Yamanaka, Y., Mizushima, H., Nozaki, M., Horio, T., and Okunuki, K. (1959), *J. Biochem. 46,* 121–132.
Yamashita, S., and Racker, E. (1969), *J. Biol. Chem. 244,* 1220–1227.
Yu, C. A., Fong, F. C., Yu, L., and King, T. E. (1971), *Biochem. Biophys. Res. Commun. 45,* 508–513.
Yu, C. A., Yu, L., and King, T. E. (1972), *J. Biol. Chem. 247,* 1012–1019.

Replacement of Acyl and Alk-1-enyl Groups in *Clostridium butyricum* Phospholipids by Exogenous Fatty Acids†

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ABSTRACT: The effect of exogenous unsaturated fatty acids on the acyl and alk-1-enyl group composition of the phospholipids of *Clostridium butyricum* has been examined. Unsaturated fatty acids support the growth of this organism in the absence of biotin. When cells were grown at 37° in media containing oleate or linoleate and a Casamino acid mixture containing traces of biotin, the exogenous fatty acids were found mainly in the alk-1-enyl chains of the plasmalogens with less pronounced incorporation into the acyl chains. However, at 25° in this medium, both the acyl and alk-1-enyl chains contained substantial amounts of the 18:1 supplement plus the C_{19}-cyclopropane chains derived from it. Alk-1-enyl chains in all the major phosphatide classes showed a uniformly high substitution by the oleate supplement in cells grown at 37°. The oleate and C_{19}-cyclopropane content of the acyl chains was more variable among the phosphatide classes. At 37°, *trans*-9-octadecenoic acid (elaidic acid) also supported growth and was incorporated into both acyl and alk-1-enyl chains at a high level. When cells were grown on oleate at 37° in media containing biotin-free Casamino acids, both the acyl and alk-1-enyl chains had a high level of 18:1 plus C_{19}-cyclopropane chains. In the cells grown at 37° with oleate substantial changes were seen in the phospholipid class composition. There was a large decrease in the ethanolamine plus *N*-methylethanolamine plasmalogens with a corresponding increase in the glycerol acetals of these plasmalogens. The glycerol phosphoglycerides were also significantly lower with the appearance of an unknown, relatively nonpolar phospholipid fraction.

There is now substantial evidence that phospholipids play a major role in the structure and function of biological membranes (Cronan and Vagelos, 1972; Singer and Nicolson 1972). The influence of the physical state of membrane lipids on membrane assembly and membrane function has been studied extensively in microbial unsaturated fatty acid auxotrophs in which the fatty acid composition of the membrane lipids can be manipulated by changing the fatty acid supplement in the growth medium (Machtiger and Fox, 1973). It is generally assumed that thermal phase transitions in biological membranes largely reflect the transitions of the fatty acyl chains of the membrane phospholipids, suggesting that the physical properties of the hydrocarbon chains are one of the principal factors involved in regulating membrane fluidity (Esfahani et al., 1969; Overath et al., 1970; Gitler, 1973). Several laboratories have correlated the temperature dependence of solute transport in bacteria with the physical state of the membrane lipids in cells possessing altered acyl group composition (Machtiger and Fox, 1973; Linden et al., 1973; Overath and Traüble, 1973).

Clostridium butyricum is an obligate anaerobe in which plasmalogens (derivatives of 1-alk-1′-enyl-*sn*-glycerol 3-phosphate) constitute more than half of the total phospholipids. We are seeking to determine if the plasmalogens of these anaerobic bacteria contribute to the fluid properties of the cell membrane, and, if so, what effect they have on the functions of the cell membrane. As first steps toward the alteration of the lipid composition in *C. butyricum,* we have tried to change the apolar chain composition of the membrane lipids by two approaches: (1) by growing the cells at various temperatures, and (2) by supplementing the growth medium with unsaturated fatty acids in place of biotin. The results obtained upon changing the growth temperature suggest that plasmalogens do participate in an adaptive response of the cell membrane lipids (Khuller and Goldfine, 1974). In this paper, the results of the second approach are reported, which show that both the acyl and the alk-1-enyl[1] group composition in *C. butyricum* polar lipids can be manipulated by supplementing the growth medium with unsaturated fatty acids in the absence of biotin or in the presence of suboptimal levels of biotin.

