Enzymic synthesis and reduction of malonyl semialdehyde-coenzyme A

Studies on propionate metabolism in animal tissues have shown that malonate semialdehyde is formed by the oxidation of β-hydroxypropionate in the presence of DPN^+ and β-hydroxypropionic dehydrogenase[1,2] and by the enzymic transamination of β-alanine with α-ketoglutarate[3,4]. Malonate semialdehyde has also been implicated as an intermediate in propionate oxidation by peanut mitochondria[5]. More recently VAGELOS AND EARL[6] have demonstrated that extracts of *Clostridium kluyveri* oxidize β-hydroxypropionyl-CoA to malonyl semialdehyde-CoA in the presence of TPN^+.

The fact that the purified pig-heart succinyl–acetoacetyl-CoA transferase[7] was recently shown to catalyze the transfer of CoA from acetoacetyl-CoA or succinyl-CoA to malonate with formation of malonyl-CoA[8] suggested that malonate semialdehyde might also be a substrate for this enzyme. We wish to report that malonyl semialdehyde-CoA can indeed be synthesized enzymically by transfer of CoA from succinyl-CoA (or acetoacetyl-CoA) to the carboxyl group of malonate semialdehyde in the presence of CoA transferase according to the following reactions:

$$\text{Succinyl-CoA} + \text{malonate semialdehyde} \rightleftharpoons \text{malonyl semialdehyde-CoA} + \text{succinate} \quad (1)$$

$$\text{Acetoacetyl-CoA} + \text{malonate semialdehyde} \rightleftharpoons \text{malonyl semialdehyde-CoA} + \text{acetoacetate} \quad (2)$$

Reaction (1) was followed by a direct optical assay[7] based upon the absorption band (λ_{max} about 303 mμ) possessed by β-keto thioesters, including malonyl semialdehyde-CoA[6], in alkaline solution. When highly purified CoA transferase was added to succinyl-CoA and malonate semialdehyde at pH 8.0, a rapid increase in absorbancy at 310 mμ occurred (Fig. 1, lower curve), indicating the formation of malonyl semialdehyde-CoA. After the reaction had approached equilibrium, the pH of the reaction mixture was lowered by successive additions of dilute HCl and the changes in absorbancy followed. It is seen that the absorbancy of the reaction product decreases with decreasing pH, but unlike that of acetoacetyl-CoA[9] is appreciable at acid pH. This spectral behavior has been found for synthetic malonyl semialdehyde pantetheine[6] and is further evidence for the identity of the product as malonyl semialdehyde-CoA. The pK of the enol group of the latter compound is apparently considerably less than that of acetoacetyl-CoA, suggesting that at pH 8.0 the absorbancy of malonyl semialdehyde-CoA might be greater than that of acetoacetyl-CoA and that reaction (2) could, therefore, be measured optically. As shown in Fig. 1 (upper curve), the absorbancy of a solution of acetoacetyl-CoA at pH 8.0 decreases due to spontaneous deacylation. On addition of purified CoA transferase (in the presence of malonate semialdehyde), the absorbancy increases rapidly, thereby demonstrating the formation of malonyl semialdehyde-CoA.

Further evidence for the occurrence of reaction (1) was obtained by coupling it with reaction (3), which we have found to be catalyzed by β-hydroxybutyryl-CoA dehydrogenase, and demonstrating the net reaction (4) by direct optical test.

$$\text{Malonyl semialdehyde-CoA} + DPNH + H^+ \rightleftharpoons \beta\text{-hydroxypropionyl-CoA} + DPN^+ \quad (3)$$

$$\text{Succinyl-CoA} + \text{malonate semialdehyde} + DPNH + H^+ \rightleftharpoons \beta\text{-hydroxypropionyl-CoA} + DPN^+ + \text{succinate} \quad (4)$$

Abbreviations: CoA, coenzyme A; DPN^+, DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN^+, TPNH, oxidized and reduced triphosphopyridine nucleotide.

Thus when malonate semialdehyde, DPNH, crystalline pig heart β-hydroxy-butyryl-CoA dehydrogenase[10], and CoA transferase were mixed, no reaction occurred (Fig. 2) as measured by the absorbancy of DPNH at 340 mμ. It is therefore evident that these enzyme preparations were entirely free of β-hydroxypropionic dehydrogenase. However, upon addition of succinyl-CoA, oxidation of DPNH was observed (Fig. 2, lower curve) as a result of synthesis and reduction of malonyl semialdehyde-CoA. As already shown with acetoacetyl-CoA[10], TPNH can replace DPNH in reaction 3 (Fig. 2, upper curve). This reduction of malonyl semialdehyde-CoA by a crystalline preparation of β-hydroxybutyryl-CoA dehydrogenase apparently explains the disappearance of malonyl semialdehyde-CoA catalyzed by crude extracts of pig heart and rabbit liver in the presence of DPNH (see ref. 6).

