Conformational Studies of β-Turns in Cyclic Peptides by Vibrational CD

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Received March 27, 1995[⊗]

Abstract: We report the infrared absorption and vibrational circular dichroism (VCD) spectral features of peptide β-turns observed in small cyclic peptides dissolved in nonaqueous solvents. The molecules studied, *cyclo*-(Cys-Pro-Xxx-Cys), with Xxx = Gly, Phe, D-Phe, all form 14-member rings closed by a −S−S− linkage. The VCD spectra of these molecules vary enormously when the polarity and H-bonding ability of the solvent is varied. Furthermore, type I and type II β-turns can be formed, depending on the chirality of the residue Xxx in the 3-position. VCD intensity calculations, based on standard type I and type II geometries, were carried out and found to agree well with the observed VCD spectra.

Introduction

Turns are common structural motifs in proteins, comprising on average about 25% of the residues.[1] A survey of 29 proteins[2] showed that the frequency for turns is 32%, compared to 38% for helices and 20% for β-sheets. Specific turns, linking strands of anti-parallel β-sheet structures, were first recognized over 20 years ago by Venkatachalam[3] and are referred to as β-turns. Thus it is clear that turns in general, and β-turns in particular, are an essential and fundamental class of polypeptide structures.

In globular proteins, the secondary structures are organized in specific ways to bring together functional groups from different regions of the protein sequence to create the active site. The turn structures between structurally rigid peptide sections allow the polypeptide chains to adopt the appropriate globular structures. Turns have also been portrayed as nucleation sites for the protein folding process,[4,5] and it has been suggested that turns function as recognition sites in complex immunologic, metabolic, genomic, and endocrinologic regulatory mechanisms because of the location of turns on the surface of proteins, and because of the predominance of reactive functional groups in the side chains of amino acids involved in turns.

The general definition of a β-turn is the area where a polypeptide chain reverses its overall direction. In β-turns, this area comprises four amino acid residues, whereas γ-turns involve three residues. Turns may or may not be stabilized by an intramolecular hydrogen bond. Compared to other peptide conformations, such as α-helices or β-sheets, the geometry of β-turns is less unique, and several different β-turns, categorized as type I, II, III (as well as type I′, II′, III′), are found in proteins, *cf.* Table 1. The most common β-turns in proteins are the type I, II, and III structures. The standard geometry for each type of turn, based on X-ray crystallographic data of proteins, is defined by the ϕ and ψ angles of residues at the $i+1$ and $i+2$ positions. However, due to the variations in amino acid residues in a β-turn region, the angles may vary by as much as ±30°. These large variations make the type III and type I turns indistinguishable in extreme cases, and also imply that the geometry of β-turns is less well defined than that of other secondary structures. The conformational angles in a turn depend on the environment involved, the sequence of amino acids in the β-turn, and the secondary structures connected by the β-turn: two single, β-sheet strands may be connected by a tight, U-shaped β-turn that consists of four residues. On the other hand, the turn between two bulky secondary structures, such as α-helices, would exhibit a different shape and resemble an open helical structure (it is referred to as an "open turn" in the literature). This kind of turn might have a left- or right-handed chirality depending exclusively on the side chains. In these structures, it is not necessary to have a 1→4 intramolecular hydrogen bond .

Table 1. Dihedral Angles for Hydrogen-Bonded β−Turns

	i + 1		i + 2	
turn	ϕ	φ	ϕ	φ
type I	−60	−30	−90	0
type I′	60	30	90	0
type II	−60	−120	80	0
type II′	60	−120	−80	0
type III	−60	−30	−60	−30
type III′	60	30	60	30
type VIa (cis)	−60	120	−90	0
type VIb (cis)	−120	120	−60	0

Since β-turns consist of only a few residues, the spectroscopic signatures of these turns are hard to detect in larger proteins. In addition, the large degree of variation in the conformational angles of turns, as compared to other secondary structures, makes the conformational analysis much more complicated. Therefore, the structure of turns in peptides in solution is less well understood, and novel spectroscopic results can enormously enhance our present understanding of these structures.

In this paper we present the results of conformational studies of β-turns using VCD, which is a relatively new technique for the study of peptide conformation. Therefore, this paper is

[⊗] Abstract published in *Advance ACS Abstracts*, September 1, 1995.

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aimed at establishing whether or not there are characteristic VCD patterns for β-turns, and whether or not different types of turns can be distinguished from each other by VCD. Recent results show that VCD is a particularly sensitive probe for the conformation of small peptides containing turn motifs.[6-8] It combines and enormously enhances the sensitivities of CD and FTIR spectroscopies, and overcomes some disadvantages of both techniques.

