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Stereochemistry of the methylmalonyl-CoA decarboxylation reaction

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The steric course of the decarboxylation of (*S*)-methylmalonyl-CoA to propionyl-CoA, catalyzed by the biotin-dependent sodium pump methylmalonyl-CoA decarboxylase of *Veillonella alcalescens* was determined. The decarboxylation of (*S*)-methylmalonyl-CoA in ^3H_2O yielded (*R*)-[2-^{3}H]propionyl-CoA; and the decarboxylation of (*S*)-[2-^{3}H]methylmalonyl-CoA in H_2O produced (*S*)-[2-^{3}H]propionyl-CoA. The results demonstrate retention of configuration during the decarboxylation reaction. The substrate stereochemistry of methylmalonyl-CoA decarboxylase is thus the same as that of all other biotin-containing enzymes investigated.

Methylmalonyl-CoA decarboxylase; Substrate stereochemistry; Biotin enzyme

1. INTRODUCTION

Methylmalonyl-CoA decarboxylase of *Veillonella alcalescens* is a membrane-bound biotin-containing enzyme that catalyzes active Na^+ extrusion from the cell at the expense of the free energy of the decarboxylation reaction [1]. Other enzymes of this type are oxaloacetate decarboxylase of *Klebsiella pneumoniae* [2] and glutaconyl-CoA decarboxylase of *Acidaminococcus fermentans* [3]. These sodium ion transport decarboxylases have a number of properties in common, e.g. binding to the membrane, specific activation by Na^+, and the prosthetic group biotin. These enzymes are also related by the catalytic mechanism and by their subunit composition [4]. The first step is a transfer of the carboxyl group from the substrate to enzyme-bound biotin. In oxaloacetate decarboxylase [5] and glutaconyl-CoA decarboxylase [6] this reaction was shown to be catalyzed by a distinct carboxyltransferase subunit and to be independent of Na^+. In the next, Na^+-dependent step, the carboxybiotin enzyme is decarboxylated, thereby regenerating the free biotin enzyme. This reaction may also be catalyzed by an analogous subunit in each enzyme complex.

The substrate stereochemistry of all Na^+ transport decarboxylases could also be the same. Oxaloacetate decarboxylase [7] and glutaconyl-CoA decarboxylase [8] perform the respective decarboxylation reaction with retention of configuration. This is the same stereochemical course as that of all other biotin-containing enzymes [9]. The decarboxylation of *(S)*-methylmalonyl-CoA in ^3H_2O produced *(R)*-[2-^{3}H]propionyl-CoA, and *(S)*-[2-^{3}H]-propionyl-CoA was obtained by decarboxylation of *(S)*-[2-^{3}H]methylmalonyl-CoA in H_2O. Thus, the stereochemical course of methylmalonyl-CoA decarboxylation involves retention of the configuration.

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2. MATERIALS AND METHODS

2.1. *Materials*

Pig heart propionyl-CoA carboxylase (EC 6.4.1.3) was purified through the first three steps

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as described in [10] followed by affinity chromatography on avidin-Sepharose [1]. The enzyme was crystallized with ammonium sulfate as described [10]. SDS-gel electrophoresis indicated the presence of the two subunits of the enzyme and very little contaminating polypeptides. The enzyme was free of methylmalonyl-CoA racemase (EC 5.1.99.1). Methylmalonyl-CoA decarboxylase (EC 4.1.1.41) of *V. alcalescens* (ATCC 17745) was prepared as described [1].

2.2. *Separation of CoA-derivatives by HPLC*

The chromatography was performed on a stainless-steel column (250×4 mm) filled with Lichrosorb RP 18, particle size 5 μm, flow rate 1 ml·min^{-1}. Propionyl-CoA (20.5 min), and methylmalonyl-CoA (14.4 min) were separated in 0.2 M Na-phosphate buffer, pH 5.0 with a linear gradient from 0 to 20% acetonitrile within 30 min. Under these conditions ATP (6.4 min) and ADP (7.1 min) were also separated. The CoA-derivatives were monitored at 254 nm and quantitated by comparing peak areas with those of known standards. The radioactivities of the separated compounds were determined by liquid scintillation counting.

