

The Importance of Structural Factors on the Rate and the Extent of N,O-acyl Migration in Cyclic and Linear Peptides

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The chemistry associated with the process of N,O-acyl migration was explored in both cyclic and linear peptides under aqueous acid conditions. The importance of backbone cyclization and N-methylation of the peptide bond on the kinetics of N,O-acyl migration in a series of linear and cyclic peptides related in structure to cyclosporin A (CsA) were examined. The similarity in the chemical reactivity of the cyclic peptide [MeLeu (3-OH)]¹-CsA and the corresponding linear peptide [Val-MeLeu (3-OH)-Abu], suggested that for this series, cyclization of the peptide backbone may not play an important role in controlling the kinetics of N,O-acyl migration. In contrast, the disparity in the chemical reactivity of tripeptides [Val-MeLeu (3-OH)-Abu] and [Val-Leu (3-OH)-Abu], indicated that N-methylation of amide bond significantly impacted the kinetics. Various hypothesis are proposed to account for this observation.

KEY WORDS: acyl migration; peptides; cyclosporin; stability.

INTRODUCTION

Considerable attention has been focused on the influence of factors such as pH, temperature, and buffer species on the chemical stability polypeptides (1). However, the existing literature on the importance of structural factors on the reactivity of polypeptides is limited and just evolving. The principle objective of this work was to examine the role of backbone cyclization and amide bond N-methylation on the rate and extent of N,O-acyl migration in a series of cyclic and linear peptides related to cyclosporin A (CsA).

The kinetics and the mechanism of N,O-acyl migration in CsA (Figure 1) under non-aqueous and aqueous acidic conditions have been described (2,3). The phenomenon of N,O-acyl migration was found to be acid-catalyzed with the formation of isocyclosporin A (isoCsA) as the predominant degradation product (Figure 2). In addition, the degradation kinetics of number of CsA analogs were also examined to support the selectivity for the site of N,O-acyl migration (3): The key analog studied was cyclosporin C (Figure 1, CsC). The chemical structure of CsC differs from CsA only by having L-threonine in the place of L- α -aminobutyric acid in the 2-position, thus, CsC contains two β -hydroxyl functionalities. Theoretically, both β -hydroxyl functionalities can undergo N,O-acyl migration. However, it was established that CsC undergoes N,O-acyl migration at only one specific site,

namely, the amino acid MeBmt (3). This observation underlines the specificity associated with the site of isomerization.

It has been our intention to determine some of the structural characteristics leading to this specificity. In the present study, the effect of backbone cyclization and amide bond N-methylation on the kinetics of N,O-acyl migration in CsA related polypeptides are described. Specifically, the degradation kinetics of a number of tripeptides, namely, [Val-MeLeu (3-OH)-Abu] (Figure 3), [Val-MeLeu-Abu] (Figure 3) and [Val-Leu (3-OH)-Abu] (Figure 3) are presented. Examining the chemical reactivities of these polypeptides allows one to probe some of the important structural factors that govern the kinetics of N,O-acyl migration in cyclosporin analogs and other structurally related polypeptides.

EXPERIMENTAL

Materials

The protected amino acids and the coupling reagents used in this study were obtained from commercial sources (Aldrich and Bachem Bioscience Inc.) and used without further purification. Methanol and acetonitrile were both HPLC grade. Tetrahydrofuran was distilled over sodium benzophenone. Dichloromethane and chloroform were of anhydrous grade. The water was de-ionized and glass distilled (Megapure system model mp-1, Corning). All other chemicals were of reagent grade and used without further purification.

Analyses

All melting points were determined with a capillary melting point apparatus (Mel-Temp, Laboratory Devices, Cambridge, MA). Infrared (IR) spectrum were recorded on a Perkin-Elmer 599B spectrometer (data in cm^{-1}). ¹H-NMR and ¹³C-NMR spectrum were recorded on a General Electric QE300 in CDCl₃ or D₂O. Chemical shift are reported in ppm (δ units) downfield from tetramethylsilane. Thin-layer chromatography (TLC) was performed on silica gel (Kiesel 60F₂₅₄ on aluminum sheet, Merck), and the compounds of interest were visualized by UV and/or ninhydrin spray. For column chromatography, Brinkman silica gel 60, 70-270 mesh, or basic ion-exchange resin (Dowex-1, hydroxide form) were used.