We find from the VCD results that small, cyclic peptides incorporating β-turns exhibit large conformational differences when the chemical environment is changed, although they are conformationally restricted. This change in environment was accomplished by varying the solvent polarity and hydrogen bonding ability. In spite of the perceived rigidness of the peptides reported here, we find that polar and hydrogen bonding solvents interfere with the internal hydrogen bonds between the peptide linkages, cause the polar group of the β-turn to be solvent exposed, and therewith induce conformational changes. In inert solvents, peptides tend to form intramolecular hydrogen-bonded structures and to be conformationally more restricted.

Finally, in order to understand the relationship between the observed VCD spectra and the conformations of peptides, we carried out VCD intensity simulations using the Nondegenerate Extended Coupled Oscillator (NECO) model.[9] The patterns of calculated VCD spectra for standard type I and II β-turns, based on literature conformational data, agree well with experimental data. We conclude that the NECO model is useful for interpreting VCD data in peptides of the size reported here. The coupling between the carbonyl group of the peptide linkages, which is considered the major mechanism for generating VCD intensity within the NECO model, will be different in single β-turns and in fused turns in larger peptides. We find that even these differences can be interpreted through the calculated VCD spectra.

Previous Structural Studies on β-Turns

(a) Previous Spectroscopic Methods. Because of the complicated properties of β-turns, a variety of techniques has been employed to study the conformation of β-turns. The first systematic study of β-turns was carried out by Venkatachalam[3] using conformational computations. In the 1970's, the most accurate structural information available for turns was obtained by crystallographic techniques.[10,11] In recent years the NMR, CD, and FT-IR spectroscopies have become the most popular techniques to study β-turns,[12-16] because these techniques are sensitive to conformation and conformational changes of peptides and proteins in solution.

CD is a very useful technique for conformational determination, especially for biomolecules. Because of its fast time scale, population-weighted average conformations will be measured, from which individual populations can be extracted. Thus, all existing conformer populations are observed simultaneously. For β-turns, four classes of CD spectra were established computationally by Woody[17-18] for the different subtypes. However, their direct observation proved to be difficult,[14] because the CD features of the turns are generally weak, and because there will be a population distribution of different turn subtypes. Furthermore, in model peptides and proteins, the signature of the turn was found to be overlapped by the strong CD spectrum of other conformations or side-chain and disulfide bond contributions.

Several techniques in NMR can be used to determine conformations of β-turns, such as $J-J$ coupling, ROSEY, and chemical shift temperature dependence.[10,11,15] A common and powerful method to distinguish between type II and other types of β-turns is NOE spectroscopy. β-Turns exhibit a number of NOEs, but the most significant one is the interaction of the α-proton on carbon atom C_{i+1} with the amide proton on N_{i+2} in the type II β-turn. In general, NOEs are observable when two protons are less than 3 Å apart. In a standard type II turn, the distance between the two protons, henceforth referred to as $C_{\alpha i+1}H$ and HN_{i+2}, is typically 2.1 Å, and strong NOE is observed. However, since there is a large variation of the conformational angles ϕ and ψ, the distance between $C_{\alpha i+1}H$ and HN_{i+2} can vary to such an extent that no NOE's are observable. In addition, the intensity of NOE is proportional to the population of each type of turn that may co-exist; therefore, interproton distances generated by NMR experiments may not fit a unique structure. Furthermore, the slow time scale of NMR, which is generally longer than the time scale of conformational interconversions, will further reduce the intensity of NOE's, particularly in a small peptide system.[15] Many of the peptides studied via NMR techniques were linear and cyclic tetrapeptides, which were found to exhibit different conformations in various environments. In addition, they were found to be more flexible than the turns in large peptides or proteins, in which the conformation of the turn is limited by the entire protein environment.

