The reaction of a series of azapeptides with porcine pancreatic (PP) elastase and human leukocyte (HL) elastase has been studied and a series of new inhibitors and active site titrants were found for both PP elastase and HL elastase. Azapeptide p-nitrophenyl esters acylate both HL and PP elastase to form stable acyl enzymes, which can be used for crystallographic studies. We have investigated the effect of a PI, P2, or P3 aza-amino acid residue on the reactivity of azapeptides with elastase. We have also studied the effect of changing the nature of the P1 leaving group and other portions of azapeptide structure. N-Acetyl-L-alanyl-L-alanyl-a-azanorleucine p-nitrophenyl ester, N-acetyl-L-alanyl-L-alanyl-a-azanorvaline p-nitrophenyl ester are suitable titrants for either PP or HL elastase.

HL1 elastase is suspected by many investigators to be the primary destructive agent in pulmonary emphysema (Cohen and Rossi, 1983 and other articles in this supplement). This enzyme is found in the granule fraction of human polymorphonuclear leukocytes and is a typical serine protease belonging to the same family as the more widely studied PP elastase. Since elastase inhibitors have considerable potential for use in therapy, synthetic inhibitors have been developed by ourselves and a number of other research groups (see Powers (1983) for a recent review). Some of these inhibitors have been shown to be effective in animal models of emphysema.

In the preceding paper (Gupton et al., 1984), we have shown that azapeptides are effective inhibitors of serine proteases, can be used as active site titrants, and can be used to generate stable acyl derivatives for crystallographic investigations. In this paper, we have extended the use of azapeptides to both HL and PP elastase and report a series of new inhibitors and active site titrants for both elastases. In addition, we have investigated the effect of a PI, P2, or P3 aza-amino acid residue on the reactivity of azapeptides with elastase. Finally, we have investigated the effect of changes in the nature of the P1 leaving group and other portions of the azapeptide structure. These investigations extend our knowledge of the active site of elastase and the types of molecules which will bind and react with it.

MATERIALS AND METHODS

Porcine pancreatic elastase was obtained from Whatman or Worthington and used without further purification; its substrate, Boc-Ala-ONp, was purchased from Sigma. Human neutrophil leukocyte elastase was generously provided by Drs. R. Baugh and J. Travis of the University of Georgia and by Drs. A. Janoff and G. Feinstein of S.U.N.Y. at Stony Brook.

Synthetic procedures and the synthesis of many of the azapeptides utilized in this paper are reported in the supplementary section to the preceding paper (Gupton et al., 1984). The remainder are reported in a supplementary section to this paper.

Reactions of Elastase with Azapeptides (Titration Procedure)—The reaction of porcine pancreatic and human leukocyte elastase with azapeptides was carried out in a solution which contained approximately a 50-fold excess of azapeptides over enzyme. Stock solutions of azapeptide in acetonitrile were prepared at a concentration of 0.5 mM. PP elastase stock solutions were made up in 1 mM HCl and had a concentration of ~10-14 M. HL elastase was usually obtained as solutions in a 20-50 mM, pH 5.0-6.0 phosphate or acetate buffer containing ~0.3-0.4 M NaCl. Exact enzyme concentrations were determined by absorbance at 280 nm (E280 = 20.2 for porcine pancreatic elastase (Shotton, 1970); E280 = 9.85 for human leukocyte elastase (Babul and Stellwagen, 1969)). Four buffers were prepared: pH 7.0, 0.1 M phosphate; pH 6.0, 0.1 M citrate; pH 5.0, 0.1 M citrate; pH 4.0, 0.1 M acetate. All reactions were performed at 25 °C. The reaction of elastase with azapeptides was carried out by adding 50 μl of azapeptide stock solution to 1.0 ml of buffer. Aliquots (500 μl) of this solution were added to the sample and reference microcells and a base-line was measured. The reaction was initiated by the addition of 25 μl of 1 mM HCl or water to the reference and 25 μl of enzyme stock solution to the sample cuvette.

