The Journal of Biochemistry, Vol. 63, No. 3, 1968

Metabolism of Hydroxy Fatty Acids

VI. Structure of Hydroxy Acids and the Degradation by *Escherichia coli* K-12*

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(Received for publication, May 9, 1967)

E. coli has been shown to have a specific mode of the oxidative breakdown of hydroxy acids. The structual requirements leading to the interruption of metabolism was investigated.

5-Hydroxytetradecanoic acid δ-lactone, 10-hydroxytetradecanoic acid and mixtures of 10- and 9-hydroxyoctadecanoic acids were synthesized and employed as substrates.

9-Hydroxyoctadecanoic acid was completely oxidized after transient accumulation of 7-hydroxyhexadecanoic and 5-hydroxytetradecanoic acids. 10-Hydroxyoctadecanoic acid was converted by *E. coli* K-12 to 8-hydroxyhexadecanoic, 6-hydroxytetradecanoic and 4-hydroxydodecanoic acids, further degradation was negligible. 10-Hydroxytetradecanoic acid was broken down leading to accumulation of 8-hydroxydodecanoic and 6-hydroxydecanoic acids in the culture medium.

On the basis of the results summerized in Fig. 8, it can be concluded that C_2 cleavages of hydroxy fatty acids by *E. coli* cease when the total chain length of a hydroxy acid becomes twelve to ten, and, at the same time, the hydroxy group is on the sixth, fifth or forth carbon atom of the acid.

It has been shown that ricinoleic (*1*), 12-hydroxyoctadecanoic (*2*) and 13-hydroxynonadecanoic (*3*) acids are converted by *Escherichia coli* to the specified shorter chain hydroxy acids, namely 6-hydroxydodecenoic, 6-hydroxydodecanoic and 5-hydroxyundecanoic acids, respectively, which did not seem to undergo further cleavage.

The present investigation was undertaken to study the structural requirements of hydroxy acids leading to the interruption of metabolism by *E. coli*.

* This investigation was supported by a grant in aid for scientific research from the Ministry of Education.

** Dr. S. Okui died during the course of this investigation (March 18, 1967) and the manuscript was prepared by the other authors.

MATERIALS AND METHODS

Synthesis of Mixtures of 10-Hydroxyoctadecanoic and 9-Hydroxyoctadecanoic Acids—Oleic acid prepared from olive oil in the manner described by Rubin and Paisley (*4*) was treated with peracetic acid according to the procedure of Mack and Bickford (*5*). Resulted epoxy acid was catalytically reduced to yield a crude mixture of 10-hydroxyoctadecanoic and 9-hydroxyoctadecanoic acids. Purification was carried out on a silicic acid column (*1*).

10-Hydroxyoctadecanoic Acid—Highly purified 10-hydroxyoctadecanoic acid was donated by Prof. K.

Saito of Kansai Medical College.

Synthesis of 5-Hydroxytetradecanoic Acid δ-Lactone—The lactone was prepared from *n*-decyl aldehyde and propargyl bromide as starting materials. The procedure was essentially identical with that for 5-hydroxyundecenoic acid δ-lactone as described in a previous report (*3*) except that *n*-heptyl aldehyde and Lindlar catalyst were replaced with *n*-decyl aldehyde and palladium on barium sulfate, respectively.

Synthesis of 10-Hydroxytetradecanoic Acid—Methyl 10-undecenoate (10 g) was dissolved in 30 ml of glacial acetic acid. Dried ozone was then introduced over 2 hr. The solution was diluted with 40 ml of ether, and 10 g of zinc dust and 1 ml of water were added over a period of 15 min at 0°C with vigorous agitation. The mixture was heated under reflux for 1 hr and filtered to remove zinc acetate formed by the reaction. The filtrate was washed with water, 1% sodium bicarbonate, and again with water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was subjected to vacuum distillation. A fraction (bp 40—60°C at 0.06 mmHg) was collected and redistilled.

