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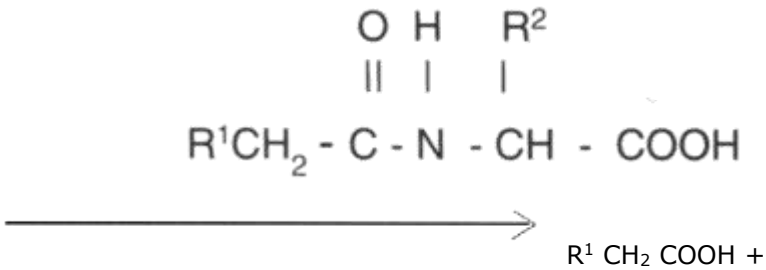
Glutamic Oxaloacetic Transaminase (AST)

Acylase 1 (Amino Acylase 1)

(N-Acylamino acid aminohydrolase; EC 3.5.1.14)

Acylase 1 (Amino Acylase 1) catalyzes the following reaction:

ACYLASE 1



$$R^1CH_2 - C(=O) - N(H) - CH(R^2) - COOH \longrightarrow R^1\ CH_2\ COOH + NH_2\ R^2\ CHOOH$$

R^1 = Cl, H, NH_2 , etc.
R^2 = L-Amino acid side chain other than L-aspartic acid

Acylase 1 is an extremely stable enzyme in the dry state. In solution, the enzyme is stable at high temperatures (70° C) at pH 7, but below pH 5 it is rapidly and irreversibly inactivated. The enzyme shows a high degree of optical specificity toward its substrates. For this reason, it has been used extensively for the resolution of racemic amino acids.

ASSAY

The assay is based on the reaction described by Mitz and Schulueter, Biochim. Biophys. Acta, 27, 168, 1958). The enzyme catalysis is followed by measuring absorbance at 238 nm.

REAGENTS

1. 0.1 M Potassium phosphate buffer, pH 7.0.

2. 0.015 M N-Acetyl-L-Methionine (2.87 mg/ml). Dissolve in 0.01 M potassium phosphate buffer. Adjust pH to 7.0 with 2 M NaOH if necessary.

3. Acylase 1 (enzyme) solution. Dilute in 0.01 M potassium phosphate buffer, pH 7.0 to yield a concentration of 1000-2000 U/ml. Prepare fresh prior to assay.

PROCEDURE

1. Set the spectrophotometer (equipped with strip chart recorder and temperature control) at 238 nm and 25° C.
2. In a cuvette place 2.9 ml of 0.015 M N-Acetyl-L-Methionine (substrate). Incubate cuvette in spectrophotometer at 25° C for 5 minutes.
3. Record absorbance at 238 nm (blank).
4. Initiate the reaction by adding 0.1 ml enzyme solution to the cuvette. Follow the reaction by measuring the absorbance at 238 nm for 5-8 minutes.
5. Calculate $\Delta E_{238nm/min}$

CALCULATION

$$\text{Activity (U/mg)} = \frac{(\Delta E_{238nm/min})(\text{Total Vol.})(\text{Enz. Diln.})(60)}{(0.018)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$