IR absorption spectroscopy has been used quite extensively to investigate turn structures, particularly in recent efforts. The assignment of absorption bands to given conformational angles, however, is not a unique one, particularly since infrared absorption peaks are broad and solvent dependent. We found in some previous studies on γ-turns, and in the present study, that the changes in infrared absorption spectra are relatively small and insignificant compared to the changes in VCD features.[6,8] However, the amide I stretching frequency of a

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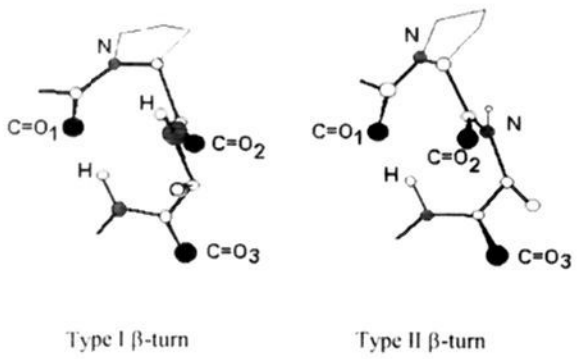

Figure 1. Geometries of the type I and type II β-turns.

proline containing peptide linkage is quite different from that for a linkage containing other amino acid residues: In a Gly-Pro peptide linkage, where the nitrogen is a tertiary amine, the $C{=}O_{(Gly)}$ stretching frequency is observed around 1640−1655 cm^{-1}, whereas in Pro-Gly, where the nitrogen is a secondary amide, the $C{=}O_{(Pro)}$ frequency is observed at 1670−1685 cm^{-1}. Thus, these frequency shifts caused by changes in the chemical environment of peptide linkages can interfere with the frequency shift generally associated with changes in secondary structure, causing the results of vibrational work to be inaccurate.[6] In this study, we use the different frequencies of the secondary and tertiary amide groups to our advantage, since they allow us to differentiate between the various possible interactions of the carbonyl groups.

(b) β-Turn Structural Considerations. Type I and type II β-turns are composed of four residues. In the rest of this discussion, we refer to the amino acids in the turn, or specific groups of them, interchangeably as $C{=}O_i$ and $C{=}O_1$, $C{=}O_{i+1}$, and $C{=}O_2$ *etc.* In the case of a tight β-turn, the $C{=}O_i$ can form an intramolecular hydrogen with HN_{i+3}. The $C{=}O_{i+1}$ is approximately perpendicular to the plane of the ring, pointing either down (type I) or up (type II), *cf.* Figure 1. It is able to interact with solvent molecules and exhibits large conformational flexibility. Thus, the geometry of a β-turn is determined by the arrangement of the three carbonyl groups $C{=}O_i$, $C{=}O_{i+1}$, and $C{=}O_{i+2}$. The advantage of VCD as a structural tool is that it monitors the orientation of these three probe groups.

The peptides reported in this study are cyclic tetrapeptides closed by a disulfide bond and a cyclic pentapeptide. The cyclization reduces the flexibility of the peptides, and the disulfide bond will constrain the distance between $C_{\alpha i}$ and $C_{\alpha i+3}$. This latter factor may determine whether the particular peptide sequence will prefer a type I or type II turn. The peptides all have a proline residue at the $i + 1$ position, because proline is well-known to play a key role in β-turn conformations of proteins.[2] On the basis of X-ray crystallographic data in proteins, proline is the most frequently occurring residue in the $i + 1$ position of β-turns. In addition, the sequence Pro-Y, in position $i + 1$ and $i + 2$, with Y= Ser, Asp, Asn, or Glu, is found almost exclusively in type I or III turns, whereas the Pro-Gly sequence may occur both in type I(III) and type II turns. The limitation on conformational freedom is due to the constraint of the ϕ angle of proline.

In this study, we concentrate on solution conformations derived from the amide I′ vibration of the peptide linkage. This vibration, which can be described mostly as the carbonyl stretching motion of the amide moiety, occurs between 1630 and 1700 cm^{-1} and has a dipole transition moment of 0.29 D. The VCD spectra reflect the dipolar coupling among the different C=O groups of neighboring peptide linkages which depends on the distance between C=O groups and their dihedral angles. Since the turn geometry is directly relatable to the orientation of the carbonyl groups, the VCD results in this spectral region are particularly sensitive to the peptide conformation.