Experimental Section

Materials. *cis*-9-Octadecenoic, *trans*-9-octadecenoic, and linoleic acids were obtained from Nu-chek Prep, Elysian, Minn. Pentadecanoic acid and NIH-D standard fatty

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[1] In this paper alk-1-enyl chains of total phospholipids include the glycerol-substituted alk-1-enyl chains in the glycerol acetals of the ethanolamine and *N*-methylethanolamine plasmalogens. These, like the ordinary alk-1-enyl chains, are released as aldehydes on acid hydrolysis. For acyl groups and alk-1-enyl groups, the number before the colon is the chain length and after, the number of double bonds. Cyc indicates a cyclopropane-containing chain.

Table I: Phospholipid Acyl and Alk-1-enyl Group Composition (wt %): *Clostridium butyricum* Grown on Oleic Acid with Suboptimal Levels of Biotin.[a,b]

	Acyl Chains		Alk-1-enyl Chains	
	37° (6)[b]	25° (3)	37° (6)	25° (3)
12:0	0.2 ± 0.2	0.5 ± 0.5		
14:0	7.5 ± 2.1	4.6 ± 4.3		
14:1	0.3 ± 0.1	0.2 ± 0.1		
16:0	34.6 ± 5.0	13.6 ± 5.6	7.9 ± 6.5	5.6 ± 2.1
16:1 + 17:cyc	17.7 ± 6.1	9.1 ± 2.3	11.0 ± 4.2	6.0 ± 4.2
18:0	0.6 ± 0.2	0.6 ± 0.1		
18:1 + 19:cyc	39.2 ± 8.3	71.5 ± 12.1	81.0 ± 7.2	88.3 ± 4.2
Total saturated	43.0 ± 3.7	19.1 ± 10.1	7.9 ± 6.5	5.6 ± 2.1
Total unsaturated plus cyclopropane	57.1 ± 3.7	80.8 ± 10.3	92.0 ± 6.5	94.3 ± 2.1

[a] Cells were grown in the presence of "vitamin free powder, salt free" Casamino acids. [b] The numbers in parentheses represent the number of separate samples analyzed. Values are means ±SD.

acids were obtained from Applied Science Laboratories Inc., State College, Pa. *cis*-9,10-Methylenehexadecanoic acid was purchased from Analabs, Inc., North Haven, Conn. *cis*-11,12-Methyleneoctadecanoic acid was a gift from Dr. K. Hofmann. Palmitaldehyde was purchased from Aldrich Chemicals as the bisulfite addition product, which was converted to the aldehyde by alkali treatment. *cis*-9,10-Methyleneoctadecanal, *cis*-9,10-methylenehexadecanal, *cis*-9-16:1 aldehyde, and *cis*-9-18:1 aldehyde were obtained by hydrolysis of the dimethylacetals, which were prepared in this laboratory (Goldfine and Panos, 1971). *cis,cis*-9,12-Octadecadienal was prepared by oxidation of linoleyl mesylate, obtained from Nu-chek Prep, Elysian, Minn., as described by Mahadevan et al. (1966). Glass-distilled solvents were obtained from the Anspec Co., Ann Arbor, Mich. Casamino acids, "vitamin free, powder, salt free" and "10% sterile solution" were obtained from Nutritional Biochemicals, Cleveland, Ohio. The former contained traces of biotin according to the manufacturer, and the latter was suitable for microbiological assay of biotin.

Methods. Clostridium butyricum (ATCC 6015) cells were grown anaerobically with Casamino acids, in the medium described by Broquist and Snell (1951) but without biotin. Fatty acids, 10 mg/l., were added as supplements in 0.1–0.25 ml of 95% ethanol. Although some of the fatty acids were soluble at this concentration, 0.002% of Brij 35 (poly(oxyethylene)-23-lauryl ether) was added routinely to minimize the turbidity resulting from the fatty acids. Methods for growing these cells at different temperatures have been described (Khuller and Goldfine, 1974). Cells were harvested in the late log phase of growth between Klett (66 filter) values of 120–150 and were washed once with 50 m*M* phosphate buffer (pH 7.2). Methods for extraction, washing, and separation of the phospholipids from the nonpolar fractions are described elsewhere (Goldfine and Bloch, 1961; Baumann et al., 1965). The polar lipids were fractionated on silica gel G plates with the solvent system $CHCl_3$–MeOH–7*N* NH_4OH (60:35:5, v/v) (Baumann et al., 1965). Alk-1-enyl groups, lysophosphatides, and diacyl phosphatides were isolated as described (Khuller and Goldfine, 1974). Fatty acids were obtained by saponification of lipids (Goldfine and Bloch, 1961) and were methylated with diazomethane. Fatty acid methyl esters and aldehydes were analyzed by gas–liquid chromatography on a 10% EGSS-X on Gas Chrom P column at 180°. Proportions of fatty acids and aldehydes were determined with a Model CR-208 Infotronics digital electronic integrator. The fatty acids and aldehydes were identified by comparing the relative retention times with those of authentic standards. Separation of saturated and cis and trans monoenoic acids as their methyl esters was accomplished by preparative silver nitrate (10%) thin layer chromatography (silica gel G) with a developing solvent system of petroleum ether–diethyl ether (95:5). The methyl ester spots were visualized under uv light after spraying with 0.05% Rhodamine 6G and were eluted with diethyl ether. Cis and trans fatty acids were quantitated by gas–liquid chromatography using an internal standard of pentadecanoic acid. Lipid phosphorus was determined by the method of Bartlett (1959).

