

Elastase from human neutrophils

Other names: Lysosomal elastase, leukocyte elastase

Leukocyte elastase is one of several hydrolytic enzymes contained in the azurophil granules of human neutrophils. Physiologically, it is involved in the degradation of foreign material ingested during phagocytosis. Damage to connective tissue caused by leakage of enzymes is normally prevented by proteinase inhibitors. It has been suggested that insufficient levels of these inhibitors leads to pulmonary emphysema, and elastase in particular has been implicated in abnormal lung connective tissue turnover^(1,2,3). It is also responsible (at least in part) for arthritis and inflammatory conditions⁽⁴⁾.

Structure:

Neutrophil elastase is a serine proteinase and consists of a single basic polypeptide chain of 218 amino acid residues, joined together by four disulfide bonds. It contains 2 asparagine linked carbohydrate side chains, and is in fact synthesized as a series of isoenzymes each containing different amounts of carbohydrate⁽⁵⁾. The isoforms do obviously not differ in catalytic activity and are immunologically identical^(6,7). The amino acid composition is known. The N-terminal amino acid sequence is strongly homologous with that of porcine pancreatic elastase⁽⁸⁾. On the other hand, there is only moderate sequence homology between the two enzymes. While pancreatic elastase is generated from the inactive precursor proelastase by tryptic cleavage of an N-terminal activation peptide, such a zymogen is not known for the leukocyte elastase⁽⁶⁾.

Specificity:

Leukocyte elastase has a very narrow specificity. It cleaves preferentially Val-X bonds and to a lesser extent Ala-X links, which are preferred by pancreatic elastase. It has been shown to degrade elastin, cartilage proteoglycans, several collagens and fibronectin⁽⁶⁾.

Physical and Chemical Properties

Mr:	29500
pI:	Isoelectric points of isoenzymes range from 8.77-9.15 ⁽⁶⁾
Optimum pH:	8.5 ⁽⁶⁾
Activators:	Leukocyte cathepsin G stimulates the rate of solubilization of elastin by leukocyte elastase ⁽⁹⁾ . The activity of elastase is also enhanced by high salt concentrations and hydrophobic solvents ⁽¹⁰⁾
Inhibitors:	α_1 -proteinase inhibitor (α_1 -antitrypsin) forms a 1:1 complex with the enzyme ⁽¹¹⁾ . Other protease inhibitors include soybean Kunitz inhibitor, turkey ovomucoid and α_2 -macroglobulin. It is reversibly inhibited by some longchain fatty acids, polysaccharide sulfates, and elastinal. PMSF inactivates leukocytes elastase eight times more efficiently than it does pancreatic elastase ⁽⁶⁾ .

Stability and Storage:

Leukocyte elastase is unusually stable. The esterase activity of the enzyme is unaffected by exposure to 8 M urea, but is reduced to 22 % in the presence of 1 % SDS⁽¹²⁾. The enzyme undergoes autolysis in SDS and 2-mercaptoethanol. At low ionic strength, leukocyte elastase precipitates or adheres to glass surfaces.

To prevent this, it is advisable to add 0.05% Triton X-100 to the incubation mixture. Recommended long-term storage temperature: -20°C

Applications:

For research use only in biochemistry and clinical chemistry.

Assay method:

The activity of leukocyte elastase is assayed by the use of a synthetic chromogenic peptide substrate: methoxysuccinyl-ala-ala-pro-val-4-nitroanilide. The amount of yellow 4-nitroanilide released is measured by the increase in adsorbance at 405 nm⁽¹³⁾.

Unit definition:

1 U releases 1 mole 4-nitroanilide per minute from methoxysuccinyl-ala-ala-pro-val-4-nitroanilide at 25 °C, pH 8.0.

References

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