Fatty Acid Compositions of *Corynebacterium simplex* Grown on 1-Alkenes[†]

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The utilization of 1-alkenes by *Corynebacterium simplex* ATCC 6946 was studied with respect to the characteristic fatty acid profiles resulting from the growth at the expense of these substrates.

It was indicated that the synthetic pathways of the cellular fatty acids in *Corynebact. simplex* grown on various *n*-alkanes or 1-alkenes changed markedly according to the chain lengths of the substrates. From shorter chain hydrocarbons (C₁₂, C₁₄) the fatty acids were found to be synthesized mainly *via de novo* synthesis pathway in a similar manner to those from glucose, while chain elongation and intact incorporation occurred to a very small extent. On the other hand, an intact incorporation mechanism was preferential in the cells grown on longer ones (C₁₆, C₁₈). When *n*-pentadecane or 1-pentadecene was used as the substrate, these three mechanisms seemed to operate simultaneously.

Our previous paper¹⁾ revealed the fatty acid profiles of *Corynebact. simplex* grown on *n*-alkanes having various chain lengths and indicated the relationship between the chain lengths of substrate and those of fatty acids. That is, when a long chain *n*-alkane (C₁₆₋₁₈) was used as the substrate, the fatty acid of the same chain length as that of the substrate was the most abundant component and fatty acids having longer chain lengths, except for methyl-branched acids, were not detected. On the contrary, from shorter chain *n*-alkanes (C₁₂₋₁₄) longer chain fatty acids, especially C₁₈ acids (including tuberculostearic acid), were mainly synthesized. When *n*-pentadecane was used as a substrate, both tendencies mentioned above were observed. These facts indicate that *de novo* synthesis, chain elon-

gation and intact incorporation mechanisms may operate, alternatively or con-comitantly, during the synthesis of the cellular fatty acids in *Corynebact. simplex*. In order to clarify the operation of these synthetic mechanisms mentioned above, 1-alkenes instead of *n*-alkanes were used as growth substrates in the present study since they may be useful as markers because of their carbon-carbon double bonds. The results obtained showed a clear-cut relationship between the alkene substrates and the cellular fatty acids.

MATERIALS AND METHODS

A description of bacterial strain, culture conditions, and analysis and fractionation of fatty acids are the same as those described in the previous papers.^{1,2)}

The fatty acid esters were analyzed with Yanagimoto Gas Chromatograph GCG 500-T equipped with a flame ionization detector. A non-polar column, 2.25 m × 3 mm (inner diameter) stainless steel tubing packed with 10% Apiezon L on 60/80 mesh Celite 545 was employed.

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RESULTS AND DISCUSSION

When *Corynebact. simplex* was grown on hydrocarbons, 1-alkenes as well as *n*-alkanes having the chain lengths of more than 10 carbons served as good substrates and maximal cell yields were obtained on those with the chain lengths of 16~18 carbons.

Table I shows the fatty acid compositions of *Corynebact. simplex* grown on *n*-alkanes and glucose. The characteristics of these cellular fatty acids was as follows: All the unsaturated fatty acids contained in these cells were Δ^9 -monounsaturated acids, and 10-methyl branched-chain acids having various chain lengths were also present. Under certain cultural conditions, the occurrence of α -hydroxy fatty acids was also demonstrated.^{3,4)}

Figures 1 and 2 show the fatty acid profiles of *Corynebact. simplex* cultivated on 1-dodecene and 1-tetradecene, respectively. These were apparently similar to those from *n*-dodecane- and *n*-tetradecane-grown cells. However, fractionation with mercuric acetate⁵⁾ has revealed that the unsaturated acids having

FIG. 1. Fatty Acid Profile of *Corynebact. simplex* Grown on 1-Dodecene.

the same or shorter chain lengths than that of the substrate were detected in the place of the saturated acids of the cells grown on *n*-alkanes. The occurrence of these unsaturated acids is considered to be resulted from an intact in-

TABLE I. PERCENTAGE OF CELLULAR FATTY ACIDS OF *Corynebacterium simplex* GROWN ON GLUCOSE AND ON *n*-ALKANES

Growth substrate	Glucose	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈
Fatty acid				(%)				
C ₁₀	—	2.6	0.3	0.6	—	0.2	—	—
C ₁₁	—	—	0.8	—	0.8	—	0.4	—
C ₁₂ +t-C ₁₃	0.4	8.5	1.5	4.2	—	1.5	—	0.3
C ₁₃	—	—	8.4	—	4.6	4.6	1.3	—
C ₁₄ +t-C ₁₅	2.0	0.7	0.3	24.4	0.4	6.3	0.5	1.4
C ₁₅ +t-C ₁₆	1.3	1.1	2.1	2.2	45.4	0.2	15.3	14.1
C ₁₆ +t-C ₁₇	18.2	24.0	11.5	21.0	15.8	80.4	0.6	32.8
C ₁₇ +t-C ₁₈	10.9	15.4	23.5	7.1	18.2	—	81.7	3.4
C ₁₈ +t-C ₁₉	41.5	44.9	50.9	38.5	12.7	—	—	48.0
iso-branched	20.0	1.4	0.7	1.8	2.1	—	—	—
unknown	5.7	1.4	—	0.2	—	6.8	0.3	—

t-Acids refer to tuberculostearic acid homologues (10-methyl branched-chain fatty acids).

