INHIBITION OF HUMAN LEUKOCYTE ELASTASE BY PEPTIDE CHLOROMETHYL KETONES

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1. Introduction

Recently, an elastolytic protease has been isolated from human polymorphonuclear (PMN) leukocyte granule fractions and purified by affinity chromatography [1]. This human leukocyte elastase is known to degrade human lung elastin, arterial walls and basement membrane, digest proteins of bacterial cell walls in vitro, induce cellular surface changes correlated with loss of growth control, and undergo inhibition by α_1-antitrypsin and peptide chloromethyl ketones [2]. A growing body of experimental evidence has suggested that this elastase may be involved in pathological processes associated with elastic tissue damage in pulmonary emphysema and acute arteritis, and with the tumor-producing action of cocarcinogenic substances [3]. Peptide chloromethyl ketones are well-known inhibitors of serine proteases which have proved quite useful for structural studies, including X-ray crystallographic investigations, of the active site regions of this group of enzymes [4]. A series of alanine tri- and tetrapeptide chloromethyl ketone inhibitors was synthesized by us and two other groups to probe the nature of the extended binding site and catalytic activity of porcine pancreatic elastase (EC 3.4.21.11) and related enzymes [5,6]. In this paper we report a study of the inhibition of human leukocyte elastase by a series of peptide chloromethyl ketones, which demonstrates that the human leukocyte enzyme is distinct from the porcine pancreatic enzyme. The results also define the structural features necessary for an effective inhibitor and may lead to a treatment for emphysema and related diseases.

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2. Materials and methods

Human leukocyte elastase was 85% pure as determined by a gel densitometry scan and contained one major and two minor elastase isoenzymes. Inhibition reactions were performed by rapidly mixing elastase and inhibitor solutions in 0.1 M phosphate, pH 6.5, at 25°C, and allowing the reaction to proceed through at least two half-lives. Final concentrations were: elastase, 10 μM; tripeptide inhibitor, 1 mM (or tetrapeptide inhibitor, 0.2 mM); $CaCl_2$, 0.1 mM; methanol, 5% v/v; in a total volume of 0.31 ml. These conditions were chosen to minimize the enzyme consumed and to facilitate kinetic measurements. Enzyme assays were periodically performed on aliquots containing 12.5 μg elastase using the BOC-Ala-ONp spectrophotometric assay method at 345 nm [7]. Kinetic results for each inhibitor were calculated from the equation

$$V_{\text{inhibition}} = k_{\text{obsd}}(\text{E}) = k_{2\text{nd}}\,(\text{I})\,(\text{E})$$

with the aid of a line-fitting computer program, and are presented in table 1. The second-order rate constant $k_{\text{obsd}}/(\text{I})$ is suitable only for purpose of approximate reactivity comparisons among the inhibitors, since $k_{\text{obsd}}/(\text{I})$ becomes non-linear with (I) when (I) becomes close to K_{I}, the dissociation constant of the enzyme–inhibitor complex [5]. Due to the small amount of enzyme available we were unable to carry out concentration-dependent inhibition studies to determine K_{I} values. Slight problems of reproducibility arose from a lack of enough enzyme for duplicating all inhibition runs, but the correlation coefficients for all runs were greater than 0.99.

Table 1
Inhibition of human leukocyte elastase with peptide chloromethyl ketones

Inhibitor	(I)	$10^4 \cdot k_{obsd}$	$10 \cdot k_{obsd}/(I)$	$k_{obsd}/(I)$	$k_{obsd}/(I)$*
P_4 P_3 P_2 P_1	(mM)	(sec^{-1})	($M^{-1} sec^{-1}$)	(rel.)	(rel.)
Ac–Ala–Ala–$AlaCH_2Cl$	1.0	2.8**	2.8	1.0	1.0
Ac–Ala–Pro–$AlaCH_2Cl$	1.0	10.9	10.9	4.0	1.6
Ac–Pro–Ala–$AlaCH_2Cl$	1.0	0	0	0	0
Z–Gly–Leu–$AlaCH_2Cl$	1.0	20.2	20.2	7.3	1.9
Z–Gly–Leu–$PheCH_2Cl$	1.0	0	0	0	–
Ac–Ala–Ala–Ala–$AlaCH_2Cl$	0.2	2.0**	9.7	3.5	18
Ac–Ala–Ala–Pro–$AlaCH_2Cl$	0.2	8.7**	43.6	15.8	71
Ac–Ala–Pro–Ala–$AlaCH_2Cl$	0.2	4.4	22.2	8.1	0
Ac–Ala–Ala–Phe–$AlaCH_2Cl$	0.2	2.6	13.2	4.8	16
Ac–Phe–Gly–Ala–$LeuCH_2Cl$	0.2	1.2	6.0	2.2	–

* Data for porcine pancreatic elastase [5].
** Average of two runs.
Elastase concentration 10 μM, at pH 6.5, 25°C, 5% methanol.

