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POSITIONAL SPECIFICITY OF CYCLOPROPANE RING FORMATION FROM *CIS*-OCTADECENOIC ACID ISOMERS IN *ESCHERICHIA COLI*

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Summary

An unsaturated fatty acid auxotroph of *Escherichia coli* was grown with a series of *cis*-octadecenoate isomers in which the location of the double bond varied from positions 3 to 17. Each of these fatty acid isomers was incorporated into the cellular lipids, but cyclopropane derivatives were formed to at least a 3-fold greater extent from the *cis*-9 and *cis*-11 isomers than from any other positional isomers. The extent of cyclopropane acid formation was observed to be highly dependent on the rate of shaking of the culture. A culture shaking at 340 rev./min converted 8.7% of its oleate to the cyclopropane derivative at stationary phase, whereas a parellel culture shaken at 110 rev./min converted 66% of the oleate to a cyclopropane acid.

The inability to observe selectivity or form derivatives from isomers other than the *cis*-9 and *cis*-11 isomers seems to be due to enzyme specificity rather than a secondary affect of the abnormal unconverted fatty acids on the cell, because the *cis*-9 isomer is converted to its cyclopropane derivative even in cells grown with abnormal unreactive positional isomers.

The preferred substrates for cyclopropanecarboxylic acid formation contained a *cis* ethylenic bond at either the 9 position or the (*n*-7) position. In combination with results of previous studies the specificity reported here supports a concept that two different enzymes may participate in cyclopropane ring synthesis. One enzyme activity may recognize its substrate by the distance from the π-bond to the carboxyl group and the other by the distance to the methyl group.

Introduction

Bacteria form cyclopropane derivatives of unsaturated fatty acids by transfer of a methylene group from *S*-adenosylmethionine to the double bond [1]. The

two major naturally occurring unsaturated fatty acids of *Escherichia coli*, namely palmitoleic and vaccenic acids, are converted to *cis*-9,10-methylenehexadecanoic acid and *cis*-11, 12-methyleneoctadecanoic acid. The existence of a fifteen-carbon cyclopropanecarboxylic acid has also been suggested by reports from several laboratories [2—4]. The cyclopropane derivatives do not appear to be essential for growth of *E. coli* as evidenced by the isolation of mutants unable to form cyclopropanecarboxylic acids due to a defect in *S*-adenosylmethionine synthetase [5]. Cyclopropanecarboxylic acids are observed under most conditions of growth and their relative abundance has been reported to increase during the transition from logarithmic to stationary phase [2,6]. The function of the cyclopropane derivative is as yet unknown, although some reports have suggested that it may play a role in amino acid transport and catabolite repression [5,7].

Several aspects of the specificity of the synthesis of the cyclopropane ring have been reported for bacteria both in vivo and in vitro. These studies indicated that cyclopropane synthesis occurred only with unsaturated fatty acids esterified to phospholipids [8,9]. No cyclopropane ring formation has been observed when the unsaturated fatty acid had the *trans* rather than the *cis* configuration [10]. In addition, several substrates which have more than one double bond (e.g. 20 : 2 and 20 : 3) * were not methylenated in vivo [10]. Marinari et al. [11] have investigated the specificity for ring formation with *cis*-6, *cis*-7, *cis*-9, *cis*-10 and *cis*-11 hexadecanoic fatty acids and found that only the *cis*-9, *cis*-10 and *cis*-11 isomers were appreciably methylenated in vivo.

The availability of both an *E. coli* strain requiring unsaturated fatty acid and a series of *cis*-octadecenoic fatty acid isomers of varied double bond position has allowed a detailed comparison of the specificity in vivo of the synthesis of cyclopropanecarboxylic acids with regard to the position of the double bond.

