Prodrugs of Peptides. 13. Stabilization of Peptide Amides Against α-Chymotrypsin by the Prodrug Approach

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Various derivatives of the C-terminal amide group in N-protected amino acid and peptide amides were synthesized to assess their suitability as prodrug forms with the aim of protecting the amide or peptide bond against cleavage by α-chymotrypsin. Whereas N-acetylation, N-hydroxymethylation, and N-phthalidylation did not afford any protection but, in fact, accelerated the terminal amide bond cleavage, condensation with glyoxylic acid to produce peptidyl-α-hydroxyglycine derivatives and, to a minor extent, N-aminomethylation were found to improve the stability of the parent amides. Besides protecting the terminal, derivatized amide moiety toward cleavage by α-chymotrypsin, α-hydroxyglycine derivatization resulted in a significant protection, by a factor ranging from 5 to 75, of the internal peptide bond in various N-protected dipeptide amides. These derivatives are readily bioreversible, the conversion to the parent peptide or amino acid amide taking place either by spontaneous hydrolysis at physiological pH, as demonstrated for the *N*-Mannich bases, or by catalysis by plasma, as for peptidyl-α-hydroxyglycine derivatives.

KEY WORDS: peptides; prodrug; α-chymotrypsin; peptidyl-α-hydroxyglycine derivatives; *N*-Mannich bases; enzymatic hydrolysis.

INTRODUCTION

A major challenge in peptide and protein drug delivery is to overcome the enzymatic barrier that limits peptide and protein drugs to be delivered to their targets of action in effective amounts (1–3). The enzymatic degradation usually begins at the site of administration and can be so extensive that it completely hinders any absorption of intact peptide or protein.

In recent years, studies in our laboratory have been performed with the aim of solving or diminishing the problem of proteolytic degradation of peptide drugs by the prodrug approach (4). Thus, it may be imagined that suitable bioreversible derivatization may protect peptides against degradation by enzymes present at the mucosal absorption barrier and also render hydrophilic peptides more lipophilic and hence facilitate their absorption. To be a useful approach, however, the derivatives should be capable of releasing the parent peptide spontaneously or enzymatically in the blood following their absorption. The potential utility of the prodrug approach has recently been demonstrated with the tripeptide thyrotropin-releasing hormone (5,6) and by N-α-hydroxyalkylation or N-α-acyloxyalkylation (7,8) of the peptide bond. This derivatization was shown to make small peptides completely resistant to cleavage by carboxypeptidase A (7).

The aim of the present work was to develop prodrug derivatives capable of protecting peptides against degradation by another pancreatic proteolytic enzyme, α-chymotrypsin. This intestinal enzyme is an endopeptidase (serine protease) which catalyzes the hydrolysis of peptide bonds in which the reactive carbonyl group belongs to the L-amino acids tryptophan, tyrosine, phenylalanine, and, to a lesser extent, leucine and methionine (9). It also catalyzes the hydrolysis of various simple esters and amides (10–12). Several peptide drugs are rapidly degraded by α-chymotrypsin, such as ACTH-(1-10) (1), oxytocin (13), [D-Ala[1]]-peptide T amide (14), and various renin inhibitors (15).

In this work, we have prepared a number of prodrug derivatives of the carboxyl-terminal α-amide group in various N-protected amino acid and dipeptide amides, used as α-chymotrypsin-reactive model compounds, and compared their reactivity toward chymotrypsin with that of the parent amides. The prodrugs studied include *N*-Mannich bases, glyoxylic acid adducts, and *N*-acyl, *N*-hydroxymethyl, and *N*-phthalidyl derivatives. The structure of the compounds appears in Table I.

MATERIALS AND METHODS

Apparatus

High-performance liquid chromatography (HPLC) was done with a system consisting of a Shimadzu pump Model LC-6A, a Shimadzu SPD-6A variable wavelength UV detector, and a Rheodyne 7125 injection valve with a 20-μl loop. The column used was a deactivated Supelcosil LC-8-DB reversed-phase column (33 × 4.6 mm, 3-μm particles) in conjunction with a Supelguard column (Supelco Inc., USA). Microanalysis was performed at Leo Pharmaceuticals, Ballerup, Denmark.

