ENZYMATIC SYNTHESIS INVOLVING CHYMOTRYPSIN

by
Lynda Jun-San Yang
University Undergraduate Fellow, 1986-1987

Department of Chemistry
Texas A&M University

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Fellows Advisor: Chi Huey Wong Date: 4/20/87

Honors Director: [signature] Date: May, 1987

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ABSTRACT

Protease-catalyzed peptide synthesis, involving α-chymotrypsin as catalyst and unusual amino acids as acyl acceptors, has been studied. The irreversibility of peptide bonds formed by this method has been explained by employing kinetic studies accompanied by computer-aided molecular modelling of the enzyme's active site. Furthermore, the variety of peptides that may be synthesized by this technique has been increased by utilizing unusual amino acids as acyl donors as well as acceptors. Because of the instablity of the native enzyme under the conditions of synthesis, preliminary studies on an alkali-stable $Met(O)_{192}$-chymotrypsin have also been undertaken.

INTRODUCTION

In the synthesis of peptides, it has been recognized that enzymes are able to catalyze regio- and stereospecific reactions with an incredible enhancement in rate. A number of enzymes have been shown to be potentially useful synthetic catalysts.[1] However, the existence of technical problems may have limited the development and popular use of this technique. An enzyme, by nature, exists in physiological conditions; therefore, in order to perform at maximum efficiency, it must be surrounded by aqueous conditions at physiological pH and temperature and offered the substrate for which it is specific. Consequently, enzymes are often too unstable and expensive to be exploited as a practical catalyst. However, the advantages of utilizing proteases in catalyzing the synthesis of peptides containing D-amino acid residues seem to outweigh the disadvantages.

Some advantages, common to all protease catalyzed synthesis, may be detected by comparing enzymatic synthesis to chemical synthesis of peptides. Although chemical methods for the synthesis of peptides have been well developed, the benefits of using the enzymatic technique include (a) general freedom from racemization, (b) less need for the protection and deprotection of functional groups because of protease specificity, and (c) immobilization of the enzyme allows for the regeneration of the catalyst thereby providing an economic advantage over chemical, stoichiometric methods. However, advantage (b) can be a limiting factor

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[1] Whitesides, G.M.; Wong, C.-H., *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 617 and references therein. Fruton, J. S. *Adv. Enzym.* **1982**, *53*, 239. Chaiken, I. M.; Komoriyaa, A.; Ohno, M.; Widmer, F. *Appl. Biochem. Biotech.* **1982**, *7*, 385. Homandberg, G.; Mattis, J,; Laskowski, M., Jr. *Biochemistry* **1978**, *17* , 5220. Glass, J. D. *Enzyme Microb. Technol.* **1981**, *3*, 2. Inouye, K.; Watanabe, K.; Marihara, K.; Tochino, Y.; Kanaya, T.; Emura, J.; Sakakibara, S. *J. Am. Chem. Soc.* **1979**, *101*, 751. Wong, C.-H.; Chen, S.T.; Wang, K. T. *Biochim. Biophys. Acta* **1979**, *576*, 247. Jakubke, H.-D.; Kuhl, P.; Konnecke, A. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 85. Widmer, F.; Bayne, S.; Houen, G.; Moss, B.A.; Rigby, R. D.; Whittaker, R. G.; Johansen, J. T. In U. Ragnarsson: *Peptides 1984*; Ragnarsson, U., Ed. Almquist and Wiksell: Stockholm, 1984; p 193. West, J. B.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1986**, 417.

because the protease specificity tends to limit the types of peptides that can be synthesized by the protease.

Several reasons for the interest in D-amino acid containing peptides are also worthy of mention. A number of antibiotic peptides,[2] synthetic peptides enhancing neural or hormonal activity[3], and many prodrugs used in chemotherapy[4] contain D-amino acid residues. A possible route to these desired peptides involves recombinant DNA technology; however, this technique is limited to the production of peptides containing L-amino acids.[5] Therefore, enzymatic methods to attain these peptides have been explored, and indeed, it has been shown that α–chymotrypsin is a reasonbly efficient catalyst in the irreversible formation of peptides containing D-amino acids.[6]

The history of α–chymotrypsin as catalyst in synthetic systems is relatively recent due to a report in 1977 that cited α-chymotrypsin to be a poor catalyst in the synthesis of dipeptides using D-Leu-NH_2 as the nucleophile.[7] Consequently, until 1984, when Petkov and others illustrated the practicality of the system in a

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[2] Lipmann, F. *Acc. Chem. Res.* **1973**, *6*, 361. Akers, H. A.; Lee, S. G.; Lipmann, F. *Biochemistry* **1977**, *16*, 5722. Griesbach, H. *Adv. Carbohydr. Chem. Biochem.* **1978**, *35*, 80. Hash, J. H. *Methods Enzymol.* **1975**, *42*. Loennechen, T.; Bergan, T.; Sydnes, L. K.;Aasen, A. J. *Acta Chem. Scand., Ser. A* **1984**, *38*, 647. Abbot, B. J. *Adv. Appl. Microbiol.* **1978**, *24*, 187. Baxter, R. L.; Thomson, G. A.; Scott, A. I. *J. Chem. Soc., Chem. Commun.* **1984**, 32. Wolfe, S.; Demain, A. L.; Jensen, S. E.; Westlake, D. W. S. *Science (Washington, D. C.)* **1984**, *226*, 1386. Shoji, *J. Adv. Appl. Microb.* **1978**, *24*, 187. Roberts, S. M. *Chem. Ind. (London)* **1984**, 162. Baldwin, J. E.; Adlington, L. M.; Turner, N. J.; Domayne-Hayman, B. P.; Ting, H. H.; Derome, A. E.; Murphy, J. A. *J. Chem. Soc., Chem. Commun.* **1984**, *26*, 1146.

