MALONYL CoA - A NEW INTERMEDIATE IN THE FORMATION OF MEVALONIC ACID*

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Received May 7, 1962

Prior to the present report, the only known reaction sequence for the biosynthesis of HMGCoA (β-hydroxy, β-methyl glutaryl coenzyme A) and mevalonic acid from acetate or acetyl CoA involved the formation of acetoacetyl CoA from two molecules of acetyl CoA via the reversal of the thiolase catalyzed cleavage of acetoacetyl CoA (Rudney, 1957; Brodie and Porter, 1960). Acetoacetyl CoA was then condensed with acetyl CoA to form HMGCoA (Rudney, 1957; Rudney and Ferguson, 1959).

The establishment of acetyl and malonyl CoA as intermediates in the biosynthesis of fatty acids (Brady, 1958; Wakil and Ganguly, 1959; Lynen, 1959) suggested the possibility of an alternate route, with a favorable equilibrium, for the formation of acetoacetyl CoA. However, acetoacetyl CoA formation by this condensation has not been observed. Instead the formation of acetoacetyl-S-enzyme has been reported (Lynen, 1961a; Alberts and Vagelos, 1961). This finding has led to the suggestion that the initial steps in the synthetic processes involved in sterol and fatty acid synthesis are separate (Lynen, 1961b).

*This investigation was supported by research grants, 61G7 from the American Heart Association, and RF 61:334 from the Wisconsin Alumni Research Foundation.

The results reported in this communication show that malonyl CoA is incorporated into HMGCoA by a new pathway which appears to be identical to the early steps in the synthesis of fatty acids. Therefore, we suggest that the proposed distinction between sterol and fatty acid synthesis (Lynen, 1961b) may be unwarranted.

Studies with an enzyme system obtained from pigeon liver and purified through chromatography on DEAE-cellulose have shown that fatty acid synthesis may be obtained in the presence of only enzyme, acetyl CoA, malonyl CoA and TPNH (Porter, et al., 1962). Removal of TPNH from the system results in the formation of 4 chromatographically-separable compounds. One of the major products migrates with an R_f of 0.53 on chromatography on paper in a system of isobutyric acid-ammonia. This product has been characterized as HMGCoA through R_f value, light absorption (adenine) and formation of a di-p-bromophenacyl ester of the HMG portion of the molecule. The product was further identified through preparation of a hydroxamic acid derivative. This compound and authentic HMG mono-hydroxamate migrate with R_f values of 0.43 in a water-saturated butanol system. The isolated HMGCoA was also shown to be biologically active through conversion to mevalonic acid on incubation with TPNH and a partially purified pigeon liver HMGCoA reductase.

Proof of the incorporation of C^{14} of malonyl CoA into HMGCoA via a route other than conversion to acetyl CoA is given in the results presented in Table I. In this experiment the incubation mixture contained only the substrates in the amounts stated, enzyme and phosphate buffer, pH 7.0. Samples were incubated at 38° for one hour and then deproteinized with heat. Coenzyme A esters of each incubation mixture were hydrolyzed and di-p-bromophenacyl esters of HMG were formed and crystallized to constant specific radioactivity. Table II reports the actual values obtained on each crystallization. A nineteen-fold in-

crease in the quantity of non-radioactive acetyl CoA increased the incorporation of C^{14} of malonyl CoA into HMGCoA by a factor of five.

TABLE I

PARTICIPATION OF MALONYL CoA IN THE BIOSYNTHESIS OF HMGCoA

Substrate*	HMGCoA	
	c/min.	mμMoles
1-C^{14}-Acetyl CoA	1150	0.6
1-C^{14}-Acetyl CoA + Malonyl CoA, .10 μMoles	6500	5.0
2-C^{14}-Malonyl CoA	7000	2.0
2-C^{14}-Malonyl CoA + Acetyl CoA, .15 μMoles	9000	8.0
2-C^{14}-Malonyl CoA + Acetyl CoA, 1.05 μMoles	12500	11.0
2-C^{14}-Malonyl CoA + 1-C^{14}-Acetyl CoA	20900	11.0

*The amount of 1-C^{14}-acetyl CoA was 100 mμMoles and 65,600 c/min; the amount of 2-C^{14}-malonyl CoA was 55 mμMoles and 61,000 c/min.

TABLE II

SPECIFIC RADIOACTIVITIES OF THE DERIVATIVES OF HMG

Recrystallization No.	Incubation mixtures*					
	1	2	3	4	5	6
	c/min/mg					
1	8.0	37.0	30.3	38.9	45.7	73.5
2	3.6	26.6	26.9	29.5	40.2	----
3	2.8	20.5	21.6	25.8	38.1	60.3
4	3.2	18.8	20.1	25.6	35.9	59.9
5	2.8	19.0	19.3	23.3	36.2	57.2

* From Table I.

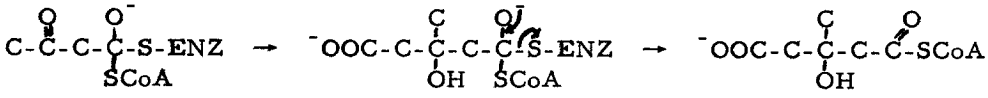
It may be noted that the effect of malonyl CoA on the formation of HMGCoA is even more impressive when one corrects for the disparities in labeling as shown in the last column of Table I. Thus in samples 1 and 3 the HMG is expected to be triply labeled. The enzyme system contains a small amount of a malonyl CoA decarboxylase, and therefore malonyl and acetyl CoA were available in sample 3 for the formation of HMGCoA. In sample 2 the HMGCoA would be expected to be doubly labeled and in samples 4 and 5 it would be expected to be singly labeled.

It might be anticipated that acetyl and malonyl CoA would condense to form acetoacetyl CoA. However, preliminary experiments have yielded no evidence for this product. Appreciable C^{14} was not found in a trapping pool of non-radioactive acetoacetyl CoA on incubation of 2-C^{14}-malonyl CoA, acetyl CoA and enzyme. This result would seem to indicate that the C_4 intermediate in the conversion of malonyl CoA to HMGCoA is enzyme bound and that this compound might also be intermediate in the formation of fatty acids.

The enzyme system used in these studies does not contain HMGCoA deacylase activity, and no evidence has been obtained for the formation of free HMG during the synthesis of HMGCoA. In the ammonia-isobutyric acid chromatographic system used in these studies, HMG chromatographs with HMGCoA. Reaction of the peak containing HMGCoA with hydroxylamine followed by chromatography in a system which separates the hydroxamate of HMG and free HMG yielded only the single peak of HMG hydroxamate.

Our inability to detect any free HMG under conditions in which appreciable amounts of HMG would be expected due to hydrolysis of an enzyme - HMG thioester lead us to consider the possibility of other intermediate structures. One possibility is that of an enzyme bound intermediate which retains the CoA

of malonyl CoA. Such a compound might be stabilized on the enzyme surface as follows:



The possibility also exists, however, that the enzyme bound intermediate may not retain CoA. If so, the HMGCoA would necessarily be formed through a specific enzymatic displacement of HMG from the enzyme by $CoAS^-$.

Further details of the mechanism of conversion of malonyl CoA to HMGCoA are under investigation.

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