To 4.2 g of the methyl 9-formylnonanoate thus obtained and dissolved in 20 ml anhydrous ether, an ethereal solution of Grignard reagent prepared with 0.5 g of magnesium ribbon and 3 g of *n*-butyl bromide was added dropwise with stirring. After gentle reflux, the Grignard complex was decomposed by addition of diluted hydrochloric acid. The resulting methyl 10-hydroxydecanoate was extracted with ether and hydrolyzed with 10% sodium hydroxide. The resulting 10-hydroxytetradecanoic acid was purified by silicic acid chromatography (*1*).

3-Hydroxytetradecanoic Acid, 4-Hydroxydecanoic Acid γ-Lactone and 4-Hydroxyundecanoic Acid γ-Lactone—These were obtained commercially and used as substrates after purification when necessary by silicic acid (*1*) or neutralized aluminum oxide (*3*) column chromatography.

Culture Method—The cells of *E. coli* K-12 were cultivated in a liquid medium containing 0.02% substrate and 2% meat extract (pH 7.2). The metabolites were extracted with ether according to the procedure described in an earlier paper (*1*) and analyzed by gas-liquid chromatography (*2*).

RESULTS

Metabolism of 10-Hydroxyoctadecanoic and 9-Hydroxyoctadecanoic Acids by E. coli K-12—A typical gas-chromatogram of the metabolites of the mixture of 10-hydroxyoctadecanoic and 9-hydroxyoctadecanoic acids is illustrated in

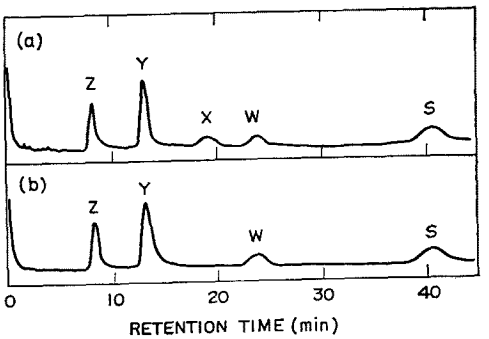

FIG. 1. Gas chromatograms of the metabolites of hydroxy fatty acids produced by *E. coli* K-12.
a: Mixture of 9- and 10-hydroxyoctadecanoic acids.
b: Pure 10-hydroxyoctadecanoic acid.

A Shimadzu GC-1B gas chromatograph equipped with a hydrogen flame ionization detector was used. The column was 1.5 m×4 mm packed with 5% diethyleneglycol succinate polyester coated on 80—100 mesh Celite-545 (Temp. 198°C). The definition of letters is in the text.

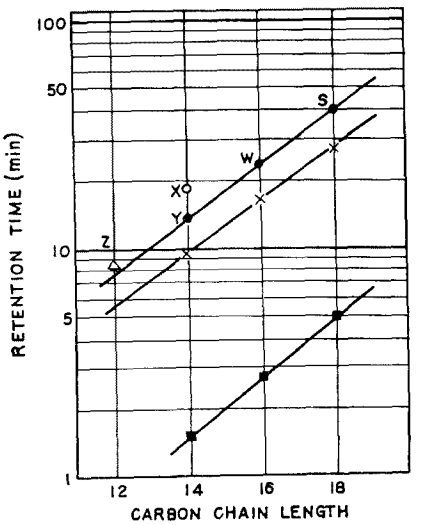

FIG. 2. Relation between carbon chain length and retention times of the metabolites of the mixture of 9- and 10-hydroxyoctadecanoic acid by *E. coli* K-12.

The definition of letters is in the text.
—●—, —○—, —△— Metabolites (treated with CH_2N_2).
—×— Metabolites after acetylation.
—■— Normal saturated fatty acid methyl esters.

Fig. 1-a, which shows four peaks denoted as W, X, Y and Z in the decreasing order of their retention times, besides the peak of substrate (S).

As shown in Fig. 2, the retention times of S, W and Y are on a straight line almost parallel to the line drawn through the retention times of normal, saturated fatty acids. Retention times were shifted in a uniform fashion following acetylation (*1*), indicating that W and Y were hydroxy fatty acids of sixteen and fourteen carbon-chain length, respectively.