[3] Morley,m J. S. *Annu. Rev. Pharmacol. Toxicol.* **1980**, *20*, 81. Iversen, L. L. *Ibid.* **1983**, *23*,1. Sawyer, T. K.; Sanfilippo, P. J.; Hruby, V. J.; Engel, M. H.; Heward, C. B.; Burnett, J. B.; Hadley, M. E. *Proc. Natl. Acad. Sci. U. S. A.* **1980**, *77*, 5754. Mauer, R.; Gaehwiler, B. H.; Buescher, H. H.; Hill, R. C.; Roemer, D. *Ibid.*, **1982**, *79*, 4815. Humphries, J.; Wan, Y. P.; Folkers, K. *J. Med. Chem.* **1978**, *21*, 120. Sawyer, T. *Ibid.* **1982**, *25*, 1022. Folkers, K.; Horig, J.; Rosell, S.; Bjorkroth, U. *Acta Physiol. Scand.* **1981**, *111*, 505. Yajima, H. *Chem. Pharm. Bull.* **1980**, *28*, 1935. Gacel, G.; Fournie-Zaluski, M. C.; Rogues, B. P. *FEBS Lett.* **1980**, *118*, 245. Monahan, M. W.; Amoss, M. S.; Anderson, H. A.; Vale, W. *Biochemistry* **1973**, *12*, 4616. Coy, D. H.; Vilchez-Martinez, J.A.; Coy, E. J.; Schally, A. V. *J. Med. Chem.* **1976**, *19*, 423. Pert, A.; Chang, J. K.; Fong, B. T. W. *Science (Washington, D. C.)* **1976**, *194*, 330. Sasaki, Y.; Matsui, M.; Taguchi, M. Suzuki, K.; Sakurada, S.; Sato, T.; Sakurada, T.; Kisara, K. *Biochem, Biophys. Res. Commun.* **1984**, *120*, 214. Manavlan, P.; Momany, F. A. *Ibid.* **1982**, *105*, 847. Blank, J. P.;Kaiser, E. T. *J. Biol. Chem.* **1984**, *259*, 9549.

[4] Chakravarty, P. K.; Carl, P. L.; Weber, M. J.; Katzenellenbogen, J. A. *J. Med. Chem.* **1983**, 26, 638.

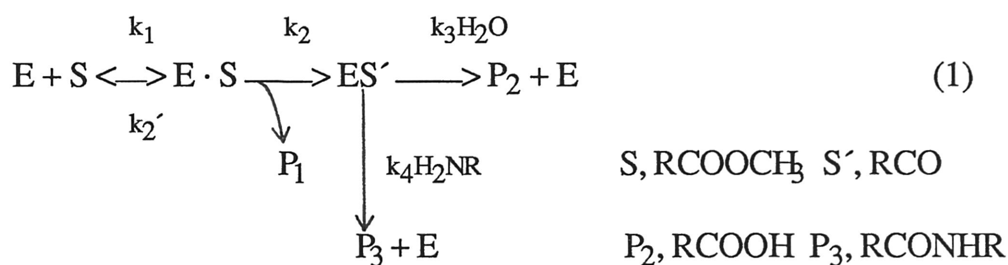
[5] For a general review, see: *Science (Washington, D. C.)* **1980**, *209*, 4463; **1983**, *219*, 4585; **1985**, *229*, 1193.

[6] West, J. B.; Wong, C.-H. *J. Org. Chem.* **1986**, *51*, 2728.

[7] Morihara, K.; Oka, T. *Biochem. J.* **1977**, *163*, 531.

kinetically controlled approach, α-chymotrypsin was not used for preparative synthesis; however, the reaction was slow, and deactivation of the enzyme occurred quickly under the conditions used. Since then, the generality and optimization of this type of unusual catalysis has been investigated.[8]

Improvement of the reaction rate using a D-amino acid ester as nucleophile can be achieved by utilizing the kinetically controlled approach to peptide synthesis.[9] In our previous syntheses, the acyl donor to the peptide bond was the ester, Z-L-Tyr-OMe. Since the rate-determining step in the reaction of an ester with an enzyme is the deacylation of the acyl-enzyme complex, the use of an ester as the acyl donor facilitates the formation of the acylenzyme. Deacylation of the acylenzyme involves a nucleophilic attack on the appropriate atom; if the nucleophile is water, the corresponding acid of the acyl donor is formed (eq 1).[10] However, if the nucleophile is another amino acid, a dipeptide is formed; therefore,



synthetic efficiency must parallel hydrolytic efficiency. Since the synthesis is kinetically controlled, secondary hydrolysis of the newly formed peptide can be

[8] Reference 6.

[9] Kullman, W. *J. Biol. Chem.* **1980**, *255*, 8234.

