CD and Fourier transform IR spectroscopic studies of peptides. II. Detection of β-turns in linear peptides

ARTICLE *in* BIOPOLYMERS · FEBRUARY 1994

Impact Factor: 2.39 · DOI: 10.1002/bip.360340204 · Source: PubMed

8 AUTHORS, INCLUDING:

Zsuzsa Majer
Eötvös Loránd University
118 PUBLICATIONS **1,234** CITATIONS
SEE PROFILE

Anna Magyar
Eötvös Loránd University
32 PUBLICATIONS **235** CITATIONS
SEE PROFILE

András Perczel
Eötvös Loránd University
233 PUBLICATIONS **4,935** CITATIONS
SEE PROFILE

CD and Fourier Transform IR Spectroscopic Studies of Peptides. II. Detection of β-Turns in Linear Peptides

M. HOLLÓSI,[1] ZS. MAJER,[1] A. Z. RÓNAI,[1] A. MAGYAR,[1] K. MEDZIHRADSZKY,[1] S. HOLLY,[2] A. PERCZEL,[1,3] and G. D. FASMAN[3,*]

[1]Department of Organic Chemistry and Research Group of Peptide Chemistry, Eötvös University, 1117 Budapest, Hungary; [2]Central Research Institute for Chemistry of the Hungarian Academy of Sciences, 1525 Budapest, Hungary; and [3]Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254 USA

SYNOPSIS

Comparative CD and Fourier transform ir (FTIR) spectroscopic data on N-Boc protected *linear peptides* with or without the (Pro-Gly) β-turn motif (e.g., Boc-Tyr-Pro-Gly-Phe-Leu-OH and Boc-Tyr-Gly-Pro-Phe-Leu-OH) are reported herein. The CD spectra, reflecting both backbone and aromatic contributions, were not found to be characteristic of the presence of β-turns. In the amide I region of the FTIR spectra, analyzed by self-deconvolution and curve-fitting methods, the *β-turn band* showed up between 1639 and 1633 cm^{-1} in trifluoroethanol (TFE) but only for models containing the (Pro-Gly) core. This band was also present in the spectra in chloroform but absent in dimethylsulfoxide. These findings, in agreement with recent ir data on cyclic models and 3_{10}-helical polypeptides and proteins in D_2O [see S. J. Prestrelski, D. M. Byler, and M. P. Thompson (1991), *International Journal of Peptide and Protein Research,* Vol. 37, pp. 508–512; H. H. Mantsch, A. Perczel, M. Hollósi, and G. D. Fasman (1992), *FASEB Journal,* Vol. 6, p. A341; H. H. Mantsch, A. Perczel, M. Hollósi, and G. Fasman (1992), *Biopolymers,* Vol. 33, pp. 201–207; S. M. Miick, G. V. Martinez, W. R. Fiori, A. P. Todd, and G. L. Millhauser (1992), *Nature,* Vol. 359, pp. 653–655], suggest that the amide I band, with a major contribution from the acceptor C=O of the 1 ← 4 intramolecular H bond of β-turns, appears near or below 1640 cm^{-1}, rather than above 1660 cm^{-1}. In TFE, bands between 1670 and 1660 cm^{-1} are mainly due to "free" carbonyls, that is, C=O's of amides that are solvated but not involved in the characteristic H bonds of periodic secondary structures or β-turns. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

There is a growing interest in the conformation and folding patterns of small and midsized (<30 residues) polypeptides, e.g., peptide hormones. Based on recent x-ray crystallographic studies, it is very likely that T-cell epitopes recognized by MHC class I and II molecules are in this sequence size.[1] Thus, there is an impetus to search for new methods that can be used effectively for conformational characterization of small peptide molecules in this size range.

CD spectroscopy is a classical tool for investigating the conformation of polypeptides in solution. CD spectra reflect not only the type of basic secondary structures (α-helix, β-sheet, β-turn, aperiodic) but also possible distortions.[2]

β-Turns are known to play a crucial role in recognition processes and protein folding.[3] Recent studies (see Refs. 4 and 5 and related references therein) support the idea that the two most frequently occurring types of β-turns (Figure 1), type I and II, give significantly different spectra. Type I β-turns generally give a class C CD pattern,[2,6] which is a slightly blue-shifted, helix-like CD spectrum with decreased band intensities [typical λ_{max} ($[\theta]_{MR}$) parameters of a C spectrum, measured in water or triflouroethanol (TFE), are 216 nm (−4,600), 205 nm (−6,200), and 186 nm (6,700)].[4] Type II β-turns, encompassing the -Pro-Gly- segment, are characterized by a class B CD spectrum (a negative band

Biopolymers, Vol. 34, 177–185 (1994)

CCC 0006-3525/94/020177-09

* To whom correspondence should be addressed.