Results

As observed by Broquist and Snell (1951) good growth of *C. butyricum* can be obtained in media containing either biotin or unsaturated fatty acids alone or with a detergent. In our initial work cells were grown on Casamino acids "vitamin free, powder, salt free" without added biotin and were subcultured until their growth ceased. It was observed that three subcultures were necessary to deplete the cells of biotin. In one experiment calls were subcultured three times in the presence of oleate at 37° without added biotin and each of the subcultures was analyzed for the acyl and alk-1-enyl chain composition of the phospholipids. The results demonstrated a gradual increase in the proportion of the fed fatty acid in the lipids with each subculture. Among the alk-1-enyl chains there was a substantial increase in the proportions of 18:1 and 19:cyc with each subculture, attaining a value of 85% of the total. An increase of 18:1 and 19:cyc in the acyl chains was also observed; however, this increase was not as marked as in the alk-1-enyl chains, going no higher than 30% of the total (Goldfine et al., 1975).

The acyl and alk-1-enyl group compositions of the phospholipids of cells harvested after four subcultures with oleate and suboptimal biotin at 37° are presented in Table I. The acyl group composition was not markedly different from that of cells grown with biotin at 37°, but the alk-1-enyl chains were substantially more unsaturated. The 18:1 plus 19:cyc in the acyl chains represented 39.2% of the total; however, the alk-1-enyl chains were 81% 18:1 plus 19:cyc. When the cells were grown at 25° with oleate and suboptimal biotin, a pronounced increase in the degree of unsaturation of the acyl chains was observed, with 80.9% of the fatty acids unsaturated; including 71.5% of 18:1 plus

Table II: Acyl Group Composition (wt %) of the Major Phosphatides: *Clostridium butyricum* Grown on Oleic Acid at 37° with a Suboptimal Level of Biotin.[a,b]

	Chain Length						
	12:0	14:0	14:1	16:0	16:1 + 17:cyc	18:0	18:1 + 19:cyc
Total phospholipids	0.4	10.2	0.4	27.9	18.7	0.5	41.9
Glycerol acetal of ethanolamine plus *N*-methylethanolamine plasmalogens	0.3	9.5	0.7	30.0	26.7	0.3	32.6
Ethanolamine plus *N*-methylethanolamine plasmalogens	0.2	7.2	0.1	34.7	15.3	1.2	41.1
Diacylphosphatidylethanolamine plus phosphatidyl-*N*-methylethanolamine	0.4	10.5	0.1	25.9	10.8	1.8	50.4
Glycerol phosphoglyceride: diacyl plus plasmalogens	0.2	6.4	0.1	24.0	12.9	0.6	55.8

[a] Values are averages of two separate runs with the same sample. [b] Cells were grown with Casamino acids, "vitamin free, powder, salt free".

Table III: Alk-1-enyl Group Composition (wt %) of the Major Phosphatides: *Clostridium butyricum* Grown on Oleic Acid at 37° with a Suboptimal Level of Biotin.[a,b]

	Chain Length			
	16:0	16:1 + 17:cyc	18:0	18:1 + 19:cyc
Total phospholipids	3.1	12.0		84.9
Ethanolamine plus *N*-methylethanolamine plasmalogens	2.6	12.2	0.2	85.0
Glycerol acetal of ethanolamine plus *N*-methylethanolamine plasmalogens	3.3	11.6		85.1
Glycerol phosphoglycerides: plasmalogens	1.8	12.3		85.9

[a] Values are averages of two separate runs with the same sample. [b] Cells were grown on Casamino acids, "vitamin free, powder, salt free".