[10] Fastrez, J.; Fersht, A. R. *Biochemistry* **1973**, *12*, 2025.

prevented by rapidly quenching the reaction. When D-amino acids are incorporated into the C-terminal end of the newly formed dipeptide, hydrolysis of the peptide bond occurs slowly, if at all[11]; consequently, peptide synthesis becomes virtually irreversible.

In order to understand the enzyme catalyzed incorporation of D-amino acids into peptides, we have utilized computer-assisted molecular modelling and investigated the kinetics of synthetic reactions catalyzed by α–chymotrypsin. We have also extended the types of peptides that can be synthesized by α-chymotrypsin by using D-amino acids as both acyl donors and acceptors, since we have previously reported that nucleophiles differing from those of known P1′ specificity can also be used in synthesis[12]. Since the native enzyme is unstable under the alkaline conditions necessary to obtain maximum efficiency in synthesis, we have begun studies on an alkali-stable chymotrypsin with Met_{192} being modified to Met sulfoxide.

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[11] Morihara, K.; Oka, T.; Tasuzuki, H. *Biochem. Biophys. Res. Commun.* **1969**, *35*, 210. Morihara, K.; Oka T. *Arch. Biochem. Biophys.* **1977**, *178*, 188.

[12] Reference 6.

RESULTS AND DISCUSSION

Determination of K_I for Z-L-Tyr-D-Met-OMe. The kinetic data are summarized in Table 1. Under both conditions, the D-Met containing peptide exhibited competitive inhibition against the substrate, with the K_I being approximately 20 to 30 times the K_M of the substrate. These numbers indicate that the binding of the dipeptide to the enzyme was much weaker than the binding of the substrate. In all cases, constants and inhibition type were determined via Lineweaver-Burk plots. Condition A is representative of the conditions of typical kinetic studies for this enzyme, whereas condition B mimics the synthetic conditions. Subsequently, computer-assisted molecular modelling was employed to present a mechanistic rationale of the observed irreversible bond formation when D-amino acids were used.

Figure 1 shows the active site of α-chymotrypsin, in which natural hydrolysis of peptides normally occurs, with the molecule of Z-Tyr-Met-OMe fitted in. In (a), the LL isomer of the dipeptide is pictured in the active site. Examination of the active site model developed through X-ray techniques provides justification for the hydrolysis of the enzyme-specific substrate.[13] Conversely, in (b) the LD isomer is positioned in the active site. Since the n region is quite flexible and can accomodate different groups, it was supposed that D-amino acid derivatives could enter this region and act as slow substrates; however, very little hydrolysis of the LD

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[13] Cohen, S. G. *Trans. N. Y. Acad. Sci.* **1969**, *31*, 705. Blow, D. M. *Acc. Chem Res.* **1976**, *9*, 145. Tsukada, H.; Blow, D. M. *J. Mol. Biol.* **1985**, *184*, 703. Jones, J. B.; Beck, J. F. In *Applications of Biochemical Systems in Organic Chemistry*; Jones, J. B., Sih, C. J., Perlman, D. Eds.; Wiley: New York, 1976; p 107.

dipeptide was observed.[14] As with the LL isomer of the dipeptide, the LD dipeptide posesses the large bulky aromatic group specific for the enzyme. However, with the LL-form, the bend in the substrate chain correctly positions the side chain of the methionine residue in the n region and brings the scissile NH-CO bond close to the His 57 and Ser 195 so that the proposed "charge relay system" can catalyze the hydrolysis. On the other hand, with the LD isomer, the bend in the substrate chain is opposite that of the LL isomer. This unusual bending propels the side chain of the methionine residue into the disulfide linkage (Cys 42 to Cys 58) bordering the n region. Consequently, because of steric reasons, the scissile NH-CO bond is prohibited from approaching the "charge relay system" and the peptide bond is left intact.

Determination of K_M for D-Met-OMe. Since it was shown that peptide synthesis is possible, it was hypothesized that D-Met must bind competitively in the P′ site. Therefore, classical kinetic studies were undertaken to confirm the hypothesis. Reactions were typically run at pH 9 to insure that the majority of the nucleophile was unprotonated, with the assumption that the charged species would bind less well to the enzyme (however, reactions at approximately pH 7 were also run). Reactions were run in both high (50%) and low (10%) organic cosolvent. Initial results, seemingly showing competitive inhibition at nucleophile concentrations of approximately 0.5M, showed no such inhibition when repeated under conditions of carefully controlled ionic strength. No significant peptide synthesis was found under the conditions of the reactions.