Chemicals

Amino acids and N-protected amino acids and peptides (all of L-configuration) were purchased from Bachem AG, Bubendorf, Switzerland, or Sigma Chemical Co., MO. α-Chymotrypsin (Type II; from bovine pancreas) and carboxypeptidase A (Type I; from bovine pancreas) were obtained from Sigma Chemical Co.

Preparation of Peptide Derivatives (Table I)

The preparation of the peptidyl-α-hydroxyglycine derivatives **2**, **9**, **13**, **15**, **17**, and **20** (Table I) has been described elsewhere (16). The *N*-Mannich bases (**3**, **4**, **11**, and **18**) were prepared by refluxing a mixture of 2 mmol of the N-protected amino acid or peptide amide, 3 mmol of formaldehyde (0.24 ml 37% aqueous solution), 3 mmol of morpholine or piperidine, and 3 ml of ethanol for 24 hr (17,18). The resulting solution was evaporated under reduced pressure and the residue obtained triturated with ethyl acetate, filtered off, and recrystallized from ethanol-ether or ethyl acetate, m.p.

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Table I. Rate Data for the Decomposition of Various Peptides and Their Prodrug Derivatives in the Presence of α-Chymotrypsin and in Buffer Solution at 37°C

Compound[a]	Half-life: Buffer, pH 7.4[b]	Half-life: Buffer, pH 7.4,[b] with α-chymotrypsin (0.5 mg ml^{-1})
(1) N-Ac-Phe-NH_2		3.8 hr (7.6 hr)[c]
(2) N-Ac-Phe-NH-CH(OH)COOH	8.1 hr (37 hr)[c]	6.7 hr (31 hr)[c]
(3) N-Ac-Phe-NH-CH_2-N⟨O⟩	51 hr (51 hr)[c]	9.5 hr (15.7 hr)[c]
(4) N-Ac-Phe-NH-CH_2-N⟨⟩	1.1 hr (2.7 hr)[c]	0.9 hr (2.0 hr)[c]
(5) N-Ac-Phe-NH-$COCH_3$	26 hr	24 min
(6) N-Ac-Phe-NH-CH_2OH	280 hr	2.1 hr
(7) N-Ac-Phe-NH-CH_2-⟨O, O⟩	12 hr	0.7 min
(8) N-Bz-Phe-NH_2		10 min
(9) N-Bz-Phe-NH-CH(OH)COOH	6.6 hr	48 min
(10) N-Ac-Trp-NH_2		1.1 hr
(11) N-Ac-Trp-NH-CH_2-N⟨O⟩	44 hr	26 hr
(12) N-Ac-Phe-Tyr-NH_2		1.0 min
(13) N-Ac-Phe-Tyr-NH-CH(OH)COOH	8.3 hr	51 min
(14) N-Z-Phe-Gly-NH_2		11 min
(15) N-Z-Phe-Gly-NH-CH(OH)COOH	7.7 hr	1.3 hr
(16) N-Z-Phe-Ala-NH_2		0.9 min (1.7 min)[c]
(17) N-Z-Phe-Ala-NH-CH(OH)COOH	13.3 hr (48 hr)[c]	20 min (42 min)[c]
(18) N-Z-Phe-Ala-NH-CH_2-N⟨⟩	1.1 hr	0.3 min
(19) N-Z-Tyr-Leu-NH_2		0.2 min
(20) N-Z-Tyr-Leu-NH-CH(OH)COOH	10.0 hr	15 min

[a] Abbreviations: Ac, acetyl; Bz, benzoyl; Z, benzyloxycarbonyl.
[b] Phosphate buffer, 0.1 *M*.
[c] The data in parentheses are half-lives in 0.1 *M* phosphate buffer of pH 6.70 with α-chymotrypsin.

168–169°C (3), 171–172°C (4), 201–203°C (11), and 172–173°C (18).

