POSSIBLE ROLE OF NADPH-DEPENDENT ENOYL COENZYME A REDUCTASE IN β-OXIDATION OF UNSATURATED FATTY ACIDS*

Michinao Mizugaki and Mitsuru Uchiyama

Pharmaceutical Institute, Tohoku University
Sendai, Japan

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Summary
An 2-enoyl CoA reductase from rat liver mitochondria catalyzes the reduction of both oct-cis-2-enoyl CoA and its trans isomer in the presence of NADPH as a specific electron donor. This reductase is solubilized from mitochondria by sonication. The possible role of the reductase in the β-oxidation pathway of the unsaturated fatty acids is discussed.

It is generally accepted that cis-2-enoates, intermediates during the succesive oxidation of unsaturated fatty acids by the β-oxidation system, are converted by a hydratase to D(-)-hydroxy derivatives which are subsequently converted by an epimerase to L(+)-hydroxy compounds to re-enter the β-oxidation cycle (1).

In a series of previous studies on degradation of hydroxy fatty acids by some microorganisms, the cis-2-enoate forming in the course of β-oxidation was noted to fairly efficiently reduced (2) in the presence of NADPH as an electron donor by water-solubilized enzyme preparations (3). The results of these microbial studies seem to suggest the physiological function of the reductase is implicit in its capacity to catalyze the reduction to saturated fatty acids of cis-2-enoates which form in the process of β-oxidation.

The present communication deals with some properties of the NADPH-dependent reductase prepared by sonication of rat liver mitochondria.

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MATERIALS AND METHODS

Preparation of Substrates: Synthesis of oct-cis-2-enoic acid was accomplished by a Grignard's reaction (4) of 1-heptyne (Tokyo Kasei Co. Ltd., Tokyo) and CO_2, followed by semi-hydrogenation of the triple bond with Lindlar's catalyst (5). The resulting acid was purified through a chromatographic column of silicic acid (6).

Oct-trans-2-enoic acid was synthesized by a condensation of n-hexylaldehyde and malonic acid in pyridine with subsequent decarboxylation (7).

The CoA derivatives were prepared by the interaction of reduced CoA with the mixed anhydride derivatives of the enoates according to the method of Wieland and Koppe (8). Amounts of CoA ester were determined by its hydrolysis with neutral hydroxylamine and by assay for sulfhydryl groups by the procedure of Ellman (9).

Enzyme Preparation: Male wistar rats (150 - 180 g) that had been fed standard laboratory chow (MF, Oriental Yeast Industry Co. Ltd., Tokyo) for a few weeks were used. Mitochondria fraction was separated from the liver according to the method of Parsons (10). The fraction was suspended in a small volume of 0.02 M potassium phosphate buffer, pH 7.2, and the suspension sonicated for a total of 60 seconds or for 15 seconds four times with 1-minute intervals between with a Tomy Sonifier model UR 150-P, at the maximal power setting. The irradiated suspension was spun at 105,000 x g for 60 min. The supernatant protein was precipitated by 80 % ammonium sulfate saturation.

Protein assays were carried out by the biuret method (11) and by the method of Lowry et al (12).

Assay of the Reductase Activity: Since the reduction is NADPH-specific, the decrease in absorbance at 340 mu provides a convenient enzymic assay using mainly oct-cis-2-enoyl CoA as substrate. A typical assay mixture contained 40 μmoles of potassium phosphate (pH 7.4), 125 mμmoles of NADPH, 0.1-1 μmole of substrate and water added to a final volume of 0.8 ml. The reaction was initiated by the addition of sufficient amounts of the reductase. All reaction mixtures were assayed at 37°.

Detection of the Reduced Fatty Acids by Gas Chromatography: The reaction mixture (three times large scale) of the enzyme

assay was incubated at 37° for 20 min. The reaction was terminated by the addition of 0.5 ml of 2N KOH and the mixture allowed to stand overnight at room temperature, then acidified with 2N H_2SO_4. Fatty acids were extracted by three cycles of treatment with ether. The combined ether extracts, after washing with small amounts of water, were concentrated and fatty acids methylated with CH_2N_2. Aliquots of the methyl esters were analyzed with a Shimazdu gas-chromatograph GC-5A equipped with a hydrogen flame ionization detector. A glass column (2m × 3mm) packed with 30 % diethyleneglycol succinate polyester coated on 60 - 80 mesh Neopak AS was used.