We propose three possible reasons for the lack of inhibition that D-Met-OMe offers to the hydrolysis of Z-Tyr-ONp: (1) there is little binding of the nucleophile; however, if this were true, it would be difficult to explain the different synthetic efficiencies of different amino acid nucleophiles. (2) The inhibition constant is

[14] Reference 6.

significantly higher than 0.5M. (3) The nucleophile does bind to the enzyme, but it binds in such a way that it does not significantly interfere with the binding of the specific substrate. Furthermore, it has been reported that L-Val-NH_2 offers no inhibition to the hydrolysis of Z-L-Phe-nitoanilide.[15]

Partitioning Ratio Analysis. Although we have detected no competitive inhibition by D-Met-OMe, it is difficult to rationalize a mechanism where the nucleophile has no interaction with the enzyme. If this were the case, no difference in the synthetic efficiency of the differing amino acid nucleophiles would be observed. An alternate approach for quantifying the affinity of the nucleophile for the enzyme has been developed by Jakubke and co-workers[16]; this kinetic model is similar to that of Kasche[17], who assumed nucleophilic binding to the enzyme. In their analysis, the acyl enzyme may be deacylated by water regardless of bound water. A schematic representation of the kinetic model is given (eq. 2).

$$\text{Ac-X} + \text{EH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\text{EH}\cdot\cdot\text{Ac-X}] \xrightarrow[\text{HX}]{k_2} \text{Ac-E} \underset{k_{-4}}{\overset{[\text{N}]k_4}{\rightleftharpoons}} [\text{Ac-E}\cdot\cdot\text{N}] \qquad (2)$$

$$\text{Ac-E} \xrightarrow{k_3'} \text{Ac-OH} + \text{EH} \qquad [\text{Ac-E}\cdot\cdot\text{N}] \xrightarrow{k_{3,N}'} \text{Ac-OH} + \text{EH} \qquad [\text{Ac-E}\cdot\cdot\text{N}] \xrightarrow{k_5} \text{Ac-N} + \text{EH}$$

An efficiency parameter, p, was defined by these workers (eq. 3) where [H]

15 Riechmann. L.; Kasche, V. *Biochem Biophys. Acta* **1985**, *830*, 164.
16 Schellenberger, V.; Jakubke, H.-D. *Biochem. Biophys. Acta* **1986**, *869*, 54.
17 Riechmann, L.; Kasche, V. *Biochem. Biophys. Res. Comm.* **1984**, *120*, 686.

$$[H]/[P]\ \{(k_3{'}k_{-4}/k_4 + k_{3,N}{'}[N])/k_5[N]\} = p/[N] \qquad (3)$$

represents the concentration of the hydrolysis product; [P], the concentration of the peptide poroduct; and [N], the concentration of the nucleophile. Equation 3 can be rewritten in the form of y = mx + b to facilitate analysis (eq. 4).

$$p = k_{3,N}{'}[N]/k_5 + k_3{'}k_{-4}/k_5k_4 \qquad (4)$$

Therefore, plotting p versus [N] will yield a straight line with a slope of $k_{3,N}{'}/k_5$ which describes the ratio of the formation of the hydrolysis product and the peptide product if the enzyme is saturated with the nucleophile. The y-intercept represents the affinity of the enzyme for the nucleophile.

We are currently applying this model in our inhibition studies involving D-Met-OMe as nucleophile.

Synthesis. The results of further enzymatic syntheses are summarized in Table 2. The effectiveness of nucleophiles ranged from amino-acetaldehyde dimethyl acetal to sarcosine methyl ester. It was observed that the replacement of the carboxyl ester functionality by the acetal has only a slight effect on the ability of the molecule to act as an acyl acceptor. Furthermore, peptides containing a-alanine in the P1´ position have been shown to bind the enzyme but resist cleavage.[18] It can be presumed that the unusual residue in the S1´ subsite prevents the peptide from positioning itself for hydrolysis. The systems employing 6-amino-caproic acid methyl ester and (D,L)-4-amino-3-hydroxy-butyric acid methyl ester as nucleophiles were studied. It was found that the former reaction required greater than six-fold excess of the acyl acceptor for moderate yield, but the peptide formation was irreversible for the time scale examined. The latter nucleophile was

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[18] Obara, M.; Karasaki, Y.; Ohno, M. *J. Biochem.* **1979**, *86*, 461.

somewhat better, but the peptide formed was susceptible to slow secondary hydrolysis.

Met(O)$_{192}$-chymotrypsin. In this study of unusual catalysis, examples investigated proved to be successful, although slow. The instablilty of the enzyme, under the conditions used to obtain maximum synthetic efficiency, led to the preliminary investigation of an alkali-stable chymotrypsin. The native enzyme is known to deactivate at high pH due to the deprotonation of the amino group of Ile 16, resulting in the destruction of the ionic bridge with Asp 194. The modified enzyme contains an oxidized methionine residue at position 192 (Met sulfoxide), and it has been reported that this derivative exists mostly in the active conformation up to pH 9. This derivative is easily prepared and purified by dialysis and by gel filtration on Biorad P-2 media. Although enzyme assays indicate that the modified enzyme is more alkali stable than the native enzyme (Figure 2), the presence of the organic cosolvent, DMSO, tends to reduce the efficiency of the enzyme (Figure 3). We are currently furthering our studies on the use of the Met(O)$_{192}$-chymotrypsin in peptide synthesis.

In summary, we have explained, using classical kinetics and molecular modelling, that the irreversibility of peptide bond formation is due to the incorrect positioning of the D-amino acid-containing peptide in the enzyme active site. We have also extended the types of peptides that may be synthesized by α–chymotrpysin with the usage of unusual amino acids as both acyl donors and acceptors. These peptides are useful intermediates for a variety of peptides with neural activities. Furthermore, preliminary studies on the alkali-stable Met(O)$_{192}$-chymotrypsin indicate that the modified enzyme may be a viable alternative to the native enzyme in certain synthetic systems.