The N-acylated amide derivative 5 was obtained by heating a mixture of 3 mmol of N-Ac-Phe-NH_2, 6 mmol of acetic anhydride, and 50 μl of concentrated sulfuric acid at 130°C for 5 min (19). Upon cooling the reaction solution was poured into 10 ml of water and extracted with ethyl acetate. Following drying over anhydrous sodium sulfate the extract was evaporated *in vacuo* to give compound 5 as an oil.

The N-hydroxymethyl derivative 6 was prepared by stirring a mixture of N-Ac-Phe-NH_2 (1.23 g, 6 mmol), 40 mg of potassium carbonate, and 2.2 ml of 37% aqueous formaldehyde at 60°C for 20 min (20). The resulting solution was cooled to 4°C and the solid formed filtered off and recrystallized from water, m.p. 136–138°C.

The N-phthalidyl derivative 7 was prepared using the general procedure described by Wheeler *et al.* (21). A mixture of N-Ac-Phe-NH_2 (0.62 g, 3 mmol) and phthalaldehydic acid (0.45 g, 3 mmol) was kept at 120°C for 30 min. The solid obtained upon cooling to 20°C was recrystallized from ethanol-water, m.p. 212–214°C.

All compounds showed elemental analysis (C, H, and N) within ±0.4% of the calculated values and ^{1}H-NMR spectra in agreement with their structures. The compounds were further characterized by their hydrolysis to the parent peptides as revealed by HPLC analysis.

Kinetic Measurements

The rates of degradation of the various peptides and prodrug derivatives were followed by using reversed-phase HPLC procedures capable of separating the compounds from their products of degradation. The HPLC column was eluted with mobile phase systems consisting of 5–25% (v/v) acetonitrile in 0.02 *M* acetate buffer of pH 4.0 or in 0.1% (v/v) phosphoric acid with triethylamine added at a concentration of 10^{-3} *M* to improve peak shape. The concentration of acetonitrile was adjusted for each compound to give an appropriate retention time (3–8 min). The flow rate was 1.5 ml min^{-1} and the column effluent was monitored at 215 nm. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

The degradation reactions in buffer solutions were ini-

tiated by adding 100 μl of a stock solution of the derivatives in acetonitrile or ethanol–water to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 10^{-4} *M*. The solutions were kept in a water bath at 37 ± 0.2°C, and at appropriate intervals samples were taken and chromatographed immediately. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual derivative against time.

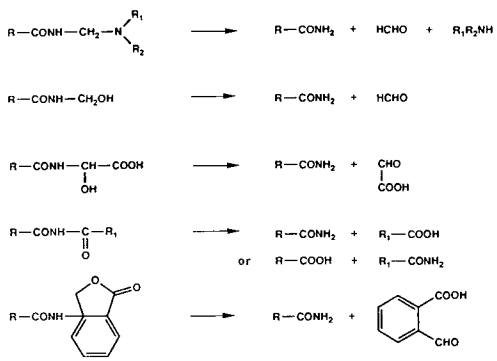
The stability of the compounds in the presence of α-chymotrypsin was examined at 37°C in a 0.1 *M* phosphate buffer solution of pH 7.40 or 6.70 containing the enzyme at a concentration of 0.25–1.0 mg ml^{-1}. The initial concentration of the compounds was 2×10^{-4} *M*. The reaction solutions were kept at 37°C, and at various intervals samples of 250 μl were withdrawn and added to 250 μl of a 5% (v/v) aqueous solution of perchloric acid in order to stop the reaction and deproteinize the samples. After mixing and centrifugation for 3 min at 13,000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above. The studies with carboxypeptidase A were performed in a similar way, the enzyme concentration being 5 or 50 U per ml.