Materials and Methods

Linolenic acid [18 : 3 (*n*-3)], methyl *cis*-9, 10-methylenehexadecanoate and methyl *cis*-9, 10-methyleneoctadecanoate were purchased from Applied Science. [1-^{14}C] Oleic acid was purchased from New England Nuclear and found to be > 95% pure by thin-layer and gas chromatography. The series of *cis*-octadecenoic acids was prepared in the laboratory of Dr. F.D. Gunstone. Their synthesis, properties and purity have been described [12]. The methyl esters of *cis*-octadecenoic acids and *cis*-9,10-methylenehexadecanoic acid were converted to acids by hydrolysis of 5—25 mg of each ester with 5 ml of 5% KOH in 75% ethanol at 80° C for 60 min. This mixture was acidified with 0.8 ml of 6 M HCl and extracted three times with 3 ml portions of benzene, and the solution was washed twice with 5 ml of 0.01 M HCl. Completeness of the hydrolysis was verified by gas chromatographic analysis. Less than 1% methyl ester was found in all cases. For use as supplements to the growth media, each fatty acid

* Number notations for fatty acids in this report represent the number of carbon atoms in the chain followed by the number of double bonds. The positions of the double bond relative to the carboxyl terminus is given after the configuration and the position relative to the methyl terminus is given in parentheses as in *cis*-9-16 : 1 (*n*-7). Cyclopropane acids are represented by the number of carbons and a designation of the cyclic methylene arrangement as 17 : Cy or 19 : Cy.

was added in microliter amounts as the ammonium salt in ethanol. These were prepared by adding a drop of NH_4OH to the neat acid, evaporating the excess ammonia with nitrogen and then dissolving the salt in 80% ethanol. The ammonium salts were stored in 5—50 mM solution at —4° C. Concentrations were determined by gas chromatographic analysis after boron trifluoride-catalyzed methylation.

Organism and growth conditions

The mutant *E. coli* 30E βox^- was obtained as a gift from Dr. Carol Linden and C.F. Fox (Department of Microbiology, UCLA). This mutant is defective both in the ability to synthesize unsaturated fatty acids (fab B) and in the ability to degrade fatty acids (fad E) [13]. The liquid growth medium consisted of Medium A salts [14] to which were added 0.15 gm thiamine hydrochloride, and 5 gm caseamino acids per liter of water. Glucose was added at a concentration of 10 g/l after autoclaving.

Cultures were stored on agar slants or petri dishes which contained the above medium (without caseamino acids) plus the following additions per l: 15 g agar, 1.0 ml Tween 40, 0.25 ml technical oleic acid, 4.0 gm disodium succinate and 1 gm glucose. Colonies on petri dishes were verified for inability to grow without unsaturated fatty acid supplementation by replica plating onto the above solid medium which omitted oleic acid. Only colonies so verified were selected for experiments.

Cells were grown in 5—10 ml of liquid medium in 18 × 150 mm culture tubes. These were shaken at a 50° angle at 250 rev./min (unless otherwise noted) in a rotary shaker maintained at 37° C. Absorbance of the cultures was read at 660 nm using the culture tubes directly in a Bausch and Lomb Spectronic 20 colorimeter. Cell density was determined by a relationship between absorbance and cells/ml established from dilution plating and microscope counts (10^8 cells/ml = $-0.004 + 4.5\ A_{660} + 34\ A^2_{660} - 67\ A^3_{660} + 42\ A^4_{660}$). For cultures grown with limiting concentration of unsaturated fatty acids, the cells per ml per A_{660} was observed to be independent of the stage of growth. The effect of different shaking rates on the relationship between absorbance and cell density was not determined and thus cell densities for cultures shaken at rates other than 250 rev./min are expressed as cell equivalents/ml. Determination of the amount of protein per cell by the method of Herbert et al. [15] yielded a value of $3.1 \pm 0.3 \cdot 10^{-13}$ g/cell ($n = 11$).

Extraction and analysis of lipids

Cultures were harvested by centrifugation for 10—15 min in 12-ml graduated centrifuge tubes in a desk top centrifuge. Chloroform (1—5 drops) was added to the cell pellet and the pellet stored at —4° C until extraction. Lipids were extracted by adding 4—5 ml of chloroform/methanol (2 : 1, v/v) to the pellet and this mixture was sonicated with a probe for 15—30 s, during which time the solution was allowed to become warm.

