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# Comparative Effects of Methylmalonyl Coenzyme A on Fatty Acid Synthetase Derived From Rat and Man[1] (39895)

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Of the two coenzyme forms of vitamin $B_{12}$ known to have a biochemical role in humans, adenosylcobalamin (AdoCbl) is a critical cofactor in the oxidative pathway of propionate (propionate → propionyl CoA → methylmalonyl CoA $\xrightarrow[\text{mutase}]{\text{AdoCbl}}$ succinyl CoA). Recent measurements of these coenzyme A intermediates in the circumstance of vitamin $B_{12}$ deficiency have provided, as previously anticipated, evidence of increased *in vivo* tissue levels of methylmalonyl coenzyme A in the deprived state (1).

Methylmalonyl CoA has been shown to be a competitive inhibitor for fatty acid synthetase in yeast systems (2). Previous studies from this laboratory (3) have confirmed the observation by Forward and Gompertz that methylmalonyl CoA was also inhibitory to fatty acid synthetase in rat liver and did not serve to effect measurable *de novo* fatty acid synthesis or chain length elongation (4). The true physiologic role of methylmalonyl CoA may differ depending upon the species or tissues involved. For instance, Buckner and Kolattukudy (5, 6) provided recent evidence that fatty acid synthetase isolated from the uropygial gland of the goose catalyzed the incorporation of methylmalonyl CoA into the appropriate multimethyl branched-chain fatty acids.

Since vitamin $B_{12}$ deficiency has its primary expression in deranged pathophysiology in man, the present study was directed toward an evaluation of the effect of methylmalonyl CoA on fatty acid synthetase activity in the liver of man in an attempt to define its correlative relationships to these studies in other species.

*Materials and methods. Sources of fatty acid synthetase.* Rat hepatic fatty acid synthetase was obtained from normal, chow-fed Sprague-Dawley rats maintained in our colony as previously described (7). The rats were killed by decapitation and a 5-g portion of liver was promptly removed and placed in cold 0.9% NaCl. Human hepatic fatty acid synthetase was obtained from liver slices obtained during autopsy examination of two trauma victims within 6 and 11 hr, respectively, of their time of death. No underlying disease was suggested by the previous clinical data and no non-trauma-associated pathologic changes were identified during the post mortem studies in either case.

*Extraction and partial purification of fatty acid synthetase.* The liver (5-g aliquots) from each source was homogenized in 5 vol (25 ml) of cold 0.25 *M* sucrose containing 0.01 *M* potassium phosphate buffer, pH 7, 0.5 m*M* EDTA, and 2 m*M* dithiothreitol. The homogenate was centrifuged at 27,000*g* for 10 min, the pellet was discarded, and the supernatant solution was then centrifuged at 100,000*g* for 1 hr. The clear, particle-free supernatant solution was used in the studies. The ammonium sulfate fractionation on this supernatant solution was performed as described by Burton *et al.* (8). The fraction obtained from the 20-33% saturated ammonium sulfate precipitation was dissolved in 0.5 *M* potassium phosphate buffer, ph 7, 1 m*M* EDTA, and 7 m*M* 2-mercaptoethanol and then dialyzed overnight at 4° against the same buffer. The enzyme was subsequently maintained at 0-4°.

*Assay for fatty acid synthetase activity.* En-

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zyme activity was assayed by a modification of the method of Collins *et al.* (9), with final concentrations of 200 m*M* potassium phosphate buffer, pH 7, 3 m*M* EDTA, 2.5 m*M* dithiothreitol, 0.1 m*M* NADPH, 0.07 m*M* acetyl CoA, and 0.1 m*M* malonyl CoA in a final volume of 1.0 ml. The reaction was begun by the addition of malonyl CoA and the rate of NADPH oxidation was measured on a Gilford recording spectrophotometer (Model 250) at 340 nm. One milliunit of activity was defined as 1 nmole of NADPH oxidized/min at 25°. The coenzyme A derivatives were obtained from P and L Biochemicals (Milwaukee, Wis.) as the lithium salts and were dissolved in distilled water and standardized as previously described (1).

*Immunologic studies.* Ouchterlony double-immunodiffusion studies were performed as previously described (10). The partially purified fatty acid synthetase from the different sources was utilized as antigen and the center well contained anti-rat fatty acid synthetase antibody (kindly provided by Dr. John W. Porter, Metabolism Laboratory, Veterans Administration Hospital, Madison, Wis.). This antibody had been raised in rabbits utilizing enzyme purified by sucrose density-gradient centrifugation and has been shown to yield a single precipitin line against crude or purified fatty acid synthetase preparations from rat liver.

*Other procedures.* Protein assays were performed by the method of Lowry *et al.* (11).