RESULTS AND DISCUSSION

Stability of Prodrug Derivatives

The C-terminal primary amide group in a number of N-protected amino acid and dipeptide amides was derivatized in various ways to evaluate whether one or another kind of modification would stabilize the amide or a neighboring peptide bond toward chymotrypsin-catalyzed cleavage. All the derivatives studied belong to derivative types previously developed as potential prodrug forms of amides (Scheme I). Thus, *N*-Mannich bases (N-aminomethylated amides) and *N*-hydroxymethyl derivatives (22) are spontaneously hydrolyzed to the parent amide in aqueous solution at rates dependent on pH, whereas the hydrolysis of α-hydroxyglycine derivatives (glyoxylic acid adducts) (16,23), N-acylated amides (19), and *N*-phthalidyl derivatives (24) is also catalyzed by plasma enzymes. These conversions proceed quantitatively except for the N-acylated amides, where a competing reaction may be hydrolysis of the "wrong" imide moiety (Scheme I) (19).

The half-lives observed for the hydrolysis of the prodrug derivatives studied at 37°C in 0.1 *M* phosphate buffer of pH 7.40 are listed in Table I. The values obtained are in the range expected on basis of the behavior of related derivatives described in the above-mentioned references. All compounds hydrolyzed with quantitative formation of the parent amide as revealed by HPLC analysis except for compound **5**, which produced N-Ac-Phe-OH in addition to N-Ac-Phe-NH_2.



Scheme I

The pH–rate profiles for the decomposition of the peptidyl-α-hydroxyglycine derivatives **2**, **9**, **13**, **15**, **17**, and **19** at 37°C have been reported elsewhere (16). In short, these derivatives show a pH-independent rate of hydrolysis at pH 1–6 and an apparent hydroxide ion-catalyzed cleavage at pH > 6. In the present study the rate of hydrolysis of the *N*-Mannich bases **3** and **4** derived from *N*-acetyl-L-phenylalaninamide was determined at various pH values. The pH–rate profiles obtained are shown in Fig. 1. The shapes of these profiles are similar to those of the corresponding *N*-Mannich bases derives from benzamide (17) and can be accounted for by assuming spontaneous decomposition of the free base species and their conjugate acids:

$$k_{obs} = \frac{k_1 K_a}{a_H + K_a} + \frac{k_2 a_H}{a_H + K_a} \quad (1)$$

where k_{obs} is the observed pseudo-first-order rate constant, a_H is the hydrogen ion activity, K_a is the ionization constant of the protonated *N*-Mannich base, and k_1 and k_2 are the apparent first-order rate constants for the spontaneous degradation of the free base and protonated form, respectively. Values of pK_a and the rate constants k_1 and k_2 are listed in Table II.

The stability of the peptidyl *N*-Mannich bases **3** and **4** is almost identical to that of analogous *N*-Mannich bases of benzamide (17), thus indicating a lack of influence of the peptidyl structure upon the reactivity.

Stability Toward α-Chymotrypsin

The stability of the various N-protected amino acid and peptide amides and their prodrug derivatives was examined at 37°C in a 0.1 *M* phosphate buffer solution of pH 7.40 containing 0.5 mg ml^{-1} of α-chymotrypsin, corresponding to 2×10^{-5} *M*. The substrate concentration was about 10^{-4} *M*. Under these conditions all compounds were found to degrade according to strict first-order kinetics. The half-lives of degradation are listed in Table I along with the half-lives observed in the same buffer solution without α-chymotrypsin.

The reaction of α-chymotrypsin with esters, amides,

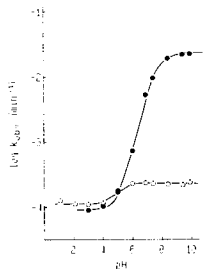


Fig. 1. The pH–rate profiles for the decomposition of the *N*-Mannich bases 3 (○) and 4 (●) in aqueous solution (μ = 0.5) at 37°C.