After standing approximately 10 min, 1.0 ml of 0.1 M NaCl in 0.1 M HCl was added to the chloroform/methanol mixture and the two phases were mixed vigorously with a test tube mixer and allowed to stand 5—10 min, and then the phases were separated by centrifugation. Most of the lower phase (measured

using the graduations of the centrifuge tube) was drawn off and the solvent evaporated on a warm sand bath under a stream of nitrogen. The lipid extract was transesterified by the addition of 2 ml of 0.5 M sodium methoxide in methanol with an internal standard of methyl pentadecanoate. The reaction was allowed to proceed for 2—5 min at room temperature after which the methyl esters were extracted by the addition of 4 ml light petroleum, 0.2 ml 3 M H_2SO_4 and 4 ml water, mixing after each addition.

The light petroleum phase was drawn off, dried over sodium sulfate/sodium bicarbonate (2 : 1) and then evaporated. The residue was taken up in 10—50 μl of a mixture of carbon disulfide and pentane and 0.5—2 μl was injected into the gas chromatograph. The methyl esters were separated in 3 mm (internal diameter) × 20 cm glass columns packed with 10% polyester copolymers of either ethyleneglycol succinate or diethyleneglycol succinate. Occasionally, the nonpolar liquid phases OV 101 or OV 109 (Ohio Valley Laboratories) were used. The column was maintained at 180—200°C, the injection port at 220—240°C and the detector at 240—270°C. The carrier gas was nitrogen and the flow rate was 20—40 ml/min. The column effluent was detected by a flame ionization detector and peak areas were quantitated either by the method of Brandt and Lands [16] or by a digital integrator. Peaks were identified by comparing their equivalent chain lengths [17] to either standards or published values.

The amount of each fatty acid contained in the sample was determined by comparing peak areas relative to the methylpentadecanoic acid internal standard. Methylundecanoic acid and methylheneicosanoic acid internal standards were also added after methylation as a control for loss of short-chain methyl esters during evaporation.

Ozonolysis

The fatty acid methyl esters were ozonized by a modification of the method of Beroza and Bierl [18]. Ozone was generated using a Supelco micro-ozonizer. Evidence for excess ozone was obtained by bubbling the effluent gas (after it had bubbled through the sample solution) into a starch/KI solution [19]. The fatty acid methyl esters were dissolved in 200—600 μl of a mixture of carbon disulfide/pentane/methanol (approximately 5 : 5 : 1) and ozone was bubbled through the solution first at room temperature for 2—3 min, then at —70°C for 2—4 min, and then the solution was allowed to warm to room temperature while bubbling ozone for 2—4 min. Finally, 400—600 μl of a —70°C solution of carbon disulfide/pentane (1 : 1), which was blue from ozone saturation, was mixed with the room temperature methyl ester solution. The solvents were then evaporated, and the residue taken up in carbon disulfide and injected into the gas chromatograph.

Collection of gas chromatograph effluent for scintillation counting

For the determination of the specific activity of octadecenoic and *cis*-9,10-methleneoctadecanoic acid, the effluent of the gas chromatographic column was split inside the detector oven with a brass Swagelok T joint. Stainless steel tubing (1/16 inch) led out of the detector over through a half inch aluminum rod, which served to conduct heat through the insulated wall of the gas chro-

matograph. Without this heat conductor, tailing of ^{14}C activity occurred, apparently indicating some effluent condensed in the outlet tubing before it could be collected.

The effluent emerging from the heated outlet was collected in glass tubes (2 mm internal diameter × 20 cm) to which a curved telfon tube (6—8 cm) containing a loose plug of glass wool was attached. A series of such collection tubes, which could be rapidly changed (in 1—2 s), was used sequentially during each run. Radioactive material collected in each tube was rinsed into counting vials with 20 ml of toluene/Triton X-100 scintillation fluid. Recovery of the injected radioactivity by this method was 75—90%.

Results

The 19-carbon cyclopropane fatty acids were identified on the basis of the three following criteria.

