L-TRANS-2,3-EPOXYSUCCINATE. A NEW SUBSTRATE FOR FUMARASE[1]

Fred Albright and G. J. Schroepfer, Jr.

Division of Biochemistry, Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois, 61801

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Summary: Fumarase (fumarate hydratase, EC 4.2.1.2) has been found to catalyze the stereospecific hydration of the L-isomer of trans-2,3-epoxysuccinate to yield meso-tartrate. The product was characterized by various forms of chromatography and by combined gas-liquid chromatography-mass spectrometry. Attempts to dissociate the fumarate-hydrating activity of the enzyme from the epoxide-hydrating activity were unsuccessful.

Our finding that a soluble enzyme extract from a pseudomonad which catalyzes the stereospecific hydration of the Δ^9-double bond of oleic acid (Schroepfer, 1965; Niehaus and Schroepfer, 1965; Schroepfer, 1966; Niehaus et al. (1970) also catalyzes the stereospecific hydration of the epoxide function of cis- and trans-9,10-epoxystearate (Niehaus and Schroepfer, 1967; Niehaus et al. 1970) led us to consider that other enzymes which catalyze the addition of the elements of water across a carbon-carbon double bond might also catalyze the hydration of the corresponding epoxides. With this possibility in mind we directed our attention to the enzyme fumarase (fumarate hydratase, EC 4.2.1.2) which catalyzes the reversible interconversion of fumarate and L-malate (Alberty, 1961). Hydration of the corresponding epoxy-acid (trans-2,3-epoxysuccinate) corresponding to fumarate, would, by an analogous stereochemical course, yield meso-tartrate. Moreover, the occurrence of such a process might conceivably be of physiological significance since L-trans-2,3-epoxysuccinate is a known natural product (Martin and Foster, 1955; Sakaguchi and Inoue, 1938,

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1940) and since meso-tartrate is known to be a potent competitive inhibitor of fumarase (Wigler and Alberty, 1960).

To investigate this matter samples of [1-^{14}C]-DL-trans-2,3-epoxy-succinate (specific activities 10 μCi per mmole and 5.85 mCi per mmole; radiopurity in excess of 99%) were prepared from [1-^{14}C]-fumarate by a modification of the method of Payne and Williams (1959) for use as a substrate. Fumarase from swine heart muscle was obtained from 3 sources: Calbiochem (Los Angeles, California), Mann Research Laboratories (New York, New York), and Nutritional Biochemicals Corporation (Cleveland, Ohio). These enzyme preparations were solubilized for use as described by Robinson et al. (1967). Incubation of the labeled DL-trans-2,3-epoxy-succinate with fumarase yielded meso-tartrate. Boiled enzyme controls were inactive. The product was characterized as meso-tartrate on the basis of (1) identical mobility of the product with that of authentic meso-tartrate on paper chromatographic analysis (Reio, 1958), (2) identical mobility of the dimethyl ester with that of authentic dimethyl meso-tartrate on thin-layer analysis on silica gel plates, (3) the same chromatographic behavior of the dimethyl ester of the product and that of dimethyl meso-tartrate on gas-liquid chromatographic analysis on a 5% diethyleneglycol succinate on Chromosorb W column, (4) the same chromatographic behavior of the bis-trimethylsilyl ether derivative of the dimethyl ester of the product with that of bis-trimethylsilyl-dimethyl meso-tartrate on gas-liquid chromatographic analysis on 3 different columns (5% diethylene glycol succinate on Chromosorb W; 3% SE-30 on Chromosorb W; and 3% QF-1 on Gas-Chrom Q[2]), and (5) the same mass spectrum of the bis-trimethylsilyl ether derivative of the dimethyl ester of the product as that observed with authentic

2. Using this column, the observed retention times (relative to that of methyl myristate) for the derivative of the product and that of the bis-trimethylsilyl-dimethyl ester derivatives of meso-tartrate, D-tartrate, and L-tartrate were 0.37, 0.37, 0.41, and 0.42, respectively.

bis-trimethylsilyl-dimethyl meso-tartrate when analyzed by combined gas-liquid chromatography-mass spectrometry[3].

The enzymatic reaction is characterized by notable stereospecificity. Upon prolonged incubation of [1-^{14}C]-DL-trans-2,3-epoxysuccinate with fumarase, the reaction proceded only to the extent of ∿ 45% utilization of the incubated racemic substrate (Figure 1)[4]. In addition, the racemic