EXPERIMENTAL SECTION

Kinetic Studies Involving Z-L-Tyr-D-Met-OMe as Inhibitor. Inhibition studies were done using Z-L-Tyr-ONp as substrate and monitoring the release of p-nitrophenol (ε 18.5 $mM^{-1}cm^{-1}$) with time by the increase of absorption at 405 nm. One assay buffer contained 0.2M Tris-HCl/dioxane (pH 6.8), 0.1M $CaCl_2$, and 10% dioxane. The other buffer contained 0.2M Tris-HCl (pH 9.0), 0.1M $CaCl_2$, and 60% DMSO. Substrate concentration was 2.4 mM Z-Tyr-ONp in dioxane. Typical assays contained 2.8 mL of buffer, 0.01 to 0.1 mL of substrate, various concentrations of inhibitor, and 0.02 mL of a1mg/mL enzyme solution in 0.001M HCl.

Molecular Modelling Studies. Computer-assisted molecular modelling was performed on a VAX 11/780 equipped with an Evans and Sutherland P330 graphics terminal. The program used was FRODO6.

Kinetic Studies Involving D-Met-OMe as Inhibitor. Inhibition studies were done using Z-L-Tyr-ONp and Z-L-Phe-nitroanalide as substrates and monitoring the release of p-nitrophenol (ε 18.5 $mM^{-1}cm^{-1}$) at 405 nm and nitroaniline (ε 22.65 $mM^{-1}cm^{-1}$) at 350 nm, respectively. A variety of assay buffers were used: (a) 0.1M Tris-Mal (pH 8.5), 0.1M $CaCl_2$, 10% dioxane; (b) 0.2M Tris-Mal (pH 6.8), 0.1M $CaCl_2$, 10% dioxane; (c) 0.2M Tris-HCl (pH 9), 0.02M $CaCl_2$, 60%DMSO; (d) 0.2M Tris-HCl (pH 7.5), 0.1 $CaCl_2$, 60% DMSO. Typical assay solution contained 2.8 mL buffer with substrate concentration of 0.01 to 0.1 mM and inhibitor concentrations of 0.1 mM to 500 mM. Generally, 0.02mL of a 1mg/mL enzyme solution was used.

Synthesis. The D-methionine, D-valine, L-tyrosine, glycine, 6-amino caproic acid, (D,L)-4-amino-3-hydeoxy butyric acid, amino-acetaldehyde dimethyl

acetal, (S-benzyl)-L-cysteine, N-Boc-(S-benzyl)-L-cysteine, N-Cbz-L-Tyrosine 4-nitrophenyl ester, N-Cbz-L-phenylalanine 4-nitroanilide, benzyl chloroformate, and phenyl-hydrazine were products of Aldrich and used as received. Picolinic acid, α–chymotrypsin (Type II, 3x recrystallized), and sarcosine were products of Sigma. D-Ala was a product of Chemical Dynamics.

Enzymatic peptide syntheses were carried out in DMSO/0.1M phosphate buffer (pH 8.0 before addition of organic cosolvent) (6/4, v/v). The pH was subsequently adjusted to 8.5 with 2N NaOH. Reactions were 4 x 10^{-8} M in chymotrypsin. For isolation, the reaction was quenched by the addition of ten volumes of MeOH, followed by centrifugation, removal of solvent in vacuo, and recrystallization in ethanol/water.

Preparation of $Met(O)_{192}$-chymotrypsin.[19] One gram of chymotrypsin was dissolved in 50 mL of water, and the solution was adjusted to pH 3.4 with 2N HCl. To this solution, 2 mL of a 50 mM solution of trichloromethane-sulfonyl chloride in acetone was added. The solution was stirred for 15 minutes, then refrigerated. Approximately 80% of the sample was dialysed (Spectrapor 2, MW cutoff 12,000) against cold, 1mM HCl for 72 hours, with three chenges of dialysis buffer. The dialysate was collected and lyophilized, yielding a white, flocculent material. The remainder of the material was chromatographed on a 50cm x 2cm Biorad P-2 column eluted with 1 mM HCl. Fractions containing active enzyme were detected by rapid color changes in a standard Z-L-Tyr-ONp containing solution. Elution volumes were subsequently lyophilized.

Enzyme Stability Studies. Enzyme stability in various buffers was assayed using Z-L-Tyr-ONp as substrate. The activity was assayed photometrically by measuring the absorbance increase at 405 nm due to the release of p-nitrophenol (ε 18.5 $mM^{-1}cm^{-1}$) from the substrate. Substrate solution was 2.4 mM Z-Tyr-ONp in