The burst of p-nitrophenol was used to obtain the concentration of active enzyme (245 nm, ε = 8250 at pH 6.0). Assays were performed...
The titrations of PP and HL elastase were carried out at concentrations of 6.5-0.33 μM (92-5 μg in 550 μl) and 2.2-0.8 μM (31-11 μg in 550 μl), respectively. A concentration of 0.33 μM gives a ΔA of 0.002 which is not very reproducible with a noisy or low sensitivity spectrophotometer.

**Kinetic Parameters for the Elastase-catalyzed Hydrolysis of Azapeptides**—With those azapeptides that were substrates, enzymatic rate of hydrolysis was measured by adding 100 μl of the enzyme stock solution to a solution containing 2 ml of 0.1 M citrate buffer (pH 6.0) and 0.1 ml of azapeptide in acetonitrile. The increase in the absorbance at 540 nm was followed (εmax = 8260 at pH 6.0). The kinetic constants were determined from the initial rate of hydrolysis by the Lineweaver-Burk method and Cleland’s iterative fitting method (Cleland, 1967; 1979), and are based on duplicate rate determinations at six substrate concentrations. Correlation coefficients were greater than 0.98. The noncatalyzed hydrolysis rates of the azapeptides were measured similarly except that no enzyme was added.

**Inhibition of Human Leukocyte Elastase with Tripeptide Azapeptides and Related Compounds**—Inhibition of human leukocyte elastase by the azapeptide was carried out in solution which contained at least 10-fold excess of inhibitor over enzyme. Stock solutions of azapeptide in acetonitrile were prepared at a concentration of 0.07-14 mM. Enzyme stock solutions had a concentration of ~7 μM. The reactions were carried out by mixing 50 μl of inhibitor solution and 50 μl of enzyme solution with 1 ml of buffer; 100-μl aliquots were removed from the reaction mixture at regular time intervals and the residual enzymatic activity was measured using the Boc-Ala-ONp spectrophotometric assay (Visser and Blout, 1972). The final concentration of inhibitors and enzyme in the reaction mixture is shown in Table V.

**RESULTS**

The kinetics of the reaction of serine proteases with azapeptides is described in the preceding paper (Gupton et al., 1984). The background hydrolysis rates at pH 6.0 of representative azapeptide p-nitrophenyl esters are shown in Table VI. The hydrolysis rates for all of the azapeptides were quite similar except for two derivatives in which the P1, NH was substituted with a methylene group or replaced by a CH2 group. These two derivatives had much slower rates than the others.

**Reaction of Porcine Pancreatic Elastase with Azapeptides**—Table I lists the results obtained upon reaction of 10 azapeptides with PP elastase. All of the azapeptides except for Ac-Ala-Ala-Agly-ONp and Ac-Ala-Ala-MeAala-ONp acylated the enzyme very rapidly and no attempt was made to measure the acylation rates. In addition to the compounds listed in the table, we checked Ac-Aphe-ONp, Ac-Ala-Aphe-ONp, and Ac-Ala-Ala-ONp at pH 5.0, 5.8, and 6.5 and observed no acylation of the enzyme. In most cases where an azapeptide acylated elastase, the acylation rate was greater than 0.20 s⁻¹, our detection limit. However, Dr. Antonio Baici, Kantonsspital Zurich, Switzerland, has measured the acylation rate using a stopped flow apparatus. With HL elastase and Ac-Ala-Ala-Anva-ONp at pH 6.0, he observed a pseudo-first order rate constant of 0.51 s⁻¹ (half-life = 1.35 s).

**Hydrolysis of Triapeptide Analogues by PP Elastase and HL Elastase**—Table III shows the kinetic results obtained when each Ala residue of Ac-Ala-Ala-ONp is substituted with an aza-alanine residue in turn. Ac-Ala-Ala-Ala-ONp is a good substrate for HL elastase and substitution of the α-CH of either the P1, P2, or P3 residue with a nitrogen reduced the kcat/Km. The profound effect of the P1, Ala residue is reflected in both kcat and Km.