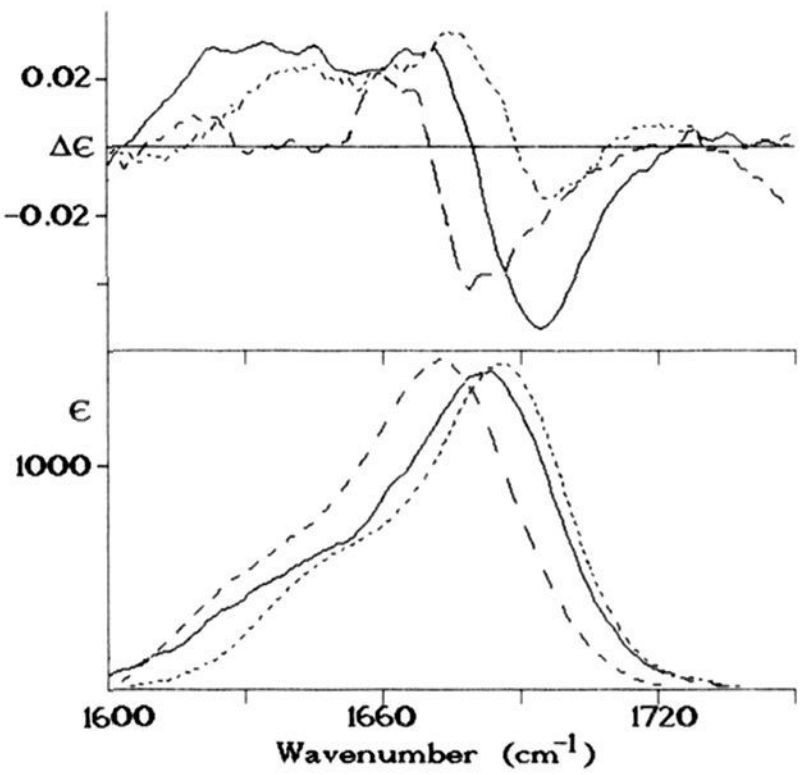

Figure 2. Infrared VCD (top) and absorption (bottom) spectra of *cyclo*-(Cys-Pro-Gly-Cys) in DMSO/$CDBr_3$ (solid trace), DMSO (short dashes), and DMSO/D_2O (long dashes).

Materials and Methods

All data presented in this paper were collected on the broad-band (800−1800 cm^{-1} spectral range), dispersive VCD spectrometer at Hunter College described previously.[19] Several improvements were implemented since the earlier description of the instrument. Mostly, these improvements deal with further lowering the artifact levels. Thus, VCD spectra with amplitudes of about 3×10^{-7} ΔA units can now be measured reliably. Details of the modifications will be reported at a later date.

Sample handling in VCD is entirely analogous to that in infrared spectroscopy. Samples are contained between CaF_2 plates, separated by Teflon spacers of appropriate thickness, typically 25 μm. Sample volumes of 10−20 μL, at peptide concentrations of 5−20 mg/mL, were utilized. These peptide concentrations result in peak absorbances of about 0.15 at the path length indicated. To check for association effects, VCD data of some peptides at lower concentration were collected as well. At present, the lowest absorbance level at which VCD can be collected reliably is about 0.06 absorbance unit. At these lower concentrations, no change in the VCD spectra was observed in the cyclic peptides, although the linear precursors did show concentration-dependent VCD features.

The cyclic peptides *cyclo*-(-Cys-Pro-Gly-Cys-) [*c*CPGC], *cyclo*-(-Cys-Pro-Phe-Cys-) [*c*CPFC], and *cyclo*-(-Cys-Pro-D-Phe-Cys-) [*c*CPdFC] were prepared by standard F-Moc solid-phase synthetic methods as described previously.[8] The cyclic molecules were obtained from the linear precursors by oxidative ring closure in the presence of H_2O_2. *cyclo*-(-Gly-Pro-Gly-D-Ala-Pro-) [*c*GPGdAP] was provided by H. Wyssbrod.[7] All peptides were lyophilized from D_2O prior to dissolving them in the solvents discussed for each experiment. Thus, all reported C=O stretching vibrational frequencies will be for the deuteriated peptide linkage, referred to as the amide I′ vibration.

Molecular modeling, the calculation of atomic coordinates, and the generation of structures shown were carried out using the program HyperChem running on a personal computer equipped with a 90-MHz Intel Pentium processor. The simulated VCD and infrared absorption spectra were obtained using exciton-type calculations allowing for nondegenerate frequencies of prolyl and glycyl carbonyl groups.[8,9]

Results

The observed IR and VCD spectra of three cyclic peptides, *c*CPGC, *c*CPFC and *c*CPdFC, in three different solvent systems are shown in Figures 2−4. The solvent systems DMSO/$CDBr_3$