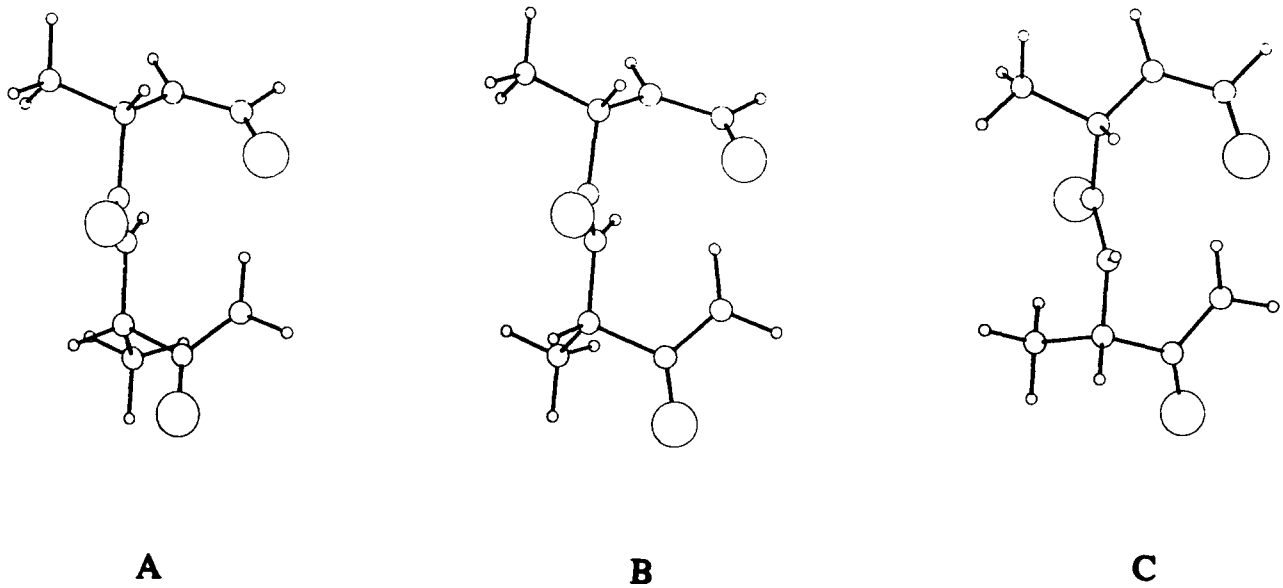

Figure 1. The β-turn conformations of For-Ala-Ala-NH_2 (For = formyl) A: type I ($\phi_2 = -60°$, $\psi_2 = -30°$, $\phi_3 = -90°$, $\psi_3 = 0°$); B: type III ($\phi_{2,3} = -60°$, $\psi_{2,3} = -30°$); C: type II ($\phi_2 = -60°$, $\psi_2 = 120°$, $\psi_3 = 80°$, $\psi_3 = 0°$). Drawings are based on standard Venkatachalam geometry[30] using CHARMM[21] parametrization in QUANTA (POLYGEN), version 3.2 on a Silicon Graphics Computer.

between 230 and 220 nm, a stronger positive band above 200 nm, and a second negative band near 190 nm).[2,6] There are several indications in the literature that type II′ and type III β-turns also give a class C CD spectral pattern.[2,3,7,8] Type II β-turns with a D residue in the second corner position result, in many cases, in class C′ CD spectrum (a mirror image of class C).[2,9,10] Thus, CD spectroscopy can distinguish between type I and type II β-turns, but only if their population is sufficiently high, and the C or B spectra are not overlapped by the strong CD spectra of extensive periodic (α-helix or β-sheet) conformations, or obscured by the chiral contribution of side-chain chromophores (e.g., aromatic amino acids).

Fourier transform ir (FTIR) spectroscopy has become a valuable technique for investigating polypeptide secondary structure.[11] Infrared studies have mainly focused on the amide I region (1700–1620 cm^{-1}). In solution the frequency of the C=O stretching vibrations is sensitive to the H-bonding characteristics of the main conformational forms and to the environment (solvation, interaction with metals, etc.) of the amide groups. Resolution-enhanced techniques are used to mathematically narrow broad amide I bands so that the individual component bands[11] can be better visualized and evaluated.

The examination of polypeptide conformation, through the analysis of their ir spectra, is somewhat limited by the lack of correlations between specific backbone conformations and individual component bands. The structure-spectral correspondence is best understood for proteins in D_2O solution, mainly due to the systematic efforts by Byler and Susi.[12] Bands centered between 1658–1650 cm^{-1} are associated with α-helical segments, whereas aperiodic (nonordered) segments are associated with an ir band around 1644 cm^{-1} (1648–1640 cm^{-1}). The latter band is generally broad, suggesting that it represents a composite of a number of closely spaced but unresolved components. The ir bands between 1640 and 1620 cm^{-1} have been assigned by many authors to β-pleated sheets. The appearance of a weak additional band between 1695–1670 cm^{-1} is indicative of the antiparallel orientation of the β-strands.[11,13] Generally, there are more weak bands in this region of the ir spectrum, which are associated with turns (see Ref. 11 and references therein).

According to comparative X-ray[14] and FTIR[15] studies on α-lactalbumin, the band that appears near 1640 cm^{-1}, in D_2O, is due to 3_{10}-helices. Unlike regular α-helices, 3_{10}-helices are usually short (the mean length is 3.3 residues, i.e., one "turn" of helix),[16] which suggests that the acceptor carbonyl of the H bond in a type III β-turn (the building unit of the 3_{10}-helix) also should result in a band near 1640 cm^{-1}.

The cyclic pseudohexapeptides cyclo[Gly^1-Pro^2-X^3-Gly^4-NH-$(CH_2)_n$-CO] [X = Gly, Ser(O^tBu), Ser; n = 4, 2] have been shown to contain one or

two 1 ← 4 intramolecular H bonds, which stabilize β-turns.[4,5] The nature of these β-turn has been established by one-dimensional and two-dimensional (2D) nuclear Overhauser effect (NOE) studies in a variety of solvents.[4,5] The presence of a type I β-turn in the Pro-Ser(O^{t}Bu) model was also verified by x-ray analysis.[5] FTIR spectroscopic studies have shown that an ir band near 1640 cm^{-1} can be correlated with the acceptor C=O of the strong 1 ← 4 intramolecular H bond of the β-turns in these cyclic peptides.[17,18] In the solution spectrum of these cyclic models, the "β-turn band" appears at 1640 ± 2 cm^{-1}. Cyclic peptides are ideal models of β-turns because the folding of the molecule is dictated by the cyclic structure. It is questionable whether the 1 ← 4 intramolecular H bond in linear peptides can approach the strength of the H bond in cyclic peptides and whether the population of H bonded turns can be high enough to result in a detectable "turn band" in the ir spectrum.

To address these questions, comparative CD and FTIR spectroscopic studies were performed on N-protected linear peptides that, based on their amino acid sequence, should tend to form H-bonded β-turns (Table I). Herein the results of spectroscopic studies and molecular dynamics simulations of the conformation of peptides *1–4* are reported. Peptides *1* and *2* have significant antagonist effect against [Met^5]-enkephalin in the mouse vas deferens bioassay.[19] The relationship between conformation and biological activity will be discussed elsewhere.