FIG. 2. Fatty Acid Profile of *Corynebact. simplex* Grown on 1-Tetradecene.

corporation of the alkene substrate after the oxidation of the terminal methyl group and from subsequent α -oxidation. This means that these acids are Δ^{ω} -unsaturated acids. The major fatty acids were tuberculostearic, palmitic and oleic acids, as shown in Figs. 1 and 2. The former two acids were all saturated and the last one contained a double bond between carbon 9 and 10. This fact is consistent with that obtained from glucose-grown *Corynebact. simplex* and shows that these longer chain acids were synthesized *de novo*.

Figures 3 and 4 show the fatty acid profiles of *Corynebact. simplex* grown on 1-hexadecene and 1-octadecene, respectively. The gas chromatograms of total fractions revealed analogous patterns to those from the cells grown on *n*-hexadecane and *n*-octadecane. The fatty acids corresponding to the alkene substrates were the most abundant com-

FIG. 3. Fatty Acid Profile of *Corynebact. simplex* Grown on 1-Hexadecene.

ponents. However, most fatty acids, even 10-methyl branched-chain ones, were fractionated into the unsaturated fractions. This fact was drastically different from not only those from *n*-hexadecane- and *n*-octadecane-grown cells but also those from 1-dodecene- and 1-tetradecene-grown cells. The ratio of unsaturated fatty acids to saturated ones reached to 75~85% when the cells were grown on 1-hexadecene. Most fatty acids, although showed the same retention time as that corresponding to palmitic or stearic acids on a column packed with Apiezon grease L, were fractionated into the unsaturated fractions. The saturated and Δ^{ω} -unsaturated acids may be indivisible by gas chromatography equipped with the above-mentioned column since the double bond at ω -position would scarcely contribute to the physical properties of fatty acids. However, the unsaturated acids were successfully separated from the corresponding

FIG. 4. Fatty Acid Profile of *Corynebact. simplex* Grown on 1-Octadecene.

FIG. 5. Fatty Acid Profile of *Corynebact. simplex* Grown on 1-Pentadecene.

saturated ones when a column packed with diethyleneglycol succinate was used. Furthermore, most 10-methyl fatty acids were shown to be unsaturated. These facts indicated that *Corynebact. simplex* attacked preferentially the terminal methyl group of alkene substrate and incorporated the Δ^{ω} -unsaturated fatty acid thus formed as well as shorter ones produced from it *via* β -oxidation pathway. Methylation at 10-position may occur after this incorporation as suggested in our previous paper.²⁾ It has also been reported that Δ^{ω} -unsaturated fatty acids, ordinarily not naturally occurring fatty acids, were detected in some microorganisms cultivated on 1-alkenes.^{4, 6~10)}

The high ratio of unsaturated (especially unsaturated at ω -position) to saturated acids and the absence of the acids having longer carbon-chains than that of the alkene used, except for 10-methyl branched-chain acids,

indicated that an intact incorporation mechanism was mainly operating in the formation of higher fatty acids from the higher alkenes or higher alkanes in this bacterium. The higher fatty acids thus incorporated may inhibit the operation of *de novo* synthesizing systems of fatty acids.¹¹⁾

Figure 5 shows the fatty acid profile of *Corynebact. simplex* grown on 1-pentadecene. The pattern of the total fraction was also similar to that from *n*-pentadecane-grown cells. However, several striking features were observed. That is, most fatty acids of C_{17} group (C_{17} and t - C_{18} acids) were fractionated into the unsaturated fraction, comprising 16-heptadecenoic, 9,16-heptadecadienoic and 10-methyl-16-heptadecenoic acids. On the contrary, the components of C_{16} and C_{18} group acids were essentially the same as those from *n*-alkane- or lower alkene-grown cells.

The fatty acids of C₁₈ group were composed of oleic, stearic and 10-methyl stearic (tuberculostearic) acids, suggesting that these longer chain acids would be synthesized *via de novo* synthetic pathway. The sum of these acids with even carbon-chain lengths attained to ca. 30% of the total fatty acids. The most abundant component of the cells grown on 1-pentadecene was 14-pentadecenoic acid, although a small amount of pentadecanoic acid was present.