Syntheses

Synthesis of ($\pm$)-threo-N-methyl- β -hydroxyleucine

This amino acid was synthesized by N-methylation of ethyl trans-5-isopropyl-2-oxazoline-4-carboxylate to form the N-methylated imidate, followed by hydrolysis in dilute HCl and ion exchange chromatography (Dowex-1, hydroxide form) as described by Rich and co-workers (4) in 55% yield. Recrystallization from aqueous acetone gave an off-white powder. mp 237–239°C dec; ¹H-NMR (D₂O) δ 0.96 (d, 3 H, CHCH₃CH₃), 0.97 (d, 3 H, CHCH₃CH₃), 1.84 (m, 1 H, CHMe₂), 2.75 (s, 3 H, N-CH₃), 3.57 (d, 1 H, H-2), 3.69 (dd, 1 H, H-3).

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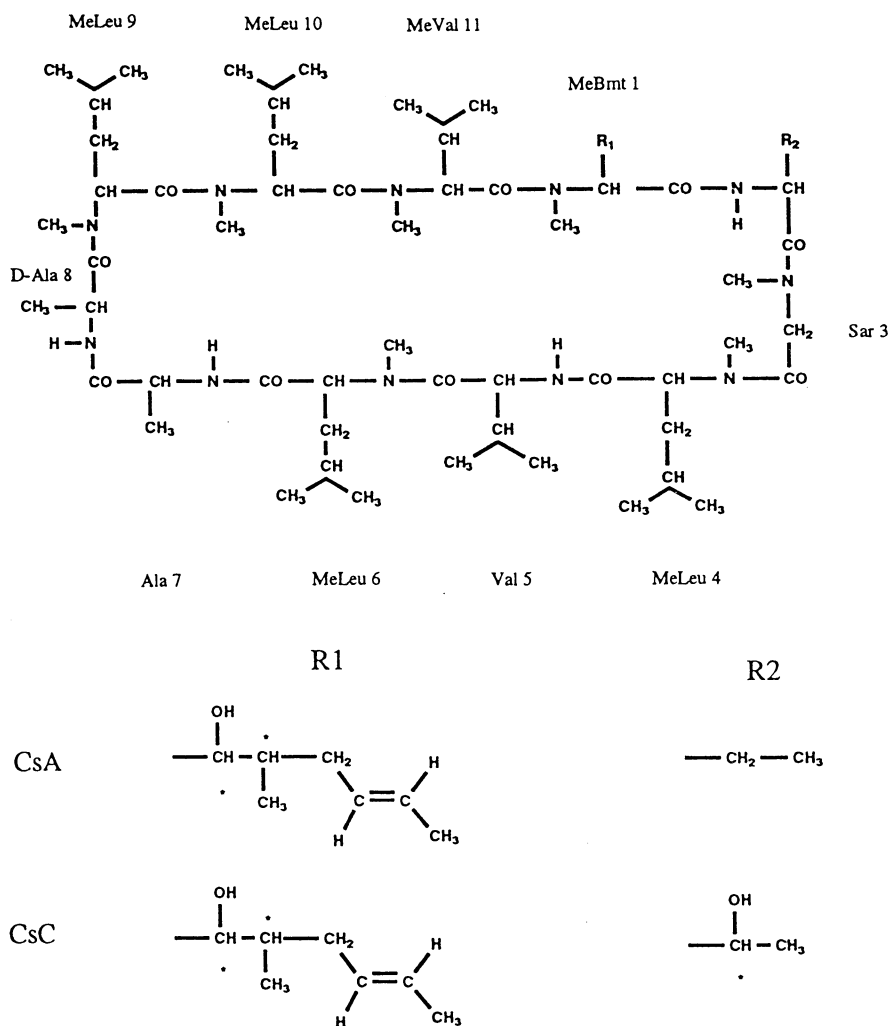


Fig. 1. Chemical structure of cyclosporin A (CsA) and cyclosporin C (CsC).