RESULTS AND DISCUSSION

Cofactor Requirement: Reduction of oct-cis-2-enoyl CoA and its trans isomer by the enzyme preparations was found to yield saturated compounds only in the presence of NADPH but not in the presence of NADH, as demonstrated by gas chromatography. In view of this assay for reductase activity in the ensuing experiments were performed by spectrophotometric estimation of the decrease in absorbance at 340 mμ of reaction mixtures containing NADPH.

Location of Reductase Activity: After fractionation of rat liver homogenates by usual procedure into three principal cellular fractions, viz. mitochondrial, microsomal and soluble, the first two fractions were treated sonically, spun at 105,000 x g, and the resultant supernates assayed for reductase activity. The supernatant fluid from the mitochondrial fraction proved to contain a predominantly high reductase activity (Table I-A).

Effect of Starvation: As can be seen from the data presented in Table I, there was no significant difference noted to exist in this respect between the rat starved overnight before liver homogenation and that not starved.

Optimal pH: The enzyme preparation displayed a rather broad range of optimum pH from 7.0 to 8.5 in catalyzing the reduction of oct-cis-2-enoyl CoA.

Effect of SH Reagents: The reductase has proven, as shown in Table II, to be considerably inhibited by thiol alkylating reagents.

TABLE I

Effect of Starvation and Distribution of the Reductase Activity

	Mitochondria	Microsomes	Supernatant
A. Normal	0.82	0.31	tr.
B. Starved	0.83	0.16	tr.

unit: nmoles/min/mg

TABLE II

Effect of SH binding Reagents on the Reductase Activity

Inhibitor	Conc. (M)	Inhibition (%)
None	-	0
p-Hydroxymercuribenzoate	5×10^{-4}	100
	1×10^{-4}	30
Iodoacetamide	1×10^{-3}	25
N-Ethylmaleimide	2×10^{-3}	50

Effect of Substrate Concentrations: Figure 1 shows the initial rates of reduction exhibited by reaction mixture of the reductase and various concentrations of oct-cis-2-enoyl CoA, wherein the observed or apparent Km value was determined to be 1.2×10^{-3} M by calculation from double reciprocal plots of the data (Fig. 1, inset). The real substrate concentration might probably be considerably lower in that the relative high, observed Km value might represent in part possible effects of some interactions of various components contained in the rather crude enzyme preparation with the 2-unsaturated substrate. Similar NADPH-dependent reaction of oct-trans-2-enoyl CoA was in evidence with the enzyme preparation.

With the previously reported finding taken into account that, toward cis-2-enoyl CoA, enoyl CoA hydratase shows only 1/3 of its activity on the trans-form (13), the experimental

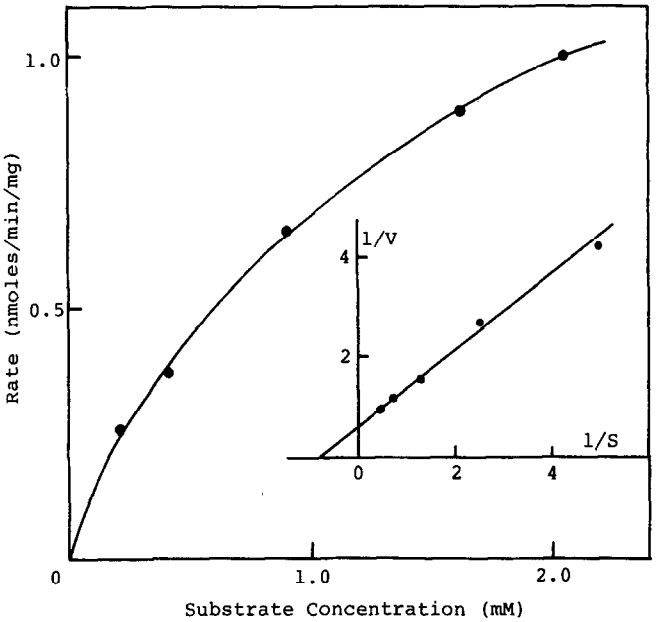

Figure 1. Rate of reduction of oct-cis-2-enoyl CoA as a function of substrate concentration. Inset, Lineweaver-Burk plot of the same data.

findings heretofore obtained suggest the possible existence of a pathway of the β-oxidation of the cis-2-form in which the reductase is involved.

Langdon (14) and Seubert et al. (15) have reported a NADPH-dependent (trans-2)-enoyl CoA reductase which is involved in the "chain elongation" system in the mitochondrion. Experiments to establish the locus in the mitochondrion and for purification of the enzyme are in progress at this laboratory with a view of elucidation of the interrelationship between the β-oxidation system of unsaturated fatty acids and the chain elongation system in mitochondria.

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