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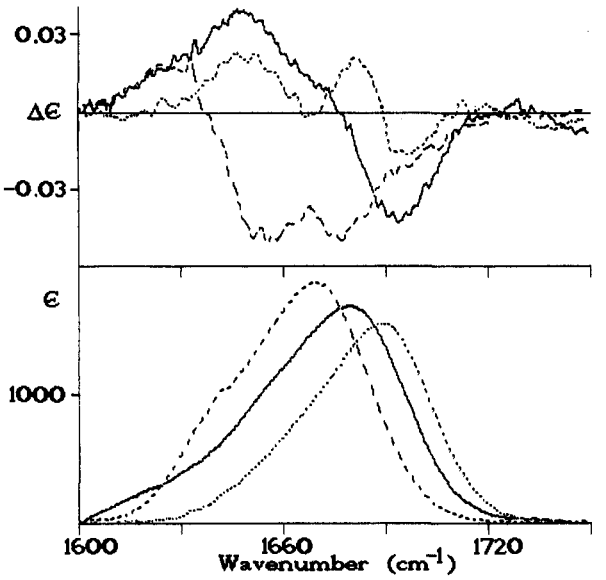

Figure 3. Infrared VCD (top) and absorption (bottom) spectra of *cyclo*-(Cys-Pro-Phe-Cys) in DMSO/$CDBr_3$ (solid trace), DMSO (short dashes), and DMSO/D_2O (long dashes).

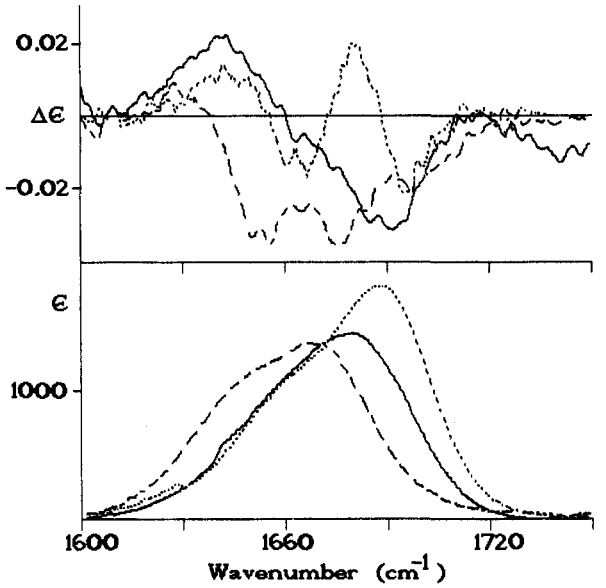

Figure 4. Infrared VCD (top) and absorption (bottom) spectra of *cyclo*-(Cys-Pro-D-Phe-Cys) in DMSO/$CDBr_3$ (solid trace), DMSO (short dashes), and DMSO/D_2O (long dashes).

(1:1, v/v) and DMSO and DMSO/D_2O (1:2, v/v) were selected to represent different polarity and hydrogen bonding ability. In principle, the pure solvents $CDBr_3$, DMSO, and D_2O would have been preferable since polarity and dielectric constants may be reproduced more accurately, but low solubilities prevented us from using the pure solvents. Figure 5 shows the observed VCD and infrared absorption spectra of the pentapeptide *c*GPGdAP in bromoform.

(a) Absorption Spectra. The changes in the absorption spectra in Figures 2–4 are relatively small. The general band shapes are very similar for the three peptides in the three different solvents, even if the amino acid at the 3-position is changed. However, the choice of solvent influences the frequency of the absorption maximum: there is a trend toward lower wavenumber of the peak absorbance between DMSO, DMSO/$CDBr_3$, and DMSO/D_2O. The solvent may affect the absorption spectra in two ways: either by a direct interaction between the carbonyl groups and the solvent or by inducing a conformational change. The direct interaction usually lowers

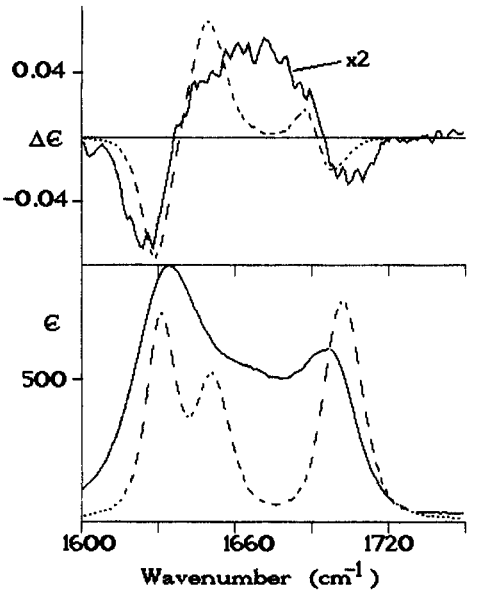

Figure 5. Infrared VCD (top) and absorption (bottom) spectra of *cyclo*-(Gly-Pro-Gly-D-Ala-Pro) in $CDCl_3$ (solid trace) and calculated from NMR solution structural data (dashes traces).