Table II. Rate Data for the Decomposition of the *N*-Mannich Bases 3 and 4 of *N*-Acetyl-L-Phenylalaninamide in Aqueous Solution at 37°C (μ = 0.5)

Compound	k_1 (min^{-1})	k_2 (min^{-1})	pK_a
(3) N–Ac–Phe–NH–CH_2–N⟨O⟩	2.3×10^{-4}	1.2×10^{-4}	5.0
(4) N–Ac–Phe–NH–CH_2–N⟨⟩	2.3×10^{-2}	9.0×10^{-5}	7.5

and peptides follows a three-step process. In the first step an enzyme–substrate complex is formed, next the substrate acylates the active site in the enzyme, and finally, the acylated enzyme is solvolyzed to liberate a carboxylic acid and regenerate the enzyme (12). For hydrolysis of the amide bond the rate-determining step is the acylation of the enzyme (12). It has been shown that the most meaningful kinetic parameter for comparing the specificity of various substrates to α-chymotrypsin is the ratio V_{max}/K_m, also called the specificity constant, where V_{max} is the maximum rate of substrate consumption and K_m is the Michaelis constant (apparent binding constant) (25). When the substrate concentration is lower than K_m the enzymatic reaction is first order, with the rate constant being equal to V_{max}/K_m. Such conditions are prevailing in the present study, and accordingly, the half-lives given in Table I refer directly to the specificity constant:

$$t_{1/2} = 0.693/(V_{max}/K_m) \quad (2)$$

Inspection of the rate data in Table I reveals a widely different pattern of reactivity of the C-terminal amide modified peptides. Using *N*-acetyl-L-phenylalaninamide as a model, neither N-acetylation, N-hydroxymethylation, or N-phthalidylation is seen to afford any protection of cleavage of the terminal amide bond. In fact, such derivatization makes the amide bond more susceptible to α-chymotrypsin-catalyzed hydrolysis as can be seen by comparing the reactivities of the compounds 5–7 with that of compound 1. The *N*-phthalidyl derivative 7 is, in particular, a very good substrate, having a 325-fold higher reactivity than 1. The reactivity of the *N*-hydroxymethyl derivative 6 was unexpected because of the known very low reactivity of N-methylated *N*-acylamino acid amides (26,27). This was confirmed in the present study with *N*-acetyl-L-phenylalaninmethylamide. When this compound was incubated with α-chymotrypsin at pH 7.40, less than 5% hydrolysis was found to occur after 24 h.

In contrast to these modifications N-aminomethylation, i.e., *N*-Mannich base formation, and conversion of the amide NH_2-group into an α-hydroxyglycine residue are useful procedures to achieve protection against α-chymotrypsin albeit to varying degree. The *N*-Mannich bases 3 and 11 are seen to stabilize the parent N-acylated amino acid amides by a factor of 3 and 25. The *N*-Mannich base 4 formed with piperidine is chemically unstable but its stability is only slightly diminished in the presence of α-chymotrypsin at pH 7.4 or 6.7.

The glyoxylic acid adduct 2 formed with N-Ac-Phe-NH_2 was found to be completely stable toward α-chymotrypsin as seen from the data in Tables I and III. Whereas the rate of hydrolysis of the parent amide increases proportionally with the α-chymotrypsin concentration, the rate of degradation of compound 2 remains at the same value, corresponding to that in the absence of enzyme (Table III). Monitoring the products of degradation of compound 2 by HPLC analysis also revealed a complete resistance toward α-chymotrypsin. Kinetic analysis of the data shown in Fig. 2 in the usual way for consecutive reactions showed that the decomposition of the derivative proceeds with the quantitative formation of N-Ac-Phe-NH_2, which subsequently is hydrolyzed by the enzyme to N-Ac-Phe-OH (Scheme II).

This result obtained with compound 2 does not imply, however, that glyoxylic acid adduct formation of an amide can be considered as a means totally to protect the amide against α-chymotrypsin in general. The degree of protection obtained may depend on the structure and/or enzymatic susceptibility of the parent amide. Thus, the glyoxylic acid derivative 9 formed with the more susceptible *N*-benzoyl-L-phenylalaninamide is seen to protect the parent amide only by a factor of 5.

