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Cell Growth with *trans* Fatty Acids Is Affected by Adenosine 3′,5′-Monophosphate and Membrane Fluidity

Abstract. *Two positional isomers (9 and 11) of* trans *octadecenoates did not support growth on glucose of an* Escherichia coli *mutant that requires unsaturated fatty acids. However, the* trans *fatty acids provided sufficient fluidity to produce much higher cell yields when the concentration of adenosine 3′,5′-monophosphate was raised. The effectiveness of the* trans *acids rose from 0 to 1 cell per femtomole to 15 to 20 cells per femtomole as the concentration of adenosine 3′,5′-monophosphate was increased. The corresponding* cis *positional isomers supported high yields (35 to 40 cells per femtomole) independent of supplementation. The enhanced growth with adenosine 3′,5′-monophosphate supplementation is not due to an increased uptake and incorporation of the* trans *isomers relative to the* cis *isomers, since the 9-*trans *isomer was incorporated more rapidly than the 9-*cis *isomer into the membrane phospholipids under all growth conditions and represented 21 ± 2 mole percent of the acids. The finding that cells growing with* trans *fatty acid isomers have a higher requirement for adenosine 3′,5′-monophosphate may indicate that some fatty acids can alter the metabolic regulation normally exerted by the cyclic nucleotide.*

Although *trans* fatty acids are minor components of most naturally occurring lipids, they provide interesting models in which a structural difference produces physical (*1*) and metabolic (*2*) properties that are clearly different from those of the *cis* acids. These properties may have practical significance in human metabolism, since American, Polish, Canadian, and German margarines may contain as much as 55 percent *trans* isomeric fatty acids (*3*, *4*). Although no toxic effect has been proved for moderate dietary intake of *trans* acids, some physiological and long-term pathological consequences have been reported (*5*, *6*). Enig *et al.* (*7*) suggested that the *trans* fatty acid content of the diet could explain the significant positive correlation between increases in dietary fat intake and mortality over a 60-year period for either total fat or vegetable fat. Studies with yeast mutants showed that *trans* fatty acids could stop phospholipid synthesis and lead to an accumulation of triglyceride and free fatty acid (*8*). The present study describes the influence of the Δ9 and Δ11 positional isomers of *cis*- and *trans*-octadecenoate on the growth of an *Escherichia coli* double mutant (*9*) that was defective in synthesis of unsaturated fatty acid and in β oxidation. Although *trans* acids were inadequate to fully support cell growth in glucose, adenosine 3′,5′-monophosphate (cyclic AMP) could reverse the inadequacy. The fluidity of cell membranes has often been thought to be lowered by the presence of lipids containing the higher melting *trans* acids. Our results show that the effect of *trans* fatty acids on growth depends not only on the fluidity they give to the cellular membrane but also on their effects on cellular metabolic states.

Table 1. Efficiencies of octadecenoate isomers in supporting cell growth. The efficiency of each isomer, defined as the number of cells produced per femtomole of fatty acid, was obtained from the slope of cell yield plotted against fatty acid concentration (*11*). Medium A (*20*) was supplemented as indicated. Cell numbers were determined turbidimetrically at 660 nm by using an empirical equation that correlated absorbance with cell number: cell/ml $\times 10^{-8} = -0.004 + 4.5\ A + 34\ A^2 - 6.7\ A^3 + 42\ A^4$. Temperature was maintained at 37°C.

Supplement	Cells per femtomole			
	trans-9	*trans*-11	*cis*-9	*cis*-11
Glucose (0.5 percent)	1	0	43	32
Glycerol (1.0 percent)	18	2	43	
Glucose + cyclic AMP	19	18		
Glycerol + cyclic AMP	16	15	43	32

The growth response of the unsaturated fatty acid auxotroph in media having a different carbon source with or without cyclic AMP supplementation is listed in Table 1. Both *cis* isomers supported cell growth in all media. But neither *trans* isomer could support growth on glucose, and this inability could be corrected to a certain degree by switching to glycerol as the carbon source. Cells grown on glycerol are known to have higher intracellular cyclic AMP levels than cells grown on glucose (*10*). Addition of 2 m*M* cyclic AMP to cultures with either carbon source also enhanced the effectiveness of growth with the *trans* isomers. Apparently, *trans* fatty acids support cell growth fully only in the presence of sufficient cyclic AMP; the lower yield in glycerol cultures with the 11-*trans* isomer (Table 1) indicates that cells may have a higher requirement for cyclic nucleotide with the 11-*trans* isomer than with the 9-*trans* isomer. This was confirmed experimentally; the results are shown in Fig. 1.

