Regulation of Membrane Lipid Synthesis in *Escherichia coli* after Shifts in Temperature†

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ABSTRACT: The fatty acid composition of cellular lipids synthesized from acetate in resting cells of *Escherichia coli* was affected by both the incubation temperature and the growth temperature of the cells; the ratio of unsaturated fatty acids to saturated fatty acids synthesized varied from 0.56 in cells grown at 10 °C to 16 in those grown at 40 °C. Moreover, cells grown at these two temperatures synthesized different proportions of unsaturated and saturated fatty acids when incubated at the same temperature. During a lag period after shifting cells grown at 40 to 10 °C, the fatty acids synthesized from acetate changed qualitatively and the ratio of unsaturated to saturated fatty acids decreased significantly. A fatty acid synthetase system from *E. coli* produced various proportions of unsaturated and saturated fatty acids in vitro, depending upon the incubation temperature; the ratio of unsaturated fatty acids to saturated fatty acids varied from 2.3 at 40 °C to 25 at 10 °C. Acyltransferase systems incorporated various proportions of oleate and palmitate into phosphatidate in vitro, depending upon the incubation temperature. However, the ratio of oleate to palmitate incorporated only varied from 0.80 at 40 °C to 1.56 at 10 °C. Acyltransferase preparations obtained from cells grown at 15 and 43 °C showed similar profiles of temperature-dependent incorporation of oleate and palmitate, and their activities with various acyl-CoAs were also quite similar. Both systems incorporated various ratios of acyl-CoAs, depending upon the ratio of acyl donors available. Thus, the effect of temperature shifts appears to reflect primarily the temperature dependence of the fatty acid synthetase system.

The fatty acid composition of *Escherichia coli* cells was shown by Marr and Ingraham (1962) to depend on the growth temperature. During a lag in growth after a shift of cells grown at a higher temperature to 10 °C, an alteration of fatty acid composition occurred without synthesis of DNA, RNA, or protein (Shaw and Ingraham, 1965, 1967). The fatty acid synthetase system and the de novo phosphatidate-synthesizing system were shown to be involved in the adaptation to such a temperature shift (Okuyama, 1969), whereas acyl-CoA:lysophospholipid acyltransferase (Proulx and van Deenen, 1966) did not appear to play a significant role (Bright-Gaertner and Proulx, 1972, Aibara et al., 1972). Later, Sinensky (1971) and Kito et al. (1975) showed that the acyltransferase systems incorporated various proportions of unsaturated and saturated fatty acids into phosphatidate in vitro, depending on the incubation temperature. These authors concluded that the temperature-dependent changes in the cellular fatty acids reflected simply the temperature dependence of the acyltransferases. However, the temperature dependence of the acyltransferases observed in vitro does not seem to be large enough to explain the synthesis of phospholipids enriched with *cis*-vaccenic acid during the adaptation period after a shift of growth temperature (Okuyama, 1969).

Recently, Cronan (1974, 1975) showed that the ratio of saturated fatty acids that accumulated in glycerol auxotrophs of *E. coli* during starvation depended upon the temperature. Cronan also showed that in a strain with an increased level of β-hydroxydecanoyl thioester dehydrase the enzyme decreased to the normal level within a few hours after a shift from 42 to 15 °C. These observations indicate that the fatty acid synthetase system is affected by temperature. However, this conclusion appears to conflict with the proposal of Cronan and Gelman (1975) and others (Ray et al., 1970, Sinensky, 1971) that the acyltransferases are highly specific for saturated fatty acids in acylation at the 1 position of glycerophosphate, and for unsaturated acids in acylation at the 2 position.

The present experiments were undertaken to evaluate the relative contributions of the fatty acid synthetase system and acyltransferases to the adaptative processes after temperature shifts, and to clarify the correlation of the temperature-dependent changes in fatty acid composition with the mechanism of phosphatidate synthesis proposed earlier by our group (Okuyama and Wakil, 1973; Okuyama et al., 1976).