With PP elastase substitution of an Ala for Ala has a similar effect on kcat/Km values. Again, the P1, Ala had the most effect on kcat and Km, although there was less change in kcat/Km when compared with HL elastase.

We investigated the reactivity of Ac-Ala-Ala-NA with PP elastase and found it to be a very poor substrate. No burst was observed. If this compound had given a burst with elastase, we intended to synthesize the 7-amino-4-methylcoumarin derivative. This would have increased the sensitivity of elastase titrations since 7-amino-4-methylcoumarin can be detected by fluorescence. We also tried to synthesize the 4-methylumbelliferone derivative of Ac-Ala-Ala-Aaia for the same purpose, but found the compound to be too unstable toward hydrolysis.

**Inhibition of HL Elastase with Azapeptides**—Table IV shows the results of inhibition of several azapeptide esters with HL elastase. Both Ac-Ala-Ala-Anva-ONp and Ac-Ala-Ala-Anle-ONp acylated HL elastase and gave acyl-enzymes which turned over very slowly. Therefore, we investigated the corresponding derivatives with different leaving groups as potential inhibitors. All inhibited elastase much more slowly than the p-nitrophenyl esters. The azapeptides with the better...
leaving groups (lower pK<sub>a</sub> of the alcohol) had the higher <i>k<sub>on</sub></i> values. The order of reactivity was -ONp > -OPh > -OCH<sub>2</sub>CF<sub>3</sub> > -OEt with the ethyl esters showing no irreversible inhibition of the enzyme during the time course of our study. Preliminary studies indicate that both the phenyl and trifluoroethyl esters inhibited PP elastase at higher rates than they inhibited HL elastase. The last inhibitor in the table is one in which the NH of a P<sub>1</sub>Aala residue has been replaced by a methylene group and where the P<sub>2</sub> residue is Aala. This turned out to be an extremely poor inhibitor. Concentration dependence studies were carried out with the phenyl and trifluoroethyl esters and we found that <i>k<sub>on</sub></i>/[I] values were constant in the inhibitor range studied. This indicates that

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Reactivity of Azapeptides with Elastase

**Kinetic parameters for the hydrolysis of azapeptides by HL elastase and PP elastase**

| Peptide                  | pH | $K_M$ (mM) | $k_{cat}$ (s⁻¹) | $k_{cat}/K_M$ (M⁻¹ s⁻¹) |
|--------------------------|----|------------|----------------|-------------------------|
| Ac-Ala-Ala-Ala-ONp*       | 6.0| 0.66       | 83             | 1.3 x 10⁶               |
| Ac-Ala-Ala-Ala-ONp*       | 6.0| 0.023-0.230| 4.8            | 0.006-0.64              |
| Ac-Ala-Ala-ONp            | 6.0| 0.64       |                |                         |
| Ac-Ala-Ala-ONp            | 6.0| 0.01-0.128 | 32.4           |                         |
| Ac-Ala-Ala-OCH₂CF₃        | 6.0| 0.064-0.64 | 1.5            |                         |
| Ac-Ala-Ala-OCH₂CF₃        | 6.0| 0.64       |                |                         |
| Ac-Ala-Ala-ONp            | 6.0| 0.64       |                |                         |
| Ac-Ala-Ala-CH₂₅N(CH₃)CO-ONp| 6.5| 0.005      | 0.21           |                         |

* No inhibition in 1 h.
* pH 6.5, 0.1 M phosphate, 0.94 μM elastase.

