VITAMIN B_{12} AND METHYLMALONYL CoA ISOMERASE

Joseph R. Stern and Daniel L. Friedman

Department of Pharmacology, School of Medicine
Western Reserve University, Cleveland, Ohio

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Smith and Monty (1959) have presented substantial evidence that the activity of methylmalonyl-CoA isomerase (Beck, Flavin and Ochoa 1957, Beck and Ochoa 1958) is greatly diminished in liver homogenates from vitamin B_{12}-deficient rats. We wish to report that a coenzyme form of vitamin B_{12}, 5,6 dimethylbenzimidazolyl cobamide coenzyme (DBCC) (Weissbach, Toohey and Barker 1959), stimulates the activity of methylmalonyl-CoA isomerase in ox liver fractions, thereby confirming the results of Smith and Monty.

The enzyme used was a dialyzed 30-60% saturated ammonium sulfate fraction of ox liver extract. Methylmalonyl-CoA isomerase activity was measured by coupling it with endogenous propionyl-CoA carboxylase (Flavin and Ochoa 1957, Flavin, et al. 1957) according to the following reactions:

(1) Propionyl-CoA + ATP + CO_2 $\rightleftharpoons$ methylmalonyl-CoA + ADP + P

(2) Methylmalonyl-CoA $\rightleftharpoons$ succinyl-CoA

C^{14}-methylmalonyl-CoA was generated by the carboxylase in the presence of $C^{14}O_2$ and the distribution of radioactivity between the methylmalonyl-CoA and succinyl-CoA formed was employed as a measure of the isomerase activity. The reaction mixture contained (in μmoles): Tris buffer pH 7.0 (100), $MnCl_2$ (1), ATP (5), glutathione (5), $KHC^{14}O_3$ (10),

124,000 counts per minute per μmole, propionyl-CoA (0.5) and 4.6 mg. of enzyme protein, final volume, 1.0 ml. After 12-20 minutes incubation at 30°, the reaction was terminated with perchloric acid. A portion was counted directly to determine the total radioactivity fixed; counting another portion after permanganate treatment (Flavin and Ochoa 1957), which destroys methylmalonate, gave an approximate measure of the radioactivity due to succinate. The major portion was subjected to alkaline hydrolysis followed by addition of carrier methylmalonic and succinic acids in equimolar amounts (50 to 100 μmoles). The acids were extracted with ethanol and chromatographed on a celite column. Methylmalonic and succinic acids were eluted as separate peaks with coincidence of C^{14} content and acid titration values.

As shown in Table I, the ox liver fraction contains

Table I

Effect of B_{12} and DBCC on Methyl Malonate and Succinate Formation from Propionyl-CoA and $C^{14}O_2$

150 μg. vitamin B_{12}; 20 μg. DBCC; incubation 20 minutes

Additions to complete system	Radioactivity		Specific Radioactivity		Di-COOH acids as succinate
	Total	Succinate*	MeMAL	SUCC	
	c.p.m.	c.p.m.	c.p.m./μmole		per cent
None	42,800	16,700	269	237	46.9
Vitamin B_{12}	45,000	16,830	268	176	39.5
" + DBCC	40,600	35,200	122	416	77.3
None+	44,300	16,600	308	193	38.6
None**	33,050	9,890	533	220	29.2
DBCC**	31,180	15,700	419	363	46.6

*After permanganate treatment.
+Enzyme was exposed to 100 watt tungsten lamp for 90 min. at 0°
**Incubation 12 minutes.

