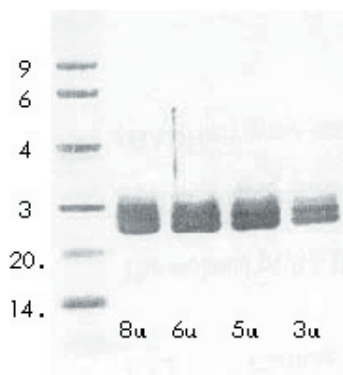


INTRODUCTION

Leukocyte elastase is one of several hydrolytic enzymes contained in the azurophil granules of human neutrophils exhibiting a relative molecular weight of 29.5 kDa, differs from pancreatic elastase in specificity on synthetic substrates and in inhibitor sensitivity. The enzyme is also a serine proteinase, but shows only moderate sequence homology with pancreatic elastase. It has a very narrow specificity, cleaving Val-X bonds preferentially, and Ala-X links to a lesser extent. It has been shown to degrade elastin, cartilage proteoglycans, several collagens and fibronectin. Physiologically, it is involved in the degradation of foreign materials ingested during phagocytosis. Damage to connective tissue caused by leakage of enzymes is normally limited 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.



MOLECULAR FORM

Human neutrophil elastase (EC 3.4.21.37, synonyms: Lysosomal elastase, neutrophil elastase, bone marrow serine protease, medullasin, polymorphonuclear leukocyte elastase; elastase; elaszym; serine elastase; lysosomal elastase; granulocyte elastase) from human neutrophils. Prepared from whole human blood shown to be non-reactive for HbsAG, antiHCV, anti-HBc, and negative for anti-HIV1 2 by FDA approved tests.

The protein consists of a single polypeptide chain of 218 amino acids I30 ... Q247 with a relative molecular weight of about 29.5 kDa (Sinha et al., 1987). The enzyme is dissolved in 10 mM acetate buffer pH 5.5.

CONCENTRATION

200 µg/mL (Pierce – BCA), 10 mM acetate buffer pH 5.5

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PURITY	Neutrophil elastase appears in SDS-PAGE as a broad protein band at a Mr of 29 kDa.
SPECIFIC ACTIVITY	Activity of human neutrophil elastase is assayed employing the synthetic chromogenic peptide Methoxysuccinyl-Ala-Ala-Pro-Val-4-nitroanilide (0.3 mM final concentration) in 0.1 M Tris-HCl pH 8 at 25°C. One unit will release 1 μmole of 4-nitroanilide per minute from the substrate at 25°C, pH 8.0. Under the specified conditions the specific activity is >20 U/mg. The enzyme is approximately 90% active by titration with α1-antitrypsin.
INHIBITORS	Human neutrophil elastase is inhibited by Aprotinin, N-(methoxysuccinyl)-Ala-Ala-Pro-Val-chloromethyl ketone, diisopropyl fluorophosphate (DFP), phenylmethanesulfonyl fluoride (PMSF), 3,4-dichloroisocoumarin, Elastinal, eglin C fragment 60-63 methyl ester, N-acetyl-eglin C and α2-macroglobulin.
STABILITY & STORAGE	Stability and storage: Human neutrophil elastase is stable until the expiry date given on the label if stored at -70 °C or lyophilized. The enzyme can be kept at -20 °C for several month and at 4 °C for 1 week without significant loss of activity. Repeated freezing and thawing should be avoided.
APPLICATIONS	<ul style="list-style-type: none"> • Degradation of extracellular matrix components such as elastin, cartilage proteoglycans, collagens and fibronectin. • Screening and characterization of inhibitors • Therapeutical implications: Neutrophil elastase is a drug target for emphysema, cystic fibrosis, adult respiratory stress syndrome and other diseases.
CONTAMINANTS	No contaminants are detectable
REFERENCES	<ul style="list-style-type: none"> • Barrett, A.J.; Leukocyte elastase (1981); <i>Methods Enzymol</i> 80; 581-588. • Harper, J.W. et al.; Active site mapping of the serine proteases human leukocyte elastase, cathepsin G, porcine pancreatic elastase, rat mast cell proteases I and II, bovine chymotrypsin A, and <i>Staphylococcus aureus</i> I.7: Human neutrophil elastase protease V-8 using tripeptide thiobenzyl ester substrates (1984); <i>Biochemistry</i> 23; 2995-3002. • Stein, R.L. et al.; Catalysis by human leukocyte elastase: mechanistic insights into specificity requirements (1987); <i>Biochemistry</i> 26; 1301-1305. • Bode, W. et al.; Human leukocyte and porcine pancreatic elastase: X-ray crystal structures, mechanism, substrate specificity, and mechanism-based inhibitors (1989); <i>Biochemistry</i> 28; 1951-1963. • Horwitz, M. et al.; Leukemia in severe congenital neutropenia: defective proteolysis suggests new pathways to malignancy and opportunities for therapy (2003); <i>Cancer Invest.</i>; 579-87; Review • Scaglioni, PP. et al.; Medicine: Taking apart a cancer protein (2003); <i>Nature</i> 426; 512-513. • Sinha, S.; Primary structure of human neutrophil elastase (1987); <i>Proc. Nat. Acad. Sci.</i> 2228-2232.