Materials and Methods

Fatty acyl-CoAs were prepared as described previously (Okuyama et al., 1969); [^{3}H]glycerophosphate was prepared by the method of Chang and Kennedy (1967) and 1-acyl-*sn*-glycerol 3-phosphate (1-acyl-GP)[1] was prepared by hydrolysis of 1-acylglycerylphosphorylcholine from egg yolk lecithin with phospholipase D, as described by Long et al. (1967). Acetyl-CoA, malonyl-CoA, and [^{14}C]malonyl-CoA were products of P. L. Biochemicals (Milwaukee, Wisc.), sodium [^{14}C]acetate (1-3 mCi/mmol) was from Daiichi Pure Chemicals Co. Ltd. (Tokyo), and chloramphenicol was from Sankyo Co. Ltd. (Tokyo). Phospholipase C was obtained from culture filtrates of *Bacillus cereus* by fractionation with $(NH_4)_2SO_4$ and DEAE-cellulose column chromatography

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[1] Abbreviations used are: acyl-GP, acyl-*sn*-glycerol 3-phosphate; diacyl-GP, diacyl-*sn*-glycerol 3-phosphate; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; DEAE, diethylaminoethyl; NADP, nicotinamide adenine dinucleotide phosphate.

TABLE I: Effects of Chloramphenicol and Incubation Temperature on the Composition of Fatty Acids Synthesized in Vivo from [^{14}C]Acetate.[a]

Add of CM	Incub temp (°C)	Incub time (min)	Distribution of radioactivity (%)			U/S[b] ratio	Total incorp. (cpm)
			Saturated acids (S)	Unsaturated acids (U)	Hydroxy acids		
−	40	5	37.2	53.9	8.9%	1.4	17 400
+	40	5	38.6	52.9	8.5	1.4	19 800
+	40	20	32.4	60.9	6.7	1.9	31 200
−	10	20	6.2	84.5	9.3	14	5 900
+	10	20	4.6	86.4	9.0	19	7 300

[a] Cells were grown at 40 °C to the early-logarithmic phase in lactate medium (see Materials and Methods). They were preincubated for 10 min at the indicated temperatures with or without 100 μg/mL of chloramphenicol (CM). Then 10 μCi of [^{14}C]acetate was added per 50 mL of medium and incubations were continued for the indicated periods. Fatty acids were analyzed as methyl esters by $AgNO_3$–Silica Gel G thin-layer chromatography, as described in the text. Values are averages of two separate determinations. [b] U/S, unsaturated/saturated.

(Zwaal et al., 1971); the final preparation dissolved in 50% glycerol showed about 1 IU of activity/mg of protein with phosphatidylcholine as substrate.

Growth of Bacteria. Bacterial growth was followed turbidimetrically at 650 nm with a Shimadzu Baush and Lomb spectrophotometer. *Escherichia coli* K12, W3110, was grown in medium containing per liter: 7 g of K_2HPO_4, 2 g of KH_2PO_4, 0.5 g of trisodium citrate·$2H_2O$, 1 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 1 mg of vitamin B_1, 10 g of casamino acids, and 2.5 g of glucose. The in vitro fatty acid synthetase system was obtained from cells harvested in the late-logarithmic phase. A stock strain of *E. coli* B was grown in medium containing per liter: 2 g of NH_4Cl, 3 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of Na_2SO_4, 0.11 g of $CaCl_2$, 0.02 g of KH_2PO_4, 7.3 g of Tris, and either 5 g of sodium lactate (named lactate medium), or 5 g of glucose (named glucose medium). The pH was adjusted to 7.4 with HCl. Cells in the early-logarithmic phase (optical density 0.25) were used for experiments on [^{14}C]acetate incorporation, and cells in the late-logarithmic phase (optical density 1.0) in lactate medium with 1 g/L of KH_2PO_4 were used for preparation of acyltransferases.

Analysis of the Products of the Fatty Acid Synthetase System. The incubation system was essentially as described by Silbert and Vagelos (1967). After incubation, the mixture was saponified and fatty acids were extracted and analyzed as methyl esters by $AgNO_3$–Silica Gel G thin-layer chromatography with hexane–ethyl ether (9:1, v/v) as solvent. Spots of material were located with 2,7-dichlorofluorescein and scraped off into scintillation vials, and the radioactivity was counted directly in toluene scintillation fluid (4 g of 2,5-diphenyloxazole, 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, and 1 L of toluene).

Assay of Acyltransferases. Membrane preparations were obtained as described previously (Okuyama and Wakil, 1973). Unless otherwise stated, the conditions for enzyme assay were as described previously (Okuyama et al., 1976). Reactions were followed either spectrophotometrically (Lands and Hart, 1965) or by using labeled substrates. The radioactivities at the 1 and 2 positions of diacyl-GP were analyzed as described elsewhere (Okuyama et al., 1971). A toluene–Triton X-100–water system was used as scintillation fluid (Okuyama and Wakil, 1973).