*Results. Fatty acid synthetase activity.* The mean specific activities of fatty acid synthetase determined in the 100,000*g* supernatant fractions obtained from the human and rat livers were quite similar, 5 and 6 mU/mg of protein, respectively. This activity was similar to that previously reported for rat liver from this laboratory (10). The mean total activities, expressed per wet weight of liver, were 270 mU/g of liver for the human and 420 mU/g of liver for the rat.

The specific activities on the partially purified enzyme were 60 mU/mg of protein for the human liver preparation and 45 mU/mg of protein for the enzyme from the rat, a 12- and 7.5-fold purification, respectively.

*Comparative catalytic and kinetic studies.* The activities of fatty acid synthetase derived from the human and rat livers with varying substrate additions are shown in Table I. Propionyl CoA effectively replaced acetyl CoA as primer in both the human and rat liver, with 90 and 95%, respectively, of the activity obtained in the complete assay system. Methylmalonyl CoA in the absence of malonyl CoA and with either acetyl CoA or propionyl CoA as primer failed to demonstrate any activity over a wide range of concentrations (see Table I).

Kinetic studies (Table I, Figs. 1 and 2)

TABLE I. COMPARATIVE STUDIES OF FATTY ACID SYNTHETASE DERIVED FROM HUMAN AND RAT LIVER.[a]

| Substrate additions | Human % | Rat % |
|---|---|---|
| Acetyl CoA (0.07 m*M*) + malonyl CoA (0.1 m*M*) | 100 | 100 |
| Propionyl CoA (0.07 m*M*) + malonyl CoA (0.1 m*M*) | 90 | 95 |
| Acetyl CoA (0.07 m*M*) + ——— | 0 | 0 |
| Propionyl CoA (0.07 m*M*) + ——— | 0 | 0 |
| ——— + Malonyl CoA (0.1 m*M*) | 54 | 49 |
| ——— + Methylmalonyl CoA (0.1 m*M*) | 0 | 0 |
| Acetyl CoA (0.07 m*M*) + methylmalonyl CoA (0.1 m*M*) | 0 | 0 |
| Propionyl CoA (0.07 m*M*) + methylmalonyl CoA (0.1 m*M*) | 0 | 0 |
| Acetyl CoA (0.07 m*M*) + methylmalonyl CoA (1.0 m*M*) | 0 | 0 |
| Acetyl CoA (0.07 m*M*) + methylmalonyl CoA (2.0 m*M*) | 0 | 0 |
| Kinetic studies[b] | | |
| $K_m$, Malonyl CoA (*M*) | $3 \times 10^{-5}$ | $4 \times 10^{-5}$ |
| $K_i$, Methylmalonyl CoA (*M*) | $2.4 \times 10^{-5}$ | $2.4 \times 10^{-5}$ |

[a] All studies were performed on the partially purified enzyme from each source. The specific activity for the human liver preparation was 60 mU/mg of protein and for the rat was 45 mU/mg of protein. All values, except for the $K_m$ and $K_i$, are expressed as percentage of activity obtained with the complete assay system and equivalent amounts of enzyme protein. Zero indicates no detectable activity with a limit of sensitivity of 1% of the activity of the completed system.

[b] See Figs. 1 and 2.

demonstrated that the $K_m$ for malonyl CoA with the human liver enzyme was $3 \times 10^{-5}$ $M$ and with the rat liver enzyme was $4 \times 10^{-5}$ $M$. Methylmalonyl CoA was shown to be a competitive inhibitor for the fatty acid synthetase for both sources of enzyme (Figs. 1 and 2), with an apparent $K_i$ of $2.4 \times 10^{-5}$ $M$.

*Immunologic studies.* Ouchterlony double-diffusion studies performed with the purified enzyme from both human and rat sources against anti-rat fatty acid synthetase antibody are shown in Fig. 3. Immunoreactivity was demonstrated with both enzymes, but spurring was seen with the enzyme from the human liver, supporting structural differences.

*Discussion.* The recent evidence that

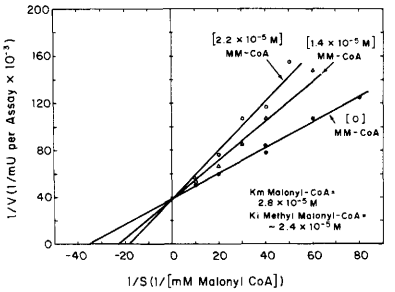


FIG. 1. Kinetic studies of fatty acid synthetase from human liver. Studies were performed on the partially purified enzyme. Acetyl CoA concentration was 0.07 m$M$. (●——●) Data in the absence of methylmalonyl CoA; (△——△) performed in the presence of 0.014 m$M$ methylmalonyl CoA; and (○——○) performed in the presence of 0.022 m$M$ methylmalonyl CoA. Each assay point represents the rate obtained utilizing 0.33 mg of protein.

