β-Hydroxypropionyl Coenzyme A

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The rate of formation of malonylsemialdehyde-CoA by β-hydroxypropionyl-CoA dehydrogenase and excess triphosphopyridine nucleotide (TPN) is directly proportional to the concentration of β-hydroxypropionyl-CoA under the experimental conditions described here[1].

Principle

β-Hydroxypropionyl-CoA dehydrogenase catalyses the TPN-dependent oxidation of β-hydroxypropionyl-CoA:

(1) β-Hydroxypropionyl-CoA + TPN^+ $\rightleftharpoons$ malonylsemialdehyde-CoA + TPNH + H^+

At neutral pH the equilibrium is to the left but at pH 9.4 it is displaced to the right. The rate of formation of malonylsemialdehyde-CoA is measured by the increase in optical density at 300 mμ. Because the enzyme preparation is not pure, the reaction cannot be followed accurately by means of TPN reduction.

Reagents

1. 2-Amino-2-methyl-1,3-propanediol *)
2. β-Hydroxypropionyl-CoA
   prepared from coenzyme A (commercial preparation, see p. 1007) and β-propiolactone **) according to [1].
3. Triphosphopyridine nucleotide, TPN
   sodium salt, $TPN\text{-}NaH_2$; commercial preparation, see p. 1029.
4. Hydrochloric acid, 1 N, A. R.
5. Potassium dihydrogen phosphate, A. R., KH_2PO_4
6. Dipotassium hydrogen phosphate, A. R., K_2HPO_4
7. β-Hydroxypropionyl-CoA dehydrogenase
   from *Clostridium kluyveri*[1], isolation, see p. 451.

Preparation of Solutions

I. 2-Amino-2-methyl-1,3-propanediol hydrochloride buffer (1.0 M; pH 9.4):
Dissolve 10.51 g. 2-amino-2-methyl-1,3-propanediol in 40 ml. distilled water, adjust to pH 9.4 with 20 ml. 1 N HCl and dilute with distilled water to 100 ml.

II. β-Hydroxypropionyl-CoA (10^{-3} M; pH 6):
For standardization, dilute the solution prepared according to [1] with distilled water.

III. Triphosphopyridine nucleotide (5×10^{-2} M β-TPN):
Dissolve 40 mg. $TPN\text{-}NaH_2$ in 1 ml. distilled water.

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*) *e.g.* from Commercial Solvents Corp., USA.
**) *e.g.* Eastman Organic Chemicals.

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

IV. Potassium phosphate buffer (0.01 M; pH 7.5):
a) Dissolve 1.74 g. K_2HPO_4 in distilled water and dilute to 1000 ml.
b) Dissolve 1.36 g. KH_2PO_4 in distilled water and dilute to 1000 ml.
Mix solutions a) and b) in ratio of 84 : 16 volumes.

V. β-Hydroxypropionyl-CoA dehydrogenase (1 mg. protein/ml.):
Dilute the enzyme solution prepared according to[1] with potassium phosphate buffer (solution IV) to give 1 mg. protein/ml.

Stability of the solutions

The enzyme solution keeps for several months at −20° C. Solutions II and III are stored frozen.

Procedure

Experimental material

The method described here was originally designed to assay the activity of β-hydroxypropionyl-CoA dehydrogenase[1]. The method is not applicable if the extract absorbs strongly at 300 mμ.

Spectrophotometric measurements

Wavelength: 300 mμ; 1 ml. quartz cuvettes, light path: 1 cm.; final volume: 1 ml.
Three test cuvettes can be prepared at the same time. Measurements are made against a blank cuvette containing distilled water instead of the sample.

Pipette into the cuvettes:

sample (containing 0.02—0.15 μmoles β-hydroxypropionyl-CoA); corresponding amount of distilled water in the blank cuvette
0.1 ml. buffer (solution I)
0.1 ml. TPN solution (III)
distilled water to 0.9 ml.

Mix well, read optical density at 300 mμ. Then add

0.1 ml. of β-hydroxypropionyl-CoA dehydrogenase (solution V)

to all four cuvettes. Read optical density at half minute intervals. Note the time interval t which is required for an increase in optical density of 0.1. The amount of enzyme taken should be such that t = 1—3 minutes. The addition of more enzyme is detrimental, since the preparation is contaminated with another enzyme which inhibits the reaction being measured.

Calculations

The rate of the reaction is directly proportional to the β-hydroxypropionyl-CoA content of the assay mixture. A standard curve is prepared for each enzyme preparation using 0.02—0.15 ml. β-hydroxypropionyl-CoA solution (II).

Sources of Error and Specificity

The 5-fold purified enzyme used here is contaminated with malonylsemialdehyde-CoA dehydrogenase, which oxidizes malonylsemialdehyde-CoA to malonyl-CoA. Therefore only sufficient enzyme should be used to obtain the optimal change of optical density.

β-Hydroxybutyryl-CoA reacts like β-hydroxypropionyl-CoA. β-Hydroxypropionyl-pantetheine gives a reaction which is insignificant in comparison to the CoA derivative.

Appendix

Isolation of β-hydroxypropionyl-CoA dehydrogenase[1)]

The enzyme is extracted from *Clostridium kluyveri* with potassium phosphate buffer (solution V) at 0°C. The purification steps include: protamine sulphate precipitation, ammonium sulphate fractionation (0.65 to 0.95 saturation) and dialysis. All attempts to purify the enzyme further (*e.g.* by fractionation with organic solvents, gel adsorption, acid precipitation, chromatography on DEAE-cellulose) have so far failed. The resulting enzyme is purified *ca.* 5-fold, in comparison to the crude extract, and is stored as a solution in 0.01 M potassium phosphate buffer (pH 7.5).