Pancreatic Elastase

EC 3.4.21.36

Structure:

Elastase is a compact globular protein consisting of a single polypeptide chain of 240 amino acids cross-linked by 4 disulfide bridges. It has a hydrophobic core and exhibits extensive sequence homology with other serine proteinases, such as trypsin and chymotrypsin [1,2]. The tertiary structure of elastase has been elucidated [1]. The enzyme is synthesized in porcine pancreas as a pre-proelastase [3]. After processing to proelastase, it is stored in the zymogen granules and later activated to elastase in the duodenum by tryptic cleavage of a single peptide bond in the inactive precursor molecule [4]. This process is closely resembling those of trypsinogen and chymotrypsinogen activation which results in the removal from the N-terminal end of the molecule of a small activation peptide, enabling the enzyme to adopt its native conformation. The existence of two forms of elastase has been reported which differ in respect to their catalytic properties [5]. Elastase contains no prosthetic groups or metal ions and is not subject to any allosteric activatory or inhibitory control. Its enzymatic activity results solely from the specific three-dimensional conformation which its single polypeptide chain adopts. Therefore activity is lost by denaturation and conformational changes [6] .

Specificity:

Elastase is a serine proteinase with broad substrate specificity. It preferentially cleaves peptide bonds at the carbonyl end of amino acid residues with small hydrophobic side chains, such as glycine, valine, leucine, isoleucine, and particularly alanine. The wide specificity of elastase for non-aromatic uncharged side chains explains its unique ability to digest native elastin, a protein rich in aliphatic side chains. Elastase is also able to digest other proteins such as fibrin, hemoglobin, and casein, but not native collagen and keratin [6,7].Due to the peculiar cross-linked structure of elastin, the rate of release of peptides into solution does not proceed linearly with the rate of hydrolysis of peptide bonds by elastase, while the conversion of synthetic peptides proceeds linearly with time [6].

Activators:

Elastase does not require any specific activators. It has been observed however that in the presence of Tris, sodium sulfate or SDS activity is greatly stimulated [1,8].

Inhibitors:

Activity is irreversibly inhibited by diisopropyl fluorophosphate and sulphonyl fluorides like PMSF [7]. It is also inhibited by elastinal, a_1-proteinase inhibitor and a_2-macroglobulin [9]. In contrast to human leukocyte elastase, pancreatic elastase is not inhibited by soybean trypsin inhibitor [9]. The elastolytic activity of elastase is significantly affected by salts: NaCl and KCl cause 50% inhibition at 50 – 70 mM and copper sulfate 50% inhibition at 0.01 mM, while millimolar concentrations of zinc, manganese, cobalt, magnesium or calcium had no effect [1].

Physical and Chemical Properties

M_r :	ca. 25900
Optimum pH	8.8
Isoelectric point	9.5 +/- 0.5

Applications:
Elastase is used in protein sequencing studies, and in preparations for releasing cells from tissues [10]

Assay Method:
The assay is based on the hydrolysis of N-acetyl- $(L\text{-ala})_3$-methyl ester, and the reaction is followed by pH stat titration at 25 °C [11].

Unit definition:
1 unit catalyzes the hydrolysis of 1 µmole of N-acetyl-L-alanyl-L-alanyl-L- alanine methyl ester per minute at 25 °C, pH 8.5.

Stability and Storage:
Elastase is soluble in water and diluted salt solutions at concentrations up to 50 mg/ml between pH 4.0 and 10.5; at 2°C, such solutions are stable for pr olonged time, if kept below
pH 6.0. In highly acid media, elastase is inactivated due to irreversible conformational changes. If incubated at room temperature at or near its optimum pH, it will rapidly autolyze to a mixture of peptides [1].
If stored at 4 °C, the ammonium sulphate suspension (cat. no. 20931) will not loose more than 5% of its activity per year. The lyophilized powder (Cat. no. 20929) should be stored desiccated at -20 °C. Under these conditions it will not lose more than 10% of its activity per year.Elastase adheres to glass surfaces. To prevent this, it is advisable to add 0.05% Triton X-100 to the incubation media.

References:

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