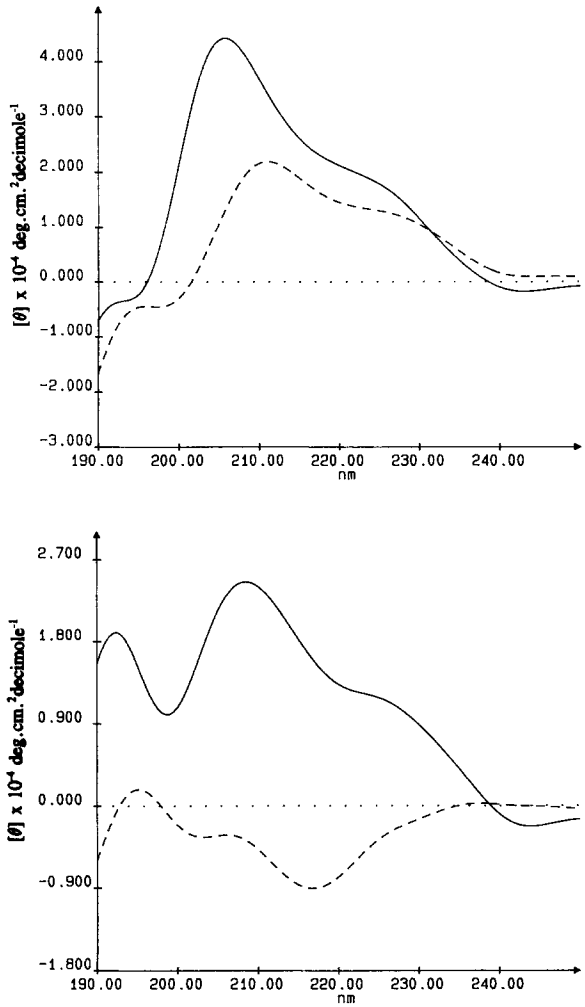

Figure 2. A: CD spectra of *1* in TFE (——) and water-TFE (60 : 40) (- - -) B: Boc-YPGFLT-OH in TFE (——) and water–TFE (85 : 15) (- - -). (For concentration, temperature, and pathlength, see Experimental.)

EXPERIMENTAL

Synthesis

Peptides *1–4* have been synthesized by standard solution methods as described earlier.[19]

CD Spectroscopy

CD studies were performed on a Jobin-Yvon Mark VI dichrograph calibrated with epiandrosterone. The nmr grade TFE (Aldrich) was used as solvent. Measurements were carried out at ambient temperature in 0.01 or 0.02 cm cells. The concentration of the samples ranged between 0.4 and 0.8 mg/mL. Molar ellipticity, $[\theta]_M$, is given in deg · cm^2/dmol. Spectra shown in Figures 2–4 are smoothed by the program of the dichrograph. The Savitzky–Golay smoothing algorithm was used.

FTIR Spectroscopy

FTIR measurements (at a resolution of 4 cm^{-1}) were performed at room temperature on a Nicolet 170SX

Table I

1	Boc-Tyr-Pro-Gly-Phe-Leu-OH, Boc-YPGFL-OH
2	Boc-Tyr-Pro-Gly-Phe-Leu-Thr-OH, Boc-YPGFLT-OH
3	Boc-Tyr-Gly-Pro-Phe-Leu-OH, Boc-YGPFL-OH
4	Boc-Tyr-Pro-Phe-Leu-OH, Boc-YPFL-OH

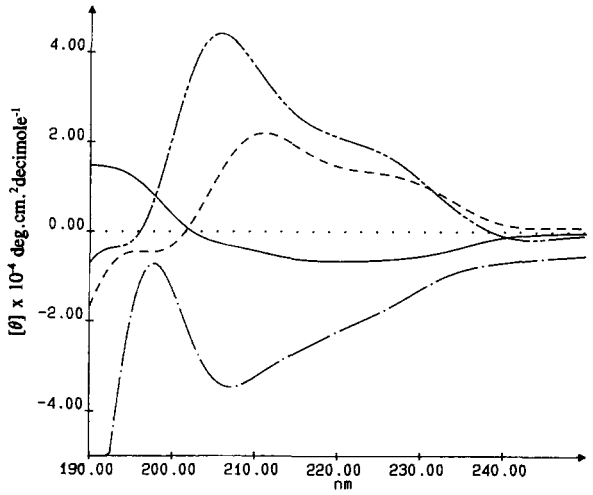

Figure 3. CD spectra of *1* in TFE (----) and water–TFE (60 : 40) (---), and *3* in TFE (——) and water–TFE (80 : 20) (-·-). (For concentration, temperature, and pathlength, see Experimental.)

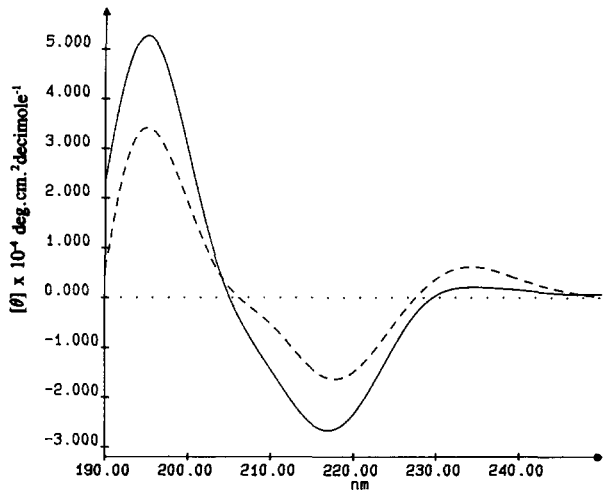

Figure 4. CD spectra of *4* in TFE (——) and water–TFE (70 : 30) (---). (For concentration, temperature, and pathlength, see Experimental.)

spectrometer. Peptide solutions (c = 0.5 mg/mL) were prepared in TFE (see above), chloroform, and DMSO (Merck, Uvasol grade). Infrared spectra of the solvents were obtained under identical conditions and subtracted from the spectra of the solutions. The bending vibrational band of water between 1700 and 1600 cm^{-1} was removed by subtracting the spectra of the solvents (containing traces of water) on the basis of the OH stretching band at 3480 cm^{-1} (in DMSO) and the combination band of OH stretching and HOH deformation at 5293 cm^{-1} (in TFE). This procedure allowed an unambiguous analysis of spectra in TFE, even in the critical 1650–1600 cm^{-1} range. KBr cells of 0.041 cm were used. The spectra were analyzed by a normalized least-squares, curve-fitting program, using products of Gaussian and Lorentzian curves (Holly et al., unpublished). The curve-fitting procedure was assisted by the Fourier self-deconvolution algorithm of Mantsch et al.[20]

Molecular Dynamics Simulations

Energy minimizations and dynamics trajectory calculations were performed on a Silicon Graphics computer using CHARMM[21] implemented in QUANTA (POLYGEN). In general, molecular dynamics simulation was achieved by heating (from 0 to 300 K), equilibration (300 K, 5 ps), and simulation steps (100 ps at 300 K). Changing the dielectric constant term (ϵ^d) from 1 to 80 did not have a significant effect on the results.