Table 2. Frequencies (cm^{-1}) of Tertiary and Secondary Amide I′ Vibrations

	tertiary amide I′	secondary amide I′	C=O_4 amide I′	solvent
cCPGC	1655	1684	1697	DMSO
cCPFC	1664	1688	1703	DMSO
cCPdFC	1661	1687	1700	DMSO
cCPGC	1648	1681	1697	DMSO/$CDBr_3$
cCPFC	1650	1679	1695	DMSO/$CDBr_3$
cCPdFC	1655	1678	1694	DMSO/$CDBr_3$

the vibrational frequency, whereas the second effect can result in a frequency shift in either direction, accompanied by a variation of intensity in the amide I′ band.

Even though the peptides are cyclic and conformationally restricted, the solvent can cause conformational changes. The most common way is by breaking the intramolecular hydrogen bond, and forming hydrogen bonds with the solvent. We believe that the shifts toward low frequency of the spectra in DMSO/D_2O are directly attributable to solvent hydrogen bonding of all four carbonyl groups, with a concomitant breaking of the intramolecular hydrogen bond.

In going from DMSO/$CDBr_3$ to DMSO solution, all C=O vibrations (except for C=O_4 in *c*CPGC, *cf.* Table 2) shift up in frequency. We believe that these frequency changes are due to conformational changes induced by breaking the 1→4 intramolecular hydrogen bond. In a low-polarity solvent, such as bromoform, peptides prefer to form an intramolecular hydrogen bond which is manifested by a shift of the tertiary amide I′ vibration by at least 6 cm^{-1}, compared to their value in DMSO (Table 2). Upon increasing the DMSO concentration, this hydrogen bond is broken by stronger solvent interactions, and a slight conformational change seems to occur. This change shifts the carbonyl peak to higher frequencies. Since the observed VCD spectra of the peptides in different solvents vary significantly, but the absorption spectra exhibit minor changes only, we conclude that the conformation changes of the β-turns cannot be detected reliably by monitoring absorption spectra alone.

(b) VCD Spectra. The VCD spectra of the peptides *c*CPGC, *c*CPFC, and *c*CPdFC show much larger variations when the solvent is varied than the corresponding absorption spectra. A

comparison between the VCD spectra of the three peptides in the same solvent reveals that the peptides actually exhibit quite similar VCD spectra. This coarse observation will lead us to believe that the changes in solution conformation caused by the solvent interaction are more severe than those caused by variation of the amino acid sequence. In *c*CPGC, a VCD spectrum with a positive/positive/negative pattern is observed in DMSO/$CDBr_3$. This pattern is similar to that observed for *c*CPGC in pure DMSO. In DMSO/D_2O, a spectral pattern is observed which is devoid of the tertiary amide (low frequency) VCD intensity and resembles the VCD of a cyclic peptide, *cyclo*-(Cys-Ala-Cys), which may include a γ-turn.[8] The peptides with a larger side group in the 3-position, *c*CPFC and *c*CPdFC, exhibit more significant changes in the VCD spectra as the solvent polarity is varied. They both exhibit large, all-negative VCD patterns in DMSO/D_2O, which we are unable to interpret at the present time. In this solvent, which is the most polar of the solvents studied, the interactions of the peptide with the solvent dominate and influence the conformation profoundly. The changes in the VCD spectra as a function of solvent polarity could correspond to changes in conformation, or a change in equilibrium populations in a mixture of different conformations. This possibility is very likely since the energies for type I and type II turns are very similar for *c*CPGC.

Discussion

Because of steric requirements of the individual amino acids in the turns, the type I β-turns are generally preferred for two L-amino acids in the $i + 1$ and $i + 2$ positions,[20] whereas type II turns are preferred for L-D sequences. A glycyl residue can be accommodated in either a type I or II turn. Among the molecules studied here, *c*CPFC should prefer a type I geometry, whereas *c*CPdFC should exist in a type II β-turn geometry. *c*CPGC can assume either type I or type II structures, and most likely exists as an equilibrium mixture of both forms. Previous NMR studied have confirmed these conformational tendencies.[21]

Since VCD is a technique which monitors short distance interaction, its conformational sensitivity is particularly pronounced and useful in the studies of small peptides. One of the major advantages of vibrational optical activity is its sensitivity to subtle structural differences of β-turns in slightly different solvent systems. Furthermore, the carbonyl groups of the tetrapeptides introduced above exhibit different amide I′ frequencies that are readily distinguishable in infrared absorption and VCD spectroscopies. These different amide I′ frequencies are due to the carbonyl group $C{=}O_i$, adjacent to the tertiary (prolyl) amine, observed at 1655 cm^{-1}, the two carbonyl groups $C{=}O_{i+1}$ and $C{=}O_{i+2}$ in the turn which are adjacent to secondary amines (1685 cm^{-1}), and the carboxylic acid $C{=}O_{i+3}$ group with a frequency of 1697 cm^{-1}. In DMSO/$CDBr_3$, these four peaks could be identified by band decomposition.