19:cyc. The major compensating decrease was in the amount of 16:0, from 34.6 to 13.6% when compared with oleate-grown cell at 37°. The alk-1-enyl groups did not change significantly in oleate-grown cells when the growth temperature was decreased to 25°.

An analysis of the acyl and alk-1-enyl groups isolated from the major phosphatide classes of cells grown at 37° with oleate and suboptimal biotin is presented in Tables II and III. It is clear from these data that there was no preferential incorporation of the fed fatty acid into the alk-1-enyl chains of a single species of phosphatide. In each phosphatide class, 85% of the alk-1-enyl chains are represented by 18:1 plus 19:cyc. However, in the acyl chains the proportion of the fed fatty acid, 18:1, and the 19:cyc derived from it, varied from 32.6 to 55%.

In the latter stages of this work, cells were grown on a hydrolysate of highly purified casein, which further reduced the level of biotin. The results obtained with these cells after four subcultures with oleate are given in Table IV. At 37° both the acyl and alk-1-enyl groups were highly unsaturated. The fatty acids were substantially more unsaturated than in cells grown with normal levels of biotin or suboptimal biotin. Decreasing the growth temperature to 25° did not further alter the fatty acid composition in biotin-free media (Table IV). Table V gives the phosphatide composition of exponential phase cells grown with oleate at 37° in biotin-free media. Compared with cells grown on biotin-containing media, significant changes were noted in the proportions of the phosphatide classes. The proportion of ethanolamine and *N*-methylethanolamine phosphoglycerides decreased by 50% with the major decrease occurring in the plasmalogens, and the glycerol phosphoglycerides were decreased by 30% (Khuller and Goldfine, 1974). A pronounced increase in the proportion of the glycerol acetals of the ethanolamine and *N*-methylethanolamine plasmologens was observed in these oleate-fed cells, and an unknown lipid species became evident.

We have also supplemented the growth media with linoleic acid and suboptimal biotin. Cells were subcultured four times at 37°, the phospholipids were isolated and the

Table IV: Phospholipid Acyl and Alk-1-enyl Group Composition (wt %): *Clostridium butyricum* Grown on Oleic Acid in Biotin-Free Medium.[a]

	Acyl Chains		Alk-1-enyl Chains	
	37° (3)[b]	25° (2)	37° (3)	25° (2)
14:0	1.1 ± 0.7	1.3 ± 0.1		
14:1	0.4	0.5		
16:0	5.6 ± 2.2	4.8 ± 1.2	0.4 ± 0.2	0.3 ± 0.02
16:1 + 17:cyc	2.4 ± 0.4	4.7 ± 3.1	1.6 ± 0.5	4.8 ± 0.4
18:0	1.8 ± 1.1	1.1 ± 0.2		0.6
18:1 + 19:cyc	88.6 ± 3.3	87.8 ± 4.7	98.1 ± 0.6	94.6 ± 0.4
Total saturated	8.6 ± 3.0	7.2 ± 1.4	0.4 ± 0.2	0.6 ± 0.4
Total unsaturated plus cyclopropane	91.4 ± 3.0	92.8 ± 1.3	99.7 ± 0.2	99.4 ± 0.4

[a] Cells were grown on Casamino acids, "vitamin free, 10% sterile solution". [b] The numbers in parentheses represent the number of separate samples analyzed. Values are means ±SD.

Table V: Composition of Phosphatides of *Clostridium butyricum* Grown at 37° on Oleic Acid in Biotin-Free Medium.[a]

Compound	Content (% of total phospholipids)
Glycerol acetals of ethanolamine plus *N*-methylethanolamine plasmalogens	51.8 ± 4.1[b]
Ethanolamine and *N*-methylethanolamine phosphoglycerides: diacyl plus plasmalogens	21.2 ± 2.9[c]
Glycerol phosphoglycerides: diacyl plus plasmalogens	15.9 ± 5.1
Unknown phospholipid	11.0 ± 1.3

[a] Cells were grown in the presence of Casamino acids, "vitamin free", 10% sterile solution." [b] Three separate batches of cells were analyzed. The values are means ±SD. [c] On hydrolysis with 90% acetic acid at 37° for 18 hr, 53.5 ± 3.5% of the mixture was converted to the corresponding lyso derivatives. This is assumed to represent the proportion of plasmalogen (Khuller and Goldfine, 1974).