2.3. *Decarboxylation of* (RS)-*methylmalonyl-CoA in ^3H_2O*

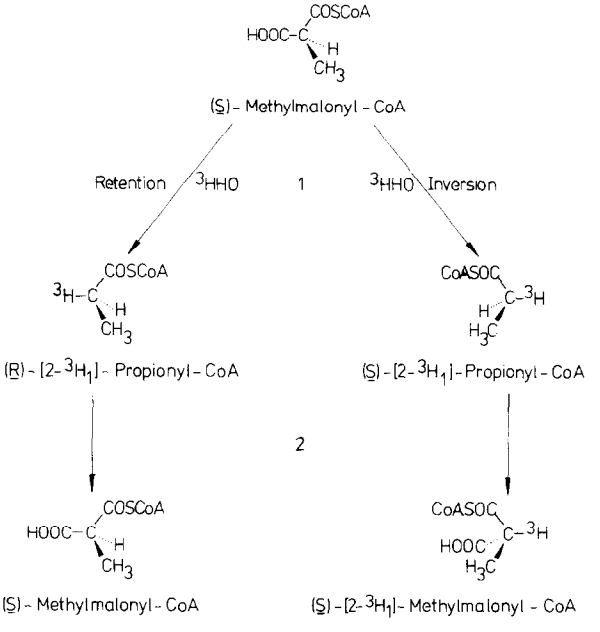
A solution containing 20 μmol K-phosphate buffer, pH 7.0, 2 μmol NaCl, and 2.3 μmol *(RS)*-methylmalonyl-CoA was lyophilized and the residue was dissolved in 100 μl ^3H_2O of specific activity 7.4×10^5 counts × min^{-1}·μmol^{-1}. The mixture was incubated at 25°C with 2 U methylmalonyl-CoA decarboxylase. The enzyme was inactivated after 1 h by adding 10 μl of 1 M HCl and the ^3H_2O was removed by lyophilization. The residue was dissolved in 100 μl H_2O and lyophilization was repeated until the distillate contained less than 1000 counts·min^{-1}. The [2-^{3}H]propionyl-CoA was purified by chromatography on DEAE-Sepharose and isolated by precipitation with ethanol [11]. Analysis of the product by HPLC indicated the presence of 1.8 μmol [2-^{3}H]propionyl-CoA with a specific activity of 6.8×10^4 counts·min^{-1}·μmol^{-1}. Residual methylmalonyl-CoA was not detectable due to the methylmalonyl-CoA racemase activity present in the methylmalonyl-CoA decarboxylase preparation (see below, section 3.1).

2.4. *Decarboxylation of [2-^{3}H]methylmalonyl-CoA in H_2O*

(S)-[2-^{3}H]Methylmalonyl-CoA was prepared from *(RS)*-[2-^{3}H]propionyl-CoA with propionyl-CoA carboxylase and isolated by HPLC. The incubation mixture contained in a total volume of 4 ml: 800 μmol K-phosphate buffer, pH 7.0, 80 μmol NaCl, 0.4 μmol *(S)*-[2-^{3}H]methylmalonyl-CoA (10^4 counts·min^{-1}·μmol^{-1}), and 0.4 U methylmalonyl-CoA decarboxylase. After 15 min incubation at 25°C, the reaction was terminated by adding 0.4 ml of 1 M HCl. The [2-^{3}H]propionyl-CoA (0.39 μmol) of specific activity 8.3×10^3 counts·min^{-1}·μmol^{-1} was isolated by HPLC and subsequently subjected to configuration analysis.