On the other hand, X and Z were uneffected by diazomethane treatment, alkaline hydrolysis or acetylation indicating that neither X nor Z contained a free carboxyl group. A mixture of the metabolites was chromatographed on a neutralized aluminum oxide column (*3*) and X and Z were recovered as ether eluates. Their infra-red spectra (Fig. 3) exhibited characteristic peaks of δ- and γ-lactones near 1725 cm^{-1} and 1765 cm^{-1}, respectively (*6*).

Using pure 10-hydroxyoctadecanoic acid as substrate, all peaks except X were detected in the medium (Fig. 1-b).

These results suggest that W is a mixture of 8-hydroxy and 7-hydroxyhexadecanoic acids and that X, Y and Z are 5-hydroxytetradecanoic acid δ-lactone, 6-hydroxytetradecanoic acid and 4-hydroxydodecanoic acid γ-lactone,

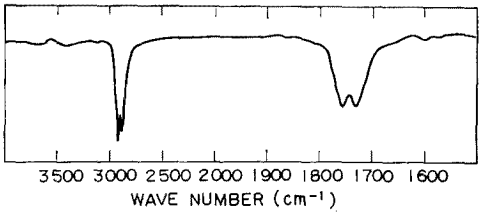

FIG. 3. Infra-red absorption spectrum of the component isolated from the metabolites of the mixture of 9- and 10-hydroxyoctadecanoic acids following neutralized aluminum oxide column chromatography.

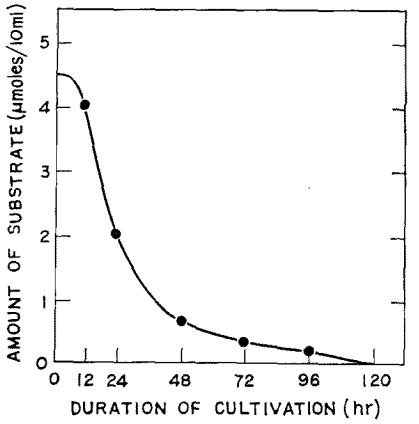

FIG. 4. Time course of degradation of 5-hydroxytetradecanoic acid δ-lactone in the culture medium of *E. coli* K-12.

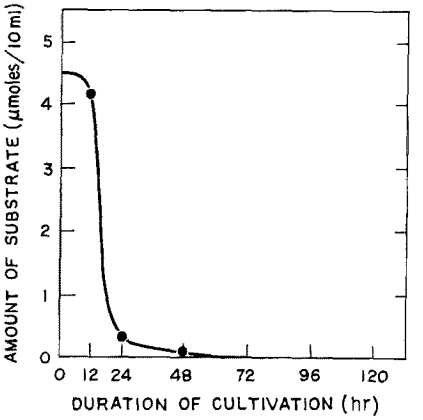

FIG. 5. Time course of degradation of 3-hydroxydodecanoic acid in the culture medium of *E. coli* K-12.

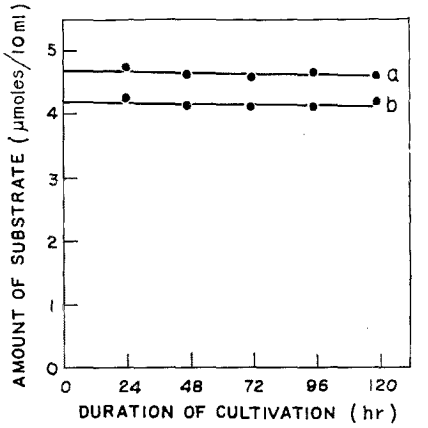

FIG. 6. Time course of the conversion of 4-hydroxydecanoic acid γ-lactone (a) and 4-hydroxyundecanoic acid γ-lactone (b) in the culture medium of *E. coli* K-12.

