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Original Article

Differential Inhibition of Human Neutrophil Elastase and Bacterial Elastase by endogenous Protease Inhibitors.

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Abstract

Human Neutrophil Elastase (HNE) is released from neutrophils when foreign substances are encountered. Elastase is also released by invasive pathogenic microorganisms and is an important virulence factor. Serine protease inhibitors counter the elastase released in excess to prevent the damage of tissues. **Aim:** The aim of this study was to assess the specificity of the endogenous protease inhibitors α_1 Anti trypsin (α_1AT) and α_2 Macroglobulin(α_2MG) on Human Neutrophil Elastase and elastase released by Pseudomonas aeruginosa. **Result:** It was observed that Pseudomonal elastase exhibited resistance to inhibition in comparison to Human Neutrophil Elastase particularly by α_1 Anti trypsin. However, the inhibition patterns of these enzymes were more or less similar in both the cases by α_2 Macroglobulin. **Conclusion;** α_1AT is a less potent inhibitor of Pseudonomas aeruginosa elastase and might require exogenous inhibitory substances to control the damaging effects of this enzyme.

Keywords: α_1 Anti trypsin, α_2 Macroglobulin, Human Neutrophil Elastase, Pseudomonas aeruginosa

Introduction

Inhibition of enzymes is one of the major control mechanisms in biological systems and a knowledge of the inhibitor enzyme interactions is important for drug discovery.[1] A simple quantitative assessment of an inhibitor's potency can be obtained by measuring the concentration of an inhibitor required to bring about 50% reduction in enzyme activity under a specific set of conditions which is referred to as IC_{50}.[2]

HNE is released in response to microbial invasion and its uncontrolled action is negated by the Serine protease inhibitors (Serpins), principally, $\alpha_1$antitrypsin(α_1AT), α_2 macro globulin (α_2MG), elafin and secretory leukocyte inhibitor.[3] α_1AT is the most abundant proteinase inhibitor in plasma and physiologically the most important inhibitor of neutrophil elastase, released during phagocytosis.[3] In addition, α_2MG is another non-specific plasma proteinase inhibitor capable of inhibiting different proteinases of endogenous and exogenous origin.[4] Serpins, inactivates serine proteases by mimicking the three dimensional structure of the protease so that the protease binds to it. Inhibition begins by docking of the serpin and protease through a formation of a complex.[5]

Elastase released by pathogenic microorganisms has been shown to act as a virulent factor primarily involving elastolysis which has been implicated as one of the important processes in the pathogenesis of some of the bacterial diseases. The multiple virulence components of P.aeruginosa works together to inactivate the hosts' immune response.[6,7] The affinity of α_1AT is much higher for trypsin ra-

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ther than elastase and this allows elastase when released in large amounts to cause tissue destruction.[8] α_2MG also has been found to inactivate pseudomonal proteolytic enzyme.[9]

Both α_1AT and α_2MG play important roles in inhibiting the proteases released by invading bacteria and the manifestations of infection depends on the ability of the bacterial enzymes to overcome the host defense or the efficiency of the host to destroy the invasive microorganism.

Materials and Methods

Materials:

The enzyme HNE (E8140) was obtained from Sigma, USA and Pseudomonas aeruginosa (MTCC3541) elastase was purified in the laboratory to homogeneity as per the methods described by (Deena Mendez etal. [10] Inhibitors α_1 Anti Trypsin and α_2 Macroglobulin (A9024, 63013) were procured from Sigma USA. Synthetic substrate STANA (C7078). Succinyl tri alanyl p-nitroanilide) was obtained from Sigma USA. Media for culturing of pseudomonas aeruginosa was prepared using the ingredients such as beef extract, peptone agar, mannitol and yeast extract all procured from Himedia. Pseudomonas aeruginosa,(MTCC 3541) used in the study was obtained from the Microbial Type Culture Collection and Gene Bank Chandigarh, India in a lyophilized form.

Methods

Pseudomonas aeruginosa,(MTCC 3541) was rehydrated and cultivated on Nutrient Agar plates as per the suppliers' instructions and routinely used procedures.[10] The purification of the elastase enzyme from Pseudomonas aeruginosa was done as per the protocol involving ammonium sulfate precipitation followed by ion exchange chromatography. The purity of the enzyme was determined by SDS gel electrophoresis.[10]

Assay of HNE and Elastase of P.aeruginosa using STANA as the substrate

To determine elastase activity the synthetic substrate STANA was used and the enzyme activity determined by the method of Bieth et al [11]The assay system comprised the purified enzyme, 200 mM of STANA in 200 mM of Tris Hcl buffer pH 8.0. The reaction was initiated by the addition of elastase enzymes. After 15 minutes of incubation at 37^{o}C, the reaction was stopped by the addition of 1.0 ml of 30% Acetic acid. The optical density of p-nitroaniline liberated was measured at 410 nm. The assay procedure was similar for both HNE and Elastase of P.aeruginosa. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µ mole of p-nitroaniline per unit time under standard assay conditions.