(1) The equivalent chain length by gas chromatography on 10% diethyleneglycol succinate at 180°C was 19.55, which agrees with published data [20] and with the value (19.55) we obtained with commercially prepared methyl *cis*-9,10-methyleneoxtadecanoate. On 1.5% OV109 at 205°C, an equivalent chain length of 18.76 was obtained, which also agrees with the equivalent chain length (18.79) observed with commercial methyl *cis*-9,10-methyleneoctadecanoate.

(2) Resistance to ozonolysis (see Table I) was indicated by quantitative recovery of the assigmed material relative to the internal standard (15 : 0) and the other saturated acids in the extract while the unsaturated derivatives were destroyed. The results were similar when either low levels (Sample 1) or high levels (Sample 2) of the cyclopropanecarboxylic acids were examined, and they indicate that no other unsaturated acid derivatives were cochromatographing with the saturated acids in this system.

(3) ^{14}C radioactivity was observed to co-elute with the peak ascribed to *cis*-9,

TABLE I

CONFIRMATION OF FATTY ACID DESIGNATIONS BY TREATMENT OF THE LIPID EXTRACT WITH OZONE

In the examples shown, the extracts were from cells grown with 10 μM *cis*-9-16 : 1 plus 10 μM *cis*-11-18 : 1 for sample 1, and 50 μM *cis*-9-16 : 1 plus 50 μM *cis*-11-18 : 1 for sample 2. Added internal standards, in addition to 15 : 0, were 11 : 0 and 21 : 0. The values are nmol in the sample relative to the 15 : 0 standard.

Fatty acid	Sample 1		Sample 2	
	Before	After	Before	After
11 : 0	20	36	—	—
12 : 0	61	70	15	23
14 : 0	403	392	165	148
16 : 0	1014	1006	460	448
16 : 1	39	—	3	—
17 : Cy	62	61	195	182
18 : 1	113	3.3	57	—
19 : Cy	8.2	8.8	142	135
21 : 0	194	178	225	206

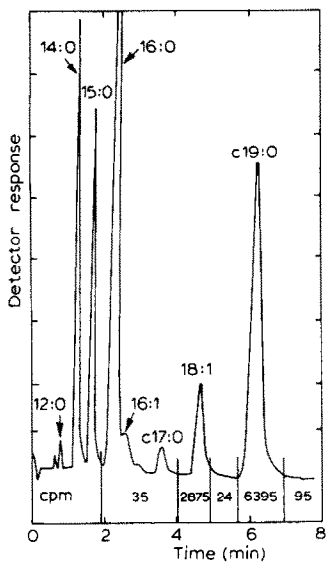

Fig. 1. Gas chromatographic profile of esterified fatty acids of *E. coli* 30E βox^- grown with 100 μM *cis*-9-[1-^{14}C] octadecenoic acid. Radioactivity recovered in the various fractions of the chromatogram is indicated below the respective fractions.

10-methyleneoctadecenoic acid after cells were grown with *cis*-9-[1-^{14}C]18 : 1 (Fig. 1). The specific activities of the eluted 18 : 1 and 19 : Cy peaks (approximately 40 and 30 cpm/nmol, respectively) affirm the concept of a close biosynthetic precursor role for the esterified 18 : 1 in forming the cyclic acid.

Positional specificity of cyclopropane synthesis

Logarithmic-phase cultures of *E. coli* 30E βox^- growing with *cis*-9,10-methylenehexadecanoic acid as the unsaturated fatty acid supplement were inocu-

TABLE II

ESTERIFIED FATTY ACID COMPOSITION OF CULTURES SUPPLEMENTED WITH ISOMERS OF *cis*-OCTADECENOIC ACID

Cultures supplemented with each isomer at 20 μM were harvested at the stationary stage, the lipids extracted, and the fatty acid composition determined by gas chromatography after sodium methoxide transmethylation. Values are the mean of the number of determinations indicated in parenthesis, given in mol%.