The above-mentioned derivatives were all formed at the amide bond that is cleaved by α-chymotrypsin. In the N-protected dipeptide amides 14, 16, and 19 the cleavage occurs entirely at the Phe–Gly (14), Phe–Ala (16), and Tyr–Leu (19) bonds as shown by HPLC analysis, revealing a quantitative formation of N-Z-Phe-OH or N-Z-Tyr-OH. In the case of N-Ac-Phe-Tyr-NH_2 (12) the α-chymotrypsin-catalyzed hydrolysis occurred predominantly (94%) at the Phe–Tyr bond, a minor

Table III. Half-Lives for the Hydrolysis of N–Ac–Phe–NH_2 (1) and N–Ac–Phe–NHCH(OH)COOH (2) in 0.1 *M* Phosphate Buffer Solutions (pH 7.40) Containing Varying Amounts of α-Chymotrypsin (at 37°C)

	Half-life (hr)		
	α-chymotrypsin (mg ml^{-1})		
Compound	0.25	0.50	1.00
(1) N–Ac–Phe–NH_2	7.8	3.8	2.3
(2) N–Ac–Phe–NH–CH(OH)COOH	6.5	6.7	6.5

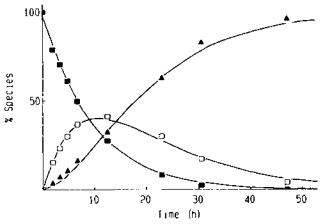
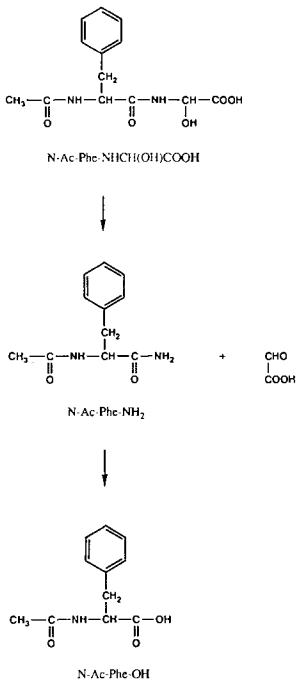


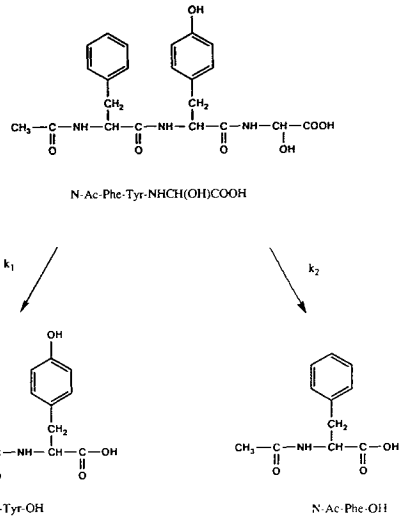
Fig. 2. Plots showing the time courses of degradation of N-Ac-Phe-NHCH(OH)COOH (2) (■) and formation of N-Ac-Phe-NH_2 (1) (□) and N-Ac-Phe-OH (▲) in 0.1 *M* phosphate buffer solution (pH 7.40) containing 0.50 mg/ml of α-chymotrypsin (at 37°C).

reaction (6%) being cleavage of the terminal amide group to yield N-Ac-Phe-Tyr-OH as revealed by HPLC analysis of the reaction solutions. Both N-Ac-Phe-OH and N-Ac-Phe-Tyr-OH were found to be inert toward α-chymotrypsin.

Whereas *N*-Mannich base derivatization of compound **16** rendered the peptide more susceptible to enzymatic cleavage, derivatization with glyoxylic acid afforded a marked degree of protection. As seen from the data in Table I, compound **13** is 51 times more stable than the parent compound **12**, whereas compounds **15**, **17**, and **20** are 7, 22, and 75 times, respectively, more stable than the parent dipeptide amides. This degree of protection was the same at pH 6.7 as shown for compound **17**. The products of decomposition of the derivative **13** was found to be N-Ac-Phe-Tyr-OH, formed in a 60% yield, and N-Ac-Phe-OH, formed in a 40% yield. The apparent first-order rate constants for the two processes depicted in Scheme III have the following values: $k_1 = 8.9 \times 10^{-3}$ min^{-1} and $k_2 = 5.5 \times 10^{-3}$ min^{-1}. Comparing these values with those for the corresponding degradation routes of N-Ac-Phe-Tyr-NH_2 showed that the C-terminal amide group is cleaved 120 times slower in compound **13** relative to compound **12** and that the Phe–Tyr bond is stabilized by a



Scheme II



Scheme III

factor of about 5 through the derivatization with glyoxylic acid.