Synthesis of ($\pm$)-threo- β -hydroxyleucine

This compound was synthesized by a similar procedure as ($\pm$)-threo-N-Methyl- β -hydroxyleucine. To a stirred solution of ethyl trans-5-isopropyl-2-oxazoline-4-carboxylate (1g, 4.6 mmol) in H_2O was added 3 mL of concentrated HCl. The mixture was refluxed for 15 hr and then concentrated. The oily orange hydrochloride salt was purified by ion-exchange resin (Dowex-1, hydroxide form) column, eluting with 0.2 N acetic acid. Fractions containing the product were

pooled and lyophilized to give ($\pm$)-threo-N-Methyl- β -hydroxyleucine in 64% yield. 1H -NMR (D_2O) δ 0.98 (d, 3 H, $CHCH_3CH_3$), 1.05 (d, 3 H, $CHCH_3CH_3$), 1.79 (m, 1 H, $CHMe_2$), 3.78 (dd, 1 H, H-3), 3.85 (d, 1 H, H-2).

Synthesis of

N,O-isopropylidene-N-methyl- β -hydroxyleucine

A suspension of ($\pm$)-threo-N-Methyl- β -hydroxyleucine

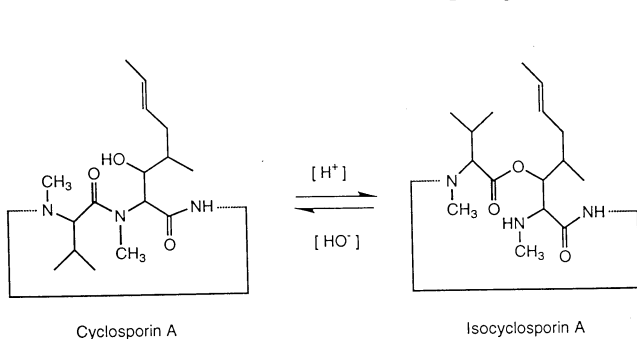


Fig. 2. Schematic representation of the isomerization of cyclosporin A to isocyclosporin A.

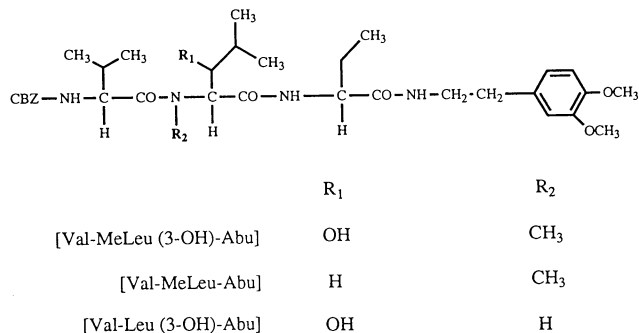


Fig. 3. Chemical structure of tripeptides [Val-MeLeu (3-OH)-Abu], [Val-MeLeu-Abu], and [Val-Leu (3-OH)-Abu].

(80 mg, dried by azeotropic distillation of H₂O with two 50 mL portions of benzene) in 50 mL of freshly distilled acetone was refluxed, as described by Rich and co-workers (4), for 24 h (in the presence of N₂ atmosphere) until a clear solution was obtained. The solvent was concentrated and was used directly in the next step.

Synthesis of *tert*-butyloxycarbonyl- β -hydroxyleucine

A solution of β -hydroxyleucine (147 mg, 1 mmol) in a 1:1 ratio of dioxane (10 mL) and water (10 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butyl pyrocarbonate (240 mg, 1.1 mmol) was added and the pH of the solution was maintained by pH 8.8–9.3 using 0.1 M NaOH. The reaction was terminated when the pH of the solution no longer fluctuated. The solution was concentrated to about 10 mL and acidified using KHSO₄ to pH 2–3 (Congo paper). The aqueous solution phase was extracted with ethyl acetate (15 mL) and the extraction was repeated. The ethyl acetate extracts were pooled, washed with water (twice, 15 mL), dried over anhydrous Na₂SO₄ and evaporated in vacuo. The oily product was used directly in the next step.

Synthesis of *tert*-butyloxycarbonyl *N* methyl-leucine

Tert-butyloxycarbonyl *N*-methyl-leucine was obtained by alkylation of the *t*-Boc protected leucine utilizing the procedure described by McDermott and Benoiton [5]. m.p. 55–56°C; ¹H-NMR (CDCl₃), multiple conformations (cis-trans) δ 0.9 (t, 6 H, CHCH₃CH₃), 1.4 (s, 9 H, Boc protons), 1.5 (m, 1 H, CHMe₂), 1.65 (t, 2 H, H-3), 2.75 (s, 3 H, N-CH₃), 4.6 (dd, 1 H, H-2).