Table VI: Phospholipid Acyl and Alk-1-enyl Group Composition (wt %): *Clostridium butyricum* Grown on Linoleic Acid at 37° with Suboptimal Biotin.[a]

	Acyl Chains (6)[b]	Alk-1-enyl Chains (6)
14:0	10.0 ± 2.8	
14:1	0.5 ± 0.4	
16:0	40.0 ± 8.7	7.0 ± 3.8
16:1	11.0 ± 4.4	13.0 ± 6.1
17:cyc	3.6 ± 1.1	5.8 ± 2.4
18:0	0.8 ± 0.3	
18:1	4.7 ± 1.7	5.0 ± 3.2
18:2	28.2 ± 13.7	62.7 ± 8.8
19:cyc	1.5 ± 0.7	6.6 ± 1.1
Total saturated	50.8 ± 8.7	7.0 ± 3.8
Total unsaturated plus cyclopropane	49.2 ± 8.7	93.0 ± 3.8

[a] Cells were grown on Casamino acids, "vitamin free, powder, salt free". [b] The numbers in parentheses represent the number of separate samples analyzed. Values are means ±SD.

Table VII: Phospholipid Acyl and Alk-1-enyl Group Composition (wt %): *Clostridium butyricum* Grown on Elaidic Acid at 37° with Suboptimal Biotin.[a]

	Acyl Chains (5)[b]	Alk-1-enyl Chains (5)
12:0	0.8 ± 1.0	
14:0	7.2 ± 1.4	
14:1	0.4 ± 0.1	
16:0	16.2 ± 2.4	4.9 ± 1.9
16:1 + 17:cyc	10.0 ± 2.5	10.1 ± 2.2
18:0	0.3 ± 0.1	0.3 ± 0.1
18:1	64.5 ± 4.9	83.3 ± 3.4
19:cyc	0.6 ± 0.1	1.4 ± 0.4
Total Saturated	24.5 ± 3.0	5.2 ± 2.0
Total unsaturated plus cyclopropane	75.5 ± 3.0	94.8 ± 2.0

[a] Cells were grown on Casamino acids, "vitamin free powder, salt free". [b] The numbers in parentheses represent the number of separate samples analyzed. Values are mean ±SD.

results of the acyl and alk-1-enyl chain analyses are given in Table VI. It is interesting to note that linoleate, which is not normally present in this organism, is incorporated into the membrane lipids. Again, we observed that the alk-1-enyl chains became substantially more unsaturated than the acyl chains. The increase in unsaturation in alk-1-enyl chains was mainly due to octadecadienal, which represented 62.7% of the total.

Cells were also grown with *trans*-9-18:1, elaidic acid, and suboptimal biotin. After four subcultures at 37° in the presence of elaidic acid the cells were analyzed for acyl and alk-1-enyl group composition. Table VII shows that there was considerable replacement of both the acyl and alk-1-enyl chains. Among the acyl chains, saturated fatty acids represented 24.5% of the total, whereas the alk-1-enyl chains were only 5.2% saturated. Since gas chromatography did not distinguish between the geometric isomers of the unsaturated fatty acids, the fatty acids of the total phospholipids of cells grown on elaidic acid were separated into cis, trans, and saturated by thin layer chromatography on silver nitrate, silica gel G plates and were subsequently analyzed by gas chromatography. Approximately 90% of the phospholipid 18:1 fatty acids was *trans*-18:1.

Discussion

The ability of unsaturated fatty acids to support the growth of *C. butyricum* in the absence of biotin was demonstrated by Broquist and Snell (1951). Goldfine and Bloch (1961) showed that the fatty acid supplement was incorporated into the acyl chains of the cellular lipids and that endogenous fatty acid synthesis from acetate was greatly diminished under these conditions. Since then, we have shown that this organism is rich in alk-1-enyl ether lipids (Goldfine, 1964; Baumann et al., 1965; Hagen and Goldfine, 1967) and that exogenous fatty acids are incorporated into both the acyl and alk-1-enyl chains (Baumann et al., 1965). The latter studies were done on cells grown in biotin-containing media with trace amounts of labeled fatty acids, and no important changes in the composition of the cellular acyl and alk-1-enyl chains were detected.