[^{14}C]Acetate Incorporation into Cellular Lipids. Cells grown to the early-logarithmic phase (optical density 0.25) at 40 or 10 °C in the lactate medium or glucose medium described above were transferred to a beaker at 10 or 40 °C. When indicated, 5 mg of powdered chloramphenicol was added per 50 mL of medium. Mixtures were preincubated for 10 min to allow the chloramphenicol to dissolve and the temperature to become steady and then 10 μCi of [^{14}C]acetate (1–3 mCi/mmol) was added and incubation was continued for the indicated period. For analysis of total fatty acids, reactions were terminated by adding 10 mL of 50% methanolic KOH and the mixtures were kept at 70 °C for 60 min; then 20 mL of 6 N HCl was added, and total fatty acids were extracted with hexane. Methyl esters were prepared by treatment with BF_3–CH_3OH and were analyzed either by $AgNO_3$–Silica Gel G thin-layer chromatography, as described above, or by a radio-gas chromatograph equipped with a proportional counter (Yanagimoto Co. Ltd., Tokyo). For analysis of the molecular species of phospholipids, the total lipids were extracted with chloroform–methanol by the method of Bligh and Dyer (1959).

Analysis of Molecular Species of Phospholipids. The labeled lipids extracted from 50 mL of incubation medium as described above were mixed with 1 mL of diethyl ether, 1 mL of phospholipase C solution (1 IU), and 1 mL of 2 mM zinc chloride–0.2 M borate (adjusted to pH 6.3 with NaOH). The mixture was shaken for 1 h at 37 °C. In this way, more than 90% of the phospholipids was hydrolyzed. The hydrolysate was extracted with chloroform–methanol and 1,2-diglyceride was purified by 0.4 M boric acid–Silica Gel H thin-layer chromatography with chloroform–acetone (96:4, v/v) as solvent. The purified 1,2-diglyceride was subjected to $AgNO_3$–Silica Gel G thin-layer chromatography with chloroform–methanol (98:2, v/v) and the radioactivities of the spot of material obtained were determined as described above.

Results

Acetate Incorporation into Cellular Lipids in Resting Cells. When cells were grown at 40 °C and incubated with [^{14}C]acetate at the same temperature, the ratio of the unsaturated to saturated fatty acids synthesized from acetate was 1.4 (Table I), which was similar to the unsaturated/saturated ratio of fatty acids extracted from cells grown at 40 °C. Neither this ratio nor the total incorporation was affected significantly by the presence of sufficient chloramphenicol to inhibit protein synthesis (100 μg/mL). When cells grown at 40 °C were shifted to 10 °C, and acetate was added after 10 min of preincubation at the latter temperature, the unsaturated/saturated ratio of the fatty acids they synthesized was tenfold of cells incubated continuously at 40 °C. Saturated fatty acids constituted less than 7% of the total fatty acids of cells shifted down to 10 °C, whereas they constituted more than 40% of the

TABLE II: Changes in the Composition of Fatty Acids Synthesized from [^{14}C]Acetate in Resting Cells during the Lag Period After Temperature Shifts.[a]

Time after shift to 10 °C (h)	Incub conditions	Distribution of radioactivity (%)			U/S[b] ratio	Total incorp. (cpm)
		Saturated acids (S)	Unsaturated acids (U)	Hydroxy acids		
0	30 min at 10 °C	3.7	85.0	11.3	23	92 200
2		8.2	84.9	6.8	10	62 300
5		14.2	76.1	9.7	5.4	22 800
0	5 min at 40 °C	53.4	39.9	6.7	0.75	90 000
2		53.9	35.7	10.4	0.66	60 100
5		62.9	26.9	10.2	0.43	31 100

[a] The incubation systems were as for Table I in the presence of chloramphenicol, except that glucose medium (see Materials and Methods) was used and the incubation time at 10 °C was 30 min. Values are averages of two separate determinations. [b] U/S, unsaturated/saturated.

