and 14% 19:cyc alk-1-enyl chains, whereas stationary phase cells had 8% 18:1 and 21% 19:cyc chains. The decrease in the sum of these chains reflected an increase in the proportion of 16:0 from 19.5 to 34% of the alkenyl chains in stationary phase.

Comparisons of the relative amounts of saturated and unsaturated plus cyclopropane acyl chains at different stages of growth at 30 and 42.5°C revealed no discernable differences, except for the more rapid conversion of 18:1 to 19:cyc at the higher growth temperature (Fig. 3). Acyl chains were also compared for batch cultures grown at four temperatures between 27 and 42.5°C to the early stationary phase of growth (Table 6). Unsaturated plus cyclopropane acyl chains constituted more than 70% of the total at all temperatures studied, and there was little change at the lower growth temperatures. At 27°C the content of cyclopropane acyl chains was decreased markedly, with a concomitant increase in mono-unsaturated species. It should be noted that at 22 h cells grown at 42.5°C contained 33.5% saturated chains, suggesting a slight trend to lower unsaturation in the acyl chains of stationary-phase cells at higher growth temperatures.

The compositions of the alkenyl chains of *M. elsdenii* grown at four temperatures to the early stationary phase of growth are also shown in Table 6. As in the acyl chains, there was no discernable trend in the ratio of saturated chains to the sum of unsaturated plus cyclopropane alkenyl chains. As in the acyl chains, there was a major increase in the ratio of unsaturated chains to cyclopropane alkenyl chains at 27°C.

Phase behavior of *M. elsdenii* phospholipids. When studied by freeze-etch electron microscopy, the inner membrane of *M. elsdenii* exhibited no particle aggregation at any temperature be-

TABLE 4. Phospholipid compositions of *M. elsdenii* cells grown at different temperatures

Temp (°C)	% of total lipid phosphorus in:		% Plasmalogen[a]
	Phosphatidylethanolamine	Phosphatidylserine	
27	71.4	28.1	46
30	68.4 ± 1.8[b]	31.5 ± 1.8	57 ± 3
37	65.2 ± 3.0	34.8 ± 3.0	49.5 ± 4
42.5	64.7	35.3 ± 0.05	54 ± 2

[a] Ratio of alk-1-enyl bonds to lipid phosphorus × 100.
[b] Mean ± standard error of the mean.

TABLE 5. Gas chromatographic behavior of acyl and alk-1-enyl chains from *M. elsdenii*

Moiety	Relative retention times on:			
	DEGS-SP		SE-30	
	Unknown	Standard	Unknown	Standard
Fatty acid methyl esters				
17:cyc	1.60[a]	1.62[b]	1.32	1.32
17:1		1.58,[c] 1.55[d]		1.26[c]
19:cyc	2.84	2.85	2.5	2.47
19:1		2.87,[c] 2.78[d]		2.35[c]
iso-20:0		2.85		3.28
Aldehydes				
17:cyc	1.37[a]	1.4[b]	0.96	0.95[b]
17:1		1.35[c]		0.92[c]
19:cyc	2.42	2.46[b]	1.80	1.80[b]

[a] Retention time relative to 16:0 methyl ester.
[b] Cyclopropane chains from *C. butyricum* (7, 9).
[c] Determined from a plot of log R_t/16:0 versus chain length of known compounds.
[d] From *V. parvula*.

tween 37 and −10°C (33). We examined the *M. elsdenii* phospholipids from cells grown at 30 and 42.5°C by differential scanning calorimetry and observed no phase transitions upon heating or cooling in the range from −10 to 50°C.

DISCUSSION

Plasmalogens, mainly in the form of alkenyl acyl glycerophosphoryl-ethanolamine (31), represented from 36 to 39.4% of the total phosphoglycerides in *V. parvula* in our experiments. The most significant adaptation of lipid composition to lower growth temperatures which occurred in the plasmalogens was a major increase in monounsaturated alkenyl chains and a decrease in saturated alkenyl chains (Fig. 1). The total acyl chains were largely unsaturated at all temperatures studied (Fig. 1). An analysis of the acyl chains of the plasmalogens showed relatively little change in the proportion of the unsaturated component. Since there was little change in the total acyl chains, we assume that this is also true for the diacylphosphatides, but we have not examined these compounds separately.