RESULTS

CD Studies

Boc-YPGFL-OH (*1*) and Boc-YPGFLT-OH (*2*) show class C′ CD spectra in TFE (Figure 2). The

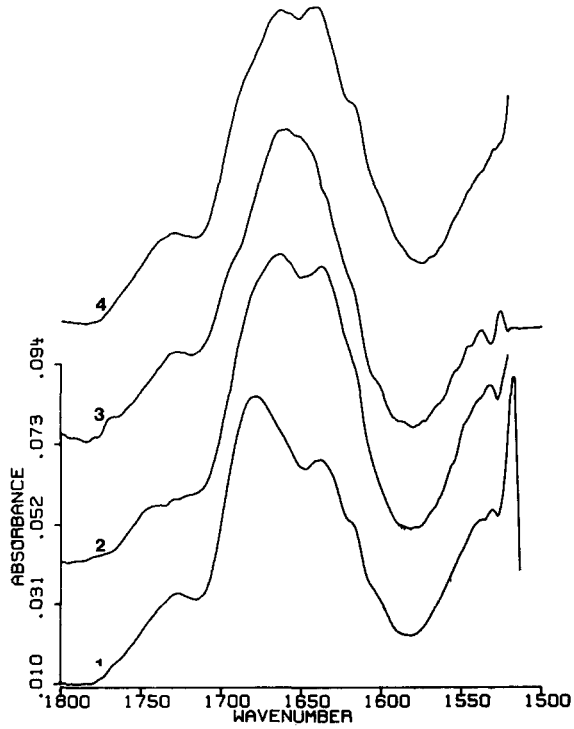

Figure 5. Amide I region of the FTIR spectra of peptides *1–4* in TFE solution. 1: Boc-YPGFL-OH; 2: Boc-YPGFLT-OH; 3: Boc-YGPFL-OH; 4: Boc-YPFL-OH.

CD spectrum of *2* is more complex than that of *1* in which the contribution of aromatic side chains overlaps the spectrum of the peptide backbone. The CD curve of *2* in TFE has a second positive band below 200 nm. In water the spectrum of *1* preserves some of its C′ character shown in TFE. The CD spectrum of *2* in a water–TFE (85 : 15) mixture reflects more conformational flexibility and shows a negative band at 217 nm, with a negative shoulder (Figure 2B) that reflects an overall conformational change of the molecule.

The CD spectra of peptides Boc-YPGFL-OH (*1*) and Boc-YGPFL-OH (*3*) in TFE and water are shown in Figure 3. Unlike the class C′ spectrum[2] of *1* and *2* in TFE, *3* shows a shapeless and weak CD curve. This indicates an averaging of the backbone and aromatic contributions of opposite sign in the predominant conformer or conformer populations of *3*. In water the increased amplitude of the negative band below 210 nm reflects a change of the dominant conformation or, most likely, a shift of the conformational equilibrium.

Boc-YPFL-OH (*4*) has a CD spectrum with a negative band at 217 nm and a positive band at 195 nm in TFE, and these features are retained in water containing at least 30% TFE (Figure 4). (Peptides *1–4* are not soluble in pure water.) In the spectrum in TFE, the shoulder near 205 nm is likely due to the overlapping contribution of the L_a band of the aromatic side chains of phenylalanine and tyrosine.[22] This "water-resistant" spectrum may reflect the predominance of a single conformer population.

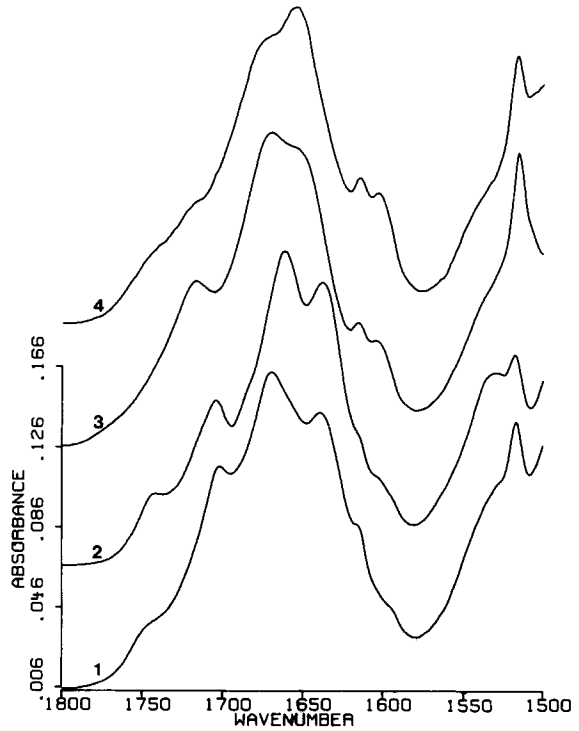

Figure 6. Amide I region of the FTIR spectra of peptides *1–4* in chloroform solution. 1: Boc-YPGFL-OH; 2: Boc-YPGFLT-OH; 3, Boc-YGPFL-OH; 4: Boc-YPFL-OH.

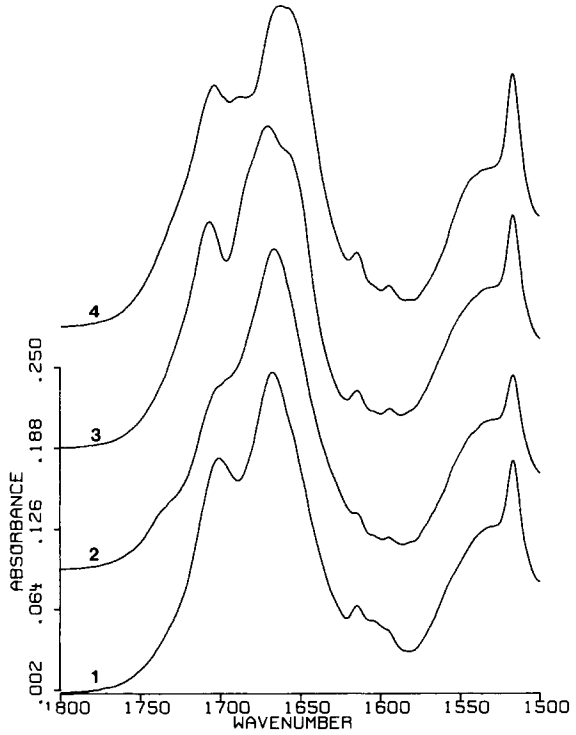

Figure 7. Amide I region of the FTIR spectra of peptides *1–4* in DMSO solution. 1: Boc-YPGFL-OH; 2: Boc-YPGFLT-OH; 3: Boc-YGPFL-OH; 4: Boc-YPFL-OH.