TABLE III: Analyses of Total Fatty Acids Synthesized in Vivo from [^{14}C]Acetate.[a]

Expt no.	Growth temp (°C)	Incub temp (°C)	14:0 12:0	Distribution of radioactivity (%)							Total S (%)	Total U (%)	U/S ratio
				14:1	16:0	16:1	18:0	18:1	Others	Hydroxy			
1	40	40	7	1	31	14	1	30	9	7	39.2	54.7	1.4
			(9)	(2)	(37)	(9)	(9)	(19)	(12)	(3)	(54.7)	(42.8)	(0.78)
2	40 → 10[b]	40	15	2	45	15	tr	14	2	7	59.5	34.0	0.57
3	10	40	10	1	48	14	1	11	8	7	59.7	33.4	0.56
4	10	10	5	2	19	22	2	36	11	3	25.7	71.2	2.8
			(8)	(1)	(26)	(17)	(9)	(36)	(2)	(1)	(42.4)	(57.2)	(1.3)
5	40 → 10	10	7	tr	7	23	tr	49	6	8	14.2	77.5	5.5
6	40	10	1	1	5	8	tr	76	6	3	5.8	91.1	16

[a] Cells were grown in glucose medium at 40 or 10 °C to the early-logarithmic phase. In experiments 2 and 5, cells grown at 40 °C were shifted to 10 °C for 5 h. Cells were preincubated with chloramphenicol (100 μg/mL) for 10 min at the incubation temperatures and then 10 μCi of [^{14}C]acetate was added per 50 mL of medium. After incubation for 10 min at 40 °C or 60 min at 10 °C, KOH solution was added. Total fatty acids were analyzed as methyl esters with a radio-gas chromatograph as described in the text. "Others" represents cyclopropane fatty acids and some unidentified components. S stands for straight-chain saturated fatty acids and U includes cyclopropane fatty acids as well as unsaturated fatty acids. The mass compositions of total fatty acids from the cells used are shown in parentheses. [b] Cells grown at 40 °C were shifted to 10 °C for 5 h.

total in cells grown at 10 °C. Thus, cells grown at 40 °C can synthesize more unsaturated fatty acids when shifted to 10 °C than those present in cells grown at 10 °C.

Fatty Acid Synthesis during the Lag Period after Temperature Shift. When cells grown at 40 °C in glucose medium were shifted to 10 °C, their total incorporation of exogenous acetate gradually decreased to about 30% of the initial value after 5 h, as shown in Table II. Their unsaturated/saturated ratio also decreased significantly. The decreases in radioactivity and the unsaturated/saturated ratio were not affected by the presence of chloramphenicol in the incubation mixture from zero time after the shift, indicating that they did not involve protein synthesis (data not shown).

The compositions of the fatty acids synthesized from acetate in cells grown at 10 and 40 °C with and without a temperature shift and the mass composition of fatty acids in cells grown at 10 and 40 °C are shown in Table III. The fatty acids synthesized at 40 °C in cells grown at 40 °C and those synthesized at 10 °C in cells grown at 10 °C were slightly different from the respective mass compositions of the fatty acids in these cells (experiments 1 and 4 in Table III). When cells grown at 40 °C were shifted to 10 °C, much *cis*-vaccenate was synthesized (76% of the total) (experiment 6), whereas cells grown continuously at 10 °C synthesized similar amounts of palmitoleate (16:1) and *cis*-vaccenate (experiment 4). When cells grown at 40 °C were shifted to 10 °C for 5 h, their fatty acid synthesis at 10 °C was significantly different from that of cells grown throughout at 10 °C (experiments 4 and 5). On the other hand, cells grown at 10 °C synthesized very little *cis*-vaccenate at 40 °C (experiment 3), whereas those grown at 40 °C synthesized a significant amount at this temperature (experiment 1).

These results suggest that the fatty acid synthetase systems from cells grown at 40 and at 10 °C are qualitatively different. Thus, the incubation temperature as well as the growth temperature of the cells affects the relative proportions of unsaturated and saturated fatty acids synthesized in resting cells. The unsaturated/saturated ratio varied from 0.56 to 16: a larger range than that of the unsaturated/saturated ratio of fatty acids extracted from cells grown at 40 and 10 °C (0.78 to 1.3).

Temperature Dependency of the Fatty Acid Synthetase System. As shown in Table IV, the in vitro fatty acid synthetase system produced different proportions of saturated, unsaturated, and hydroxy fatty acids at different incubation temperatures. The unsaturated/saturated ratio of the products was about tenfold larger at 10 than at 40 °C, but the system was slightly inactivated at 40 °C. The incubation time also affected the unsaturated/saturated ratio slightly, and the proportion of hydroxy fatty acids increased with a decrease in the total incorporation.

