ON THE MECHANISM OF THE COENZYME B_{12} DEPENDENT ISOMERISATION OF (R)-METHYLMALONYL CoA TO SUCCINYL CoA

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Methylmalonyl CoA mutase, a coenzyme B_{12} dependent enzyme, catalyzes the reversible interconversion of (R)**-methylmalonyl CoA to succinyl CoA (2-4). Several mechanistic possibilities for this unusual rearrangement have been discussed (5,6). One of them suggested by WHITLOCK (5) involves a methylmalonyl group transfer from coenzyme A to the cobalt atom of coenzyme B_{12}. In order to test this possibility we ran the reaction in the presence of free ^{14}C-coenzyme A and measured the radioactivity of the resultant equilibrium mixture of succinyl CoA and methylmalonyl CoA.

Materials and methods

^{14}C-Coenzyme A (3.10^6 cpm/umole) was prepared according to SCHWEIZER (7). A pantothenic acid heterotrophic yeast, Saccharomyces carlsbergensis, was grown on a medium containing radiactive pantothenic acid, ^{14}C-labeled in the β-alanine moiety. The radioactive coenzyme A was isolated from the cells as described earlier (7-9). The free ^{14}C-coenzyme A was liberated from benzoyl-^{14}C-coenzyme A just before running the reaction by means of transesterification in the presence of excess 2-mercaptoethylamine at pH 9 or

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**The configurative assignements according to CAHN, INGOLD and PRELOG (1) are used.

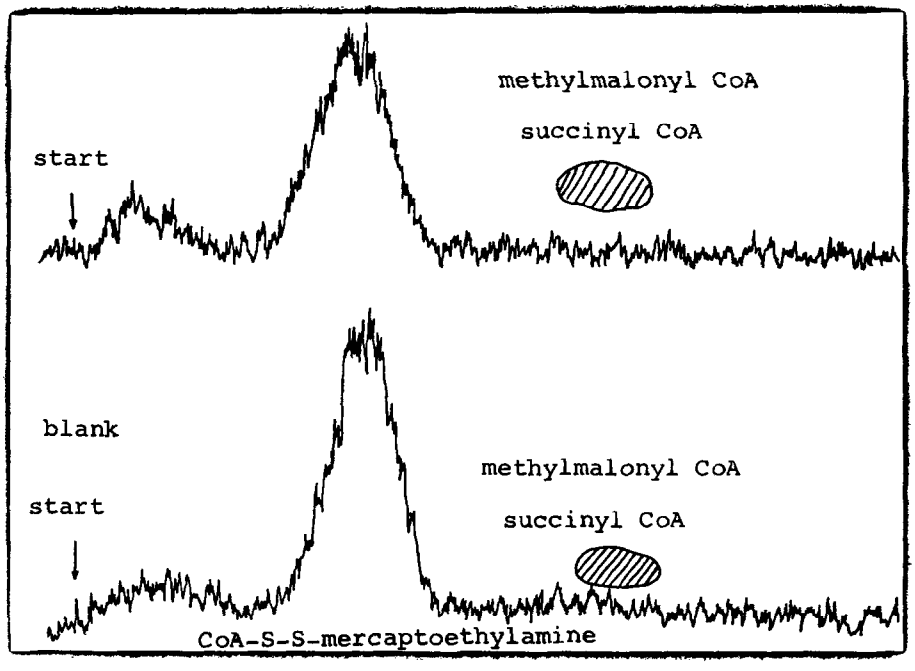
by alkaline hydrolysis and subsequent reduction with hydrogen sulfide. The radioactive coenzyme A thus obtained was fully active for the palmitoyl group exchange between CoA and ^{14}C-CoA catalyzed by the fatty acid synthetase from yeast (7).

(RS)-Methylmalonyl CoA was prepared by transesterification from (RS)-methylmalonyl octanoyl cysteamine and CoA (10). Succinyl CoA was prepared according to SIMON and SHEMIN (11). Highly purified methylmalonyl CoA mutase from Propionibacterium shermanii was a generous gift of Professor H.G. WOOD (12). The enzyme was assayed after running the exchange experiments according to FLAVIN and OCHOA (13) and OVERATH et al. (14). A specific activity of 16,7 units/mg was found at 37°. This compares favorably with the specific activity of 7,9 units/mg at 25° measured by Professor WOOD for this same enzyme sample.

Results

Conversion of methylmalonyl CoA to succinyl CoA in the presence of ^{14}C-CoA. Two experiments were run, differing only in the buffer systems used. The reaction mixtures contained: 0,25 umole (RS)-methylmalonyl CoA; 0,001 umole coenzyme B_{12}; 0,01 umole ^{14}C-CoA (30 000 cpm); 120 ug enzyme; and either 20 umoles sodium succinate buffer (pH 6,25) or 20 umoles potassium phosphate buffer (pH 6,5). Volume 0,5 ml. In both cases control experiments were run which contained all the reactants, plus 0,1 umole succinyl CoA, except the enzyme. After 20 min. incubation at 25°, the reactions were stopped by acidification to pH 1 (1 M hydrochloric acid). After the addition of 20 umoles of 2-mercaptoethylamine the reaction mixtures were oxidized by adding few drops of 5°/o methanolic iodine solution. This converts all the radioactive CoA to the CoA-2-mercaptoethylamine mixed disulfide which differs greatly in its electrophoretic properties from methylmalonyl and succinyl CoA. The inorganic salts were removed from the mixture by means of phenol extraction. The samples in centrifuge tubes were extracted three times with 1 ml portions of phenol saturated with water. The two phases were separated each time by centrifugation. After washing the united phenol extracts three times with 1 ml of water the organic phase was mixed with 6 ml of ether and the nucleotides were extracted three times

with 1,5 ml of water. The united water extracts were washed three times with 2 ml of ether and concentrated in vacuo to a volume of about 0,1 ml. The nucleotide mixture was then separated by high voltage paper electrophoresis (Fig. 1). Radiopherograms obtained after electrophoresis show no radioactivity at the spots where the mixtures of methylmalonyl and succinyl CoA were detected by the sodium nitroprusside method (15). Markers for succinyl and methylmalonyl CoA were run in parallel.



Medium; Pyridine : acetic acid : water = 90 : 10 : 900.
1500 volts, 2 hours.

Fig. 1

Discussion

The experimental results described in this paper suggest that the mechanism of enzymatic isomerisation of (R)-methylmalonyl CoA probably does not involve an acyl transfer from coenzyme A to the coenzyme B_{12} enzyme. However, there are some

examples where enzymes are able to bind normally exchangeable groups, without exchange with the medium and then transfer them back to the substrate at a later stage of the reaction (16). For this reason we cannot absolutely exclude the acyl transfer mentioned above. On the other hand several enzymatic reactions which are known to involve the intermediate release of coenzyme A, show a rapid CoA-^{14}C-CoA exchange (7,17).

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