# Acrylyl Coenzyme A

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Acrylyl-CoA is aminated by acrylyl-CoA aminase and $NH_4Cl$, with a corresponding decrease in optical density at 263 mμ. Acrylyl-CoA can be quantitatively determined with the aid of this decrease in optical density [1].

## Principle

Acrylyl-CoA aminase catalyses the amination of acrylyl-CoA by ammonium salts to form β-alanyl CoA:

(I) $CH_2=CH-C(=O)-S-CoA + NH_3 + H^+ \rightleftharpoons H_3^+N-CH_2-CH_2-C(=O)-S-CoA$

The equilibrium constant [2] of this reaction is $2.58 \times 10^{13}$ [l./mole]$^2$ at pH 7.5 and 25° C. With excess of an ammonium salt a mole of β-alanyl-CoA is formed from each mole of acrylyl-CoA. The disappearance of acrylyl-CoA is measured spectrophotometrically.

## Reagents

1. Triethanolamine, redistilled
2. Ammonium chloride
3. Acrylyl-CoA aminase
   Purified 75-fold from extracts of *Clostridium propionicum* according to [1] (protamine sulphate precipitation, ammonium sulphate precipitation, dialysis, calcium phosphate gel adsorption, a second ammonium sulphate precipitation, dialysis). The specific activity is about 55 units*)/mg. protein. It is stable for at least a month when stored at −20° C.

## Preparation of Solutions

I. Triethanolamine buffer (1.0 M, pH 7.5):
   Dissolve 14.9 g. triethanolamine in *ca.* 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 a glass electrode.

II. Ammonium chloride (1.0 M):
   Dissolve 5.3 g. $NH_4Cl$ in distilled water and make up to 100 ml.

III. Acrylyl-CoA aminase (5 units*)/ml.):
   Dilute the preparation obtained according to [1] with distilled water. Store the solution at −20° C.

## Procedure

### Experimental material

The method was designed as an assay for acrylyl-CoA aminase [1]. It should be applicable to any type of material in which acrylyl-CoA is the only compound having a strong absorption at 263 mμ.

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*) A unit is the amount of enzyme dissolved in 1 ml. which causes a decrease in the optical density at 263 mμ from 0.500 to 0.400 in 1 min.

1) *P. R. Vagelos, J. M. Earl* and *E. R. Stadtman*, J. biol. Chemistry *234*, 490 [1959].
2) *P. R. Vagelos* and *J. M. Earl*, J. biol. Chemistry *234*, 2262 [1959].

**Spectrophotometric measurements**

Wavelength: 263 mμ; 1 ml. silica cuvettes, light path: 1 cm.; final volume: 1 ml.; room temperature. Read the experimental against the control cuvette.

Pipette into the cuvettes:

| *Experimental cuvettes* | *Control cuvette* |
|---|---|
| sample (containing 0.02—0.1 μmoles acrylyl-CoA) | corresponding volume distilled water |
| 0.05 ml. buffer (solution I) | 0.05 ml. buffer (solution I) |
| 0.1 ml. $NH_4Cl$ solution (II) | 0.1 ml. $NH_4Cl$ solution (II) |
| distilled water to 0.95 ml. | distilled water to 0.95 ml. |

Mix well and read the optical densities $E_1$. Mix into all four cuvettes

0.05 ml. acrylyl-CoA aminase solution (III)

and take readings every 30 seconds until there is no further decrease. Multiply the final optical densities $E_2$ by 1.05 (dilution factor), subtract the mean from the mean of the initial optical densities. Use the difference $\Delta E = E_1 - E_2$ for the calculations.

The amount of enzyme used should be sufficient to bring the reaction to completion in 1—3 minutes. More enzyme reduces the specificity.

**Calculations**

Between 0.01 and 0.13 μmoles/ml., the decrease in optical density at 263 mμ is strictly proportional to the amount of acrylyl-CoA added. The acrylyl-CoA content of the reaction mixture is calculated from the extinction coefficient of 6.7 cm.$^2$/μmole for the thiolester bond of acrylyl-CoA[3].

$$\frac{\Delta E}{6.7} = \text{μmoles acrylyl-CoA/reaction mixture}$$

## Specificity and Sources of Error

Crude extracts of *Clostridium propionicum*, which have been grown on β-alanine instead of α-alanine, may have acrylyl-CoA aminase activity as high as 91.6 units/mg. protein[1]. Since so little protein is required, such crude extracts can be used directly in this method without risk of interference from side reactions.

Acrylyl-CoA reacts with the following compounds (in descending order of reactivity): acrylyl-CoA, crotonyl-CoA, acrylyl pantetheine, and crotonyl pantetheine[1]. It reacts slowly with acrylyl-*N*-acetyl thioethanolamine, but not at all with acrylyl-*S*-thiopropionic acid or crotonyl-*N*-acetyl thioethanolamine. The rates of the reactions differ enormously: *e.g.* crotonyl-CoA reacts at only 5% of the rate with acrylyl-CoA. Measurement of the kinetics of the reaction gives an indication which compound in the experimental material reacts. If more enzyme is used in the reaction mixture than is stated above, then the determination will be less specific and will also include the other thiolesters reacting with acrylyl-CoA aminase.

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[3] *F. Lynen*, Fed. Proc. *12*, 683 [1953].