both propionyl-CoA carboxylase and methylmalonyl-CoA isomerase. Addition of synthetic crystalline vitamin B_{12} (Merck), or DBCC, or irradiation of the enzyme fraction with visible light does not affect the total radioactivity fixed, i.e., the carboxylase. Irradiation of the enzyme or addition of vitamin B_{12} decreased the conversion of methylmalonyl-CoA to succinyl-CoA. DBCC stimulated the extent of conversion of methylmalonyl-CoA to succinyl-CoA, both in the absence or presence of vitamin B_{12}, by 60 per cent and 96 per cent respectively (column 6). In the presence of DBCC (and vitamin B_{12}) after 20 minutes, 77.3 per cent of the methylmalonyl-CoA formed was converted to succinyl-CoA. Flavin and Ochoa (1957) have shown that this conversion proceeds practically to completion, probably because of the presence of succinyl-CoA deacylase. (It should be pointed out that nothing is known of the equilibrium of the isomerization reaction(s) and its mechanism is obscure.) Apart from stimulation of the isomerase, stimulation of succinyl-CoA deacylase by DBCC could explain in part or wholly the data in Table I. The ox liver fraction does possess a weak succinyl-CoA deacylase, but its activity was not affected by DBCC.

Methylmalonyl-CoA isomerase activity was also measured by determining the total fixation of $C^{14}O_2$ into non-volatile organic acids, in the presence of succinyl-CoA and ATP, by exchange through reversal of Reactions 2 and 1. (The enzyme contained ATP-ase and adenylate kinase.) Under these conditions all the radioactivity fixed is recovered in methylmalonate and succinate. The results (Table II) show that much less radioactivity is fixed when succinyl-CoA is substituted for propionyl-CoA. In this system vitamin B_{12}

Table II

Effect of B_{12} and DBCC on Radioactivity Fixed with Succinyl-CoA and $C^{14}O_2$

Conditions as in Table I except succinyl-CoA (1 μmole) substituted for propionyl CoA; 6.6 mg. enzyme protein. Incubation 60 minutes at 30°. Values are total mμmole $C^{14}O_2$ fixed. Those in parentheses refer to light-treated enzyme.

Additions to Complete System	$C^{14}O_2$ fixed	
	Exp. 1	Exp. 2
None	19.0	12.2 (14.5)
ATP replaced by ADP and P	19.3	---
DBCC 20 μg.	18.1	--- (19.7)
Vitamin B_{12}, 150 μg.	14.8	10.3
" + DBCC	18.0	17.9
Succinyl-CoA omitted	1.2	---
NaCN, 0.1 M	11.0	---

(50-150 μg.) inhibited $C^{14}O_2$ fixation 20-50%, the inhibition being reversed by DBCC. Irradiation of the enzyme was without effect, and addition of DBCC increased fixation by untreated and irradiated enzyme (Expt. 2). Brief treatment of the enzyme with 0.1 M NaCN, which slowly inactivates DBCC (Weissbach et al. 1959) and which has little or no effect on carboxylase, decreased $C^{14}O_2$ fixation.

These experiments show that the enzymic isomerization of methylmalonyl-CoA to succinyl-CoA in liver is stimulated by DBCC. Inhibition of the isomerization by vitamin B_{12}, which is relieved by DBCC, suggests that vitamin B_{12} can displace, or compete with, the endogenous coenzyme, which is presumably DBCC or a related structure. DBCC does occur in rabbit liver (Barker et al. 1958), and is inactivated by light and by NaCN. The ox liver coenzyme was little

affected by light. Barker *et al.* have identified DBCC as a coenzyme in the isomerization of glutamate and β-methylaspartate by extracts of *Clostridium tetanomorphum*. It is now apparent that the isomerization of both glutamate and succinyl-CoA can be regarded as propionate transfer reactions in which the carbon-carbon bond of the β-carbon of the propionate (or propionyl-CoA) moiety of the straight chain structure is broken and the propionate moiety then transferred to another acceptor so that the α-carbon forms the new carbon-carbon bond of the branched chain product (*cf.* Munch-Peterson and Barker 1958). In these systems DBCC can be regarded as a coenzyme of transpropionation, and the methylmalonyl-CoA isomerase enzyme (or enzymes) as a transpropionase. From this viewpoint it is relevant that various methylmalonyl-CoA isomerase preparations do not isomerize ethylmalonyl-CoA (Stern, *et al.* 1959) to glutaryl-CoA which would involve a transbutyration reaction (Friedman and Stern).

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