2.5. *Configuration analysis of [2-^{3}H]propionyl-CoA*

The configuration of [2-^{3}H]propionyl-CoA was determined from the amount of tritium which was converted into *(S)*-methylmalonyl-CoA by propionyl-CoA carboxylase. The incubation mixtures contained at 25°C: 100 mM Tris-HCl buffer, pH 8.0, 100 mM KCl, 50 mM $KHCO_3$, 4 mM $MgCl_2$,



Scheme 1. Reactions used to determine the steric course of the methylmalonyl-CoA decarboxylation reaction. Reaction 1 is catalyzed by methylmalonyl-CoA decarboxylase, and reaction 2 by propionyl-CoA carboxylase.

2 mM glutathione, 2 mM ATP, and 90–100 μM [2-^{3}H]propionyl-CoA (3300–5800 counts · min^{-1}). The total volume was 1 ml and 4 ml, respectively, for the reaction with *(R)*- and *(S)*-[2-^{3}H]propionyl-CoA. The reactions with the *(R)* or *(S)* enantiomer were initiated with 0.1 U or 0.8 U propionyl-CoA carboxylase and terminated by transferring samples (0.2 ml or 1.0 ml) to 20 μl or 100 μl of 1 M HCl. After centrifugation, propionyl-CoA and methylmalonyl-CoA were separated by HPLC; their amounts and radioactivities were quantitated.

3. RESULTS AND DISCUSSION

3.1. *Formation of* (R)-*[2-^{3}H]propionyl-CoA by decarboxylation of methylmalonyl-CoA in ^3H_2O*

The method used to establish the steric course of the reaction catalyzed by methylmalonyl-CoA decarboxylase is shown in scheme 1. *(S)*-Methylmalonyl-CoA was decarboxylated in ^3H_2O and the configuration of the resulting [2-^{3}H]propionyl-CoA was determined with propionyl-CoA carboxylase. The stereochemistry of the carboxylase reaction is known to be retention of configuration: the pro-*(R)* hydrogen at C-2 of propionyl-CoA is replaced by the carboxyl group to yield *(S)*-methylmalonyl-CoA [12]. *(S)*-Methylmalonyl-CoA is the substrate of methylmalonyl-CoA decarboxylase [1,13]. If the decarboxylation would occur with retention of configuration, *(R)*-[2-^{3}H]propionyl-CoA would be formed; the tritium of this enantiomer would be given up to water in the propionyl-CoA carboxylation reaction. On the other hand, tritium would be retained in the product *(S)*-methylmalonyl-CoA, if the decarboxylation would involve inversion of configuration.

The preparation of methylmalonyl-CoA decarboxylase which we used in these studies was contaminated with a small amount of methylmalonyl-CoA racemase. After the fast decarboxylation