The present study was undertaken in order to measure the changes in lipid composition that could be brought about by adding higher levels of exogenous fatty acids in media with little or no added biotin. When the cells were grown with oleate in media containing a suboptimal level of biotin, a major alteration was seen in the alk-1-enyl chain composition of the polar lipids, with little change in the degree of unsaturation of the acyl chains (Table I). It appears that exogenous fatty acids do not completely suppress endogenous synthesis when suboptimal levels of biotin are present in the medium. When cells were grown on oleate in media containing a hydrolysate of highly purified casein containing little or no biotin, the acyl chains as well as the alk-1-enyl chains were highly enriched in 18:1 and 19:cyc (Table IV). Endogenous synthesis appears to have been almost entirely eliminated.

At present we have no explanation for the preferential incorporation of exogenous fatty acids into the alk-1-enyl chains and of endogenously synthesized fatty acids into the acyl chains. It is known that the initial step of phospholipid synthesis, the acylation of *sn*-glycerol-3-P, occurs with acyl derivatives of the acyl carrier protein rather than with CoA derivatives, in extracts of *C. butyricum* (Goldfine et al., 1967; Goldfine and Ailhaud, 1971). An acyl CoA reductase has been identified in extracts of this organism (Day et al., 1970), but the pathway of exogenous fatty acids into both the alk-1-enyl and acyl chains of the polar lipids is not understood (Goldfine and Hagen, 1972).

When the cells were grown with oleate and suboptimal biotin at 25° rather than at 37°, both the acyl and alk-1-

enyl chains were largely 18:1 plus 19:cyc. Apparently, the exogenous unsaturated fatty acid is preferentially utilized for acyl chains as well as alk-1-enyl chains when the cells are grown at a lower temperature. In *Escherichia coli* the acyl CoA:glycerophosphate acyltransferase responds to lower temperatures by catalyzing increased acyl transfer from unsaturated acyl CoA esters (Sinensky, 1971). Both acyl CoA and acyl ACP derivatives are utilized for the acylation of monoacyl glycerophosphate in extracts of *C. butyricum* (Goldfine and Ailhaud, 1971), and it is possible that selectivity is also operating at the level of the acyltransferases in this organism in response to lowered growth temperatures. When *C. butyricum* was grown at 25° in the presence of biotin, an increase in the degree of unsaturation of the acyl chains of the phospholipids was also observed (Khuller and Goldfine, 1974).

The incorporation of both linoleate and elaidate into the acyl and alk-1-enyl chains in *C. butyricum* lipids was also substantial when cells were grown with suboptimal levels of biotin at 37° (Tables VI and VII). Linoleate, like oleate, was preferentially incorporated into the alk-1-enyl chains. Polyunsaturated alk-1-enyl chains have not been observed in bacterial plasmalogens (Goldfine and Hagen, 1972) and are unusual even in animal tissues (Horrocks, 1972).

When cells were grown with elaidate on suboptimal levels of biotin at 37°, both the acyl and alk-1-enyl chains were largely 18:1. Argentation thin layer chromatography showed that about 90% of the 18:1 acyl chains were *trans*-18:1. A similar analysis of the alk-1-enyl chains was not performed, but it is safe to assume that the 18:1 was largely trans as evidenced by the very low level of conversion to 19:cyc (Table VII). In contrast, *cis*-9-18:1 alk-1-enyl chains are readily converted to the corresponding cyclopropane compound (Goldfine and Panos, 1971). In the experiment shown in Table IV, 89% of the *cis*-9-18:1 alk-1-enyl chains were converted to 19:cyc at a similar stage of growth (data not shown).

The observation that larger amounts of *trans*-9-18:1 than *cis*-9-18:1 were incorporated at 37° into *C. butyricum* lipids when some biotin was present is similar to the results of experiments with unsaturated fatty acid auxotrophs of *Escherichia coli*. The effect is thought to be due to the higher melting points of the trans acids, which results in higher temperatures of thermal phase transitions in the cellular membranes (Overath et al., 1970; Linden et al., 1973).