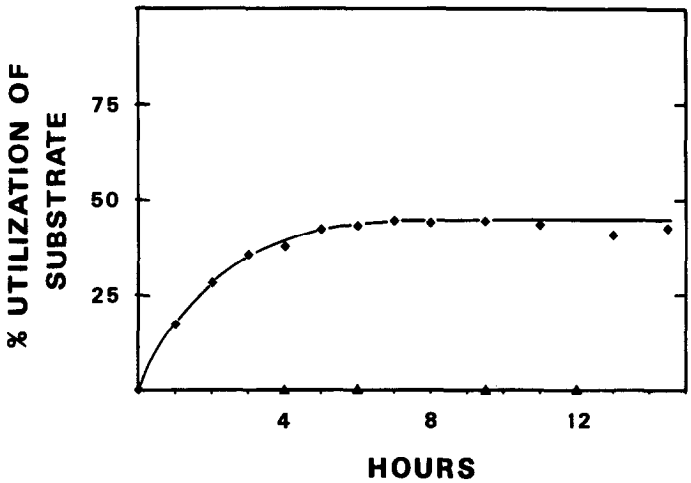

Figure 1 Time-course of the formation of meso-tartrate from racemic trans-2,3-epoxysuccinate.

Dipotassium [1-^{14}C]-DL-trans-2,3-epoxysuccinate (22.3 µmoles) in water (12 µl) was incubated with fumarate hydratase (0.13 mg; Calbiochem) in 0.01 M potassium phosphate buffer (0.6 ml; pH 7.31) at 30°. The progress of the enzymatic hydration was monitored by ascending paper radiochromatographic analysis of aliquots (5 µl) of the incubation mixture.

♦——♦, conversion of labeled epoxysuccinate to meso-tartrate.

▲——▲, boiled enzyme control.

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3. We gratefully acknowledge that this experiment was carried out in the laboratory of Dr. James McCloskey (Houston, Texas). An LKB 9000 combined instrument was used. The column used was 1% SE-30 on Gas Chrom P.

4. Moreover, in a similar experiment, the unreacted substrate was isolated after 7 hours of incubation (∿ 42% conversion of the incubated substrate to meso-tartrate) and reincubated with fumarase for 5 hours. Slight (0.5%) additional conversion of the substrate to meso-tartrate was observed.

labeled trans-epoxysuccinate was incubated with fumarase on a preparative scale. After the reaction had proceded to the extent of 35% utilization of the substrate (as judged by radiochromatographic assay), the unreacted epoxysuccinic acid was isolated and found to be optically active ($[\alpha]_D$ + 65°, c. 3.45 ethanol). Pure L-trans-epoxysuccinic acid is levorotatory ($[\alpha]_D$ - 118°, c. 1.0, ethanol; Miller, 1963). The results of these two experiments indicate the stereospecific nature of the reaction and that the absolute configuration of the enantiomer acted upon by the enzyme is L.

The catalytic activity of fumarase for the hydration of L-trans-2,3-epoxysuccinate is considerably less than that for the hydration of fumarate. The calculated value of the turnover number for the enzyme with respect to L-trans-2,3-epoxysuccinate (based upon a molecular weight for fumarase of 194,000 (Kanarek et al., 1964)) at 30° was 5.82 moles of substrate per minute per mole of enzyme. The corresponding value for the hydration of fuamrate (assayed by a modification of the method of Kanarek and Hill (1964)) under the same conditions was 77,600.

That the enzymic hydration of the L-trans-2,3-epoxystearate is due to fumarase and not to another enzyme is strongly suggested by a constant ratio of epoxide-hydrating activity to fumarate-hydrating activity: (1) in samples of crystalline fumarase from 3 different commercial sources, (2) before and after partial inactivation of the enzyme by heat, and (3) before and after acrylamide gel electrophoresis. Moreover, L-trans-2,3-epoxysuccinate acts as a competitive inhibitor of the fumarate-hydrating activity of fumarase and fumarate acts as a competitive inhibitor of the epoxide-hydrating activity of fumarase.

It is important to note that Martin and Foster (1955) have previously reported the preparation of a cell-free extract of a species of Flavobacterium (selected to grow on L-trans-2,3-epoxysuccinate as a sole carbon source) which catalyzed the conversion of the epoxide to meso-tartrate. Whether this enzymic activity is due to fumarase on another enzyme was not

established. More recently Allen and Jakoby (1969) have reported the isolation of an enzyme from Pseudomonas putida which catalyzes the quantitative conversion of both isomers of trans-2,3-epoxysuccinate to meso-tartrate.

The findings in this study constitute an extension of the substrate range of fumarase which until recently (Nakamura and Ogata, 1968; Clarke et al., 1968; Teipel et al., 1968; Nigh and Richards, 1969), was regarded as limited to the interconversion of fumarate and L-malate. We have previously reported that a crude enzyme preparation from a pseudomonad which catalyzes the stereospecific hydration of the Δ^9-double bond of oleic acid also catalyzes the stereospecific hydration of the epoxide function of cis- and trans-9,10-epoxystearate (Niehaus and Schroepfer, 1967; Niehaus et al., 1970). The present report constitutes, to our knowledge, the first description of findings which strongly suggest that a single enzyme which catalyzes the hydration of an olefin can also hydrate the corresponding epoxide.

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