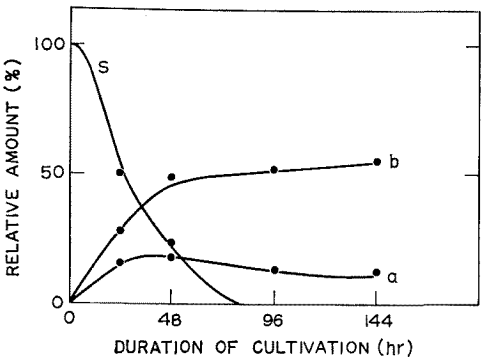

FIG. 7. Time course of the conversion of 10-hydroxytetradecanoic acid in the culture medium of *E. coli* K-12.
S: Substrate
a: 8-Hydroxydodecanoic acid
b: 6-Hydroxydecanoic acid

respectively.

Time Course of Degradation of 5-Hydroxytetradecanoic Acid δ-Lactone by E. coli K-12—Chemically pure 5-hydroxytetradecanoic acid δ-lactone synthesized from decylaldehyde and propargyl bromide had the same retention time as X on gas-chromatography. As illustrated in Fig. 4, the δ-lactone was completely metabolized by *E. coli* K-12 and no metabolite was detected at any time during the cultivation.

Time Course of Degradation of 3-Hydroxydodecanoic Acid, 4-Hydroxydecanoic Acid γ-Lactone and 4-Hydroxyundecanoic Acid γ-Lactone by E. coli K-12—3-Hydroxydodecanoic acid was degraded by *E. coli* K-12 and disappeared from the medium very rapidly (Fig. 5), while the two γ-lactones were virtually unmetabolized (Fig. 6).

The retention times of hydroxydecanoic acid γ-lactone, 4-hydroxyundecanoic acid γ-lactone and Z mentioned above were on one straight line when that of Z was plotted in the position of carbon-chain length of twelve, suggesting that Z was 4-hydroxydodecanoic acid γ-lactone.

Degradation of Hydroxytetradecanoic Acid by E. coli K-12—10-Hydroxytetradecanoic acid was converted by *E. coli* K-12 to 8-hydroxydodecanoic and 6-hydroxydecanoic acids. Gas-chromatographic analysis showed that after 96 hr cultivation the amount of 6-hydroxydecanoic acid accumulated in the medium was about four times larger than that of 8-hydroxydodecanoic acid, while 10-hydroxytetradecanoic acid had almost disappeared.

DISCUSSION

Based on the results summarized in Fig. 8,

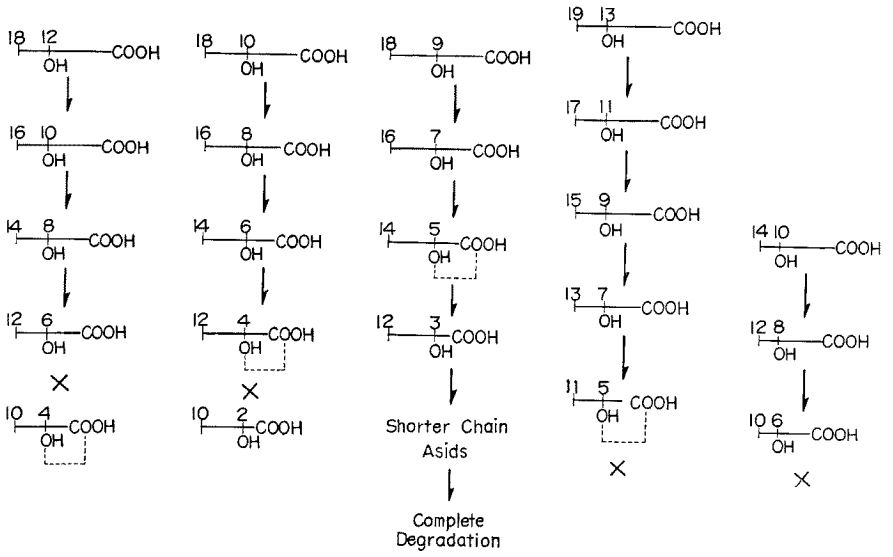

FIG. 8. The mode of degradation of some hydroxy fatty acids by *E. coli* K-12.
× indicates the interruption of degradation.

it is evident that 9-hydroxyoctadecanoic acid was completely oxidized by *E. coli* K-12 transiently accumulating two metabolites, 7-hydroxyhexadecanoic acid and 5-hydroxytetradecanoic acid (detected as its δ-lactone (*7*)).