Fourier-Transform Infrared Spectroscopic Studies

FTIR spectroscopic studies of peptides *1–4* were performed in TFE, chloroform, and DMSO (Figures 5–7). Unlike the CD spectra, which reflect both backbone and aromatic contributions, only the former is reflected in the amide I region (1700–1620 cm^{-1}) of the corresponding ir spectra. The minor band at 1615 cm^{-1}, along with the strong band at 1515 cm^{-1}, originates from the tyrosine group, while the weak band at 1610 cm^{-1} arises from the aromatic side chain of phenylalanine.

In TFE the ir spectra of the -(PG)- models, *1* and *2*, show a band at 1638 cm^{-1}, as well as a strong band centered at 1678 and 1663 cm^{-1}, respectively (Figure 5). In the curve-fitted spectra, major amide I component bands are found at 1685, 1666, and 1633 cm^{-1} for *1* (Figure 8) and at 1680, 1663, and 1634 cm^{-1} for *2* (not shown). The band below 1640

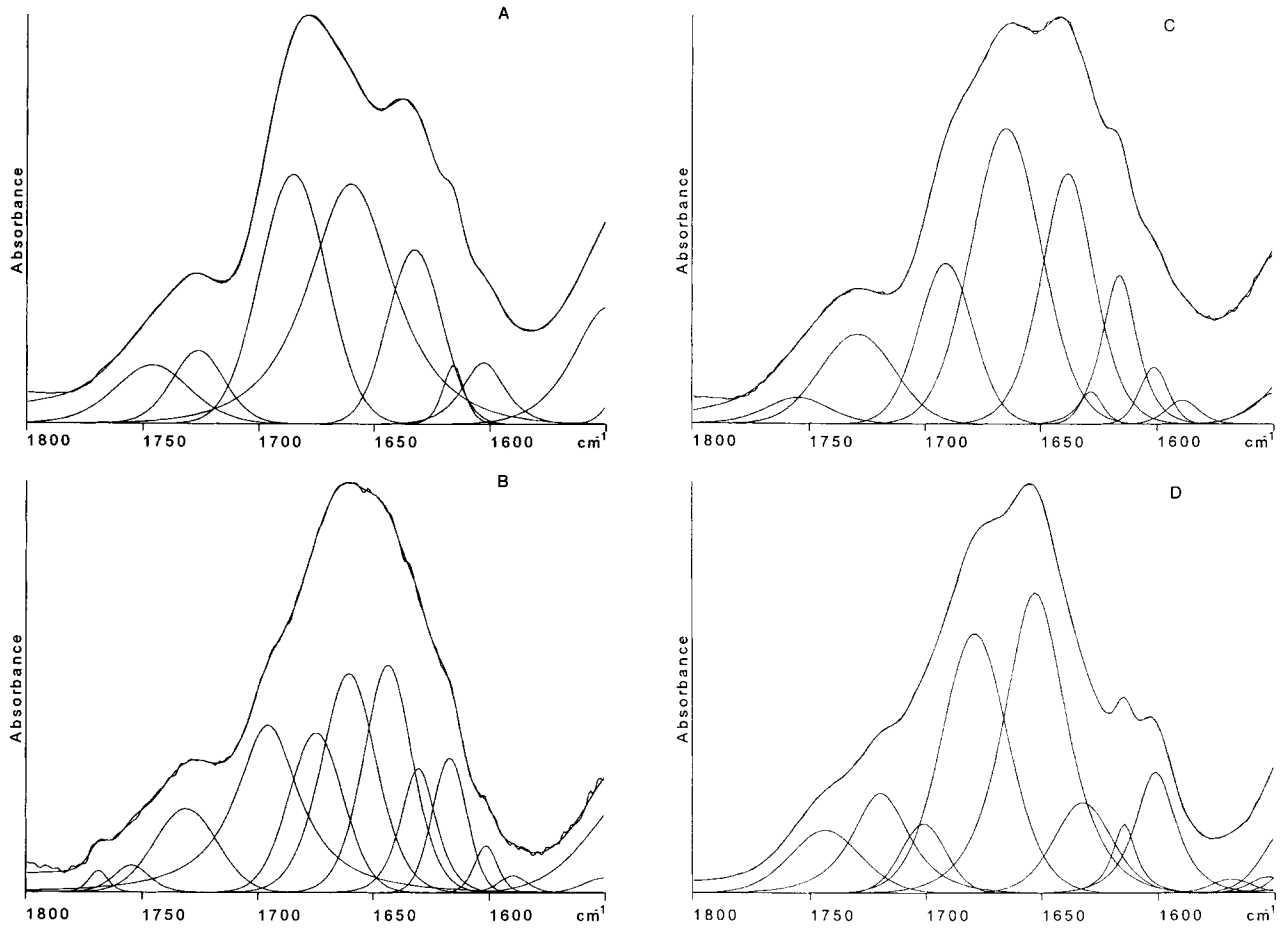

Figure 8. Decomposition of the amide I region of the FTIR spectra of (A) Boc-YPGFL-OH(*1*) in TFE, (B) Boc-YGPFL-OH(*3*) in TFE, (C) Boc-YPFL-OH(*4*) in TFE, and (D) Boc-YPFL-OH(*4*) in chloroform (also see Experimental). The ragged overlapping lines show the experimental and curve-fitted (sum) spectra. The position of component bands was checked by Fourier self-deconvolution.[20]

cm^{-1} is present in the ir spectra of *1* and *2* in chloroform (Figure 6) but absent in DMSO (Figure 7). This solvent is known to disrupt H bonds[23] that are not buried, e.g., in cyclic peptides.

In the ir spectrum of *3* and *4* in TFE, not featuring the turn-forming (PG) motif, no band is found between 1640 and 1633 cm^{-1}, as in the case of peptides *1* (Figure 8, panel A) and *2*.