3. Results and discussion

A comparison of the effectiveness of the peptide chloromethyl ketone inhibitors, based on their relative $k_{obsd}/(I)$ values, leads to several interesting relationships between the structure of inhibitors and reactivity toward human leukocyte elastase. The tetrapeptides were usually observed to be faster inhibitors than the tripeptides and were run at 5-fold lower inhibitor concentrations (I). Ac–Ala–Ala–Pro–$AlaCH_2Cl$ is the best leukocyte elastase inhibitor of the series. In general the tetrapeptides are about 3 to 4 times more reactive than the tripeptides, which is evidence for the inhibitors' interaction with an extended binding site in leukocyte elastase. The P_1 residue [8] is alanine in almost all of the inhibitors, e.g. Ac–Ala–Ala–Ala–*Ala*CH_2Cl, corresponding to the substrate specificity of elastase for alanine and other amino acids with small side chains. If P_1 is phenylalanine, as in the chymotrypsin inhibitor Z–Gly–Leu–*Phe*CH_2Cl, no leukocyte elastase inhibition is observed, whereas if P_1 is leucine, as in the potent subtilisin inhibitor Ac–Phe–Gly–Ala–*Leu*CH_2Cl, a small rate of elastase inhibition takes place. In the P_2 position, proline appears to be favored over alanine: $k_{obsd}/(I)$ for Ac–Ala–Ala–*Pro*–$AlaCH_2Cl$ is 4 to 5 times larger than for Ac–Ala–Ala–*Ala*–CH_2Cl, and this also holds true for the analogous tripeptide pair. A leucine in P_2 is also favorable as in Z–Gly–*Leu*–$AlaCH_2Cl$, but a phenylalanine in P_2 is little better than alanine: compare Ac–Ala–Ala–*Phe*–$AlaCH_2Cl$ with Ac–Ala–Ala–*Ala*–$AlaCH_2Cl$. Thus human leukocyte elastase may possibly exhibit a weak secondary specificity for inhibitors with P_2 residues possessing medium-sized alkyl side chains (4). If the P_3 residue is proline as in the tripeptide inhibitor Ac–*Pro*–Ala–Ala–CH_2Cl, no inhibition of leukocyte elastase is observed. But if P_3 is proline as in the tetrapeptide inhibitor Ac–Ala–*Pro*–Ala–Ala–CH_2Cl, elastase inhibition does occur. Ac–Ala–*Pro*–Ala–Ala–CH_2Cl is a non-inhibitor toward porcine pancreatic elastase [5]. In the P_4 position a large hydrophobic group, e.g. phenyl, appears to be beneficial although further studies will be necessary for verification. This could explain the higher than expected activity of *Z*–Gly–Leu–$AlaCH_2Cl$ and Ac–*Phe*–Gly–Ala–$LeuCH_2Cl$, both of which possess a benzyl side chain at P_4, toward leukocyte elastase. For optimum reactivity, a leukocyte elastase inhibitor should contain a P_1 alanine, a P_2 proline or leucine, no P_3 proline (in the case of tripeptides), and possibly a P_4 phenylalanine.

At this point, it is of interest to examine certain inhibition characteristics of human leukocyte elastase as compared to the more thoroughly studied porcine pancreatic elastase [5]. First of all, leukocyte elastase reacts less rapidly overall with this group of peptide chloromethyl ketones than does pancreatic elastase, as indicated by smaller values of $k_{obsd}/(I)$ for inhibition. Secondly, the increase in reactivity toward inhi-

bitors in going from tripeptides to tetrapeptides is smaller in the case of leukocyte elastase, which may indicate differences in the geometry of the extended binding sites and the effect of the individual subsites on the catalytic activity of these two enzymes. Thirdly, the derived structure–reactivity relationships are generally parallel between the inhibitors and the two kinds of elastase, with regard to the structural features required for enhancement of elastase inhibition. Finally, Ac–Ala–Ala–Pro–AlaCH_2Cl is the most effective inhibitor tested for both human leukocyte and porcine pancreatic elastase. Interestingly, its isomer Ac–Ala–Pro–Ala–AlaCH_2Cl is a relatively good inhibitor for the leukocyte enzyme but is not effective to touch the pancreatic enzyme, which is strong evidence that the leukocyte and pancreatic enzymes are distinct entities.

In conclusion, this initial kinetic study of human leukocyte elastase clearly demonstrates the value of peptide chloromethyl ketones as active-site directed inhibitors for this enzyme in providing structure–reactivity correlations. At present a number of investigators have begun in vivo enzymatic studies with these inhibitors and they should prove useful for elucidating many of the molecular pathways by which human leukocyte elastase carries out its physiological and pathological functions.

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