Although 3-hydroxydodecanoic acid was not detected when a mixture of 10-hydroxyoctadecanoic and 9-hydroxyoctadecanoic acids (Fig. 1-a), or 5-hydroxytetradecanoic acid δ-lactone (Fig. 4) was added as substrate, it was considered that 3-hydroxydodecanoic acid was formed from 5-hydroxytetradecanoic acid by C_2 cleavage and that the 3-hydroxy acid was broken down as a normal intermediate of the β-oxidation cycle. This was supported by the observation of rapid disappearance of 3-hydroxytetradecanoic acid (Fig. 5).

On the other hand, 10-hydroxyoctadecanoic acid was converted to 8-hydroxyhexadecanoic, 6-hydroxytetradecanoic and 4-hydroxydodecanoic (detected as its γ-lactone) acids. Further oxidation seemed unlikely.

Since both 4-hydroxydecanoic acid γ-lactone and 4-hydroxyundecanoic acid γ-lactone remained unaffected in the medium (Fig. 6), one might suppose that the lactone-ring was resistant to β-oxidation. However, this ring formation was not essential to terminate oxidation since the conversion of 6-hydroxydodecanoic to 4-hydroxydecanoic acid was difficult (*2*) and since 5-hydroxytetradecanoic acid δ-lactone was degraded, while 5-hydroxyundecanoic acid δ-lactone seemed not to be metabolized (*3*). Furtheremore, 10-hydroxytetradecanoic acid was broken down yielding 8-hydroxydodecanoic and 6-hydroxydecanoic acids.

In view of these facts, it seems that the degradation of hydroxy fatty acids by *E. coli* stops when,

a) chain length of a hydroxy acid is 10, 11 or 12,

b) the hydroxy group is on the sixth, fifth or fourth carbon atom of the acid.

It is uncertain whether the resistance of these medium chain hydroxy acids is due to the oxidizing enzyme system or primarily due to the permeability of *E. coli* cell walls.

It is well known that there are at least three thiokinases and acyl dehydrogenases specific for long chain, medium chain and short chain carboxylic acids (*8*). Therefore, it might be suggested that the hydroxy group offers steric hindrance to a certain enzyme (s) specific for medium chain intermediates of the fatty acid oxidation cycle.

The authors wish to express their gratitude to Prof. N. Ishida of Tohoku University School of Medicine for providing facilities for this work. Thanks are also due to Prof. K. Saito of Kansai Medical College for the gift of 10-hydroxyoctadecanoic acid.

REFERENCES

(*1*) S. Okui, M. Uchiyama, and M. Mizugaki, *J. Biochem.*, **53**, 265 (1963)

(*2*) M. Mizugaki, M. Uchiyama, and S. Okui, *J. Biochem.*, **58**, 174 (1965)

(*3*) M. Mizugaki, M. Uchiyama, and S. Okui, *J. Biochem.*, **58**, 273 (1965)

(*4*) L.J. Rubin, and W. Paisley, *J. Am. Oil Chemists' Soc.*, **37**, 300 (1960)

(*5*) C.H. Mack, and W.G. Bickford, *J. Org. Chem.*, 18, 686 (1953)

(*6*) L.J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd Ed., Methuen and Co. Ltd., London, p. 178 (1958)

(*7*) H.H. Guest, *J. Am. Chem. Soc.*, **69**, 300 (1947)

(*8*) D.E. Green, and S.J. Wakil, "Lipid Metabolism," ed. by K. Bloch, John Wiley and Sons, New York, p. 1 (1960)