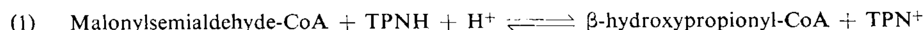
Malonylsemialdehyde Coenzyme A

P. Roy Vagelos

Malonylsemialdehyde-CoA is reduced by β-hydroxypropionyl-CoA dehydrogenase and reduced triphosphopyridine nucleotide (TPNH). The decrease in the optical density of TPNH at 340 mμ serves as a measure of the reaction[1].

Principle

β-Hydroxypropionyl-CoA dehydrogenase catalyses the reduction of malonylsemialdehyde-CoA by TPNH:



(1) Malonylsemialdehyde-CoA + TPNH + H^+ $\rightleftharpoons$ β-hydroxypropionyl-CoA + TPN^+

At neutral pH the equilibrium of this reaction is strongly in favour of the right-hand side. The reaction proceeds to completion and for each mole of malonylsemialdehyde-CoA, a mole of TPNH is oxidized.

Reagents

1. Triethanolamine, redistilled
2. Reduced triphosphopyridine nucleotide, TPNH
   sodium salt, $TPNH\text{-}Na_4$; commercial preparation, see p. 1030.
3. β-Hydroxypropionyl-CoA dehydrogenase
   purified 5-fold from extracts of *Clostridium kluyveri* according to[1] (protamine sulphate precipitation, ammonium sulphate precipitation and dialysis, see p. 451). The preparation is stable for several months at −20°C.

Purity of the enzyme preparation

The enzyme preparation obtained according to[1] is contaminated with TPNH oxidase and malonylsemialdehyde-CoA dehydrogenase. If sufficiently dilute enzyme solutions are used these impurities do not interfere. Each new enzyme preparation must be tested in a reaction mixture prepared as described under "Spectrophotometric measurements, experimental cuvette", except that the sample is replaced with distilled water. Any consumption of TPNH by this mixture is taken into account during the calculations.

Preparation of Solutions

I. Triethanolamine buffer (1.0 M; pH 7.5):
   Dissolve 14.9 g. triethanolamine in about 50 ml. distilled water, adjust pH to 7.5 with *ca.* 30 ml. 2 N HCl and dilute with distilled water to 100 ml. Check pH with glass electrode.

II. Reduced triphosphopyridine nucleotide (10^{-3} M β-TPNH):
   Dissolve 4.4 mg. $TPNH\text{-}Na_4$ in distilled water and make up to 5 ml. Store solution at −20°C.

III. β-Hydroxypropionyl-CoA dehydrogenase (0.1 mg. protein/ml.):
   Dilute the preparation obtained according to[1]. Store solution at −20°C.

Procedure

Experimental material

The method described here can be used to determine malonylsemialdehyde-CoA in any sample which does not absorb strongly at 340 mμ.

---

[1] *P. R. Vagelos* and *J. M. Earl*, J. biol. Chemistry *234*, 2272 [1959].

Spectrophotometric measurements

Wavelength: 340 mμ; 1 ml. silica cuvettes, light path: 1 cm.; final volume: 1 ml.; room temperature. Read experimental against control cuvette.

Pipette into the cuvettes:

Experimental cuvette	*Control cuvette*
sample (containing 0.02–0.1 μmoles malonylsemialdehyde-CoA)	0.2 ml. buffer (solution I)
0.2 ml. buffer (solution I)	distilled water to 0.9 ml.
0.2 ml. TPNH solution (II)	
distilled water to 0.9 ml.	

Mix the cuvette contents well, read optical density E_1. Add to both cuvettes

0.1 ml. enzyme solution (III)

and take readings every 30 seconds until the reaction stops. Multiply the final optical density E_2 by 1.1 (dilution factor).

The amount of enzyme used should be sufficient to bring the reaction to completion within 2–4 min.

Calculations

Between 0.01 and 0.15 μmoles, the decrease in optical density at 340 mμ is strictly proportional to the malonylsemialdehyde-CoA content of the reaction mixture. The amount is calculated from the extinction coefficient for TPNH (6.3 cm.^{2}/μmole).

$$\frac{E_1 - E_2 \times 1.1}{6.3} = \mu\text{moles malonylsemialdehyde-CoA/reaction mixture}$$

Specificity

Acetoacetyl-CoA reacts like malonylsemialdehyde-CoA. Malonylsemialdehyde pantetheine and acetoacetyl pantetheine also react under the conditions of the assay, but very much more slowly than the CoA derivatives.