**Table IV**

Inhibition of HL elastase by azapeptides

The conditions were: pH 6.0, 0.1 M citrate, 5% CH₃CN, 25 °C, 0.3 μM elastase, Boc-Ala-ONp as substrate.

| Inhibitor                | pH | $I/I_o$ | $k_{cat}/K_M$ (M⁻¹ s⁻¹) |
|--------------------------|----|---------|-------------------------|
| Ac-Ala-Ala-AnLe-OPh       | 6.0| 0.003-0.023| 343                    |
| Ac-Ala-Ala-OCH₂CF₃        | 6.0| 0.023-0.230| 4.8                    |
| Ac-Ala-Ala-OH            | 6.0| 0.64     |                         |
| Ac-Ala-Ala-ONp            | 6.0| 0.01-0.128| 32.4                   |
| Ac-Ala-Ala-OCH₂CF₃        | 6.0| 0.064-0.64| 1.5                    |
| Ac-Ala-Ala-OH            | 6.0| 0.64     |                         |
| Ac-Ala-Ala-CH₅N(CH₃)CO-ONp| 6.5| 0.005    | 0.21                   |

**Fig. 2**

The scheme proposed for the reaction of an azapeptide (RCO-Anle-L) with the active site of elastase. The azapeptide binds to the enzyme with the side chain (n-butyl) of the P₁ Anle residue interacting with the S₁ or primary substrate binding pocket of elastase. The acyl group is shown interacting with other portions of the extended substrate binding site of elastase. L represents a leaving group such as p-nitrophenol or trifluoromethane. The azapeptide acylates the active site serine forming a carbazyl enzyme (right).

Previously, we showed that the azapeptide p-nitrophenol ester Ac-Ala-Ala-Ala-ONp could be used as an active site titrant of PP elastase and that Z-Ala-Ala-Pro-Ala-ONp acylated both PP and HL elastase rapidly with the formation of a stable acyl-enzyme (Powers and Carroll, 1975; Powers and Gupton, 1977). In the preceding paper (Gupton et al., 1984), we studied the scope of the reactivity of azapeptides with chymotrypsin, subtilisin BNP⁺ and Carlsberg, and cathepsin G. In this paper, we have extended those studies to include PP and HL elastase.

The reaction scheme for an azapeptide with elastase is shown in Fig. 2. The azapeptide binds to the active site of elastase and then acylates the enzyme to form the acyl (carbazyl)-enzyme shown. At this stage, a certain degree of substrate recognition is required. Neither elastase was acylated by the Acpe-containing peptides Ac-APe-ONp and Ac-Ala-Ape-ONp nor by the short Ala-containing peptide Ac-Ala-Ala-ONp. This shows that both elastases recognize the side chain of the P₁ aza-amino acid residue and that an extended substrate structure is required for binding to elastase. The requirement of elastase for an extended peptide chain for recognition and binding is well known and has previously been observed with both substrates and chloromethyl ketone inhibitors. For example, dipeptide chloromethyl ketone inhibited PP elastase much more slowly than tripeptide or tetrapeptide chloromethyl ketones (Powers et al., 1977).

The NH group of the P₁ aza-amino acid residue is essential for acylation of elastase to occur. If this is substituted with a methyl group (Ac-Ala-Ala-MeAala-ONp), no reaction with either elastase occurs. In addition, replacement of this NH with a CH₂ gives a very poor inhibitor of HL elastase (Table IV). In the binding of substrates and inhibitors to serine proteases, the NH of the P₁ residue forms a hydrogen bond with the backbone peptide bond carbonyl of residue 214. In the case of PP elastase, this has been observed crystallographically in the binding of a trifluoroacetyl dipeptide anilide to elastase, although in that case the peptide is binding in a reverse direction compared to normal substrates and most other inhibitors (Hughes et al., 1989).

**Stable Acyl-enzymes**—In the previous paper, we discussed the electronic and steric effects that occur when an aza-amino acid residue is substituted for an amino acid in the P₁ site of a substrate. This substitution has a profound effect on the deacylation rate. In the case of PP elastase, the $k_{cat}$ at pH 6.0 decreases by 11,000 upon going from Ac-Ala-Ala-Ala-ONp to Ac-Ala-Ala-Ala-ONp. In the case of HL elastase, a 17,000-fold decrease is observed. Azapeptides with P₁ aza-amino acid residue give stable acyl derivatives with both elastases.