Fatty acid	Isomer				
	8 (2)	9 (3)	10 (3)	11 (3)	12 (3)
12 : 0	4.5	5	4	4	5.6
14 : 0	27	25	23	25	27
16 : 0	51	55	56	54	46
16 : 1	1.2	0.6	0.3	0.3	1.6
17 : Cy	1.3	1.8	1.4	2.0	1.6
18 : 1	16	9.8	11.9	9.3	17
19 : Cy	0.1	2.6	0.5	4.6	0.2

lated into media containing one of the *cis*-oxtadecenoic isomers at 20—100 μM concentrations. Growth was monitored at 30—60 min intervals by absorbance measurements and, when no further increase in A_{660} was observed, cells were harvested by centrifugation, lipids were extracted, and the esterified fatty acyl composition determined by gas chromatographic analysis. The observed composition from stationary-stage cultures supplemented with growth-limiting concentrations of the Δ^8—Δ^{12} positional isomers is presented in Table II. Cyclopropane derivatives were formed to a significantly greater extent from the fatty acids having the double bond in the 9 or 11 positions than from the isomers with double bonds in the 8, 10 or 12 positions. The fatty acid composition of cultures supplemented with each of the 3—17 isomers was determined, and the degree of the *cis* isomer to the cyclopropane derivative was calculated as: 100 × mol% c19 : 0/(mol% 18 : 1 + mol% c19 : 0). In Fig. 2 the mean percent of cyclopropane ring formation observed with each isomer is plotted versus the position of the double bond. In any given experiment, cyclopropane formation was always at least 3-fold greater from *cis*-9 and *cis*-11 than from any of the other isomers, and the mean values were 5-fold greater.

Since some of the isomers (e.g. *cis*-6 or *cis*-14) were incorporated into cellular lipids (> 10% of total fatty acid), but were in these experiments unable to support high cell yields (> $5 \cdot 10^8$ cells/ml), it might have been possible that inadequate growth or a disruption of some cellular process impaired the formation of cyclopropanecarboxylic acids. That this was not the case is indicated by the following two observations.

(1) When cells were grown with 100 μM *cis*-6 or *cis*-14 plus 30 μM linolenate (18 : 3 (*n*-3), high cell yields (> $15 \cdot 10^8$ cells/ml) were obtained and still less than 5% of the incorporated *cis*-6 or *cis*-14 isomers had been converted to cyclopropane derivatives.

(2) When cells were grown with ^{14}C-labelled *cis*-9-18 : 1 mixed with *cis*-6, *cis*-10 or *cis*-14, cyclopropane formation from the *cis*-9 isomer still occurred,

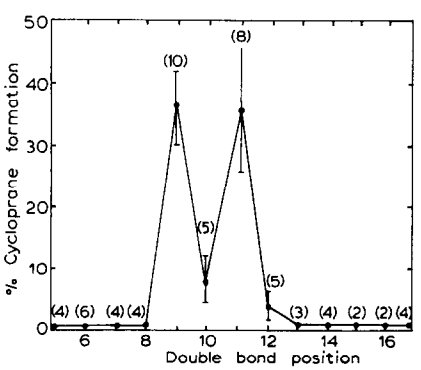

Fig. 2. Cyclopropane ring formation from the *cis*-5—*cis*-17 isomers of octadecenoic acid. The percent formation represents the value of (mol% 19 : Cy · 100)/mol% 19 : Cy + mol% 18 : 1). The mean and standard error of mean are indicated with the number of determinations given in parenthesis above the standard error. (Peaks having retention times on diethyleneglycerol succinate similar to the cyclopropanecarboxylic acids were observed for cultures grown with the 3 and 4 isomers. However, on OV-101 these peaks did not correspond with cyclopropane derivatives and the peak from the *cis*-3 culture was eliminated by ozonolysis. Further identification was not attempted.)