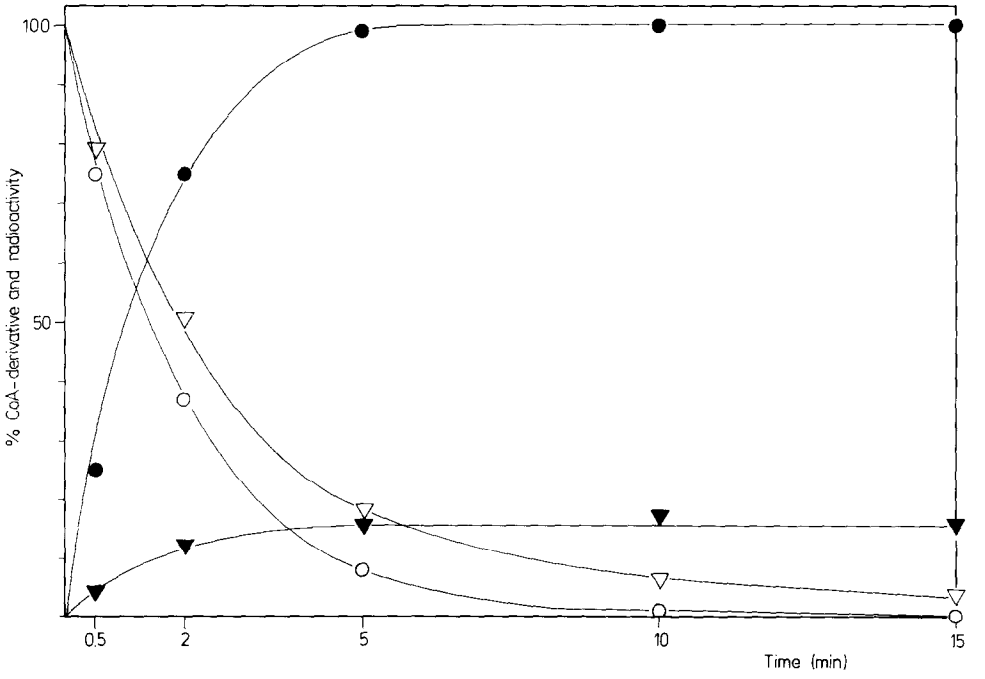


Fig.1. Release of ^3H_2O by propionyl-CoA carboxylase from [2-^{3}H]propionyl-CoA obtained by enzymic decarboxylation of *(RS)*-methylmalonyl-CoA in ^3H_2O. The carboxylation reaction was terminated after various incubation periods and the amounts and radioactivities of methylmalonyl-CoA and propionyl-CoA were determined after separation by HPLC. The values are given as percentage methylmalonyl-CoA (●) and propionyl-CoA (○) of total CoA-derivatives (100% corresponds to 86 nmol); percentages of radioactivities of methylmalonyl-CoA (▼) and propionyl-CoA (▽); 100% corresponds to 5840 counts · min^{-1}. For details see section 2.

of half of the racemic *(RS)*-methylmalonyl-CoA, the reaction was completed slowly. From these rates, the racemase activity was estimated to be about 1–2% that of the decarboxylase activity. The stereochemical purity of [2-^{3}H]propionyl-CoA derived from the decarboxylation of the *(S)* enantiomer of methylmalonyl-CoA in ^3H_2O is therefore probably not significantly affected by the comparatively low activity of the racemase. However, the conversion of the remaining *(R)*-methylmalonyl-CoA into the *(S)* enantiomer is accompanied with the incorporation of tritium from ^3H_2O into the 2-position of the substrate. Decarboxylation of *(S)*-[2-^{3}H]methylmalonyl-CoA in ^3H_2O produces *(R)* and *(S)* enantiomers of [2-^{3}H]propionyl-CoA (50% of each if the rate of tritium incorporation in the racemase and decarboxylase reaction is the same). After the complete decarboxylation of *(RS)*-methylmalonyl-CoA in ^3H_2O the stereochemical purity of [2-^{3}H]propionyl-CoA is therefore expected to be about 75%, sufficient to determine the steric course of the decarboxylation reaction.

After the incubation of 2.3 μmol *(RS)*-methylmalonyl-CoA with 2 U methylmalonyl-CoA decarboxylase for 1 h, the methylmalonyl-CoA was completely decarboxylated. The product [2-^{3}H] propionyl-CoA was isolated and carboxylated to *(S)*-methylmalonyl-CoA with propionyl-CoA carboxylase. The results of fig.1 indicate a progressive washout of tritium into water as the carboxylation proceeds. After complete carboxylation about 20% of the tritium of [2-^{3}H]propionyl-CoA were retained in *(S)*-methylmalonyl-CoA. The results thus indicate that decarboxylation of methylmalonyl-CoA occurs with retention of configuration.