Synthesis of the tripeptide [Val-MeLeu (3-OH)-Abu], 3a

The dipeptide 2a was prepared by the coupling of 1 with N,O-isopropylidene-*N*-methyl- β -hydroxyleucine followed by deprotection as indicated in Figure 4. Compound 1 had previously been prepared by coupling β -(3,4-dimethoxyphenyl)ethylamine with *tert*-butyloxycarbonyl- α -aminobutyric acid. To a stirred solution of dipeptide 2a (40 mg, 0.1 mmol), CBZ-valine (17 mg, 0.1 mmol) and *N*-methylmorpholine (20 μ L) in dichloromethane (4 mL) at 0°C was added N,N-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (BOP Chloride, 33 mg, $\approx$ 0.1 mmol). The reaction mixture was stirred for 2 hours at 0°C and 24 hours at room temperature. The reaction was diluted with CHCl₃ and filtered. The organic solution was washed with 1N HCl (2 $\times$ 15 mL), saturated NaHCO₃ (2 $\times$ 20 mL), and H₂O (2 $\times$ 15 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude material was purified by reverse phase high pressure liquid chromatography using a preparative HPLC column (Dynamax-300A, C₁₈, 12 μ m, 21.4 mm $\times$ 25 cm) and acetonitrile/H₂O (1/1) as eluent. The appropriate fractions were pooled, evaporated and lyophilized. Mass spectrum (M⁺) m/e 643; ¹H-NMR (CDCl₃) δ 0.17–1.0 (m, 15 H, CH₃ of Abu, β -OH-MeLeu and Val), 1.4–1.8 (3 H, β -CH of Abu, Val), 2.65 (t, 2 H, CONHCH₂CH₂), 3.05 (s, 3 H, N-CH₃), 3.45 (m, 2 H, CONHCH₂CH₂), 3.79 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃), 3.82 (m, 2 H, β -CH and OH of β -OH-MeLeu), 4.2–5.0 (m, 3 H, α -CH), 5.04 (m, 2 H, Ph-CH₂), 5.3 (d, 1 H CBZ NH), 5.93 (t, 1 H, NH), 6.5 (d, 1 H, NH), 6.6–6.8 (m, 3H, Ar-H), 7.3 (bs, 5 H, CBZ Ar-H).

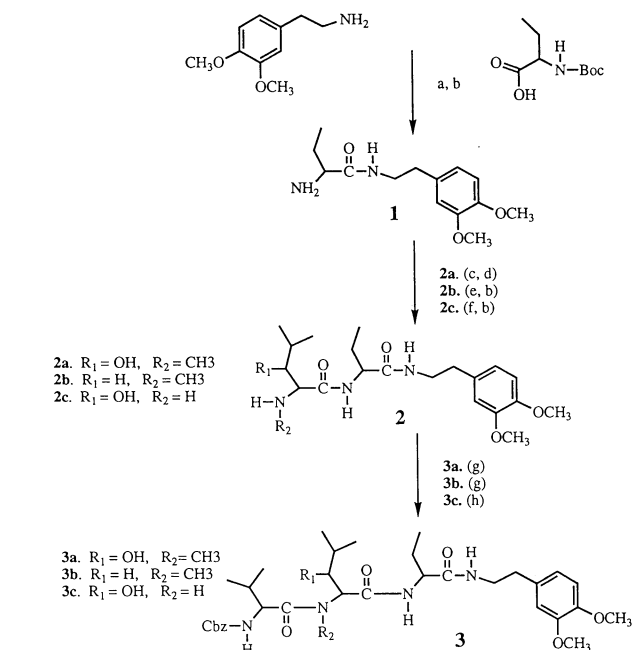


Fig. 4. Synthetic scheme for the preparation of the linear tripeptides.

(t, 1 H, NH), 6.6–6.8 (m, 3H, Ar-H), 6.9 (d, 1 H, NH), 7.3 (bs, 5 H, CBZ Ar-H).