The change in the phase behavior of the total phospholipids is consistent with the increase in plasmalogen alkenyl chain unsaturation in cells grown at 25°C. A similar lowering of the upper end of the phase separation was observed in *C. butyricum* ATCC 6015 when phospholipids from cells grown at 25°C were compared with phospholipids from cells grown at 37°C (11). However, in *C. butyricum* the modifications of phospholipid composition at the lower growth temperature were very different from those observed in *V. parvula*. The major changes in the former were increased acyl chain unsaturation and an increase in the plasmalogen form of phosphatidylglycerol at the expense of ethanolamine and *N*-methylethanolamine phosphoglycerides (19), whereas in *V. parvula* increased unsaturation of the plasmalogen alk-1-enyl chains was the predominant change. In a study of the acclimation of the goldfish nervous system to environmental temperatures, Roots and Johnston observed an increase in the proportion of 18:1 chains from approximately 19% of the total alkenyl chains at 30°C to approximately 29% at 5°C, along with a concomitant decrease in the proportion of 18:0 chains. In the goldfish nervous system, there are also increases in the degree of unsaturation of the phospholipid acyl chains (25, 26).

The means by which the alkenyl chain composition of *V. parvula* is affected by growth temperature is not known. In *C. butyricum* these chains are derived from fatty acids (13); if they are similarly derived in other anaerobes, the synthesis of the fatty acid chains represents one potential site of thermal regulation, as in *Escherichia coli* (4, 24). However, temperature-induced changes in the ratio of unsaturated fatty acids to saturated fatty acids synthesized de novo should be reflected in both the acyl and alk-1-enyl chain compositions, which was not observed. Therefore, attention must be focused on the steps leading to the formation of the alk-1-enyl bond. It is not known how these bonds are formed in anaerobic bacteria (9), but the reactions involved, including reduction of fatty acids or their derivatives to the oxidation level of aldehydes (5, 13), obviously represent other potential sites for regulation.

In contrast to *V. parvula*, in *M. elsdenii* phospholipids the total proportion of unsaturated plus cyclopropane alkenyl chains shows no consistent change in cells grown at 27 to 42.5°C (Table 6). One possible explanation for this is