Cells were grown in glucose medium supplemented with 48 μ*M* unsaturated fatty acid and different amounts of cyclic AMP. For the cultures supplemented with either 9-*trans* or 11-*trans* isomer, cell yields increased progressively above the threshold with added cyclic AMP until reaching a maximum cell yield. The maximum cell yield was independent of the exogenous cyclic AMP concentration for both 9-*cis* and 11-*cis* isomers; the maximum yields obtained with the *cis* isomers (35 to 40 cells per femtomole) were two times greater than those obtained with the *trans* isomers (15 to 20 cells per femtomole). When fatty acid supply limits growth, the maximum cell yield per femtomole of the nutrient acid is the inverse of the number of femtomoles needed to maintain membrane function of each cell, and lower yields per femtomole reflect a higher need for nutrient acid in the membranes. With adequate cyclic AMP, the maximum yield seems to be decided by the fluidity of the acid; the lower maximum yields with the *trans* isomers can be attributed to their lower fluidity. A similar relation is evident in the nutrient efficiencies (ε) of the 15 positional isomers of octadecenoate (*11*) that are closely correlated ($r = .9$) with the estimated state of expansion of their phospholipid esters: $\epsilon_{37} = 0.8\ (\Delta T) - 7$, where ΔT is the difference between the culture growth temperature (37°C) and the transition temperature (*12*) of phospholipid derived

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from the isomer. This difference is proportional to the state of expansion of the lipid and therefore to its fluidity. Since phospholipid esters of *trans* isomers have higher transition temperatures than those of *cis* isomers (*13*) and would be less expanded at 37°C, more *trans* acid would be needed per cell to maintain the minimal fluidity that supports growth. Thus lower ϵ values are predicted for *trans* isomers in accord with the equation.

The nutrient fatty acid, esterified into phospholipids, provides membrane fluidity as the cells are growing. When the supply of the nutrient acid is consumed, no more unsaturated fatty acid can be incorporated and the membrane fluidity decreases as a result of continued synthesis of saturated acids. Eventually, growth ceases as the fluidity is lowered to a value below which no further cell division is possible.

To determine the minimum amount of each unsaturated fatty acid isomer needed to support the growth of each cell, we analyzed the fatty acid composition of cells at an early stationary phase with growth-limiting levels of the nutrient fatty acid (Table 2). The threshold values (mole percent) of unsaturated fatty acids for cells grown on the same isomer in different media were fairly constant: 9-*trans*, 28 ± 3; 11-*trans*, 30 ± 1; 9-*cis*, 14 ± 1; and 11-*cis*, 14 ± 1. The relatively constant content of unsaturated acids with each nutrient isomer suggests that the consistent fluidity threshold (*14*) below which cell division is arrested is independent of carbon source or cyclic AMP content. Cells supplemented with *trans* isomers stopped growing while they had the same overall lipid content as cells grown with *cis* fatty acids (140 attomoles per cell) and twice the mole percentage of nutrient unsaturated fatty acid (21 ± 2 for both 9- and 11-*trans* isomers) as those supplemented with either *cis* isomer (9-*cis*, 9 ± 1; 11-*cis*, 8 ± 1) in all media. These results support the indication, based on ϵ values, that the lipids with *trans* isomers are less fluid than the *cis* derivatives (Table 1), and agree with the report that phospholipids containing *trans* acyl chains have a higher transition temperature than those containing *cis* acyl chains (*13*).

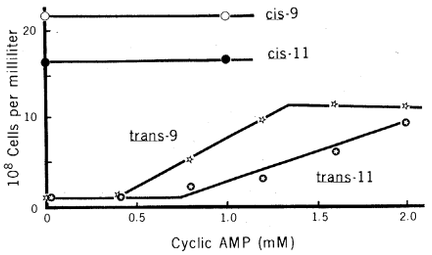

Fig. 1. Dependence of cell yield on cyclic AMP concentration in glucose-fed cultures. Methods and procedures are the same as described in the legend to Table 1. Each culture contained the nutrient fatty acid (48 μM) indicated.

For cultures with inadequate levels of cyclic AMP, two hypotheses can be considered to explain how growth with *trans* isomers was poorer than considerations of fluidity would lead one to expect: (i) *trans* acids are not transported into the cells or activated and esterified into membrane lipids and (ii) the *trans* isomers selectively exert some detrimental effect on *E. coli* metabolism. The first hypothesis takes into account poor utilization of the nutrient *trans* acids and is related to the reported need for an inducible acid:coenzyme A (CoA) ligase to transport and activate exogenous fatty acids (*15*). Perhaps with *trans* fatty acids, the ligase is inadequately induced, and increased cyclic AMP somehow reverses this inadequacy. To test this hypothesis, we measured the incorporation rates of the 9-*cis* and -*trans* isomers in either glucose or glycerol culture. Radioisotopic studies showed that in either medium, the 9-*trans* isomer was incorporated into the membrane phospholipids at a higher rate (13.9 nmole/min per 10^{10} cells) than the 9-*cis* isomer (8.3 nmole/min per 10^{10} cells). These results indicate that all three steps in incorporation of exogenous fatty acid—uptake, activation, and esterification—are operative for *trans* acids in the glucose-fed cultures. Thus an inadequate induction of the acid:CoA ligase with *trans* fatty acids is ruled out, and the poor growth in glucose medium (0 to 1 cell per femtomole) is not due to an inability of the *trans* acids to be incorporated into membrane lipids.