Considerable substrate specificity was exhibited in the deacylation step. At pH 6.0 in the series of azapeptides Ac-Ala-Ala-Ala-ONp, the deacylation rate ($k_{cat}$) varied by 37-fold with PP elastase and over 1900-fold with HL elastase as the P₁ aza-amino acid residue was changed. The order of reactivity with PP elastase was Val > Ala > Aaa > Anva > Aile > Anle, with Ac-Ala-Ala-Anle forming the most stable acyl-enzyme with PP elastase. The following preference of PP elastase for substrates has been observed: polyglycines, Ile > Val > Ala > Leu (Powers et al., 1977); tetrapeptide 4-nitroanilide substrates, Ala > Leu > Val, Ile no hydrolysis (Zimmerman and Ashe, 1977); $k_{cat}$ values for Boc-Ala-Ala-AAA-SBzil hydrolysis, Leu > Nva > Nle > Ala > Val > Ile. The order of reactivity with HL elastase was Val > Aile > Aaa > Anva > Aile > Anle. The following preference of HL elastase for substrates has been observed: polyglycines, Ile > Val > Ala > Leu (Zimmerman and Ashe, 1977); $k_{cat}$ values for Boc-Ala-Ala-AAA-SBzil hydrolysis, Ala > Leu > Nva = Nle > Val > Ile.⁶ When we examine these preferences carefully, we detect an inversion in the general order of

⁶ R. R. Cook, J. W. Harper, and J. C. Powers, unpublished results.
reactivity. When a particular P₁ amino acid residue yields a more reactive substrate, the corresponding aza-amino acid residue in a peptide has a slower deacylation rate (kₐ₋ₑ) and forms a more stable acyl-envelope and vice versa.

Electronic effects must make a relatively minor contribution to the deacylation rates since the hydrolysis rates at pH 6.0 for the two peptides Ac-Ala-Ala-Ala-ONp and Ac-Ala-Ala-Ala-Anle-ONp are very similar. These hydrolysis rates, to a first approximation, can be considered to be measures of the relative susceptibility of an aza-amino acid and an amino acid residue to nucleophilic attack. Since electronic effects have little effect on the deacylation rates, we believe that the major effect is steric. As we discussed in the preceding paper, substitution of a N atom for the α-CH in the P₁, amino acid residue will result in a change in the geometry of the azapeptide carbonyl group relative to the ideal geometry for the deacylation reaction. This would especially be the case when a well defined interaction took place between the side chain of the P₁ aza-amino acid residue and the S₁ pocket of the serine protease. We would expect the P₁ amino acid residues in the best substrates to have the strongest interaction with the S₁ pocket of elastase. This would twist the carbonyl group of the corresponding azapeptide out of a good alignment for deacylation and the most stable acyl derivatives would thus be formed. This in general was what we observed.

The deacylation rates observed with both elastases were considerably faster than those observed with chymotrypsin and cathepsin G. We believe this is caused by a strong P₁-S₁ interaction in chymotrypsin and cathepsin G which prevents the carbonyl group of the P₁ aza-amino acid from occupying the conformation necessary for deacylation. Since the S₁ binding pocket in elastase is not as deep and the side chains of good elastase substrates are not as long as they are in the case with chymotrypsin and cathepsin G and their substrates, the S₁-P₁ interaction is less effective at preventing the P₁ aza-amino acid from occasionally occupying a suitable conformation for deacylation. Thus, the deacylation rates are higher with elastase. When comparing the two elastases, HL elastase forms more stable acyl-enzymes than does PP elastase. Again this is consistent with the fact that HL elastase has a larger S₁ binding pocket as evidenced by its acceptance of substrates with longer P₁ alkyl chains than PP elastase. In comparison to the subtilisin, the elastase deacylations were in the same range. However, the most stable derivatives at pH 6.0 formed from the elastases were more stable than the most stable subtilisin acyl-enzymes.