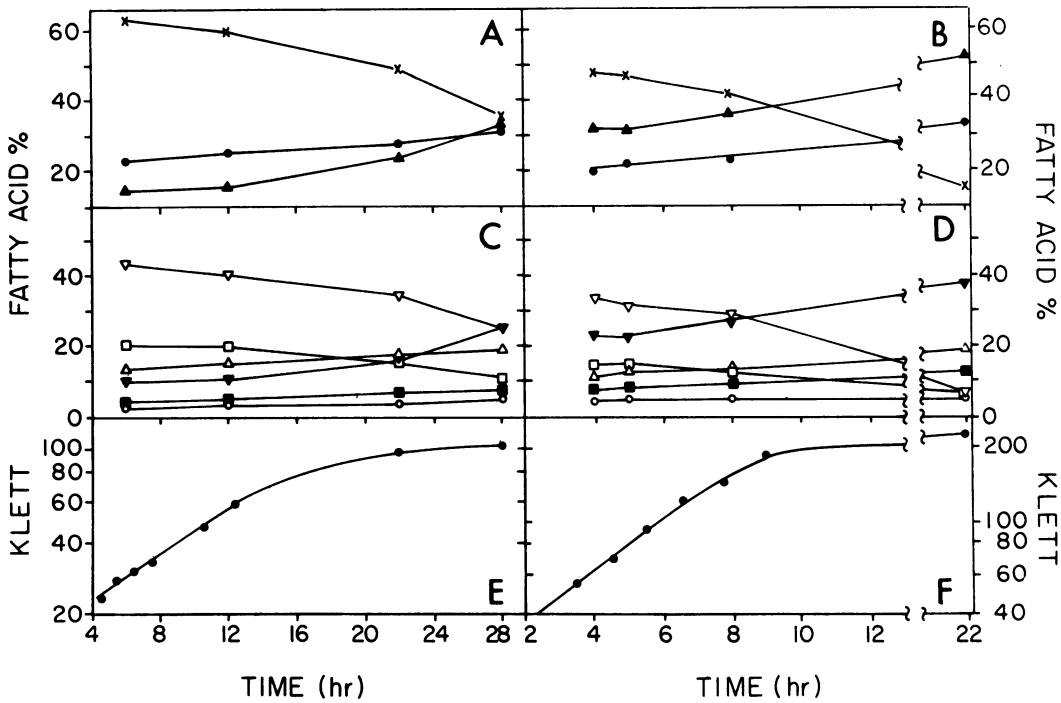

FIG. 3. Acyl chain compositions of *M. elsdenii* phospholipids during growth at 30°C (A, C, and E) and 42.5°C (B, D, and F). (E and F) growth shown as turbidity measured with a Klett colorimeter (type 660 filter). (C and D) Individual acyl chains. Symbols: △, 16:0; □ 16:1; ■, 17:cyc; ○, 18:0; ▽, 18:1; ▼, 19:cyc. (A and B) Total saturated (●), total unsaturated (×), and total cyclopropane (▲) chains.

the higher content of lower-melting unsaturated plus cyclopropane alkenyl chains even at 42.5°C in *M. elsdenii* than are present in *V. parvula* phospholipids at 25°C. The acyl chains of both of these organisms have a high proportion of unsaturated plus cyclopropane moieties at all of the growth temperatures that have been examined. Between 30 and 27°C we observed a major increase in the proportion of unsaturated acyl and alkenyl chains and a decrease in the proportion of the corresponding cyclopropane chains of *M. elsdenii* (Table 6). The relatively low rate of formation of the cyclopropane chains at 30°C (Fig. 3) is presumably accentuated at the lower temperature, resulting in an even lower rate of ring formation on the preformed phospholipids (22). Knivett and Cullen (21) observed a similar effect of temperature on cyclopropane synthesis in *E. coli* cells, and Taylor and Cronan (28) showed that the activation energy for the purified enzyme from *E. coli* is 25 kcal/mol. The results of these authors further indicated that the reaction was not affected by the order-disorder state of the lipid substrate. One effect of the failure to convert the double bonds to cyclopropane rings would be the lowering of the liquid crystalline to gel transition temperature, since phosphatidylcholines with two mono-unsaturated fatty acids melt 16°C lower than the lipid with cyclopropane chains (23, 27). Since the lipids from *M. elsdenii* appear to be well above their phase transition temperature at 27°C, the value of this failure to convert to cyclopropane fatty acids is questionable in terms of membrane fluidity.

The different natural environments of *V. parvula* and *M. elsdenii* may be relevant to the effects of growth temperature on membrane lipid composition. *V. parvula* is commonly isolated from mouths, respiratory tracts, and intestines. Therefore, it may have to adjust to greater temperature variations than *M. elsdenii*, which grows at the fairly high and relatively unvarying temperature of rumens (38 to 40°C) (16). This does not take into account the period of transmission from one animal to another, which may subject this organism to a period of lower temperatures outside the body. Little is known about this aspect of the habitat of *M. elsdenii*.

Along with our earlier studies of temperature adaptation in *C. butyricum* (19) and the studies of Roots and Johnston on the goldfish central nervous system (25, 26), this study revealed four patterns of response in plasmalogen-rich tissues or cells. In the goldfish central nervous system both acyl and alk-1-enyl chains become more

TABLE 6. Acyl and alk-1-enyl compositions of phospholipids from *M. elsdenii* cells grown at different temperatures[a]

