EVIDENCE FOR THE FORMATION OF ACETOACETATE BY DIRECT DEACYLATION OF ACETOACETYL-CoA IN LIVER MITOCHONDRIA*

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Received October 4, 1960

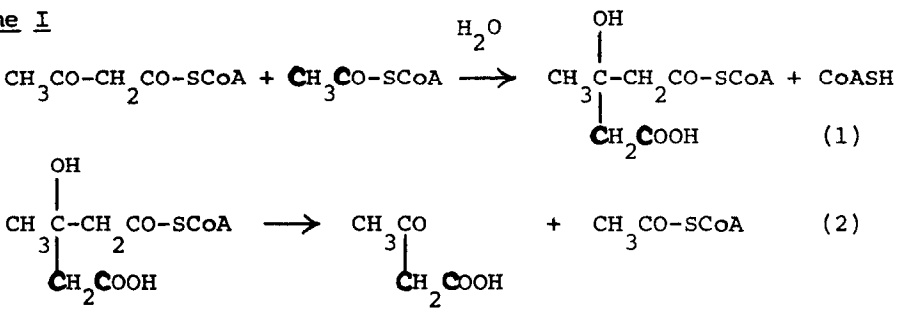
We have recently reported that there is an elevation in the activity of the enzyme system which forms acetoacetate from acetoacetyl-CoA in liver preparations from diabetic rats, and that this defect is promptly corrected by insulin administration (Segal et al. 1960).

Two pathways have been proposed for the formation of acetoacetate from acetoacetyl-CoA. In a partially purified system prepared from acetone powders of liver, Lynen et al. (Lynen et al. 1958) obtained evidence that the reaction proceeded via HMG-CoA[1] as an intermediate (Scheme I), while with a partially purified system prepared from bicarbonate extracts of liver, Drummond and Stern (Drummond and Stern 1960) reported that acetoacetate formation from acetoacetyl-CoA took place under conditions where the HMG-CoA pathway was not operative, presumably via a direct deacylation reaction (Scheme II).

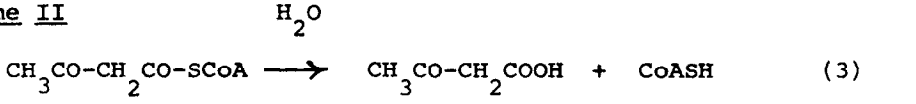
* Supported by a grant (A-3642) from the United States Public Health Service.

[1] HMG is an abbreviation for β-hydroxy, β-methyl glutaric acid.

Scheme I



Scheme II



It was considered of interest, therefore, to examine the pathway of acetoacetate formation in our hormonally-responsive liver mitochondrial system (Segal et al. 1960).

As demonstrated by Rudney and Ferguson (Rudney and Ferguson 1957), and as illustrated in Scheme I, acetoacetate formed via the HMG-CoA pathway contains carbon atoms derived from acetyl-CoA which have not gone through acetoacetyl-CoA as an intermediate. Thus, in the presence of labeled acetyl-CoA and unlabeled acetoacetyl-CoA, acetoacetate formed via the HMG-CoA pathway would contain the same specific activity as the starting acetyl-CoA, while the acetoacetyl-CoA would remain unlabeled, except for labeling introduced from acetyl-CoA as a result of contaminating β-ketothiolase action. On the other hand, acetoacetate formed directly from acetoacetyl-CoA would have specific activity no higher than that of the acetoacetyl-CoA remaining at the end of the experiment.

To distinguish between these possibilities acetyl-CoA-2-C^{14} and unlabeled acetoacetyl-CoA were incubated with liver mitochondria activated by freezing and thawing three times (Segal et al. 1960).

TABLE I

Formation of unlabeled acetoacetate in the presence of C^{14}-labeled acetyl-CoA and unlabeled acetoacetyl-CoA

	acetoacetate			acetoacetyl-CoA		
	μmoles formed enzymatically	specific activity* (c.p.m. per μmole)		μmoles recovered	specific activity (c.p.m. per μmole)	
		observed	theoretical via HMG-CoA pathway		observed	theoretical at isotope equilibrium (via thiolase)
Experiment I	0.11	1640	43,200	0.08	1980	21,200
Experiment II	0.12	750	43,200	0.28	910	21,200

* Calculated in terms of the acetoacetate formed enzymatically.

Incubation mixtures contained in a final volume of 0.75 ml: 100 μmoles Tris buffer, pH 7.9, 1.0 μmoles of acetoacetyl-CoA, 0.65 μmoles of acetyl-CoA-2-C^{14} (containing 43,200 c.p.m. per μmole), and 0.1 ml. (Experiment I) or 0.2 ml. (Experiment II) of "activated" normal mitochondrial suspension diluted to ten times the original volume with 0.25 M sucrose (ca. 1 mg. protein per ml.). Incubations were at 37° for 10 minutes (Experiment I) or 5 minutes (Experiment II). After extraction of the formazan derivative of acetoacetate into ethyl acetate for colorimetric assay and counting, an aliquot of the aqueous layer was alkalinized to hydrolyze acetoacetyl-CoA. The formazan derivative of the acetoacetate released was prepared and treated as above. The radioactivity values reported were corrected for a small amount of radioactivity carried over into the ethyl acetate layers in a control incubation in which the enzyme was added after $HClO_4$. This incubation also served as a control for the appreciable non-enzymatic hydrolysis of acetoacetyl-CoA, which amounted to 0.41 μmoles in Experiment I and 0.21 μmoles in Experiment II. No acetoacetate appeared in the absence of added CoA ester.

The acetoacetate formed was isolated as the formazan derivative of Walker (Walker 1954), assayed, and counted. In addition, the acetoacetyl-CoA remaining at the end of the experiment was converted into acetoacetate by alkaline hydrolysis, after the preformed acetoacetate had been removed, and also assayed and counted. The results are presented in Table.I. As can be seen, there was very little radioactivity in the acetoacetate formed in this system, in comparison with the calculated amount which would have arisen *via* the HMG-CoA pathway. Furthermore, even this small amount of radioactivity can be accounted for by hydrolysis of labeled acetoacetyl-CoA formed from labeled acetyl-CoA through β-ketothiolase action. The presence in these preparations of a low level of β-ketothiolase activity was confirmed by direct assay (Stern 1955).

It was further noted that the elimination of acetyl-CoA from the assay system produced no detectable effect on the rate of acetoacetate formation. This observation is also consistent with the lack of operation of the HMG-CoA pathway.

The results reported here, together with previous experiments which have shown that the bulk of the acetoacetate forming activity of liver is in the mitochondrial fraction (Segal *et al.* 1960; Bucher *et al.* 1960), lead us to the conclusion that the major, if not sole, pathway of acetoacetate formation in normal liver is *via* deacylation[2] of acetoacetyl-CoA.

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[2] Transacylation of the acetoacetyl group to another acceptor prior to hydrolysis is not ruled out by these experiments.

References

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