The significant stabilization of a peptide or C-terminal amide bond achieved by converting the C-terminal amide group into an α-hydroxyglycine moiety can most likely be ascribed to the introduction of a carboxylic acid function affording electrostatic interaction with the enzyme. Peptides with a free C-terminal carboxylic acid are usually more resistant to α-chymotrypsin-catalyzed hydrolysis than the amide or ester analogs (e.g., Ref. 28).

The possibility that stabilization of a peptide toward a specific enzyme such as α-chymotrypsin by derivatization may render it vulnerable toward other enzymes should not be overlooked. Thus, it can be imagined that the peptidyl-α-hydroxyglycine derivatives would be substrates for carboxypeptidase A due to the presence of the terminal carboxylic acid group. Carboxypeptidase A is also a pancreatic proteolytic enzyme whose primary function is that of a C-terminal exopeptidase. The enzyme catalyzes the hydrolysis of almost any peptide having a terminal free carboxyl group and a C-terminal residue of the L-configuration (29).

To investigate whether the glyoxylic acid derivatization would make the resulting derivatives susceptible to carboxypeptidase A, the stability of compound **13** was determined at 37°C and compared with that of N-Ac-Phe-Tyr-OH. While the latter acid was found to degrade to yield N-Ac-Phe-OH and tyrosine very rapidly ($t_{1/2}$ <10 sec) in the presence of carboxypeptidase A at a concentration of 5 U ml^{-1}, the α-hydroxyglycine derivative **13** showed the same stability ($t_{1/2}$ being 8.3 hr), even at a carboxypeptidase A concentration of 50 U ml^{-1}, as in plain buffer solution. Thus, the α-hydroxyglycine moiety group does not render the terminal peptide bond susceptible to cleavage by carboxypeptidase A.

Finally, the stability of the peptides **12** and **16** and their prodrug derivatives **13** and **17** was examined in a 20% rabbit intestinal homogenate (pH 7.4). The half-lives of degradation observed at 37°C were 2.9 hr (**12**), 9.2 hr (**13**), 15 min (**16**), and 2.4 hr (**17**). Thus, compound **13** was as stable as in plain buffer solution and compound **17** only slightly more unstable

but considerably more stable than the parent peptide. The degradations of the parent peptides may be due to α-chymotrypsin adhering to the gut tissue or other nonpancreatic enzymes.

CONCLUSIONS

The results described show that the prodrug approach in certain cases may be useful for protecting peptides against proteolytic cleavage by α-chymotrypsin. The peptide model compounds studied all contain a C-terminal amide group which is found in many bioactive peptides (30). The α-chymotrypsin-catalyzed cleavage of this bond can be retarded by N-aminomethylation or glyoxylic acid adduct formation, whereas N-hydroxymethylation, N-acetylation, or N-phthalidylation does not offer any protection. If the α-chymotrypsin-catalyzed hydrolysis occurs at the second peptide bond, derivatization of the terminal amide moiety with glyoxylic acid to give peptidyl-α-hydroxyglycine derivatives still affords a marked degree of protection, whereas the effect of N-aminomethylation is greatly diminished or lost. The conversion of peptidyl-α-hydroxyglycine derivatives to the parent peptide amides occurs spontaneously at pH 7.4 and 37°C with half-lives of 7–13 hr, but in the presence of human plasma this conversion is accelerated by a factor of 3–9, probably due to the occurrence of an α-hydroxyglycine amidating dealkylase enzyme in plasma (16).

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