When the cells were grown with oleate either with or without suboptimal levels of biotin, there were noticeable changes in the phospholipid class composition. The diacyl plus 1-alk-1′-enyl, 2-acyl (plasmalogen) forms of the ethanolamine plus *N*-methylethanolamine phosphoglycerides decreased, with the major decrease in the plasmalogen forms. The glycerol phosphoglycerides were also decreased. The major compensating increase was in the glycerol acetals of the ethanolamine plus *N*-methylethanolamine plasmalogens (Matsumoto et al., 1971; Khuller and Goldfine, 1974). In addition there was an increased amount of an unidentified phospholipid fraction which migrated between the glycerol phosphoglycerides and the solvent front on thin layer chromatograms. Similar changes were observed in cells grown with linoleate at 37° whereas elaidate-fed cells resembled cells grown in biotin-rich medium (data not shown).

Changes in phospholipid class composition were also noted in *C. butyricum* grown in biotin-rich medium at 25 and 37°. It was suggested that such changes may be involved in the maintenance of membrane fluidity (Khuller and Goldfine, 1974). Perhaps the changes seen in oleate-grown cells at 37° are compensatory for the large changes taking place in the apolar chains. The results of preliminary studies on the solubility of the electron spin resonance probe 2,2,6,6-tetramethylpiperidinyl-*N*-oxy in lipids extracted from oleate- and biotin-fed cells grown at 37°, indicate that these changes are in some way compensatory.

The work presented here demonstrates the existence of techniques available for the modification of the apolar chains of the polar lipids including the plasmalogens of an anaerobic bacterium. Using a variety of fatty acid supplements, including cis monounsaturated, cis polyunsaturated, and trans monounsaturated, it has been possible to alter the degree of unsaturation of both alk-1-enyl chains and acyl chains. In the presence of suboptimal levels of biotin greater changes are seen in the alk-1-enyl chains. In media without biotin, both types of chains are affected. Thus it is possible to begin to ask questions about the effects of these changes on the physical properties of the membrane lipids and on the physiological functions of the membranes.

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References

Bartlett, G. R. (1959), *J. Biol. Chem. 234,* 466.
Baumann, N. A., Hagen, P.-O., and Goldfine, H. (1965), *J. Biol. Chem. 240,* 1559.
Broquist, H. P., and Snell, E. E. (1951), *J. Biol. Chem. 188,* 431.
Cronan, Jr., J. E., and Vagelos, P. R. (1972), *Biochim. Biophys. Acta 265,* 25.
Day, J. I. E., Goldfine, H., and Hagen, P.-O. (1970), *Biochim. Biophys. Acta 218,* 179.
Esfahani, M., Barnes, Jr., E. M., and Wakil, S. J. (1969), *Proc. Natl. Acad. Sci. U.S.A. 64,* 1057.
Gitler, C. (1973), *Annu. Rev. Biophys. Bioeng. 1,* 1.
Goldfine, H. (1964), *J. Biol. Chem. 239,* 2130.
Goldfine, H., and Ailhaud, G. P. (1971), *Biochem. Biophys. Res. Commun. 45,* 1127.
Goldfine, H., Ailhaud, G. P., and Vagelos, P. R. (1967), *J. Biol. Chem. 242,* 4466.
Goldfine, H., and Bloch, K. (1961), *J. Biol. Chem. 236,* 2596.
Goldfine, H., and Hagen, P.-O. (1972), in Ether Lipids, Snyder, F., Ed., New York, N.Y., Academic Press, p 329.
Goldfine, H., Khuller, G. K., and Lueking, D. L. (1975), *Lipids 1,* (in press).
Goldfine, H., and Panos, C. (1971), *J. Lipid Res. 12,* 214.
Hagen, P.-O., and Goldfine, H. (1967), *J. Biol. Chem. 242,* 5700.
Horrocks, L. A. (1972), in Ether Lipids, Snyder, F., Ed. New York, N.Y., Academic Press, p 171.
Khuller, G. K., and Goldfine, H. (1974), *J. Lipid Res. 15,* 500.
Linden, C. D., Wright, K. C., McConnell, H. M., and Fox, C. F. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2271.
Machtiger, N. A., and Fox, C. F. (1973), *Annu. Rev. Biochem. 42,* 575.
Mahadevan, V., Phillips, F., and Lundberg, W. O. (1966), *Lipids 1,* 183.

Matsumoto, M., Tamiya, K., and Koizumi, K. (1971), *J. Biochem. (Tokyo) 69,* 617.
Overath, P., Schairer, H. U., and Stoffel, W. (1970), *Proc. Natl. Acad. Sci. U.S.A. 67,* 606.
Overath, P., and Traüble, H. (1973), *Biochemistry 12,* 2625.
Sinensky, M. (1971), *J. Bacteriol. 106,* 449.
Singer, S. J., and Nicolson, G. L. (1972), *Science 175,* 720.