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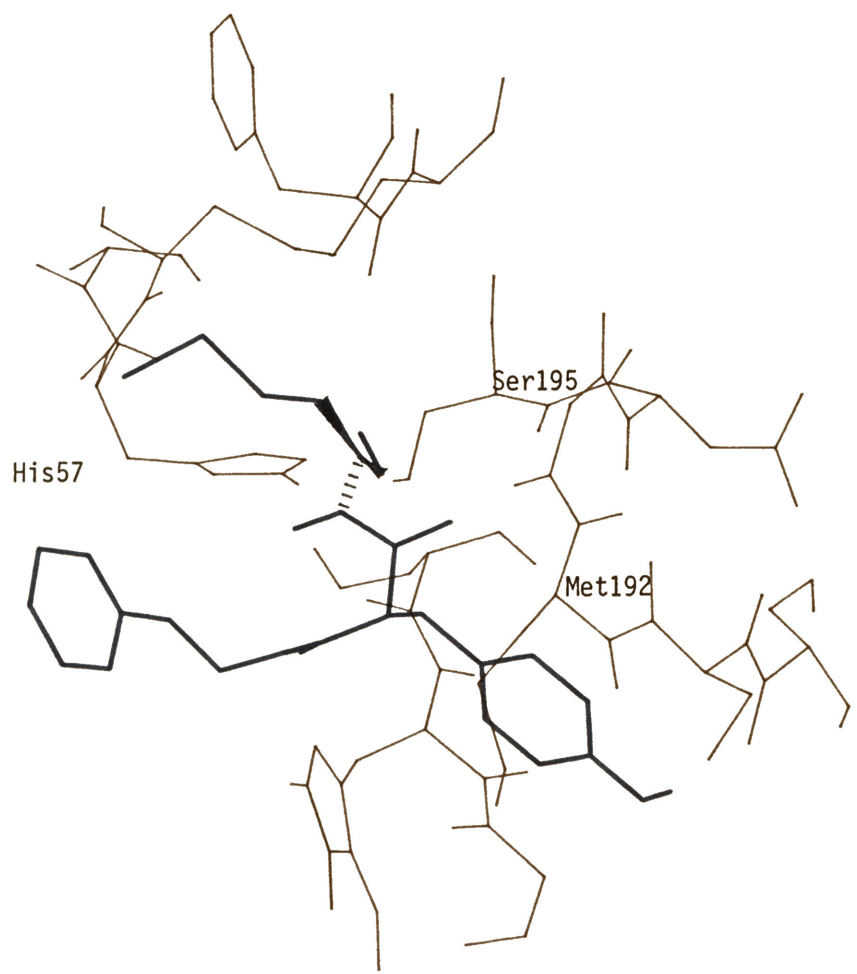
[19] Taylor, R. P.; Vatz, J. B.; Lumry, R. *Biochemistry* **1973**, *15*, 293.

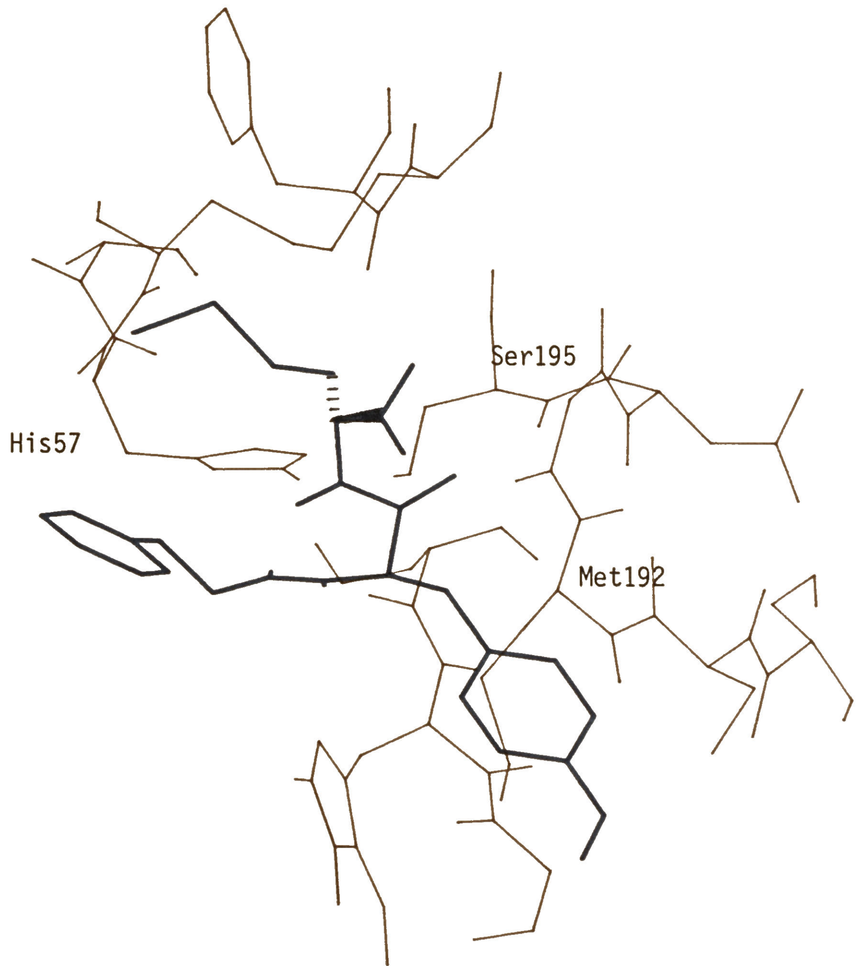
dioxane. Typical assay contained 2.8 mL of buffer, 0.1 mL of substrate, and 0.02 mL of the enzyme solution (1mg/mL) being assayed.

APPENDIX: Figures and Tables.

Figure 1. Computer-assisted molecular modelling of (a) the LL and (b) LD isomers of Z-Tyr-Met-OMe binding to the active site of a-chymotrypsin.



