THE REVERSIBLE HYDRATION OF OLEIC ACID TO 10D-HYDROXYSTEARIC ACID[1]

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The conversion of oleic acid to 10-hydroxystearic acid by a Pseudomonas species (NRRL-B-2994; Wallen et al., 1962) provides a unique example of the stereospecificity of enzymatic reactions at an isolated double bond in an acyclic compound. The 10-hydroxystearic acid, which is formed in this reaction, is optically active (Schroepfer and Bloch, 1963, 1965) and has the D-configuration (Schroepfer and Bloch, 1965). Incubation of this organism with oleic acid in a medium enriched with deuterium oxide yielded 10-hydroxystearic acid containing one stably-bound deuterium atom (Schroepfer, 1965). Moreover, this deuterium was shown to be on carbon atom 9 and in the L-configuration. These findings are compatible with a stereospecific hydration of the olefinic bond of oleic acid analogous to the hydration of fumaric acid catalyzed by the enzyme fumarase (Gawron and Fondy, 1959, Englard, 1964). However, the possibility that the overall conversion of oleate to 10-hydroxystearate proceeds by an initial epoxidation of the olefin followed by reductive opening of the epoxide ring cannot be excluded by our previous studies with the intact organism. We now wish to report findings which render the latter possibility extremely unlikely and which provide additional information concerning the mechanism of this reaction.

Soluble (105,000 x g) extracts of the organism have been prepared which catalyze the reversible conversion of oleic acid to 10-hydroxystearic acid. The reaction proceeds anaerobically as well as in an atmosphere of air. Moreover, neither DL-cis-9,10-epoxystearic acid nor DL-trans-9,10-epoxystearic acid serve as a precursor of 10-hydroxystearic acid (or oleic acid) in this enzyme system.

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Experimental and Results

Preparation of Bacterial Extract

The organism was grown for 24 hours at 30° in a medium (pH 8.0) containing inorganic salts, glucose, and potassium oleate. The cells were collected by centrifugation, suspended in 0.02 M Tris buffer (pH 8.0) containing 0.002 M mercaptoethanol, and subjected to sonic oscillation. The supernatant fluid, obtained by centrifugation at 15,000 x g for 20 minutes, was recentrifuged for 90 minutes at 105,000 x g. The resulting supernatant fluid, which catalyzed the conversion of oleic acid to 10-hydroxystearic acid, retained enzymatic activity upon storage for several months at -10°.

Conversion of Oleate to 10-hydroxystearate

Oleic acid-1-^{14}C (Tracerlab, purified by silicic acid column chromatography) was diluted with unlabeled oleic acid (Hormel Institute), neutralized with KOH, mixed with a solution of bovine serum albumin (Pentex), and the pH of the resulting solution (10 mg oleic acid per ml; 85 mg albumin per ml) was adjusted to pH 8. The substrate was incubated with 0.1 ml of the enzyme extract in 1.0 ml Tris buffer (0.02 M; pH 8.0) at 30°. Incubations were terminated by the addition of hot 15% ethanolic KOH. After heating for 3 hours on a steam bath, the incubation mixtures were acidified to pH 1, and the fatty acids were extracted with ether and applied to silica gel G thin layer plates. After development of the plates (pentane, ether, acetic acid - 90:15:1), 1 cm increments of the silica gel were scraped into counting vials and assayed for radioactivity by the method of Snyder (1964). Figure I shows the amounts of 10-hydroxystearate formed when 3.55 μmoles of substrate were

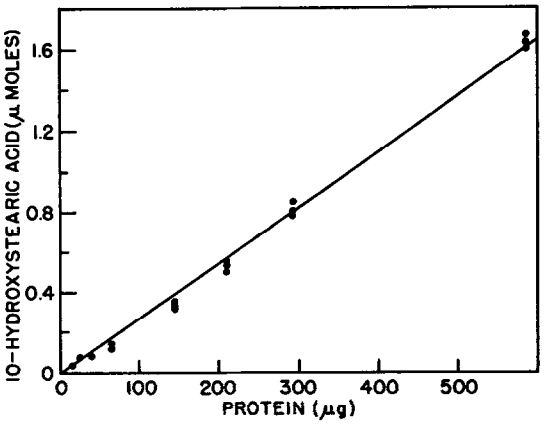

Figure 1. Enzymatic conversion of oleic acid to 10-hydroxystearic acid.