Procedure of inhibitory assay of P. aeruginosa elastase and HNE by α_1AT and α_2MG

Varying concentrations of the inhibitors α_1AT (0.5-4.0µg/ml) and α_2MG (4.0-20.0µg/ml) were preincubated with fixed concentration of P.aeruginosa elastase for 20 minutes. After 20 minutes preincubation, the reaction was initiated by 1ml of the substrate. It was then kept for incubation for 15 minutes at 37^{0}C. After this, the reaction was stopped by the addition of 1 ml of 30% Acetic Acid. The residual activity was determined by estimating the amount of p. nitroaniline liberated at 410nm.

Similarly, a fixed concentration of HNE was taken (concentration equivalent to 10U/ml). The same assay procedure was followed with varying concentration of the inhibitors ie. α_1AT (0.5-10.0µg/ml) and α_2MG (4.0-25.0µg/ml). The pre incubation period was 20 minutes and the reaction was initiated by addition of the substrate STANA. After stopping the reaction by the addition of 1 ml 0f 30% Acetic acid, the residual activity was calculated by measuring the p-nitroaniline released at 410nm. Suitable controls were also run without the inhibitor

The inhibitory activity was determined by the difference between activity of the bacterial elastase, HNE without inhibitor and then the residual activity of the same enzyme solution after adding the inhibitor. The percentage

of inhibition was calculated using Inhibition (%) = [1-(B/A)] X100 where A is the activity of the enzymes without inhibitor and B is the activity in presence of inhibitor .IC_{50} was determined for both elastase of P. aeruginosa and HNE with varying concentrations of the inhibitor required to bring about 50% inhibition and further to achieve 100% inhibition.

Results

The IC_{50} was calculated by performing the enzyme assay with varying concentrations of the inhibitors (α_1AT and α_2MG) and from the residual activity of both HNE and P.aeruginosa. The $IC_{50}$for HNE against α1AT was 1.8 µg/ml while for Elastase of P. aeruginosa the value was 4.1 µg/ml. Complete inhibition of HNE was brought about at a concentration of 4.5 µg/ml while 10.0µg/ml was required for complete inhibition of elastase of P. aeruginosa. Similarly, the IC_{50} value for HNE against α_2MG was 10.8 µg/ml which was marginally lower than the value observed for that of P.aeruginosa elastase which was 12 µg/ml. Complete inhibition of HNE was brought about by 22µg/ml while 24µg/ml was required for complete inhibition of P.aeruginosa elastase. Linearity of inhibition was observed upto 90% of the inhibition in both the cases. The IC_{50} values from α_1 AT and α_2 MG are represented in Table 1

Table 1. IC_{50} µg/ml of HNE and P. aeruginosa elastase with α_1AT and α_2MG

Enzyme	Inhibitor	IC_{50} µg/ml
HNE	α_1 AT	1.8
P. aeruginosa elastase	α_1 AT	4.1
HNE	α_2 MG	10.8
P. aeruginosa elastase	α_2 MG	12

Discussion

Elastase is a proteolytic enzyme capable of degrading elastin, a major protein in the basement membrane of various tissues. This enzyme is produced by various organisms and it has been studied extensively for its ability to act as a virulence factor in pathogenic organisms.[8] In view of this critical role, it attracts attention with regard to its role in the progression of tissue damage in infection. The present study was taken up to assess the comparative efficacy of α_1 AT and α_2 MG to inhibit the elastase of human and bacterial origin. This was aimed in order to know the probable differential effects of these enzymes on host tissue, as the inhibition of these enzymes are critical in controlling the tissue damage and progression of the disease.

This study was designed to arrive at IC_{50} value of α_1 AT and α_2 MG on HNE and P.aeruginosa elastase enzymes. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of molecules in inhibiting an enzyme and it is the simplest way to assess the efficacy of an inhibitor. The results obtained indicate that when faced with its target protease HNE, α_1AT inhibited HNE in preference to P.aeruginosa elastase thus performing its physiological role. Literature survey shows that the protease and the inhibitor form an irreversible complex which is physiologically destroyed [8]. α_1AT has been shown to have preferential inhibition on HNE.[8] However, not much was known about the comparative inhibitory action of α_2MG on HNE and P.aeruginosa.

Studies have shown that α_2MG a large spectrum protease inhibitor targets both endogenous and exogenous proteases and it is known to inactivate Pseudomonal proteolytic enzymes.[4,9] Recent studies have shown that P. aeruginosa synthesizes a structural homolog of human α_2 MG capable of binding to HNE.[12] This emphasizes the over powering role of P.aeruginosa elastase as a virulent factor. In a study by the same authors, on the differential inhibition of HNE and P.aeruginosa elastase by ethanol and aqueous extract of the leaves of Psidium guajava it was found that the ethanol leaf extract preferably inhibited bacterial elastase over that of HNE.[10] It is evident from the literature that pathogenic bacteria have inherent capacities to overcome the host resistance. Therefore, control or stoppage of devastating effects of P. aeruginosa elastase could only be effectively achieved by exogenous molecules.

Conclusion

α_1AT preferentially inhibits HNE over P. aeruginosa elastase as seen from the IC_{50} values for inhibition. α_2MG acted as a nonspecific inhibitor as the IC_{50} values were almost similar for human and bacterial elastase. These observations reiterate that endogenous protease inhibitors may not be very effective molecules in the stoppage of elastase enzyme of P. aeruginosa.

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