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Propionate-Induced Synthesis of Odd-Chain-Length Fatty Acids by *Escherichia coli*[1]

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Exogenous propionate is incorporated in vivo by *Escherichia coli* as a primer to produce lipids with fatty acids of odd chain lengths. This provides a method for the specific labeling of the three terminal carbons in the fatty acyl chains of phospholipids.

Lipids containing fatty acids of odd chain lengths are produced by *Escherichia coli* K-12 during growth in glucose/Luria broth (6) supplemented with sodium propionate (Table 1). These abnormal fatty acids were identified by using a DuPont 21-491 mass spectrometer interfaced to a Varian 2700 gas chromatograph as 15:0, 17:0, and 17:1. Although growth in the presence of other carboxylic acids caused changes in fatty acid composition, no unusual fatty acids were produced.

All carboxylic acids tested caused an increase in the proportion of saturated fatty acids. Short-chain-length carboxylic acids have been shown to increase membrane fluidity in model systems (4). The observed increase in the proportion of saturated fatty acids would decrease membrane fluidity and may represent an adaptive response.

In vitro studies of fatty acid synthesis (3, 7) have shown that propionate can compete with acetate both as a primer and as a substrate for carboxylation. Thus it seemed likely that propionate was being incorporated in vivo to make odd-chain fatty acids. Odd-chain fatty acids could be produced from propionate in three ways: (i) by utilization of propionyl-acyl carrier protein (ACP) as a primer; (ii) by utilization of methylmalonyl-ACP (from the carboxylation of propionate) for elongation an odd number of times; or (iii) by utilization of propionyl-ACP as a primer coupled with the use of methylmalonyl-ACP for elongation an even number of times. The hypothesis that propionate is incorporated intact to produce odd-chain fatty acids as well as the three possible routes can be readily examined experimentally by using ^{13}C-enriched propionate and ^{13}C nuclear magnetic resonance (NMR). Cells were grown into stationary phase in glucose/Luria broth (6) supplemented with 0.035 M [1-^{13}C]propionate (greater than 90% enriched; Bio-Rad Laboratories, Richmond, Calif.). Phosphatidylethanolamine was isolated from the extracted lipids by thin-layer chromatography as described by Ames (1). For comparison, cells were also grown in the presence of unenriched propionate, and the phosphatidylethanolamine was isolated in a similar fashion. ^{13}C NMR spectra of purified lipid (in deuterochloroform) were taken, using a Varian XL-100 NMR spectrometer equipped with a Nicolet Fourier transform accessory. Trimethyl silane was added as a reference. Peak assignments agree with previous studies (Table 2; 2).

The ω, ω-1, ω-2, α-cyclopropane, 3, 2, and 1 (carbonyl) carbons were clearly resolved (Fig. 1B). The largest peak in the unenriched sample presented the middle carbons in the chain (m + n, Table 2). The incorporation of [1-^{13}C]propionate both as a primer and in elongation would result in enrichment of the carbonyl carbon, ω-2 carbon, α-cyclopropane carbon, and the mixed peak (m + n). The incorporation of [1-^{13}C]propionate during elongation only would result in enrichment at all of these except the ω-2 carbon. The incorporation of [1-^{13}C]propionate as a primer only would result in enrichment of the ω-2 carbon only. Figure 1A shows enrichment only at the ω-2 carbon, indicating the direct incorporation of propionate as a primer only. Based upon a comparison of peak height ratios, the ω-2 carbon in lipids from cells grown with [1-^{13}C]propionate was enriched 12-fold.

^{13}C-labeled fatty acids have been used in NMR studies to probe membrane organization and protein-lipid interactions (2, 8). Growth of *E. coli* in the presence of [^{13}C]propionate offers

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TABLE 1. *Fatty acid composition of cells grown in the presence of n-monocarboxylic acids*[a]

Supplement	Conc (M)	Fatty acid composition (% of total)							
		12:0	14:0	15:0	16:0	16:1	17:0	Δ17	18:1
None		3.9	3.7		32.8	31.7			27.8
Acetate	0.096	2.0	1.7		43.4	23.3		6.0	23.7
Propionate[b]	0.049	3.5	2.7	8.9	21.9	26.3	5.8	5.8	25.2
	0.096	3.6	1.6	11.5	19.9	21.1	8.2	9.3	24.8
	0.142	4.0	1.6	12.4	17.6	16.3	9.5	14.3	24.3
Byturate	0.096	2.9	2.9		44.5	17.5		7.5	24.7
Pentanoate	0.049	3.2	2.2		43.7	14.7		12.7	23.5
Hexanoate	0.025	3.1	3.7		35.5	29.0		4.1	24.6
Heptanoate	0.018	3.4	3.6		35.7	31.0		1.4	25.0

[a] Lipids were extracted from cells in exponential phase and analyzed as described previously (5). Composition is expressed as percentage of total fatty acids.