The ir spectrum of (*4*) in TFE shows a resemblance to the spectra of *1* and *2*. Component bands appear at 1729 (urethane), 1691 (COOH), 1665, 1638, and 1616 cm^{-1} (Figure 8, panel C). The component band at 1638 cm^{-1} may be indicative of some folded conformation. However, the significant spectral differences (Figure 8) suggest different features of the major conformers of *1* (or *2*) and *4* in TFE.

The assignment of bands in the ir spectrum of *4* in chloroform (Figure 6) is more challenging. Component bands at 1665 and 1638 cm^{-1} in the curve-fitted ir spectrum in TFE (Figure 8, panel C) are replaced by bands at 1679, 1653, and 1632 cm^{-1} (panel D). The strongest one shows up at 1653 cm^{-1} (at 1654 cm^{-1} in the original spectrum, Figure 6). The component bands, near or above 1700 cm^{-1} in the curve-fitted spectrum of *4* in chloroform, are also weaker (cf. Figure 8, panels C and D). This finding, together with the appearance of the dominant band at 1653 cm^{-1}, may be the sign of the adoption of a unique H-bonded conformation of *4* in chloroform.

The ir spectra of *3* in chloroform and DMSO show similarities (Figures 6 and 7). The predominant band appears in both solvents at 1670 cm^{-1}, accompanied by a shoulder near 1655 cm^{-1}. The band at 1670 cm^{-1} (Figures 6 and 7) can be attributed to free weakly solvated amide carbonyls, while the shoulder, which is more expressed in the chloroform spectrum, is attributed to carbonyls involved in some H-bonded conformation.

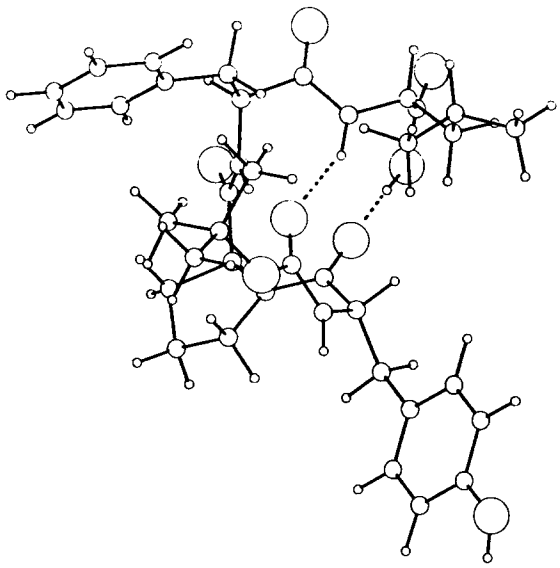

Figure 9. Proposed conformation of *4* in nonpolar solution. Molecular dynamics simulation was started from a conformation with two intramolecular H bonds between $C{=}O_{urethane}\cdots H\text{-}N_{Leu}$ and $C{=}O_{Tyr}\cdots H{-}O_{COOH}$ ($d_{O\ldots H}$ = 2.2 Å before simulation).

Molecular Dynamics Simulation

For *1* molecular dynamics simulations were started from type I and type II β-turn geometries. Surprisingly, when starting from a type I structure, a jump in the ψ angle of proline was observed, from $-15° \pm 10°$ to $100° \pm 10°$, an indication of the higher stability of the type II β-turn-like structure. In the case of Boc-YPFL-OH *4*, the calculation was started from a structure with 1 ← 5 H bonds between $C{=}O_{urethane}\cdots H{-}N_{Leu}$ and $C{=}O_{Tyr}\cdots H{-}O_{COOH}$; $d_{O\ldots H} = 2.2 \pm 0.3$ Å (see FTIR studies). The supposed H bonds, as shown in Figure 9, were preserved during the simulation. After geometry optimizations, the $d_{O\ldots H}$ distances were found to be 1.81 Å ($C{=}O_{Tyr}\cdots H{-}_{COOH}$) and 1.99 Å ($C{=}O_{urethane}\cdots H{-}N_{Leu}$).

DISCUSSION

Conformation of Peptides *1–4*

In the case of small peptides containing aromatic amino acid residues, CD spectroscopy cannot assign the conformation of the peptide backbone unambiguously. However, its sensitivity toward subtle differences in the steric structure makes this method indispensible in investigating peptides. Based on the CD spectra, peptides *1* and *4* could be divided into three conformational groups. FTIR spectroscopy yielded a more detailed characterization of the backbone conformation of the major conformers.

1. The class C′ CD spectrum of peptides *1* and *2* in TFE is compatible with the adoption of a type II β-turn conformation (Figure 2). The band below 1640 cm^{-1} in the ir spectrum of *1* and *2* in TFE and chloroform (Figures 5 and 6) supports the presence of a 1 ← 4 intramolecular H bond, which stabilizes the β-turn structure.[17,18] The type II character of the turn is in agreement with recent nmr (nuclear Overhauser effect) data on linear (Pro-Gly)-models[10,24] showing significant type II β-turn conformer populations in solution. Molecular dynamics simulations also suggest that *1* has lower energy if it adopts a type II β-turn conformation. The hexapeptide model (*2*), elongated with a C-terminal threonine, has more conformational freedom, as indicated by the marked change of its CD spectrum measured in TFE and water/TFE mixtures (Figure 2). The difference in backbone flexibility is also reflected in the FTIR spectra in TFE of *1* and *2* (Figure 5).

2. The CD spectra of *3* in TFE and water exclude the predominance of a single conformer population, while the corresponding ir spectrum in pure TFE (Figure 5) does not support the occurrence of β-turn populations (no band is present at about 1638 cm^{-1}).

3. The CD spectrum of *4* in TFE differs significantly from those of peptides *1–3* (Figures 2–4). The ir spectrum of *4* in TFE (Figures 5 and 8) also reflects conformational differences. The component band at 1638 cm^{-1} in the curve-fitted ir spectrum (Figure 8, panel C) is compatible with conformer population(s) featuring a 1 ← 4 intramolecular H bonding. (Because of the presence of the bulky side chain of phenylalanine, the adoption of a type II β-folded conformation is unlikely.) The characteristic difference between the spectra in TFE and chloroform suggests a dramatic change of the intramolecular system of H bonds. In the ir spectrum of *4* in chloroform (Figure 6), the urethane C=O band is shifted to lower frequencies, which is likely due to its involvement in intramolecular H bonding. These findings permit the adoption of two 1 ← 5 H bonds ($C{=}O_{urethane}\cdots H{-}N_{Leu}$ and $C{=}O_{Tyr}\cdots H{-}O_{COOH}$), which may stabilize a short α-helical segment (Figure 9).