3.2. *Formation of* (S)-*[2-^{3}H]propionyl-CoA by decarboxylation of* (S)-*[2-^{3}H]methylmalonyl-CoA in H_2O*

The steric course of the methylmalonyl-CoA

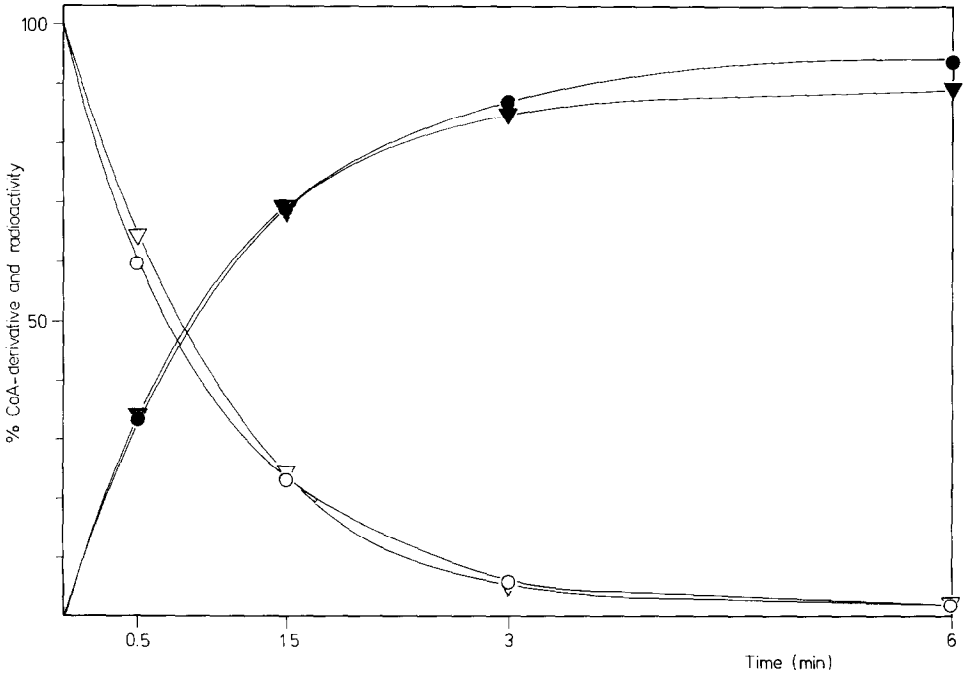


Fig.2. Retention of tritium by propionyl-CoA carboxylase catalyzed carboxylation of [2-^{3}H]propionyl-CoA formed by enzymic decarboxylation of *(S)*-[2-^{3}H]methylmalonyl-CoA in H_2O. The carboxylation reaction was terminated after various incubation periods and the amounts and radioactivities of methylmalonyl-CoA and propionyl-CoA were determined after separation by HPLC. The values are given as percentage methylmalonyl-CoA (●) and propionyl-CoA (○) of total CoA-derivatives (100% corresponds to 390 nmol); percentages of radioactivities of methylmalonyl-CoA (▼) and propionyl-CoA (▽); 100% corresponds to 3300 counts · min^{-1}. For details see section 2.

decarboxylation reaction with retention of configuration was confirmed with the decarboxylation of *(S)*-[2-^{3}H]methylmalonyl-CoA in H_2O. This reaction yielded *(S)*-[2-^{3}H]propionyl-CoA, as shown by the retention of tritium in *(S)*-methylmalonyl-CoA produced in the subsequent carboxylation with propionyl-CoA carboxylase (fig.2).

The substrate stereochemistry of methylmalonyl-CoA decarboxylase is the same as that of the related biotin-dependent Na^+ transporting decarboxylases oxaloacetate decarboxylase [7] and glutaconyl-CoA decarboxylase [8]. In the first step of the reaction sequence of these enzymes which is catalyzed by the carboxyltransferase activity, a carboxyl residue is abstracted from a methylene or methine group of the substrate and replaced by a proton under retention of the configuration. A carboxyltransferase reaction is also involved in the two other classes of biotin enzymes (carboxylases and transcarboxylase); it always occurs with retention of configuration. These data therefore suggest that the carboxyltransferase reactions of all biotin enzymes occur by a common mechanism.

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