Benzoyl Coenzyme A

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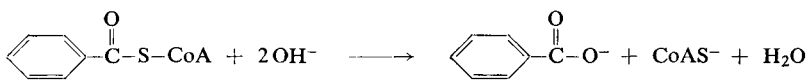
Principle

After it has been hydrolysed at alkaline pH, the benzoyl derivative of coenzyme A (Bz-CoA) can be determined with one of the enzymatic assay methods for CoA. The hydrolysis corresponds to the well-known alkaline saponification of esters:

(1) 

Generally, the thioesters of acids with low pK values are hydrolysed more rapidly in alkali than those with high values[1]. The half-life time for the hydrolysis of the thioesters of the saturated fatty acids with chain lengths from C_2 to C_8 (pK = 4.7–4.9) is only 1 to 2 min. at 30° C (in 0.1 N NaOH)[2]. The half life of benzoyl-CoA (pK = 4.17) may be slightly longer. However, the hydrolysis time of 20 min. used here is sufficient. CoA-SH is easily oxidized to CoA-S-S-CoA, therefore Bz-CoA is determined as the sum of the CoA-SH + CoA-S-S-CoA with the HOADH assay (see p. 513).

Reagents

See p. 513. In addition:

1. Sodium hydroxide, A. R.
2. Hydrochloric acid, A. R., sp. gr. 1.19, *ca.* 36% (w/w)

Preparation of Solutions

See p. 514. In addition:

I. Sodium hydroxide (1 N):
Dissolve 4.0 g. NaOH in doubly distilled water and make up to 100 ml.

II. Hydrochloric acid (*ca.* 1 N):
Dilute 8.3 ml. HCl (sp. gr. 1.19) to 100 ml. with doubly distilled water.

Procedure

Weigh out exactly 10 to 15 mg. Bz-CoA and dissolve in 8. 0 ml. 0.1 N NaOH (solution I). Allow the solution to stand for 20 min. at room temperature, then adjust to pH 9.0 with 1 N HCl (solution II) and dilute to 10.0 ml. with doubly distilled water. Determine the total CoA content of this solution with HOADH, see p. 515. In the calculations insert the molecular weight of benzoyl-CoA (870.6).

Example

11.2 mg. Bz-CoA was dissolved in 8 ml. NaOH (1.40 mg./ml.) and this solution was analysed. The following optical densities were measured at 366 mμ: $E_0 = 0.300$; $E_1 = 0.178$; $E_2 = 0.183$; therefore $\Delta E = E_0 - E_1 = 0.122$; $\Delta E_{HOADH} = 0.005$ and $\Delta E_{corr.} = 0.127$.

According to p. 515

$0.127 \times 9.10 \times 870.6 = 1005$ μg. Bz-CoA/ml. sample solution after the preliminary treatment for the CoA assay.

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[1] *T. Wieland*, quoted by *E. R. Stadtman* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1957, Vol. III, p. 934.

[2] *F. Lynen*, quoted by *E. R. Stadtman* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1957, Vol. III, p. 934.

The additions before the assay (thioglycollic acid, diketene and KOH) resulted in a final dilution of the sample to 10.30 ml. Therefore the concentration of Bz-CoA in the original 8.0 ml. of solution is

$$1005 \times 10.30/8.00 = 1295 \text{ μg. Bz-CoA/ml.}$$

The purity of the sample is obtained from the weight of sample taken:

$$\frac{1295}{1400} \times 100 = 92.5\%.$$

Specificity and Sources of Error

What has been said about the HOADH assay of CoA (p. 512, 516) applies here also.