Moiety	Composition at:			
	27°C	30°C	37°C	42.5°C
Acyl chains				
12:1	1.7 ± 0.2	3.0 ± 2.5	4.7 ± 0.9	4.3 ± 0.9
14:0	2.7 ± 0.3	1.4 ± 0.3	1.9 ± 0.05	1.5 ± 0.05
15:0	<1	<1	1.1 ± 0.05	<1
16:0	16.3 ± 0.4	18.4 ± 0.7	18.9 ± 0.6	14.1 ± 0.05
16:1	27.1 ± 0.9	8.5 ± 0.5	7.4 ± 0.7	10.6 ± 0.3
17:0	Trace	1.4 ± 0.1	2.4 ± 0.4	1.0 ± 0.05
17:cyc	4.3 ± 0.1	9.9 ± 0.2	9.7 ± 0.4	9.3 ± 0.5
18:0	3.7 ± 0.3	6.4 ± 0.9	6.8 ± 0.3	5.6 ± 0.2
18:1	38.4 ± 1.4	20.2 ± 0.1	13.8 ± 1.8	24.4 ± 1.0
19:cyc	5.7 ± 0.4	29.3 ± 1.7	31.5 ± 1.1	27.4 ± 0.1
Total saturated	22.7	27.6	29.4	22.8
Total unsaturated	67.2	31.7	25.9	39.3
Total cyclopropane	10.0	39.2	41.2	36.7
Alk-1-enyl chains				
16:0	17.9 ± 0.0	20.4 ± 2.0	24.8 ± 0.2	17.6 ± 0.6
16:1	16.7 ± 0.2	5.9 ± 0.3	7.0 ± 0.7	8.3 ± 0.8
17:0	Trace	1.6 ± 0.1	1.4 ± 0.05	Trace
17:cyc	7.9 ± 0.1	19.1 ± 0.7	20.9 ± 0.5	20.1 ± 0.1
18:0	7.8 ± 0.0	9.5 ± 0.8	7.7 ± 1.0	7.9 ± 0.4
18:1	43.2 ± 0.2	26.5 ± 1.1	20.0 ± 1.1	25.7 ± 0.3
19:cyc	6.2 ± 0.1	16.9 ± 0.6	18.0 ± 0.5	20.3 ± 0.8
Total saturated	25.7	31.5	33.9	25.5
Total unsaturated	59.9	32.4	27.0	34.0
Total cyclopropane	14.1	36.0	38.9	40.4

[a] All values are weight percentages ± standard errors and represent analyses of two separate batches of cells, except for the analysis at 27°C, which was done once. The acyl and alk-1-enyl chains of each batch of cells were analyzed twice by gas chromatography.

unsaturated at lower environmental temperatures. In *C. butyricum* only the acyl chains become more unsaturated. In *V. parvula* the acyl chains have a high proportion of unsaturated components at all temperatures studied, but the alk-1-enyl chains become more unsaturated at lower growth temperatures. Finally, *M. elsdenii* phospholipids have a high content of unsaturated plus cyclopropane acyl and alk-1-enyl chains at all growth temperatures studied, and there is little if any change at lower growth temperatures.

Although it is difficult to explain the differences between our analyses of the acyl and alkenyl chains of *M. elsdenii* and those of Verkley et al. (33), these differences do not seem to be due to strain differences, since we found the same C_{17} and C_{19} cyclopropane chains in the lipids of strain B159, the strain studied by Verkley et al. (33). As noted above, the retention times of 17:1 and 17:cyc and of *iso*-20:0 and 19:cyc are essentially the same on polar phases, such as EGSS-X, which was used by Verkley et al. (33), and DEGS, which we used. The fact that neither *iso*-20:0 nor 19:cyc would be reduced during hydrogenation in hexane with the Adams catalyst could also lead to confusion concerning the identities of these moieties. However, it is difficult to understand the apparent hydrogenation of the 17:cyc fraction observed by Verkley et al. (33). Although we did not isolate these cyclopropane acyl and alkenyl chains, we believe that the results of hydrogenation in acetic acid and the highly diagnostic nuclear magnetic resonance signals offer strong evidence for their identity. Reciprocal increases in the proportions of the cyclopropane acyl chains concomitant with decreases in the proportions of the corresponding mono-unsaturated chains have been observed in many organisms containing cyclopropane fatty acids during entry of batch cultures into the stationary phase of growth (3, 22). This phenomenon has also been observed in the alkenyl chains of *C. butyricum* (7, 9). Another difference between our results and the results of previous work was our inability to find the longer *iso*-22:0 alkenyl chains reported by Verkley et al. (33).

ACKNOWLEDGMENTS

We thank Michael C. Phillips for the use of the scanning calorimeter. This work was supported by Public Health Service grant AI08903 from the National Institutes of Health. Nuclear magnetic resonance spectra were taken at the Middle

Atlantic NMR Facility, which is supported by Public Health Service grant RR542 from the National Institutes of Health to the University of Pennsylvania.

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