Schultz et al. (1969) reported that addition of salts to the

TABLE IV: Effects of Temperature and Time of Incubation on the Composition of Fatty Acids Synthesized in Vitro.[a]

Incub conditions		Distribution of radioactivity				
Temp (°C)	Time (min)	Saturated acids (S) (%)	Unsaturated acids (U) (%)	Hydroxy acids (%)	U/S ratio	Total incorp (cpm)
40	20	29.6	67.8	1.7	2.3	35 400
30	20	19.3	77.3	3.0	4.0	36 700
20	20	6.2	80.5	12.8	13	21 600
20	40	13.4	84.2	2.1	6.3	34 900
10	40	2.2	55.5	41.9	25	14 200

[a] The incubation mixture was essentially as described by Silbert and Vagelos (1967) and contained 15 nmol of acetyl-CoA, 30 nmol of [^{14}C]malonyl-CoA (0.03 μCi), 2.5 μmol of glucose 6-phosphate, 0.75 μmol of $NADP^+$, 2 μmol of mercaptoethanol, 0.65 IU of glucose-6-phosphate dehydrogenase, 23 μg of acyl carrier protein in 93 μg of protein, and the fatty acid synthetase preparation (1 mg of protein) in 0.5 mL of 0.1 M potassium phosphate buffer (pH 7.0). Fatty acids labeled with [^{14}C]malonyl-CoA were analyzed as methyl esters by $AgNO_3$–Silica Gel G thin-layer chromatography.

TABLE V: Effect of Incubation Temperature on the Selectivity of Acyltransferase Systems.[a]

Incub temp (°C)	Incorp into diacyl GP (nmol min^{-1} (mg of protein)$^{-1}$)			Acyl-CoA remaining
	[^{3}H]Oleate	[^{14}C]Palmitate	^{3}H/^{14}C	^{3}H/^{14}C
40	2.46	3.07	0.80	0.7
30	3.52	3.80	0.93	0.8
20	2.03	1.79	1.14	0.7
10	1.10	0.70	1.56	0.7

[a] The incubation mixture contained 25 μM [^{3}H]oleoyl-CoA, 25 μM [^{14}C]palmitoyl-CoA, 3 mM glycerophosphate, 0.4 M KCl, 1 mg/mL enzyme protein (P_1), and 0.1 M Tris-HCl (pH 8.5). Incubations were carried out for 1 min at the indicated temperatures. The ^{3}H/^{14}C ratio is shown as a molar ratio. Cells grown at 37 °C in lactate medium were used. Values are averages of two separate determinations.

incubation mixture affected the activity of an in vitro fatty acid synthetase system. However, under our conditions addition of 1% KCl did not affect the unsaturated/saturated ratio, or its influence by temperature (data not shown).

Molecular Species of Phospholipids Synthesized after Temperature Shifts. The molecular species of total phospholipids labeled with [^{14}C]acetate after shift-up or shift-down of temperature was analyzed. The molecular species was measured as the ratio of the radioactivities in three molecular species; those with two unsaturated fatty acids (UU), those with one saturated and one unsaturated fatty acid (SU), and those with two saturated fatty acids (SS). When cells grown at 40 °C were shifted to 10 °C the proportions were 75, 23, and 2%, respectively, whereas when cells grown at 10 °C were shifted to 40 °C the proportions were 4, 62, and 34%, respectively. Cells grown at 37 °C synthesize mainly 1-saturated acyl-2-unsaturated acylglycerophospholipids (Kito et al., 1975).

Acyltransferase Systems in Vitro. Table V shows results on the temperature dependency of formation of diacyl-GP from glycerophosphate by the acyltransferase systems. Equivalent amounts of oleoyl-CoA and palmitoyl-CoA were added as substrate and the enzyme preparation was obtained from cells grown at 37 °C. The ratio of oleate to palmitate incorporated into diacyl-GP varied from 0.80 at 40 °C to 1.56 at 10 °C. The absolute values were slightly different from those reported by Sinensky (1971), but about twofold difference between the ratios obtained at 40 and 10 °C was consistently observed, although the acyltransferase systems were slightly inactivated at 40 °C.