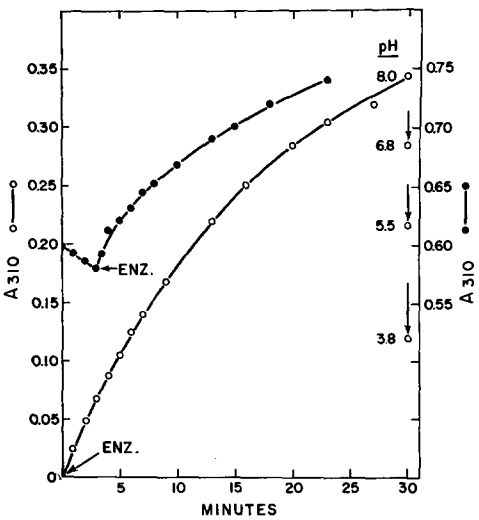

Fig. 1. The reaction mixture initially contained sodium pyrophosphate, 200 μmoles; malonic semialdehyde, 10 μmoles; and acetoacetyl-CoA, 0.27 μmoles (upper curve) or succinyl-CoA, 1.0 μmole (lower curve). Purified pig heart CoA transferase (3.2 μg; specific activity, 1000) was added at zero time (lower curve) and after 3 min (upper curve), to start the reaction. The final pH was 8.0. Volume, 1.50 ml, d = 1.0 cm, temperature, 22°. In the experiment represented by the lower curve after 30 min acid was added in increments to achieve the pH values shown and the resultant A_{310} values are indicated by the open circles. Thioester was omitted from the reference cuvette.[2]

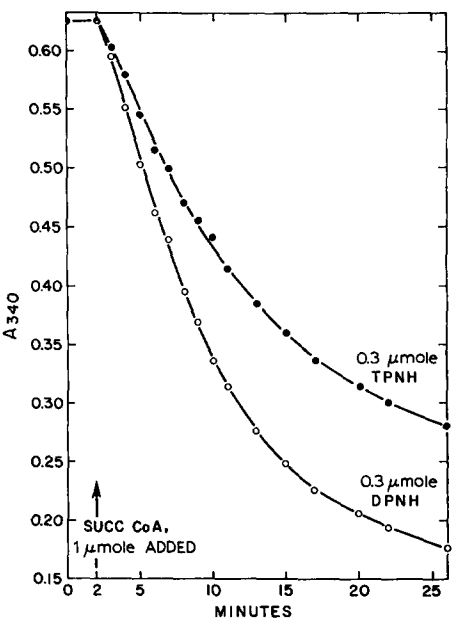

Fig. 2. The reaction mixture initially contained potassium phosphate buffer, pH 7.5, 100 μmoles; malonic semialdehyde, 10 μmoles; TPNH (upper curve) or DPNH (lower curve), 0.3 μmole; highly purified pig heart CoA transferase (8 μg; specific activity, 1000); and recrystallized pig heart β-hydroxybutyryl-CoA dehydrogenase (11.6 μg; specific activity, 149). At zero time the reaction was started by addition of 1 μmole succinyl-CoA to the experimental cuvette. Volume, 1.5 ml, d = 1.0 cm, temperature, 22°.

The evidence presented establishes an enzymic route for the conversion of malonic semialdehyde to its CoA ester and thereby provides additional support for the occurrence in animal tissues of a metabolic pathway for the β-oxidation of propionate. The function of such a pathway (other than in the formation of β-alanine) is not yet clear, particularly since the oxidation of malonyl semialdehyde-CoA to malonyl-CoA, although shown in a bacterial enzyme preparation[11], has not yet been demonstrated

in animal enzyme preparations. It may be noted that the β-oxidation of propionate to malonyl-CoA would provide a means for the formation of the latter compound, an intermediate in fatty acid synthesis, other than by acetyl-CoA carboxylation[12–15].

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Dissociation of pyruvic kinase in urea solutions

Crystalline pyruvic kinase prepared from rabbit muscle according to the method for "fluorokinase" of TIETZ AND OCHOA[1], dissociates in concentrated urea solutions into smaller fragments. In 2.5–3 M urea both forms (undissociated and dissociation products) are clearly separated in the ultracentrifuge (Fig. 1).

The sedimentation coefficient ($S_{20,w}$) of the undissociated form drops from 10.4 S in phosphate buffer (pH 6), to 7.5 S in 3 M urea, as a result of an increase in the friction coefficient. In 6 M urea the dissociation is complete, and the product sediments in the ultracentrifuge as a single, fairly symmetrical peak (Fig. 2). Its sedimentation coefficient, determined at 0.6% protein concentration in 6 M urea (dissolved in phosphate buffer, pH 6, I 0.1, for the elimination of charge effects) amounts to 2.2 S.

The diffusion coefficient ($D_{20,w}$) determined under the same conditions is $1.4 \cdot 10^{-7}$ cm^2/sec.

The molecular weight of the dissociation product calculated from the above values is 150,000, assuming the partial specific volume to be the same as for the intact enzyme, viz. 0.75 cm^3/g^2. The friction coefficient (f/f_0) is 4.2. Native pyruvic kinase has a molecular weight within the range 230,000–237,000[2,3], so it seems that its molecule is split by strong urea into two parts. Since molecular-weight determinations in