Z-L-Tyr-L-Met-OMe



Z-L-Tyr-D-Met-OMe

Figure 2. Stability of native Chymotrypsin (□) and Met(O)$_{192}$-Chymotrypsin (○) in 0.1 M Tris HCl, pH 9.0.

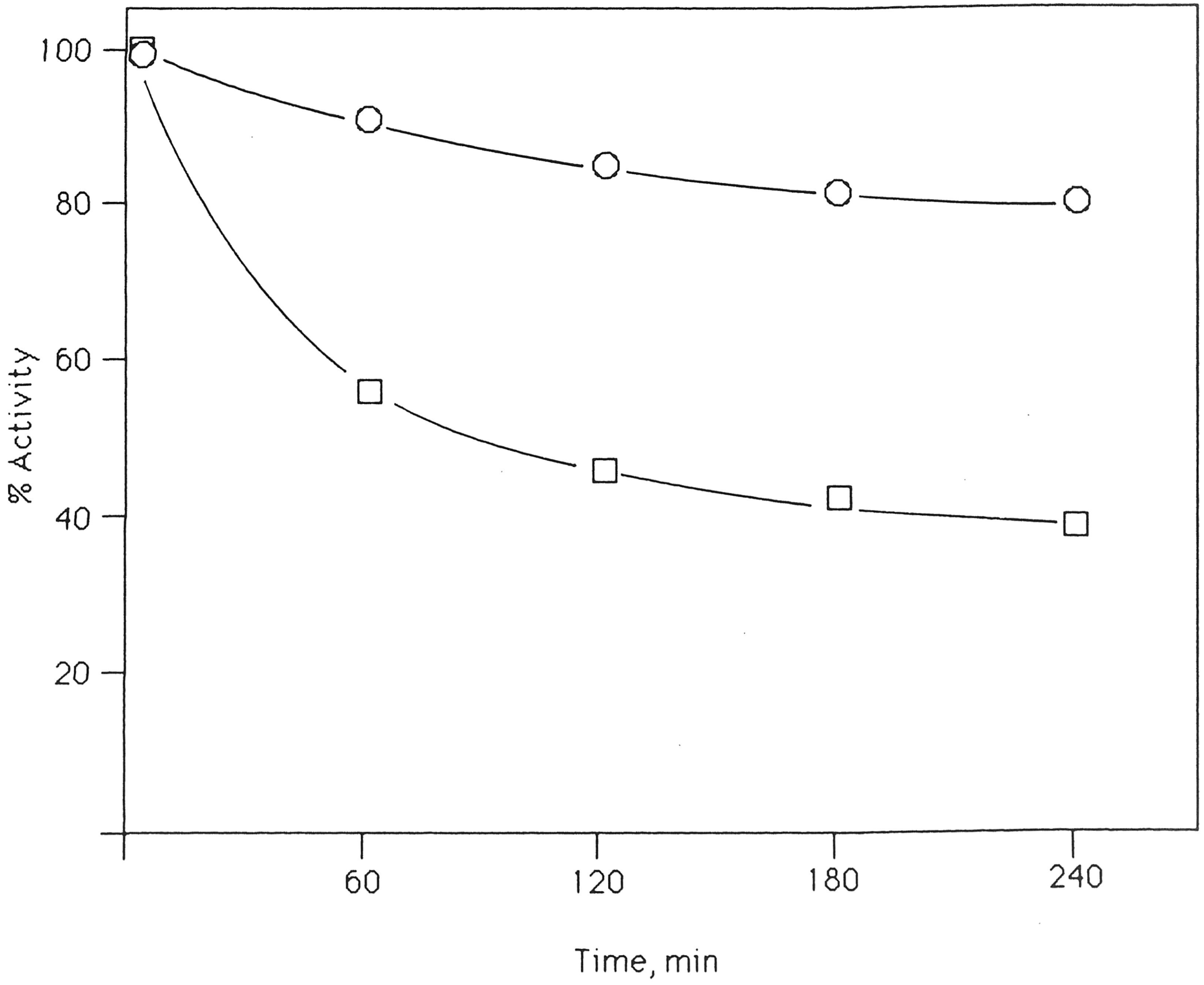

% Activity
100
80
60
40
20
60
120
180
240
Time, min

Figure 3. Stability of native Chymotrypsin (□) and $Met(O)_{192}$-Chymotrypsin (○) (purified by dialysis and gel filtration) in 0.1 M Tris-HCl/DMSO (1:1, v:v), pH 8.8.