These results strongly suggested that three different mechanisms were simultaneously operating for the syntheses of the major fatty acids of *Corynebact. simplex* growing on 1-pentadecene: Pentadecenoic acid would be formed by terminal oxidation of a methyl group of the alkene and incorporated intactly; the fatty acids of C₁₇ group (including t-C₁₈ acid) by a chain elongation pathway from 14-pentadecenoic acid primarily formed; and those of C₁₈ group (including t-C₁₉ acid) by a *de novo* synthesis mechanism. Table II summarizes

the fatty acid patterns of *Corynebact. simplex* grown on 1-alkenes of various chain lengths. The result obtained with the cells cultivated on 1-pentadecene would present the first evidence for the operation of a direct chain elongation mechanism in hydrocarbon-utilizing bacteria, although Klug and Markovetz⁸⁾ suggested involvement of a chain elongation mechanism in *Candida lipolytica* based on the formation of Δ^w -unsaturated C₁₈ acid from 1-hexadecene. They⁸⁾ and Foster¹²⁾ also mentioned an alternative pathway involving an attack on the double bond of 1-alkene, decarboxylation of the 2-hydroxy acid thus formed and subsequent chain elongation to yield longer-chain saturated fatty acids. This does not seem to be the case of *Corynebact. simplex*, judging from the above-mentioned fatty acid compositions of the bacterium grown on various alkenes.

On the other hand, pentadecanoic and tridecanoic acids were detected in the cells grown on 1-pentadecene. This fact indicates

TABLE II. MAJOR FATTY ACIDS OF *Corynebacterium simplex* GROWN ON VARIOUS 1-ALKENES

Growth substrate	Major fatty acids	Notes
1-Dodecene	Palmitic acid	<i>via de novo</i> synthesis
	Oleic acid	"
	10-Methylstearic acid	"
1-Tetradecene	13-Tetradecenoic acid	<i>via</i> intact incorporation
	Palmitic acid	<i>via de novo</i> synthesis
	Oleic acid	"
	10-Methylstearic acid	"
1-Pentadecene	14-Pentadecenoic acid	<i>via</i> intact incorporation
	Palmitic acid	<i>via de novo</i> synthesis
	16-Heptadecenoic acid	<i>via</i> chain elongation
	9,16-Heptadecadienoic acid	"
	10-Methyl-16-heptadecanoic acid	"
	Oleic acid	<i>via de novo</i> synthesis
	10-Methylstearic acid	"
1-Hexadecene	15-Hexadecenoic acid	<i>via</i> intact incorporation
	10-Methyl-15-hexadecenoic acid	"
1-Octadecene	15-Hexadecenoic acid	<i>via</i> intact incorporation
	17-Octadecenoic, 9,17-Octadecadienoic and 10-Methyl-17-octadecenoic acids	"
		"

that oxidation of the terminal double bond of the alkene would be operative in the organism. In this manner, the 1-alkene or *n*-alkane of an intermediate chain length (C_{15}) was converted into the cellular fatty acids *via* rather complex pathways in *Corynebact. simplex*.

When *n*-tridecane was used as the growth substrate, the content of C_{17} group acids in the bacterium was relatively high (Table I). The chain elongation mechanism, which was not observed when 1-alkenes or *n*-alkanes of even carbon numbers (C_{12} to C_{18}) as well as C_{17} were used as substrates, may operate in the bacterium growing on hydrocarbons of appropriate odd carbon numbers.

Finnerty and Kallio⁽¹³⁾ presented interesting results that additions of two C_1 units were involved in the synthesis of palmitic acid from *n*-tetradecane in *Micrococcus cerificans*. Furthermore, Makula and Finnerty^(10,14) observed the formation of higher fatty acids with odd carbon chains from lower *n*-alkanes of odd number in *Micrococcus cerificans*, suggesting that a synthesizing system for odd carbon fatty acids is different from the addition mechanism of C_2 unit to the original carbon skeleton. However, in our case C_2 unit is considered to be directly added to the original C_{15} skeleton.

In conclusion, the main synthetic pathways of the cellular fatty acids in *Corynebact. simplex* changed depending on the carbon chain length of *n*-alkanes or 1-alkenes. Hydrocarbons of C_{12} , C_{13} , and C_{14} were mainly converted to the fatty acids *via* degradation and the subsequent *de novo* synthesis, *n*-pentadecane and 1-pentadecene were converted *via de novo* synthesis, chain elongation and intact incorporation mechanisms operating simultaneously, and higher ones (C_{16-18}) were incorporated intactly after oxidation of their terminal methyl group. A proposed scheme for the fatty acid synthesis in *Corynebact. simplex* was illustrated in Fig. 6.

FIG. 6. Proposed Pathway of Cellular Fatty Acid Synthesis from *n*-Alkanes or 1-Alkenes in *Corynebact. simplex*.

The oxidation of terminal double bond of 1-alkene was fortunately a minor pathway, if existed, in this bacterium. α -Oxidation mechanism, the operation of which has been demonstrated by Yano *et al.*^(3,4) in *Corynebact. simplex*, did not seem to occur in our strain under the cultural conditions employed.

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