The aim of the following discussion is an attempt to interpret the conformational changes observed for the tetrapeptides between pure DMSO and DMSO/$CDBr_3$, since these spectral changes are far less drastic then the ones observed for DMSO/D_2O. Furthermore, we wish to establish whether or not any of the observed spectra represent type I and type II β-turn VCD signatures. We shall attempt to determine the effects of the conformational changes induced by the different solvents on the VCD spectra by assuming that the VCD spectra in the amide I′ region are determined mostly by the coupling among the carbonyl groups within a given geometry. In the cyclic tetrapeptides that were used to model β-turns, there are only three carbonyl groups involved in the turn itself, while $C{=}O_4$, which is outside the ring, is expected to be in an undefined geometry. As pointed out before, one of the remaining three carbonyl groups has a low frequency ($C{=}O_1$), and two of them have relatively high frequencies (*cf.* Table 2), and it is easy to assign their VCD features based on their frequencies.

We start this attempt to interpret the observed VCD data by presenting, in Figures 6 and 7, the calculated VCD and absorption spectra for standard type I and type II β-turn geometries. These calculations were carried out using the carbonyl coordinates extracted from structural data of the two turns, a constant carbonyl transition moment of 0.29 D, and 1645 and 1685 cm^{-1} transition frequencies for the tertiary

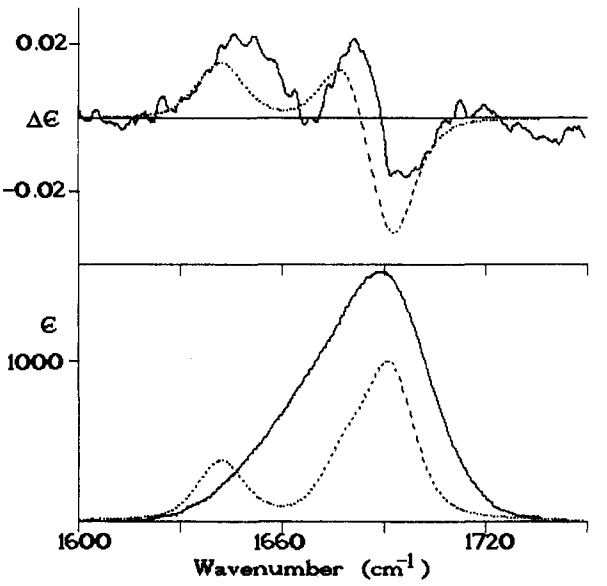

Figure 6. Infrared VCD (top) and absorption (bottom) spectra of *cyclo*-(Cys-Pro-Phe-Cys) in DMSO (solid trace) and calculated for the type I β-turn (dashes traces).

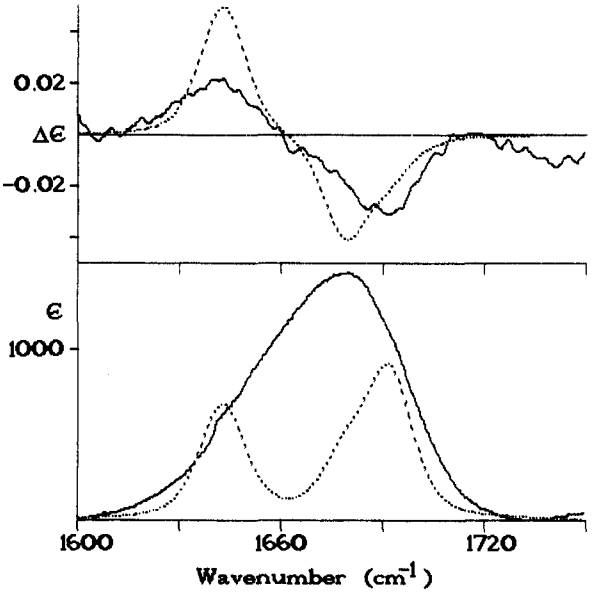

Figure 7. Infrared VCD (top) and absorption (bottom) spectra of *cyclo*-(Cys-Pro-D-Phe-Cys) in DMSO/$CDBr_3$ (solid trace) and calculated for the type II β-turn (dashes traces).