Recently, a series of heterocyclic derivatives such as N-acetyl saccharins and N-arylbenzothiazolinone 2,1,1-dioxides have been reported to be acylating inhibitors of HL and PP elastase, cathepsin G, and chymotrypsin (Zimmerman et al., 1980; Ashe et al., 1981). These heterocyclic compounds form acyl-enzymes which have considerable variability in stability. Seemingly small changes in structure can have considerable influence on the deacylation rates. We believe this is another example where the directionality and strength of the P₁-S₁ interaction impart a twist to the acyl carbonyl and determine the ease with which the various derivatives deacylate.

**Active Site Titration—Azapeptide p-nitrophenyl esters** are extremely useful for active site titration of both HL and PP elastase. Standardized elastase solutions are essential for measuring the stoichiometry of interaction of elastase with its natural protein inhibitors such as α₁-protease inhibitor, for studying the interaction of elastase with its natural substrate elastin and other connective tissue proteins, and for calculating accurate kₐ₋ₑ values for synthetic peptide substrates.

Prior to the development of azapeptide p-nitrophenyl esters, the only reported titrants for elastase were nonspecific inhibitors such as diethyl p-nitrophenyl phosphate (Bender et al., 1966) and radiolabeled diisopropyl phosphofluoridate. These compounds are characterized by low specificity and react with numerous other serine proteases. The p-nitrophenyl ester acylates elastase slowly and must be used at high pH values where the background hydrolysis of the titrant makes accurate determination of a burst difficult. In contrast, many of the azapeptide p-nitrophenyl esters which we have studied acylate elastase within the time interval required for mixing the solution and placing the cell into the spectrometer (>90% complete in 9 s, k₂ > 0.2 s⁻¹) and can be used at pH values from 4–7 where turnover of the titrant does not interfere with the titration results.

Several of the azapeptide p-nitrophenyl esters make suitable titrants for either PP or HL elastase. In our laboratory, we have frequently used Ac-Ala-Ala-Ala-ONp, Ac-Ala-Ala-Ala-Anle-ONp, and Ac-Ala-Ala-Ala-Anva-ONp for such titrations. Ac-Ala-Ala-Ala-Anle-ONp does not react with cathepsin G at pH 6.0, nor with chymotrypsin at pH 4.0 and 5.0, although it does react at pH 6.0 and 7.0. If specificity is important or if the elastase contains traces of contaminating serine proteases then this would be the titrant of choice. However, we have tended to use Ac-Ala-Ala-Anle-ONp or Ac-Ala-Ala-Anva-ONp more frequently. These two azapeptides are in the group with the lowest deacylation rates with both HL and PP elastase, are easier to synthesize in large amounts than the Aala derivative, and can also be used to titrate cathepsin G. Most titrations are carried out at pH 6.0 to minimize background hydrolysis, although this effect is cancelled by having titrant in both the sample and the reference cells and titrations can be carried out over a much wider pH range without difficulty. To minimize the use of precious enzymes such as HL elastase, we recommend the use of microcells. All three azapeptides have now been used in the laboratories of several other independent investigators with satisfactory results.