Conformation and Motion of the Choline Head Group in Bilayers of Dipalmitoyl-3-*sn*-phosphatidylcholine†

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ABSTRACT: The conformation and motion of the choline head group in lipid bilayers above and below the gel-to-liquid crystal transition point are studied by means of deuterium and phosphorus magnetic resonance. For this purpose dipalmitoyl-3-*sn*-phosphatidylcholine is selectively deuterated at various positions on the choline and glycerol constituents. The residual deuteron quadrupole couplings and the phosphorus chemical-shift anisotropy of the corresponding lipid–water mixtures yield quantitative information on the segmental motions. The choline methyl group is only slightly hindered in its movement, but the motional freedom becomes increasingly restricted the closer the segment is located to the glycerol backbone. The average value of the OC–CN bond rotation angle changes with temperature. Increasing the temperature rotates the choline methyl group into the vicinity of the phosphorus atom. The choline group as a whole is thus characterized by a flexible, temperature-dependent structure. Its orientation in space is not fixed, either parallel or perpendicular to the bilayer surface. Instead all segments execute angular oscillations with varying degrees of restriction around the normal on the bilayer surface. The gel-to-liquid crystal phase transition at 41° is clearly reflected in the deuterium and phosphorus resonance spectra of the choline moiety, while no change is observed at 34°. The calorimetric pretransition at 34° seems not to be associated with a conformational change in the choline group.

The structure and function of lipid–water systems depend in large part on the nature of the polar groups. The interaction of charged hydrophilic polar groups with each other and with water and the van der Waals attractions between the hydrophobic fatty acyl chains constitute the physical basis for the arrangement of the lipid molecules in many different structures—micelles, cylindrical rods, bilayer leaflets, etc. (Luzzati, 1968; Shipley, 1973; Parsegian, 1973). The role of the polar head groups is of particular interest in relation to the biophysical reactivity of lipid–water systems. Ion-binding or pH-induced charge alterations at the polar groups can lead to a completely different long-range or short-range order (Rand and Sengupta, 1972; Träuble and Eibl, 1974). It is also plausible, though not yet proven experimentally, that biochemical reactions or cell–cell intersurface contacts could be triggered by small changes in the conformation, orientation, or charge of the polar groups.

In looking at the properties of the polar groups with physicochemical methods the most detailed information has so far been provided by single-crystal X-ray studies of phospholipids and their constituents. A precise picture of the molecular conformation, the bond angles, and the bond distances for the choline and ethanolamine head groups in the crystalline state has been derived (Sundaralingam, 1972; Hitchcock et al., 1974; Phillips et al., 1972; Abrahamsson and Pascher, 1966; DeTitta and Craven, 1973). It has been suggested that these conformations most likely will also be exhibited in solution and in the liquid crystalline state characteristic of biological systems. For lipid molecules in solution some of these predictions have been confirmed by means of proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR) (Birdsall et al., 1972; Dufourcq and Lussan, 1972; Shaw et al., 1973; Richard et al., 1974; Lichtenberg et al., 1974). The liquid crystalline state, however, has received relatively little attention in this respect since the conditions necessary to obtain high-resolution proton or ^{13}C NMR spectra are generally not met. Here the introduction of deuterium magnetic resonance holds promise of major advances (Oldfield et al., 1971; Charvolin et al., 1973; Seelig and Niederberger, 1974a,b; Seelig and Seelig, 1974a,b; Niederberger and Seelig, 1974; Stockton et al., 1974; Fujiwara et al., 1974). If a chain segment of the polar head is specifically deuterated any anisotropic motion of this segment should give rise to a doublet splitting of the deuterium magnetic resonance signal. The separation of the two deuterium transitions is then connected in a simple way with the degree of order of the C–D bond vector. We have therefore synthesized a series of dipalmitoyl-3-*sn*-phosphatidylcholines specifically deuterated at the three carbon atoms of the choline group and also at the C-3 position of the glycerol moiety, the latter being generally considered the rigid core part of the lipid molecule. The deuterium magnetic resonance spectra of the corresponding lipid–water systems have been measured as a function of temperature. In order to elucidate the motion of the phosphate group, the phosphorus-31 nuclear magnetic resonance spectra of the same systems have also been recorded as a func-

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