TABLE III

RECOVERY OF RADIOACTIVITY IN FRACTIONS COLLECTED FROM GAS CHROMATOGRAPHIC ANALYSIS OF THE ESTERIFIED FATTY ACIDS OF CELLS GROWN WITH THE SUPPLEMENTS INDICATED

18 : 1 supplememt	Recovery of radioactivity from gas chromatograph			
	cpm 18 : 1	cpm 19 : Cy	Spec. act. 18 : 1	(cpm/nmol) c19 : 0
100 μM *cis*-9	5400	13 000	330	193
100 μM *cis*-9 + 200 μM *cis*-6	660	630	124	297
50 μM *cis*-9 + 80 μM *cis*-10	1550	4 900	50	118
100 μM *cis*-9 + 200 μM *cis*-14	1080	730	72	197

as evidenced by the recovery of 40—75% of the *cis*-9-18 : 1 radioactivity in the 19 carbon cyclopropane peak (Table III).

Results in Fig. 3 indicate that formation of cyclopropanecarboxylic acids does not necessarily occur at the stationary stage. Cells grown with 20 μM *cis*-11 (Fig. 3A) grew to a cell yield of $6.4 \cdot 10^8$ cells/ml, but showed no increase in cyclopropane content (7.9%) during growth, whereas those grown with 100 μM *cis*-11 grew to a higher cell yield ($28 \cdot 10^8$ cells/ml) and converted up to 60% of the esterified 18 : 1 to the cyclopropanecarboxylic acid (Fig. 3A). Cyclopropanecarboxylic acid formation in cells grown with 20 μM *cis*-9 was greatly stimulated by lowering the shaking rate of the culture (Fig. 3B).

Discussion

The data in Fig. 3 suggest that the increase in cyclopropane formation at the stationary stage reported by many workers is not primarily a function of the stage of growth, but more probably a result of a decrease in oxygen tension, which accompanies high cell yields, or a decrease in aeration of the culture.

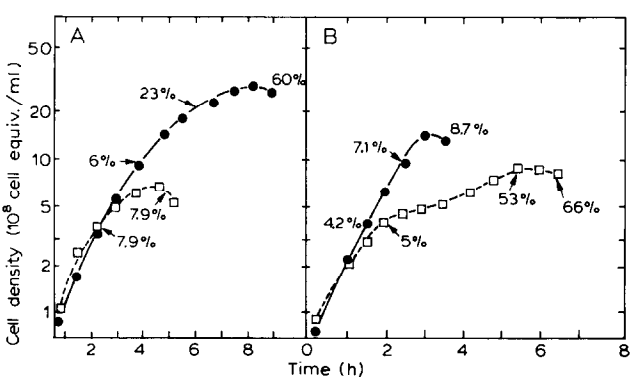

Fig. 3. Factors controlling cyclopropane ring formation. (A) Influence of cell density at shaking rate of 250 rev./min. (●) Culture supplemented with 100 μM *cis*-9-18 : 1; (□) culture supplemented with 20 μM *cis*-9-18 : 1. (B) Influence of shaking rate. (●) Shaking rate of 340 rev./min; (□) shaking rate of 110 rev./min. Both cultures were supplemented with 20 μM *cis*-9-18 : 1. Cells were harvested from the growth curves at the times indicated and the conversion of monoene to cyclopropane determined by gas chromatographic analysis of the fatty acids after methoxide transmethylation. At each arrow the numbers represent the percentage of esterified *cis*-9-18 : 1 converted to its cyclopropane derivative.

TABLE IV

SPECIFICITY OF CYCLOPROPANE FORMATION OBSERVED WHEN UNSATURATED FATTY ACID AUXOTROPHS WERE SUPPLEMENTED WITH VARIOUS MONOENOIC FATTY ACIDS

Superscript values indicate information from prior reports [10,11]. Values for the 18 : 1 isomers are taken from Fig. 2, and the results in parenthesis are from ref. 11.

Double bond position	100 · cyclo/(monoene + cyclo)		
	14 : 1 [10]	16 : 1 [11]	18 : 1
6		<0.5	<1 (1.5)
7		<0.5	<1
8			<1
9	50	80	36 (28)
10		40	7
11		10	34 (31)
12			4
13			<1

A similar hypothesis concerning the involvement of oxygen in cyclopropane ring formation was made by Knivett and Cullen [21], who found that reducing the amount of oxygen bubbled through a culture from 500 ml/min to 4 ml/min resulted in an approxumately 3-fold increase in 16 : 1 conversion to its cyclopropane derivative. Crowfoot and Hunt [22] have also noted an increase in cyclopropane ring formation upon reducing the aeration of *E. coli* cultures. Since the cells used in the present studies neither synthesize nor can oxidize than acids, the effect of dimished O_2 is not merely one of sparing the unsaturated acid.