Acyltransferase systems are embedded in membranes and their properties are affected by those of membrane lipids (Mavis and Vagelos, 1972, Mavis et al., 1972). Thus, differences in the properties of membrane lipids may influence their temperature dependence. To test this possibility, membranes were prepared from cells grown to the late-logarithmic phase at 43 and at 15 °C. The proportions of phosphatidylglycerol, phosphatidylethanolamine, and cardiolipin were 7, 84, and 8%, respectively, in membranes of cells grown at 43 °C, and 18, 74, and 4%, respectively, in those of cells grown at 15 °C. The percentages of the major fatty acids, palmitate, palmitoleate, and *cis*-vaccenate, were 67, 6, and 8%, respectively, in cells grown at 43 °C, and 25, 23, and 45% in cells grown at 15 °C. However, despite these differences in the membrane lipids, the acyltransferase systems in the two membrane preparations showed a similar temperature dependency in vitro (data not shown). Moreover, as shown in Table VI, the maximal velocities of the two preparations with various acyl-CoAs and glycerophosphate or 1-acyl-GP were not significantly different.

The temperature dependency of the activities of acyltransferase systems observed in vitro (Table V) was consistent with the difference in the fatty acid compositions observed in cells grown at 40 and at 10 °C. However, it was significantly less than the range of the ratios of fatty acids synthesized from acetate in cells under various conditions (Table III). This discrepancy could be explained by the finding that when the ratio of oleoyl-CoA to palmitoyl-CoA in the incubation mixture was varied from 4 to 0.25, keeping the total amount constant, the ratio of the amounts of oleate and palmitate incorporated in acylation of 1-acyl-GP varied from 4.8 to 0.44 (Table VII). A similar effect of the ratios of the acyl donors on glycerophosphate acyltransferase was reported previously (Okuyama et al., 1976). Thus, acyltransferase systems can use the fatty acyl donors of widely different relative proportions supplied by the acyl donor system.

Discussion

The mechanisms of temperature control of membrane lipid synthesis in *E. coli* may be explained in terms of short-term and long-term control. The short-term control was as follows. An abrupt temperature shift results primarily in great change in the proportions of unsaturated and saturated fatty acids produced by the fatty acid synthetase system (Tables I and IV). The nonspecific nature of the acyltransferase systems and their temperature dependencies (Tables V and VII) allow the assi-

TABLE VI: Comparison of the Activities of Acyltransferase Systems from Cells Grown at Different Temperatures.[a]

Membranes from cells grown at (°C)	Acceptor	Relative acyl transfer rate with 16:0-CoA	18:0-CoA	*cis*-Δ^9-18:1-CoA	*cis*-Δ^{11}-18:1-CoA
43	1-Acyl-GP	1 (43.4)	0.79	1.51	1.78
15		1 (52.5)	0.87	1.35	1.63
43	Glycero-	1 (5.1)	0.71	0.83	0.95
15	phosphate	1 (5.7)	0.79	0.90	1.01

[a] Membrane preparations (P_2) from cells grown at 43 or 15 °C to the late-logarithmic phase in lactate medium were obtained as described previously (Okuyama and Wakil, 1973). The incubation systems for glycerophosphate acyltransferase and 1-acyl-GP acyltransferase were as described elsewhere (Okuyama et al., 1976). Values in parentheses are the specific activities of the preparations expressed as nmol min^{-1} (mg of protein)$^{-1}$ at 25 °C. Averages of two separate determinations are shown.

TABLE VII: Effect of Variation in the Ratio of Oleoyl-CoA to Palmitoyl-CoA on the Selectivity of 1-Acyl-GP Acyltransferase.[a]

^{14}C-18:1/^{3}H-16:0 Molar ratio of		
Substrate	Product	Acyl-CoA remaining
4	4.8	2.9
2	2.7	1.5
1	1.7	0.7
0.5	0.86	0.4
0.25	0.44	0.2

[a] The incubation mixture (1 mL) contained 90 μmol of Tris-HCl (pH 7.5), 50 nmol of 1-acyl-GP, 0.15 mg of enzyme protein (P_2), and various amounts of oleoyl-CoA (^{14}C-18:1) and palmitoyl-CoA (^{3}H-16:0) giving a total concentration of 50 μM. Incubations were carried out at 23 °C for 1 min. The ^{14}C/^{3}H ratios were determined after purification of diacyl-GP by Na_2CO_3-Silica Gel H thin-layer chromatography. Values are averages of two separate determinations.

milation of the great variety of ratios of fatty acyl donors provided. These temperature dependencies observed in vitro might be explained thermodynamically by differences in the activation energies of the enzymes in the rate-limiting steps. It is also possible that the temperature dependency of the acyltransferase system reflects temperature-dependent changes in the physicochemical properties of the acyl-CoA substrates (Zahler et al., 1968).