Synthesis of tripeptide [Val-MeLeu-Abu], 3b

To a stirred solution of dipeptide 2b (46 mg, 0.1 mmol), prepared in a similar manner to 2a except that *tert*-butyloxycarbonyl *N*-methylleucine was used in place of N,O-isopropylidene-*N*-methyl- β -hydroxyleucine, CBZ-valine (17 mg, 0.1 mmol) and *N*-methylmorpholine (20 μ L) in dichloromethane (4 mL) at 0°C was added N,N-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (BOP Chloride, 33 mg, $\approx$ 0.1 mmol). The reaction mixture was stirred for 2 hours at 0°C and 24 hours at room temperature. The reaction was diluted with CHCl₃ and filtered. The organic solution was washed with 1N HCl (2 $\times$ 15 mL), saturated NaHCO₃ (2 $\times$ 20 mL), and H₂O (2 $\times$ 15 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude material was purified by reverse phase high performance liquid chromatography using a preparative HPLC column (Dynamax-300A, C₁₈, 12 μ m, 21.4 mm $\times$ 25 cm) and acetonitrile/H₂O (1/1) as eluent. The appropriate fractions were pooled, evaporated and lyophilized. Mass spectrum (M⁺) m/e 627; ¹H-NMR (CDCl₃) δ 0.7–1.0 (m, 15 H, CH₃ of Abu, β -OH-MeLeu and Val), 1.4–2.1 (6 H, β protons and γ -CH MeLeu), 2.65 (t, 2 H, CONHCH₂CH₂), 2.95 (s, 3 H, N-CH₃), 3.45 (m, 2 H, CONHCH₂CH₂), 3.79 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃), 4.2–5.0 (m, 3 H, α -CH), 5.04 (m, 2 H, Ph-CH₂), 5.5 (d, 1 H CBZ NH), 5.93 (t, 1 H, NH), 6.5 (d, 1 H, NH), 6.6–6.8 (m, 3H, Ar-H), 7.3 (bs, 5 H, CBZ Ar-H).

Synthesis of tripeptide [Val-Leu (3-OH)-Abu], 3c

Dipeptide 2c (46 mg, 0.1 mmol) was prepared in a sim-

ilar manner to 2a except that *tert*-butyloxycarbonyl- β -hydroxyleucine was used in place of *N,O*-isopropylidene-*N*-methyl- β -hydroxyleucine. DCC (0.5 mmol) was added to a solution of CBZ-valine (0.5 mmol), 1-hydroxybenzotriazole (0.5 mmol), *N*-methylmorpholine (0.5 mmol) in dichloromethane (5 mL). The suspension was stirred for 30 min under cooling with an ice-bath and filtered. A solution of 2c (0.5 mmol) in dichloromethane (2 mL) was added to the filtrate and the reaction mixture was stirred at 0°C for 1 hr and room temperature for 3 hr. The reaction was diluted with CHCl_3 and filtered. The organic solution was washed with 1N HCl (2×15 mL), saturated aqueous NaHCO_3 (2×20 mL) and H_2O (2×15 mL), dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified on a silica gel column, eluting with 97.5/2.5 and 90/10 chloroform-methanol as eluent to give the tripeptide 3c. Mass spectrum (M^+) m/e 629; $^1\text{H-NMR}$ (CDCl_3) δ 0.7–1.0 (m, 15 H, CH_3 of Abu, β -OH-Leu and Val), 1.5–2.1 (3 H, β -CH of Abu, Val), 2.68 (t, 2 H, $\text{CONHCH}_2\text{CH}_2$), 3.45 (m, 2 H, $\text{CONHCH}_2\text{CH}_2$), 3.72 (m, 2 H, β -CH and OH of β -OH-Leu), 3.79 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.93–4.5 (m, 3H, α -CH), 5.04 (d, 2 H, Ph-CH_2), 5.38 (d, 1 H CBZ NH), 6.6 (d, 1 H, NH), 6.6–6.8 (m, 3H, Ar-H), 7.1 (d, 1 H, NH), 7.2 (d, 1 H, NH), 7.3 (bs, 5 H, CBZ Ar-H).