The 500-MHz ^{1}H-nmr studies[25] in CD_3CN of the peptides *1* and *3* strongly support the above assignments. In the nuclear Overhauser effect spectroscopy (NOESY) spectrum of *1* in CD_3CN, characteristic cross peaks were found between the $H^{\alpha}_{Pro}/NH_{Gly}$, $H^{\alpha}_{Gly}/NH_{Gly}$, NH_{Phe}/NH_{Gly}, $H^{\alpha}_{Gly}/NH_{Phe}$, and $NH_{Phe}/H^{\alpha}_{Pro}$ protons. These

findings, together with the $J_{NH,\alpha}$ coupling constants of Gly (6.5 and 5.3 Hz) and further NOESY and ^{13}C-nmr data,[26] clearly reflect the all-*trans* backbone conformation and are diagnostic of a major type II β-turn conformation of *1* in CD_3CN.

The interpretation of NOE data in CD_3CN on *3* was obscured by the overlapping of some important NH pairs, as well as H^{α} pair signals. The strong cross peaks between the $NH_{Phe}/H^{\alpha}_{Pro}$ and $NH_{Gly}/H^{\alpha}_{Tyr}$ protons, together with $J^{Phe}_{NH,\alpha}$ (8.7 Hz) and $J^{Gly}_{NH,\alpha}$ (4.7 and 4.2 Hz), are compatible with an open β-turn or a γ-turn centered at Pro, but other conformations are also possible. Comparative ^{1}H- and ^{13}C-nmr studies on peptides *1–4* in CD_3CN and DMSO-d_6 will be published elsewhere.[26]

Detection of β-Turns by Fourier-Transform Infrared Spectroscopy

Comparative x-ray crystallographic[14] and ir spectroscopic data[15] on α-lactalbumin and lysozyme have suggested that the band at 1639 cm^{-1} in the ir spectra in D_2O of these proteins can be correlated with short 3_{10}-helical segments. More recently, the band located at 1637 cm^{-1} in the ir spectrum in D_2O of short alanine-based peptides was also attributed to a 3_{10}-helix on the basis of electron-spin resonance spectra of doubly spin-labelled derivatives.[27] The 3_{10}-helices are repeats of type III β-turns. Thus, the 1 ← 4 H bonds of type III β-turns are also expected to give an "acceptor band" near 1640 cm^{-1}, rather than above 1660 cm^{-1}. FTIR spectroscopic studies reported herein give support to our earlier proposal[17,18] that 1 ← 4 H-bonded type I and II β-turns feature an amide I C=O stretching band at 1640 ± 2 cm^{-1} in D_2O, chloroform, and DMSO solution, while in halogenated alcohols (TFE, chloroethanol) this band may be shifted as low as ~ 1633 cm^{-1}.[18] In the ir spectrum of cyclic peptides, the β-turn band, that is, the amide I band that is contributed mostly by the acceptor C=O of the intramolecular H bond, is present even in the H-bond disrupting solvent, DMSO,[23] whereas it is missing from the spectrum in DMSO of the linear turn-forming peptides *1* and *2* (Figure 7). This finding is in good agreement with recent nmr studies on linear β-turn models Ac-Val(Ala)-Pro-X-Y-NH_2 (where X = Gly, D-Ser, D-Ala, D-Val, D-Leu, and Y = His, Phe, Ile).[10] The 2D spin-locked ROESY experiments and variable temperature studies showed that in DMSO no significant type II β-turn population is present. TFE and other halogenated alcohols appear to have not only a helix but also a β-turn-stabilizing effect and result in an increase of the H-bonded β-turn conformer population.

The β-turn band was observed in the FTIR spectra in TFE of N-glycosylated peptides containing the -(Pro-Gly)- turn motif.[28]

The above data, together with recent FTIR studies on 1 ← 4 H bonds in linear and cyclic polypeptides[15,17,18,25,27] suggest that all three basic subtypes (I–III) of β-turns give rise to a characteristic β-turn ir band near or below 1640 cm^{-1}. This allows one to distinguish between α- and 3_{10}-helices but not between type I(III) and type II β-turns.

In the ir spectra of the pseudohexapeptide models cyclo[Gly-Pro-X-Gly-NH-$(CH_2)_4$-CO] [X = Gly, Ser(O^{t}Bu), Ser] bands in the range of 1676–1667 cm^{-1} were assigned to amide C=O groups not involved in intramolecular H bonds.[18] In the spectra of the linear models *1–4*, the band receiving more contribution[29] from the nonacceptor C=O's of the turn, as well as other amides in the molecule, was observed in a similar frequency range (1676–1661 cm^{-1}). The frequency of the "free" amide band is higher in DMSO and chloroform (near 1670 cm^{-1}, Figures 6 and 7) than in TFE and other strong H-bond donating solvents (1670–1661 cm^{-1}).[18]

The normal coordinate analysis treatment of the amide modes of β-turns has recently been reviewed by Bandekar.[29] The calculations have clearly shown that the coupling between transition dipoles may also lead to conformation-dependent effects in the infrared spectra.[29] The amide I band, which contains a major contribution from the acceptor C=O of the 1 ← 4 intramolecular H bond, was calculated to appear above 1650 cm^{-1} for β-turns I–III of standard Venkatachalam geometry.[30] Only high $\Delta\mu_{eff}$ values (0.45D) resulted in frequencies near 1650 cm^{-1} for types I and III, but not for type II β-turns. Apparently, further studies are required to find an explanation for the disagreement between theory[32] and experiments discussed in this paper and summarized in recent literature.[15,17,18,25,27]

CONCLUSION

While amide I bands between 1665 and 1695 cm^{-1} have been assigned to turns in general,[11,31] it now appears that the amide I band, with a major contribution from the acceptor C=O of H-bonded type I–III β-turns, may occur at much lower frequencies, i.e., 1640 ± 2 cm^{-1} or below. For short peptides, bands above 1665 cm^{-1} may arise from carbonyl vibrations of the second (central) or third (H-bond donating) amide groups. These carbonyl groups are more exposed to the solvent than the acceptor C=O group, which has an inside orientation. In larger peptides and proteins, bands above 1665 cm^{-1} are

thought to originate from C=O of amide groups that are not involved in H bonds of periodic structures (α-helix, β-sheet, etc.) and β-turns. The frequency of these amide bands reflects the solvational state and/or "degree of internalization" of the nonacceptor carbonyls, and it is not related directly to the characteristic intramolecular H bond of the β-turn.