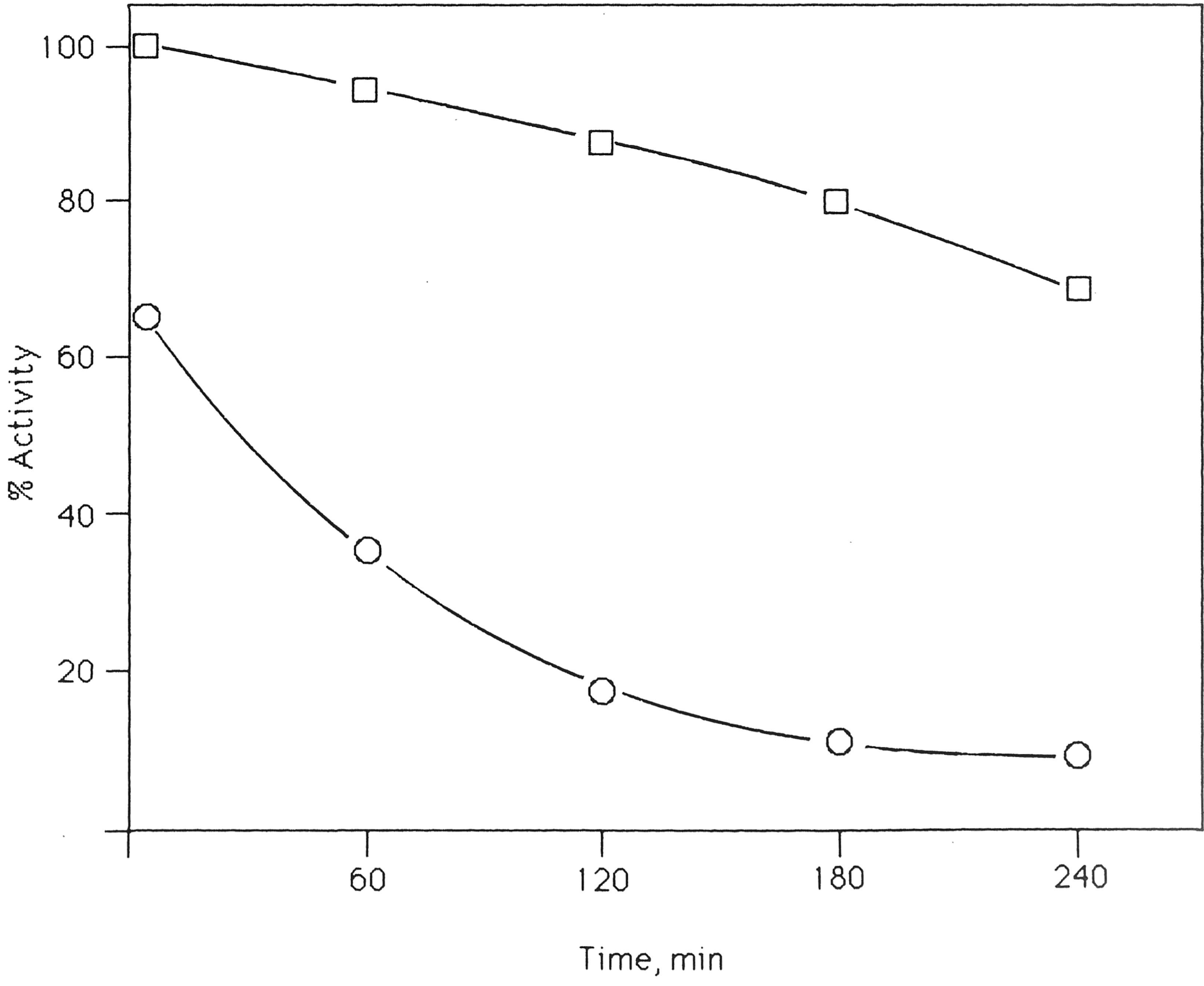

100
80
60
40
20
% Activity
60
120
180
240
Time, min

Table 1. Kinetic data for the competitive inhibition of Z-L-Tyr-D-Met-OMe on Z-L-Tyr-ONp hydrolysis.

KINETIC DATA

Substrate	Inhibitor	Conditions	$K_I (K_M)$	Inhibition Type
Z-L-Tyr-ONp	-	A	8.9×10^{-5}M	-
Z-L-Tyr-ONp	Z-L-Tyr-D-Met-OMe	A	3.5×10^{-4}M	Competitive
Z-L-Tyr-ONp	-	B	1.1×10^{-4}M	-
Z-L-Tyr-ONP	Z-L-Tyr-D-Met-OMe	B	2.5×10^{-3}M	Competitive

Conditions:

A: 0.1M Tris-HCl (pH 7.5), 0.2M $CaCl_2$, 10% dioxane. [substrate] = 0.5 - 5.3×10^{-5}M, [Inhibitor] = 4.5×10^{-4}M.

B: 0.1M Tris-HCl (pH 9.0), 0.2M $CaCl_2$, 60% DMSO.

Table 2. Results of Enzymatic Peptide Syntheses.

ENZYME CATALYSED SYNTHESIS OF UNUSUAL PEPTIDES

Peptide	Concentration		Reaction Time	Yield (%)	Irreversible?
	Acyl Donor	Acyl Acceptor			
Pic-D-Ala-Gly-$N_2H_2C_6H_5$	0.05M	0.2M	4 days	50.4	no
Z-(S-Bzl)LCys-D-Val-OMe	0.05M	0.2M	16 hrs	56.1	yes
Z-L-Tyr-6ACOMe	0.05M	0.2M	25 min	8.4	yes
Z-L-Tyr-6ACOMe	0.05M	0.4M	25 min	26.8	yes
Z-L-Tyr-GDMA	0.05M	0.2M	25 min	46.1	no
Z-L-Tyr-Sar-OMe	0.05M	0.2M	4 days	0.0	-
Z-L-Tyr-AHB-OMe	0.05M	0.2M	10 min	35.2	no