**Azapeptides As Inhibitors** — Those azapeptides which form stable acyl-enzymes can be considered to be good inhibitors of elastase. In the case of PP elastase, none of the azapeptide p-nitrophenyl esters formed acyl-enzymes which had any significant life at pH 6.0 or 7.0, although Ac-Ala-Ala-Anle-ONp formed the most stable derivative. In the case of HL elastase, this same compound formed an acyl-enzyme which appears to be stable at pH 6.0 and 7.0. In addition, Ac-Ala-Ala-Ala-Anle-ONp formed a stable derivative with HL elastase at pH 6.0. One disadvantage with p-nitrophenyl esters is their instability to hydrolysis and the possibility that they are too reactive as acylating agents. Therefore, we tested azapeptides with less reactive leaving groups (Table IV). Azapeptide phenyl or trifluoroethyl esters of Ac-Ala-Ala-Anva- or Ac-Ala-Ala-Ala- were inhibitors of HL elastase, although the trifluoroethyl esters were quite poor. Ethyl esters did not inhibit irreversibly at all. They were not tested as reversible inhibitors and it is likely that they would bind competitively to HL elastase (Dorn et al., 1977). The prospects for using azapeptides in vivo for the treatment of emphysema are probably only fair due to their hydrolytic instability and the possibility of acylating other nucleophilic groups in proteins.

**Conclusion** — Azapeptides have been shown to be extremely useful reagents for the study of elastase and other serine proteases. Azapeptide p-nitrophenyl esters acylate both HL and PP elastase to form stable acyl-enzymes. Some of these derivatives appear to have sufficient stability for crystallographic studies, especially at low temperature. Although relatively little has appeared to date, there are currently active
investigations on the structures of small molecule binding to PP elastase and a number of attempts are being made to obtain crystals of HL elastase. Azapeptide derivatives would be worth considering for such studies.

Substitution of an aza-amino acid residue into the structure of a peptide would be an excellent way of stabilizing the peptide toward protease digestion in vivo (Dutta and Giles, 1976) with the possibility of only slight changes in its interaction with its enzyme or receptor. For example, the $k_{cat}/K_m$ for the PP elastase-catalyzed hydrolysis of Ac-Ala-Ala-Ala-NA (1.7 M$^{-1}$ s$^{-1}$) is at least 700-1000-fold lower than that for Ac-Ala-Ala-Ala-NA ($k_{cat}/K_m = 745-2160$ M$^{-1}$ s$^{-1}$ at pH 8.0 depending on the organic solvent and buffer used (Bieth and Wermuth, 1973)), while there is almost no change in $K_M$ (6.6 mM (Kasafirek et al., 1974)), the major effect being in $k_{cat}$.

The results in Table III also show that substitution of an aza-amino acid residue in the P$_2$ or P$_3$ site of a substrate substantially reduces $k_{cat}/K_M$. Of course, the effects are even more pronounced at P$_1$, but it appears that substitution in regions of peptides adjacent to cleaved bonds can be an effective way of reducing the proteolysis of peptides in vivo.

Finally, azapeptides can be used as active site titrants and inhibitors for elastase and other serine proteases.

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**Section for Reaction of Azapeptides with Human Lysosomal Elastase and Porcine Pancreatic Elastase**

**A. Reaction of Azapeptides with Human Lysosomal Elastase**

| Peptide                  | Reaction Rate (U/mg) |
|--------------------------|----------------------|
| Ac-Ala-Ala-OH          | 6.0                  |
| Ac-Ala-Ala-OH             | 6.0                  |
| Ac-Ala-Ala-Ala-OH       | 6.0                  |
| Ac-Ala-Ala-Ala-Ala-OH    | 6.0                  |
| Ac-Ala-Ala-Ala-Ala-Ala-OH | 6.0                  |

**B. Reaction of Azapeptides with Porcine Pancreatic Elastase**

| Peptide                  | Reaction Rate (U/mg) |
|--------------------------|----------------------|
| Ac-Ala-Ala-OH          | 6.0                  |
| Ac-Ala-Ala-OH             | 6.0                  |
| Ac-Ala-Ala-Ala-OH       | 6.0                  |
| Ac-Ala-Ala-Ala-Ala-OH    | 6.0                  |
| Ac-Ala-Ala-Ala-Ala-Ala-OH | 6.0                  |

**Notes:**
- Reaction rates are given in U/mg, where U represents unit activity.
- The peptides listed are representative of the azapeptides used in this study.
- The rates are indicative of the enzymatic activity of human lysosomal elastase and porcine pancreatic elastase.

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