HPLC Analysis System

High-performance liquid chromatography (HPLC) was performed using a system consisting of a Shimadzu SPD-6A variable wavelength detector operating at 278 nm; Shimadzu LC-6A pumps; a Shimadzu SIL-6B auto injector; and a Shimadzu CR601 integrator for peak processing. The HPLC studies were conducted using a reverse-phase analytical column C_8 (15 cm $\times$ 3.9 mm) with mean particle diameter of 5 μm . All the analyses were performed under isocratic condition at ambient temperature. Flow rate was set at 1.0 mL/min. The mobile phase contained 50 parts aqueous, 50 parts acetonitrile and 1 mM TBA. Retention volumes for [Val-MeLeu (3-OH)-Abu], iso[Val-MeLeu (3-OH)-Abu], [Val-MeLeu-Abu], [Val-Leu (3-OH)-Abu] and iso[Val-Leu (3-OH)-Abu] were respectively 5.2, 7.3, 9.2, 4.8, 5.4 ml. Calibration curves were constructed from linear plots of peak height versus concentration.

Kinetic Procedure

The pH of aqueous buffer solutions were adjusted at the experimental temperatures by measuring the pH of the solution using a Corning pH meter which was standardized at the experimental temperature with NBS buffer solutions. The ionic strength of the solutions were adjusted to 0.15 with KCl.

Stock solutions of tripeptides were prepared in acetonitrile. One hundred microliter aliquot of stock solution was used to prepare 10 mL dilute solutions of compounds ($4\text{--}6 \times 10^{-6}$ M) in the pH-adjusted buffer solutions. At appropriate time intervals, samples were withdrawn and analyzed. The decomposition kinetics of tripeptides were studied at $50 \pm 0.2^\circ\text{C}$, pH 1–2 (KCl/HCl). Pseudo-first order rate constants for the apparent decomposition were obtained by following the disappearance of the material for at least 3 half-lives.

Due to the poor aqueous solubility of [Val-Leu (3-OH)-

Abu], the decomposition kinetics of [Val-Leu (3-OH)-Abu] and [Val-MeLeu (3-OH)-Abu] were conducted in buffer solutions containing 5% acetonitrile.

RESULTS AND DISCUSSION

The Role of the β -hydroxyl Group

The kinetics of [Val-MeLeu (3-OH)-Abu] degradation were studied in dilute aqueous solution as a function of pH. The degradation followed an apparent-first-order kinetics for at least 3 half-lives in the pH range of 1–2. A representative plot of the disappearance of [Val-MeLeu (3-OH)-Abu] is shown in Figure 5. To determine the importance of the β -hydroxyl group in the degradation of [Val-MeLeu (3-OH)-Abu], the chemical stability of structurally similar tripeptide, [Val-MeLeu-Abu] does not possess a β -hydroxyl functionality and was essentially stable within the experimental time frame (Figure 5). This observation highlights the participation of the β -hydroxyl functionality in the degradation of [Val-MeLeu (3-OH)-Abu]. In addition, the chromatographic retention property of the product was highly dependent on the pH of the mobile phase and the added concentration of the TBA, consistent with an O-peptide (2,3). Also, upon neutralizing the pH to 7, the peak corresponding to the product disappeared, followed by reappearance of the starting N-peptide. Collectively, these observations suggest that, under aqueous acidic conditions, the tripeptide [Val-MeLeu (3-OH)-Abu] undergoes *N,O*-acyl migration to afford the corresponding O-peptide.

Significance of Backbone Cyclization on the Reactivity of CsA

Changes in peptide conformation may retard or enhance the rate of a specific chemical reaction by altering the overall population of the active conformer(s) responsible for the reactivity (6). Cyclization of the peptide backbone usually leads to more conformationally restricted peptides that often adopt a single or a limited number of preferred conformations (7,8). In contrast to cyclic peptides, small linear peptides of two to three amino acids generally possess signifi-

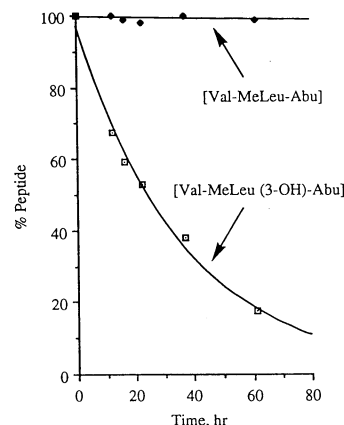


Fig. 5. Plot of disappearance of [Val-MeLeu (3-OH)-Abu] and [Val-MeLeu-Abu] at 50°C (pH 1.0 and $\mu = 0.15$ with KCl).

cant conformational flexibility and do not adopt a preferred secondary structure (9,10).