Proteins are rich in subconformations that are nonideal or distorted variants of the basic secondary structures. The family of β-turns also has nonhydrogen bonded (distorted or open) members.[3,32,33] Inverse γ-turns ($\phi = -80°$, $\psi = 80°$) and γ-turns ($\phi = 80°$, $\psi = -80°$), the other group of folded secondary structures, may also be H bonded (1 ← 3 H-bonded turns, C_7 structures[3]). Moreover, β-turns may exist in equilibrium with γ-turns. The assignment of the amide I frequencies of the acceptor carbonyls in these substructures awaits further studies.

This is a joint publication of the Department of Organic Chemistry and Research Group of Peptide Chemistry, Eötvös University, Budapest, H-1117, Hungary; Central Research Institute for Chemistry, of the Hungarian Academy of Sciences, 1525 Budapest, Hungary; and the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254, USA. This work is supported by Hungarian grants, OTKA I/3/2239 and 2245 (to MH), a joint NSF–Hungarian Academy of Sciences grant (to GDF and MH), and NSF no. DMB-9007055 (to GDF).

REFERENCES

1. Madden, D. R., Gorga, J. C., Strominger, J. L. & Wiley, D. C. (1991) *Nature* **353,** 321–325.
2. Woody, R. W. (1985) in *The Peptides,* Vol. 7, Hruby, V. J., Ed., Academic Press, New York, pp. 15–113.
3. Smith, F. A. & Pease, L. A. (1980) *CRC Crit. Rev. Biochem.* **8,** 315–399.
4. Hollósi, M., Kövér, K. E., Holly, S., Radics, L. & Fasman, G. D. (1987) *Biopolymers* **26,** 1555–1572.
5. Perczel, A., Hollósi, M., Foxman, B. M. & Fasman, G. D. (1991) *J. Am. Chem. Soc.* **113,** 9772–9784.
6. Woody, R. W. (1974) in *Peptides, Polypeptides and Proteins,* Blout, E. R., Bovey, F. A., Lotan, N. & Goodman, M., Eds., Wiley, New York, pp. 338–360.
7. Gierasch, L. M., Deber, C. M., Madison, V., Niu, C.-H. & Blout, E. R. (1981) *Biochemistry* **20,** 4730–4738.
8. Bandekar, J., Evans, D. J., Krimm, S., Leach, S. J., Lee, S., McQuie, J. R., Minasian, E., Nemethy, G., Pottle, M. S., Scheraga, H. A., Stimson, E. R. & Woody, R. W. (1982) *Int. J. Peptide Protein Res.* **19,** 187–201.
9. Hollósi, M., Perczel, A. & Fasman, G. D. (1990) *Biopolymers* **29,** 1549–1564.
10. Imperiali, B., Fisher, S. L., Moats, R. A. & Prins, T. J. (1992) *J. Am. Chem. Soc.* **114,** 3182–3188.
11. Surewicz, W. K. & Mantsch, H. H. (1988) *Biochim. Biophys. Acta* **952,** 115–130.
12. Byler, M. & Susi, H. (1986) *Biopolymers* **25,** 269–487.
13. Krimm, S. & Bandekar, J. (1986) *Adv. Protein Chem.* **38,** 181–364.
14. Acharya, K. R., Stuart, D. I., Walker, N. P. C., Lewis, M. & Phillips, D. C. (1989) *J. Mol. Biol.* **208,** 99–127.
15. Prestrelski, S. J., Byler, D. M. & Thompson, M. P. (1991) *Int. J. Peptide Protein Res.* **37,** 508–512.
16. Toniolo, C. & Benedetti, E. (1991) *TIBS* **16,** 350–353.
17. Mantsch, H. H., Perczel, A., Hollósi, M. & Fasman, G. D. (1992) *FASEB J.* **6,** A341.
18. Mantsch, H. H., Perczel, A., Hollósi, M. & Fasman, G. D. (1992) *Biopolymers* **33,** 201–207.
19. Rónai, A. Z., Botyánszki, J., Hepp, J. & Medzihradszky, K. (1992) *Life Sci.* **50,** 1371–1378.
20. Mantsch, H. H., Moffatt, D. J. & Casal, H. G. (1988) *J. Mol. Structure* **173,** 285–298.
21. Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S. & Karplus, M. (1983) *J. Comp. Chem.* **4,** 187–217.
22. Woody, R. W. (1978) *Biopolymers* **17,** 1451–1467.
23. Jackson, M. & Mantsch, H. H. (1991) *Biochim. Biophys. Acta* **1078,** 231–235.
24. Dyson, H. J., Rance, M., Houghten, R. A., Lerner, R. A. & Wright, P. E. (1988) *J. Mol. Biol.* **201,** 161–200.
25. Perczel, A., Majer, Zs., Holly, S., Machytka, D., Fasman, G. D. & Hollosi, M. (1993) *Tetrahedron: Asymmetry* **4,** 591–603.
26. Machytka, D., Hollosi, M., Perczel, A., Fasman, G. D. & Radics, L., in preparation.
27. Miick, S. M., Martinez, G. V., Fiori, W. R., Todd, A. P. & Millhauser, G. L. (1992) *Nature* **359,** 653–655.
28. Laczkó, I., Hollósi, M., Ürge, L., Ugen, K. E., Weiner, D. B., Mantsch, H. H., Thurin, J. & Ötvös, L., Jr. (1992) *Biochemistry* **31,** 4282–4288.
29. Bandekar, J. (1992) *Biochim. Biophys. Acta* **1120,** 123–143.
30. Venkatachalam, C. M. (1968) *Biopolymers* **6,** 1425–1436.
31. Surewicz, W. K., Mantsch, H. H. & Chapman, D. (1993) *Biochemistry* **32,** 389–394.
32. Chou, P. Y. & Fasman, G. D. (1977) *J. Mol. Biol.* **115,** 135–175.
33. Wilmot, C. M. & Thornton, J. M. (1990) *Protein Eng.* **3,** 479–493.

Received April 16, 1993
Accepted August 6, 1993