Table I. Reversible Conversion of Oleic Acid to 10-Hydroxystearic Acid

Substrate	% of Recovered Radioactivity Associated Chromatographically With	
	10-Hydroxystearic Acid	Oleic Acid
Oleic acid-1-^{14}C*	93,93	7,7
Oleic acid-1-^{14}C, anaerobic*	87,90	13,10
Oleic acid-1-^{14}C, 38.5 mM EDTA*	91,86	9,14
Oleic acid-1-^{14}C, boiled enzyme extract*	<0.1	>99
10-Hydroxystearic acid-1-^{14}C**	85,86,90	15,14,10
10-Hydroxystearic acid-1-^{14}C, boiled enzyme extract**	>99	<0.5
Oleic acid-1-^{14}C**	77,82	23,18

*Incubation conditions: 3.55 μmoles of substrate (in 0.1 ml of albumin solution); 0.2 ml enzyme extract (2.4 mg protein); 1.0 ml Tris buffer, pH 8.0; 1 hour; 30°.
**Incubation conditions: 3-18 milliμmoles of substrate (in 0.1 ml 95% ethanol); 0.1 ml enzyme extract (1.2 mg protein); 1.0 ml Tris buffer, pH 8.0; 2 hours; 30°.

incubated with varying amounts of bacterial extract (time of incubation 60 minutes; total volume 1.2 ml).

The product was characterized as 10-hydroxystearic acid by thin-layer chromatographic analysis, and, as the methyl ester, by gas-liquid chromatographic analysis, melting point (55-56°), and mass spectrometry.

The conversion of oleate to 10-hydroxystearate was found to proceed to the same extent anaerobically as in the presence of air (Table I). The extent of conversion was not affected by the addition of EDTA at a concentration of 38.5 mM. It is noteworthy that the addition of ATP or coenzyme A was not required.

Conversion of 10-hydroxystearic Acid to Oleic Acid

10-hydroxystearic acid-1-^{14}C (~33 μc/mg) was prepared by incubation of oleic acid-1-^{14}C with the bacterial extract. After purification by silicic acid column chromatography, the radiopurity of the hydroxy fatty acid was judged to be in excess of 99% on the basis of thin-layer chromatographic analysis. Incubation of the labeled hydroxy fatty acid with the enzyme extract yielded oleic acid (Table I) which was characterized by its chromatographic behavior on silica gel thin-layer plates, by the chromatographic behavior of the methyl ester on silica gel G-$AgNO_3$ plates, by the retention time of the methyl ester on gas-liquid radiochromatographic analysis, and by permanganate-periodate oxidation of the methyl ester (Chang and Sweeley, 1962) followed by gas-liquid radiochromatographic analysis.

Lack of Conversion of cis- or trans-epoxystearic Acid to 10-hydroxystearic Acid

DL-cis-9,10-epoxystearic acid-1-^{14}C was prepared from oleic acid-1-^{14}C by treatment with peracetic acid according to Julietti et al. (1960). DL-trans-9,10-epoxystearic acid-1-^{14}C was prepared from elaidic acid-1-^{14}C in a similar manner. The labeled elaidic acid was prepared from oleic acid-1-^{14}C by treatment with nitrous acid as described by Litchfield et al. (1965). Identity and radiopurity of the two labeled epoxystearic acids was established by thin-layer chromatographic analysis and by formation of the 9,10-dihydroxy derivatives by acid hydrolysis. The cis- and trans-epoxystearates yielded, upon acid hydrolysis, labeled threo-9,10-dihydroxystearate and erythro-9,10-dihydroxystearate respectively.

Upon incubation of the labeled epoxystearic acids with the enzyme extract, we could detect no conversion of either epoxide to 10-hydroxystearic acid or oleic acid. Oleic acid was efficiently converted to 10-hydroxystearic acid under the same conditions.

Discussion

Soluble extracts of a Pseudomonas species have been prepared which catalyze the reversible conversion of oleate to 10-hydroxystearate. The 10-hydroxystearic

acid produced from oleic acid by this organism has been shown to be optically active and its configuration has been established (Schroepfer and Bloch, 1963, 1965). Vioque and Maza (1964) have also reported the isolation of an optically active 10-hydroxystearic acid from sulfur olive oil and have suggested its possible origin from trans-9,10-epoxystearic acid, which also occurs in this oil (Vioque and Morris, 1961). The results obtained in the present study do not support a mechanism involving an epoxide intermediate in the overall conversion of oleic acid to 10-hydroxystearic acid. The conversion of oleate to 10-hydroxystearate proceeds under anaerobic conditions, and the reversibility of the reaction is readily demonstrable. These findings, coupled with the observation of the stereospecific uptake of one atom of solvent hydrogen into the 10-hydroxystearate and the lack of conversion of either the cis- or trans-9,10-epoxystearic acid to 10-hydroxystearic acid, are compatible with a mechanism involving stereospecific hydration of the double bond of oleic acid.

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