In the presence of sufficient cyclic AMP, cell yields increased as a linear function of increasing concentrations of either *trans* isomer, indicating that the *trans* fatty acids are capable of providing sufficient membrane fluidity. Thus poor growth in cultures with low cellular cyclic AMP concentrations must be due to some event other than an inability of 9- and 11-*trans* acyl chains to contribute to membrane fluidity. Since both positional isomers were extensively incorporated into the membrane even in nonpermissive media, the inadequacy cannot be in the incorporation of these isomers into membrane lipid. One possibility is that *trans* acids somehow lower the cyclic AMP level (for example, by inhibiting the adenyl cyclase activity) to such an extent that some vital biochemical reaction cannot proceed. Preliminary evidence does not support this concept (*16*). Another important possibility is that *trans* isomers diminish the effectiveness of cyclic AMP without altering its concentration. This could be achieved by reducing the interaction of the cyclic AMP

Table 2. Fatty acid composition of stationary cells with nutrient fatty acid as the growth-limiting factor. Cultures supplemented with the acids indicated were harvested at the stationary phase and extracted as described (*11*). For all cultures analyzed, the amount of nutrient fatty acid limited cell growth. The values given are the mole percent contents in cell lipids. The inocula were prepared with palmitoleate or linoleate. When cyclic AMP was added, the final concentration was 2 mM.

Carbon source	Detected fatty acid	Cell lipids with (+) and without (−) cyclic AMP (mole %)							
		9-*trans*		11-*trans*		9-*cis*		11-*cis*	
		−	+	−	+	−	+	−	+
Glucose	12:0	1.3	2.6	1.4	2.0	2.2	2.1	3.5	3.1
	14:0	18.0	14.8	17.3	15.8	20.1	17.8	20.1	18.7
	16:0	47.8	53.8	48.4	53.2	61.6	64.3	59.3	62.1
	18:0	0.6	0.5	0.4		0.4	0.7	0.5	0.5
	16:1	3.4	4.8	3.8	7.6	5.8	5.5	6.1	6.1
	17:Cy		0.1		0.4	0.1	0.1	0.3	0.5
	18:1	23.3	18.7	23.1	19.0	8.4	8.1	8.4	7.8
	19:Cy		0.2			0.1	0.2	0.2	0.1
	18:2	4.7	4.0	4.7	2.9	0.6	0.7		
Glycerol	12:0	6.3	5.6	3.3	5.1	4.9	2.8	4.3	4.6
	14:0	28.2	23.2	26.2	23.1	25.2	25.6	28.8	27.8
	16:0	39.0	45.7	40.7	42.0	56.6	59.2	51.2	53.3
	18:0	0.2	0.1		0.2	0.2	0.3	0.2	0.4
	16:1	5.4	5.2	5.4	11.0	3.0	3.6	6.1	4.6
	17:Cy	0.2	0.2	0.3	1.2	0.4	0.3	0.6	1.5
	18:1	20.0	19.9	20.3	17.5	9.8	8.2	7.8	7.1
	19:Cy		0.1				0.2		
	18:2	0.7						0.4	

with its receptor site (or sites) on various proteins (*17*). Such a negative effect might be lessened or prevented by raising the intracellular concentration of cyclic AMP.

No matter which mechanism is operating, our results clearly indicate that *trans* fatty acids do provide sufficient fluidity to cell membranes to allow growth at 37°C. More important, however, their ability to support growth depends on the state of cellular metabolism—in which cyclic AMP is also involved. It seems desirable to reinterpret previous reports of *trans* fatty acid effects on cell physiology in terms of the metabolic state of the cells at the time of exposure to the acids. The finding that other fatty acids also exhibit regulatory interactions with cyclic AMP (*18*) opens the possibility that many exogenous fatty acids may form a class of inhibitory regulating agents that influence cell metabolism. Our results indicate that conditions that decrease cellular cyclic AMP may sensitize cells so that exogenous *trans* fatty acids add to the cell physiology a burden that is analogous to "total body burden" (*19*).