The wide range in extent of conversion of a given octadecenoic isomer to its cyclopropane derivative evident in Fig. 2 probably reflects our use of cultures that had grown to different cell densities, and thus differed in oxygen tension when harvested for fatty acid analysis.

The results with the series of postional isomers (Fig. 2) suggest that the synthesis of cyclopropanecarboxylic acids in *E. coli* has a high degree of specificity for the *cis*-9 and *cis*-11 isomers of octadecenoic acid and is consistent with reports concerning other acids [10,11]. These two isomers have the same position relative to the carboxyl as found in the naturally occurring palmitoleic and vaccenic acids of *E. coli.*

When the *cis*-6, *cis*-10 or *cis*-14 isomer was provided to the cells simultaneously with *cis*-9-[1-^{14}C]18 : 1 there appeared to be a preferential formation of the cyclopropane ring from the *cis*-9 isomer, as evidenced by the higher specific activity of the cyclopropane peak relative to that for the octadecenoic acid peak during gas chromatography (Table III). Thus, the lower levels of cyclopropane derivatives formed from the 6,10 and 14 isomers most probably reflect enzyme specificity rather than a change in cell physiology resulting from the difference in fatty acyl structure.

Many examples of specific interactions between acyl chains and catalytic proteins have been investigated [23—25]. In a study of the specificity of fatty acid desaturases and hydrolases, Howling et al. [25] suggested that two desaturases may exist, one recognizing the substrate by the distance from the double bond to the carboxyl and the other recognizing the placement of the double

bond with respect to the methyl terminus. Since the unsaturated fatty acids in *E. coli* are derived from *cis*-3-decenoyl-acyl carrier protein, the only naturally occurring monoene substrates for cyclopropane synthesis in *E. coli* all have the double bond seven carbons from the methyl terminus. Thus, the cyclopropane "synthetase" might be regarded as one enzyme that recognizes the distance of the double bond from the methyl terminus of both *cis*-9-16 : 1 (*n*-7) and *cis*-11-18 : 1 (*n*-7). This hypothesis, however, is not consistent with our results in Fig. 2, which indicate that *cis*-9-18 : 1 (*n*-9) is also a good substrate for cyclopropane ring formation.

Marinari et al. [11] reported the specificity of cyclopropane formation from several hexadecenoate isomers, and their data are summarized in Table IV, together with results from this study. Marinari et al. [11] described significant (40%) cyclopropane formation from *cis*-10-16 : 1 and much less (11%) from *cis*-11-16 : 1. Thus, the positional specificities for cyclopropane synthesis appear to be different with 16-carbon substrates than with 18-carbon substrates. The *cis*-9 isomer of 14 : 1 also may be a substrate for cyclopropane formation [10]. Thus, for chain lengths of 14, 16 and 18 carbons, the isomer with a *cis* double bond 9 carbons from the carboxyl has been found to be a good substrate. This suggests that one enzyme may recognize the substrate by the distance of the ethylenic bond from the carboxyl groups in the acyl chain, rather than by the distance from the methyl carbon.

The above discussion leads to the concept that two types of selectivity may be operating in *E. coli:* one for the 9 position of an acyl chain (a "carboxyl-oriented" enzyme) and one for the *n*-7 position (a "methyl-oriented" enzyme). Palmitoleate may then serve as a substrate for both activities, whereas oleate and vaccenate would serve with only one of them. Such considerations are also in accord with the observation that palmitoleate (16 : 1) is generally more extensively converted to the cyclopropane acid (17 : Cy) (e.g. see Tables I and II) than is vaccenate (18 : 1). Although the evidence is still quite circumstantial at this time, we believe it is sufficient to warrant a careful search for two separate cyclopropane ring-forming activities in *E. coli.*

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