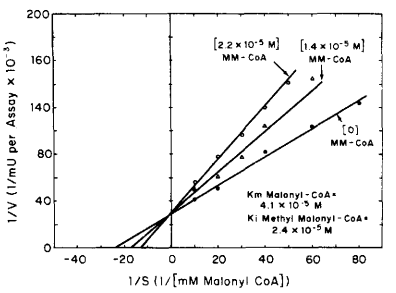


FIG. 2. Kinetic studies of fatty acid synthetase from rat liver. The conditions of the study and the additions of methylmalonyl CoA were as shown in Fig. 1. Each assay point represents the rate obtained with 0.53 mg of protein.

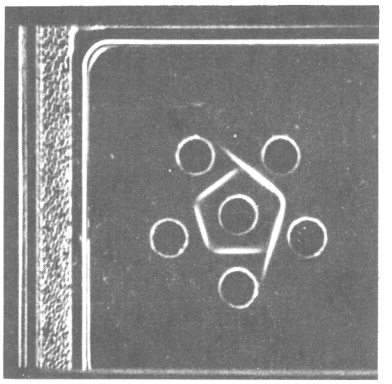

FIG. 3. Ouchterlony double-diffusion study of fatty acid synthetase derived from human and rat sources. The center well contained anti-rat fatty acid synthetase antibody. The top well and the two adjacent clockwise wells contained the partially purified human fatty acid synthetase. The final two clockwise wells contained the partially purified enzyme obtained from rat liver.

methylmalonyl CoA could serve as an effective substrate with fatty acid synthetase to produce branched-chain fatty acids has been provided for the selected circumstance of the uropygial gland of the goose (5, 6). The specificity in that tissue appears to be due to a gland-specific and substrate-specific malonyl CoA decarboxylase which renders malonyl CoA unavailable to the synthetase without significantly affecting the availability of methylmalonyl CoA (5). The result of this unique mechanism is that methylmalonyl CoA serves as the major fatty acid substrate in this select tissue. That such an event may have broader implications was suggested by Cardinale *et al.* (12) by an *in vitro* description of methylmalonyl CoA incorporation into fatty acids. However, the possibility that methylmalonyl CoA had been converted to propionyl CoA prior to incorporation had not been eliminated (12). Recently, Garton *et al.* (13) identified branched-chain fatty acid production in the livers of baboons who had been treated with an analog of vitamin $B_{12}$ which produces a functionally $B_{12}$-deficient state in these animals. These studies provided the impetus to evaluate the effect of methylmalonyl CoA on the fatty synthetase obtained from the liver of man. The present study demonstrated that methylmalonyl CoA was inhibitory to the fatty acid synthetase derived

from human liver. In fact, the pattern of inhibition was similar to that demonstrated in rat liver obtained from both normal and $B_{12}$-deprived rats (3).

The physiologic significance of such an inhibition can only be speculative. Increased fatty acid and odd-chain fatty acid synthesis has been demonstrated in both animals (3) and man (14) in the vitamin $B_{12}$-deprived state. Vitamin $B_{12}$ deficiency has been shown to produce an increased content and activity of the regulatory enzymes of fatty acid synthesis (3, 15), and *in situ* measurements of the CoA derivatives reveal increased levels of propionyl CoA as well as methylmalonyl CoA. Since propionyl CoA is an excellent substrate for fatty acid synthetase with resultant odd-chain fatty acid synthesis (15), a reasonable physiologic role for the inhibitory effect of methylmalonyl CoA might be presumed to counterbalance this sequela.

Finally, these studies in man provide further evidence that the animal model of vitamin $B_{12}$ deficiency (7) is correlative to the events in man.

*Summary.* The effect of methylmalonyl CoA on fatty acid synthetase activity was compared on enzyme derived from rats and man. Partially purified fatty acid synthetase from both sources was shown to be strikingly similar. Enzyme from both sources catalyzed propionyl CoA equally well (approximately 90% the rate of acetyl CoA at equimolar concentrations), giving rise to odd-chain fatty acids. Neither enzyme catalyzed methylmalonyl CoA in measurable activities over a wide range of concentrations. Michaelis constants for malonyl CoA were essentially the same ($K_m = 4 \times 10^{-5}$ and $3 \times 10^{-5}$ $M$ for rat and human liver, respectively). Methylmalonyl CoA was demonstrated to be a competitive inhibitor of malonyl CoA, with $K_i$ values of $2.4 \times 10^{-5}$ $M$ for the enzyme from both sources. Human liver fatty acid synthetase was immunoreactive with antiserum prepared against purified rat liver fatty acid synthetase.

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