The significance of backbone cyclization and possibly conformation of CsA on the rate of isomerization may be assessed by comparing the chemical reactivity of a small tripeptide fragment with that of CsA. The tripeptide fragment must have an identical configuration, near the reactive site, to that of CsA but lack the backbone structural characteristics. Thus, the tripeptide must have a primary sequence of Val-MeBmt-Abu, protected at both C- and N-termini to negate any possible electrostatic interactions. The amino acid residue, MeBmt, which undergoes N,O-acyl migration in CsA, presents a synthetic challenge with its three contiguous asymmetric centers and the trans olefinic functionality (11). However, the amino acid N-methyl β -hydroxyleucine is more readily prepared, since it only contains two asymmetric centers. It was previously shown (3) that [Me-Leu (3-OH)]¹-CsA undergoes N,O-acyl migration at an identical rate to that of CsA. Therefore, it seemed reasonable to replace MeBmt with N-methyl β -hydroxyleucine in studying the effect of various structural elements on the rate and extent of N,O-acyl migration. Comparing the chemical reactivity of the linear tripeptide [Val-MeLeu (3-OH)-Abu], with the corresponding cyclic peptide [MeLeu (3-OH)]¹-CsA allows for a more precise definition of the effect of backbone cyclization (for this series) on the rate of N,O-acyl migration.

Figure 6 represents a partial pH-rate profile for the degradation of [Val-MeLeu (3-OH)-Abu] at 50°C. The plot is linear with slope approximately equal to negative unity, indicating apparent hydrogen ion catalysis. Similar pH-dependency has also been reported for the kinetics of N,O-acyl migration in CsA (3). The magnitude of the isomerization rate constant at pH 1.0 $\{2.6 (\pm 0.1) \times 10^{-2} \text{ hr}^{-1}\}$ was nearly identical to that of [MeLeu (3-OH)]¹-CsA $\{2.2 (\pm 0.2) \times 10^{-2} \text{ hr}^{-1}\}$. This observation suggested that any restriction in the aqueous backbone conformation of [MeLeu (3-OH)]¹-CsA due to its cyclic nature did not play a significant role in governing the kinetics of N,O-acyl migration. There are two plausible explanations consistent with these findings. First, the aqueous backbone structure of [MeLeu (3-OH)]¹-CsA, and presumably CsA, is quite distorted and possesses significant conformational flexibility, where the conformation state(s) of the reactive site closely resemble those

of the linear tripeptide. This is consistent with the postulated conformation of CsA in aqueous system, established using various other indirect methods (12). Second, based on the nature of reaction mechanism for N,O-acyl migration, the backbone conformation(s) restriction implied in cyclic peptides have no appreciable effect on the rate or the extent of reaction. Of course, this conclusion may be dependent on the ring size of the cyclic peptide. In the absence of additional evidence, it is impossible to unambiguously rule out either one of these possible explanations.

Importance of N-methylation of the Reactive-Amide Bond

The two possible N,O-acyl transfer reactive sites of CsC differ with respect to the nature of the reactive amide bonds. The reactive amide bond at residue MeBmt in both CsA and CsC is a tertiary amide; whereas, L-threonine in CsC is a secondary amide. To assess the influence of N-methylation of the amidic unit, the chemical reactivity of the tripeptide [Val-MeLeu (3-OH)-Abu] with that of [Val-Leu (3-OH)-Abu] were compared. The structures of these two tripeptides (Figure 3) differ only in the nature of amide linkage between the amino acids Leu (3-OH) and Val.

The kinetics of [Val-Leu (3-OH)-Abu] degradation was studied in dilute aqueous solution at pH 1.0. A representative plot comparing the disappearance of [Val-MeLeu (3-OH)-Abu] and [Val-Leu (3-OH)-Abu] is shown in Figure 7. As can be seen from the plot, the rate of chemical degradation of [Val-Leu (3-OH)-Abu] is substantially slower than [Val-MeLeu (3-OH)-Abu]. Chromatographically, a single degradation product with O-peptide like characteristics was isolated and shown to convert back to the starting polypeptide upon raising the pH of the solution.