The difference between the properties of the fatty acid synthetase systems of cells grown at 40 and at 10 °C seems to indicate an ingenious means of maintaining suitable membrane fluidity on abrupt change in the environmental temperature. Although apparently paradoxical, cells grown at 40 °C contained higher *cis*-vaccenate synthetic activity than cells grown at 10 °C: on lowering the temperature to 10 °C, they synthesized more unsaturated fatty acids than cells grown continuously at 10 °C, thus effectively diluting saturated fatty acids in their membranes. Conversely, cells grown at 10 °C synthesized more saturated fatty acids at 40 °C than cells grown at 40 °C.

During the adaptational period after shift of cells from 40 to 10 °C (long-term control), the fatty acid synthetase system in the cells changed qualitatively to that of cells grown at 10 °C, losing more of the activity to synthesize unsaturated fatty acid than of that to synthesize saturated fatty acids. Thus, cells adapted to 10 °C could synthesize more saturated fatty acids on shift to 40 °C than cells grown at 40 °C throughout. In completely adapted cells or in cells after only a small temperature shift (Kito et al., 1975), the proportions of saturated and unsaturated fatty acids produced by the fatty acid synthetase system seem to coincide fairly well with the temperature-dependent incorporations of these fatty acids by the acyltransferase systems.

Two mechanisms have been proposed for synthesis of diacyl-GP from glycerophosphate in *E. coli*. Ray et al. (1970), Sinensky (1971), and Cronan and Gelman (1975) reported that the glycerophosphate acyltransferase forms 1-acyl-GP and 2-acyl-GP with saturated acyl-CoAs and unsaturated acyl-CoAs, respectively, and that the 1-acyl-GP acyltransferase system is highly specific for unsaturated acyl-CoAs forming 1-saturated acyl- 2-unsaturated acyl-GP species. However, the formations of significant amounts of molecular species with two unsaturated or two saturated fatty acids, as observed in this work and by Akamatsu (1974), are not consistert with this mechanism of phosphatidate synthesis as the temperature dependencies of the acyltransferase systems are so small. Moreover, we found previously (Okuyama and Wakil, 1973; Okuyama et al., 1976) that the pathway through 2-acyl-GP is of little significance and that the glycerophosphate and 1-acyl-GP acyltransferase systems can both utilize saturated and unsaturated acyl-CoAs to form three molecular species: 1,2-diunsaturated acyl-GP, 1,2-disaturated acyl-GP, and 1-saturated acyl-2-unsaturated acyl-GP species. Furthermore, we found that the proportions of these molecular species found in vitro varied with the acceptor concentration and the availability of acyl donors. In fact, change in the availability of acyl donors seems to be the critical factor affecting the molecular species of phospholipids synthesized after the temperature shift.

Although the acyltransferase systems can be nonspecific, they can also be highly selective for saturated and unsaturated acyl-CoAs in acylations at the 1 and 2 positions of the glycerophosphate moiety, respectively, when the acceptor concentrations are relatively low and when equal proportions of the two acyl-CoAs are provided. However, when the relative proportion of a certain acyl-CoA in the acyl donor pool is increased, this acyl-CoA can be incorporated into both the 1 and 2 positions of glycerophospholipids (Okuyama et al., 1976). Thus, depending on the composition of the intracellular acyl donor pool, the acyltransferase systems may show highly selective incorporation of saturated and unsaturated fatty acids into the 1 and 2 positions, respectively, or nonselective incorporation of various fatty acids into both positions. Furthermore, the glycerophosphate acyltransferase system may be the rate-limiting step, allowing the acyl donor pool (acyl-CoA

and/or acyl-ACP) to exert possible feedback control effects on the fatty acid synthetase system, resulting in variation in the proportions of saturated and unsaturated fatty acyl donors (Esfahani et al., 1971, Silbert et al., 1972). However, the interaction of the fatty acid synthetase system with membrane lipids in *E. coli* is probably not tight enough for membrane fluidity to exert an influence, such as that proposed recently by Martin et al. (1976) and Kasai et al. (1976) for the *Tetrahymena* system.

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