YUAN-KAI TSAO
WILLIAM E. M. LANDS
Department of Biological Chemistry, University of Michigan, Ann Arbor 48109

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Cellulose in the Cell Walls of the Bangiophyceae (Rhodophyta)

Abstract. *Mechanically isolated cell walls of the conchocelis phase of* Bangia fuscopurpurea *yield cellulose II (regenerated cellulose) upon treatment with Schweitzer's reagent. X-ray powder analysis and thin-layer chromatography of partial hydrolyzates confirm the presence of cellulose in this extract. Gas-liquid chromatographic analysis of wall hydrolyzates indicates that xylose, mannose, galactose, and glucose are major wall constituents. The presence of cellulose in the conchocelis provides evidence that this bangiophycean life cycle phase represents a transitional form or link between the two classes of red algae, Bangiophyceae and Florideophyceae. This suggests a close affinity of the two classes of the Rhodophyta and supports the hypothesis that bangiophycean algae were precursors of the Florideophyceae.*

Cell wall composition is one of four major characteristics used to distinguish algae at the class or divisional level (*1*). Although the algal division Rhodophyta has been systematically separated into two classes, the Bangiophyceae and the Florideophyceae, primarily on the basis of morphological and reproductive characteristics (*2*, *3*), the two classes also differ in the chemical composition of their cell walls. Cellulose is present in the Florideophyceae but has been thought to be absent in the Bangiophyceae (*4*).

The bangiophycean alga *Bangia fuscopurpurea* possesses two alternating life cycle phases, the bangia phase and the conchocelis phase. Although the generic phase is typically bangiophycean, the conchocelis phase possesses some morphological and ultrastructural features of the Florideophyceae (*5*). Chemical and physical analysis of mechanically isolated cell walls of the conchocelis phase reveals the presence of cellulose. The cellulosic content of the wall is low (about 3 percent, dry weight) but similar in quantity to that reported in the Florideophyceae (*4*).

We obtained a clean cell wall preparation from the laboratory-cultured conchocelis phase of *B. fuscopurpurea* (*6*) by using a modification of the techniques

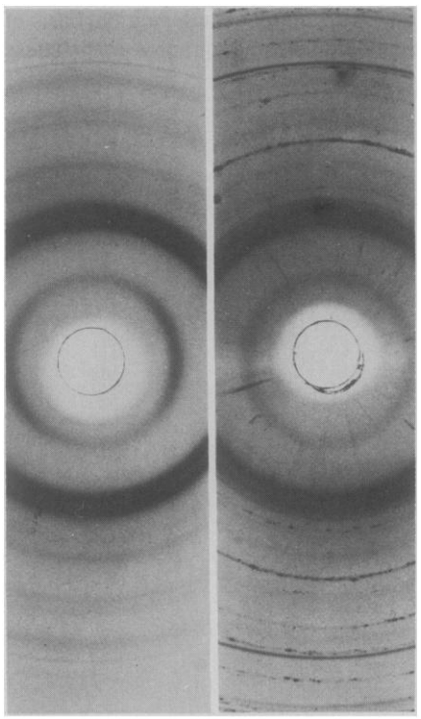
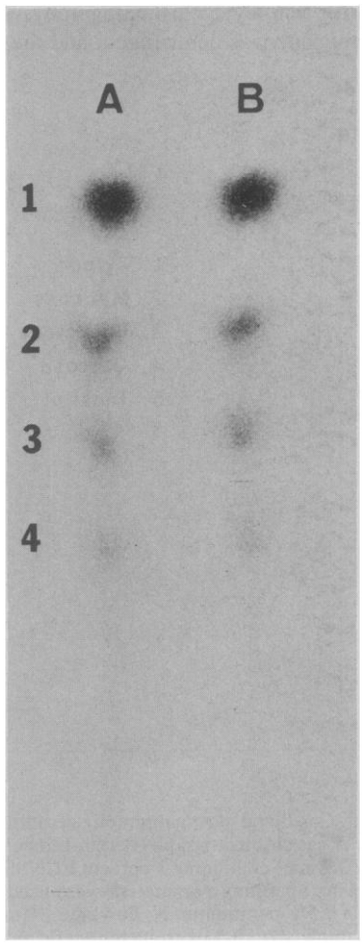


Fig. 1 (left). X-ray powder diagrams of regenerated cellulose from an Avicel standard (left) and from cell walls of the conchocelis phase (right). Fig. 2 (right). Thin-layer chromatogram comparing partial hydrolyzates of the cellulose fraction of the walls of the conchocelis phase (A) with an Avicel standard (B). Numbers denote sugars as follows: 1, glucose; 2, cellobiose; 3, cellotriose; and 4, cellotetraose.



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