The disparity in the chemical reactivity of these two tripeptides was consistent with our initial findings on the site of N,O-acyl migration in CsC i.e., in both cases, the tertiary amide bonds undergo N,O-acyl migration at a much higher rate than the secondary amide bonds. This observation was also consistent with the higher hydrolytic susceptibility of tertiary Asp-Pro amide bond than Asp-X linkages of secondary amide nature (13,14).

A number of factors affect the overall reactivity of pri-

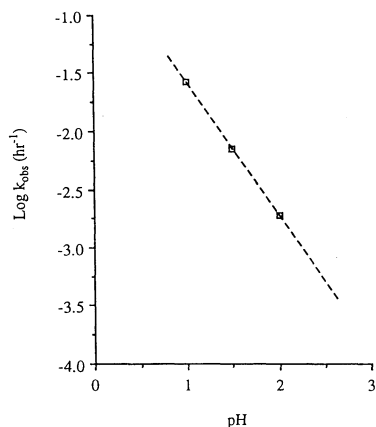


Fig. 6. Partial pH-rate profile for the degradation of [Val-MeLeu (3-OH)-Abu] at 50°C ($\mu = 0.15$ with KCl).

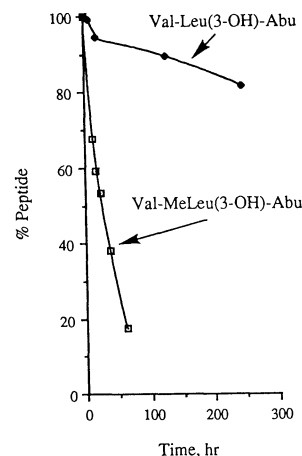


Fig. 7. Plot of disappearance of [Val-MeLeu (3-OH)-Abu] and [Val-Leu (3-OH)-Abu] at 50°C (pH 1.0 and $\mu = 0.15$ with KCl).

mary, secondary and tertiary amides. These factors include structural distortion of the amide bond, solvation, steric differences, conformational mobility of the anionic tetrahedral intermediate, the basicity of the amine leaving group and the *cis-trans* isomerization equilibrium of the amide bond. It is therefore difficult to explain the relative reactivity of primary, secondary, and tertiary amides based on a single generalization.

Structural distortion of an amide unit away from planarity has been shown to markedly alter its kinetic reactivity toward nucleophilic attack/hydrolysis (15,16). The relationship between hydrolytic reactivity and amidic distortion has been extensively studied (17). Bennet and co-workers (18), using ^{13}C and ^{15}N NMR spectroscopy, have shown that N-methylation of the amide bond increases the length of N-C(O) bond and diminishes the extent of resonance, accompanied with an accelerated hydrolysis. Therefore, the relative chemical reactivities of the tripeptides [Val-Leu (3-OH)-Abu] and [Val-MeLeu (3-OH)-Abu] may be explained in terms of the electronic and structural characteristics of the amidic units, where the N-methylation may distort and destabilize the amide bond and accordingly accelerate the rate of N,O-acyl migration.

The basicity of the amine leaving group may also play an important role in determining the relative chemical reactivity of amidic units (19). N-Alkylation of an amine group usually alters its basicity, via inductive and solvation effects, and hence leaving group ability.

Alternatively, the population of *cis* and *trans* isomers may be an important factor in determining the chemical reactivity of these tripeptides. The steric characteristics associated with each isomer might effect their propensity to undergo N,O-acyl migration. To our knowledge, there are no literature precedent supportive or consistent with this explanation.

CONCLUSIONS

In summary, the similarity in the chemical reactivity of the cyclic peptide [MeLeu (3-OH)]¹-CsA and the corresponding linear peptide [Val-MeLeu (3-OH)-Abu], suggests that cyclization of the peptide backbone may not play an important role in controlling the kinetics of N,O-acyl migration, with these two compounds. The disparity in the chemical reactivity of tripeptides [Val-MeLeu (3-OH)-Abu] and [Val-Leu (3-OH)-Abu], indicates that N-methylation of amide bond significantly impacted the kinetics of N,O-acyl migration, however, the mechanistic etiology of this phenomenon is not yet clear.

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