[b] Δ17 column represents the sum of both 17 cyclopropane + 17:1.

TABLE 2. *^{13}C resonance assignments*[a]

$$\underset{\omega}{CH_3}-\underset{\omega\text{-}1}{CH_2}-\underset{\omega\text{-}2}{CH_2}-(CH_2)_m-\underset{\alpha}{CH_2}-\underset{\alpha}{CH_2}-(CH_2)_n-\underset{3}{CH_2}-\underset{2}{CH_2}-\underset{1}{CO_2}-$$

$$\underset{\text{cyclo}}{CH_2}$$

Position	Dihydrosterculic acid[b]	Dipalmitoyl phopshatidyl-ethanolamine[b]	*E. coli* phosphatidyl-ethanolamine[c]
1a	178.82	174.03	173.40
1b		173.63	173.13
2a	34.40	34.63	34.30
2b		34.46	34.12
3	25.52	25.30	24.95
α-Cyclo	16.24		15.78
CH_2-cyclo	11.07		10.98
(m + n)	30.20	30.02	29.76
ω-2	32.48	32.26	31.98
ω-1	23.15	22.99	22.71
ω	13.94	14.11	14.12

[a] Chemical shifts are expressed in parts per million downfield from trimethyl silane. Carbons 1 and 2 have different chemical shifts when attached at the *sn*-1 (1a,2a) and *sn*-2 (1b,2b) positions.

[b] Data from reference 2.

[c] Deuterochloroform solvent.

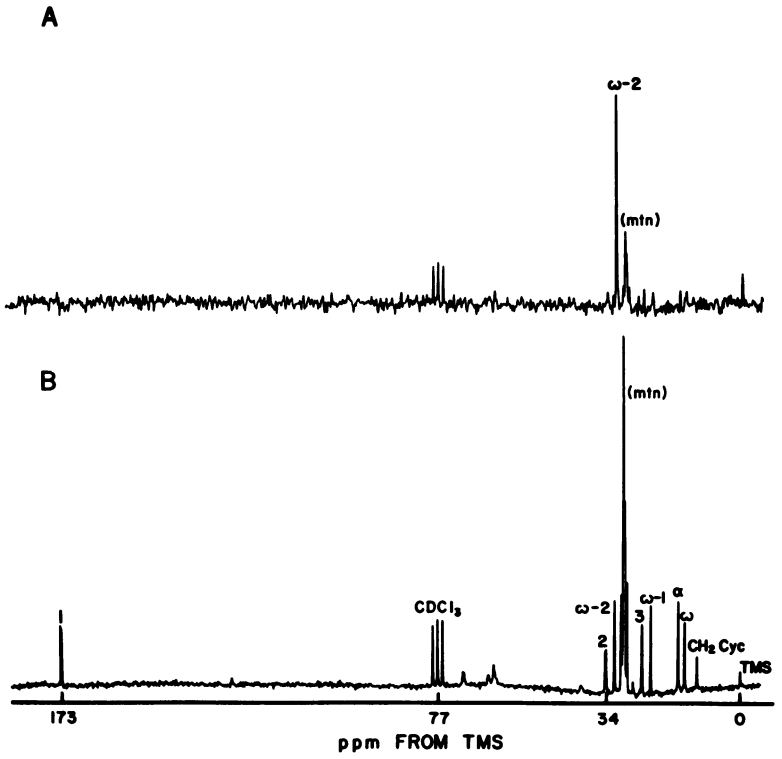

FIG. 1. *^{13}C NMR spectra of phosphatidylethanolamine isolated from stationary-phase cells of E. coli. (A) Cells grown with 1-^{13}C-enriched propionate (0.035 M); (B) cells grown with unenriched propionate (0.035 M).*

a gentle method of introducing ^{13}C specifically at the ω, ω-1, and ω-2 positions of fatty acids in biological membranes.

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