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Table 3. Dipole–Dipole Coupling Energies (cm^{-1}) between $C{=}O_1$, $C{=}O_2$, and $C{=}O_3$ for the Type I β-Turn

	V_{ij}		
	$i=1$	$i=2$	$i=3$
$j=1$		9.26	0.24
$j=2$			6.4
$j=3$			

Table 4. Eigenvectors of the V_{ij} Matrix for the Type I β-Turn

	1692.4	1679.7	1642.9
$C{=}O_1$	−0.149	−0.162	0.975
$C{=}O_2$	−0.746	−0.629	−0.218
$C{=}O_3$	−0.649	0.760	0.027

Table 5. Dipole–Dipole Coupling Energies (cm^{-1}) between $C{=}O_1$, $C{=}O_2$, and $C{=}O_3$ for the Type II β-Turn

	V_{ij}		
	$i=1$	$i=2$	$i=3$
$j=1$		−5.6	−6.4
$j=2$			5.5
$j=3$			

($C{=}O_1$) and secondary ($C{=}O_2$ and $C{=}O_3$) amide frequencies, respectively. Plotted along with the calculated VCD spectra are the observed VCD spectra of *c*CPFC in DMSO (Figure 6) and *c*CPdFC in DMSO/$CDBr_3$ (Figure 7). Similar cyclic dipeptides were found previously to exist in these solvents in type I and type II β-turn conformations.[21,22]

Next, we shall attempt to quantify the origin of the spectral patterns observed and calculated. Two kinds of VCD patterns were observed and calculated for these model peptides. One pattern (Figure 6) consists of a couplet at relatively high frequency that covers a narrow frequency range and a low-frequency positive VCD signal. The couplet is attributed to interactions between two high-frequency carbonyl groups, $C{=}O_2$ and $C{=}O_3$, and exhibits a positive/negative pattern with a zero crossing point located at the maximum absorption of the secondary amide I′ band. This $C{=}O_2/C{=}O_3$ interaction indicates a favorable distance/geometry between these groups. The VCD of the $C{=}O_1$ group is monosignate, and at lower frequency. Although monosignate VCD may be due to the inherent chirality of a chromophore, we believe that this signal is due to weak coupling of the $C{=}O_1$ group with $C{=}O_2$ or $C{=}O_3$, *cf.* Tables 3 and 4.

The calculated and observed VCD couplet shown in Figure 7 is located at lower frequency and covers a wider frequency range. Therefore, we believe it is due to the interaction of a low-frequency and a high-frequency carbonyl group, either $C{=}O_1$ with $C{=}O_2$, or $C{=}O_1$ with $C{=}O_3$, or both. Since it involves the coupling between a high- and a low-frequency carbonyl vibration, the zero crossing of the VCD spectrum is located between the absorption of the secondary and tertiary amide I′. It, too, has a positive/negative pattern, but the spectrum is much broader. Here, either the $C{=}O_1/C{=}O_2$ or the $C{=}O_1/C{=}O_3$ groups must be in a good coupling geometry.

The dipolar coupling energies V_{ij} between interacting C=O groups, listed in Tables 3 and 5, may provide a handle to the origin of the observed VCD. The V_{ij} elements depend on the distance and the orientation between the interacting carbonyl groups. To a first approximation, we may assume that a small conformational change in a cyclic molecule will alter the orientation much more than the distance; thus, the coupling energies may provide another sensitive clue to conformation.

According the exciton formalism, large rotational strengths are created for perpendicular geometry between the interacting groups. Such a geometry, however, produces the smallest coupling energy and splitting of the exciton components. On the other hand, a parallel or antiparallel geometry between the interacting groups will produce maximum splitting, but minimal rotational strengths. Thus, one may conclude that large VCD intensities are associated with intermediate values of V_{ij} if the distance between the carbonyl groups does not change much between the conformers.

For the type I β-turn, (*cf.* Tables 3), V_{23} is responsible for the mixing of the $C{=}O_2/C{=}O_3$ coordinates, whereas the large and small coupling energies V_{12} and V_{13} may prevent the formation of a couplet as discussed above: a large term because of near parallel geometry, and a small term for lack of coupling. In the type II turn, V_{12}, V_{13}, and V_{23} all are similar in magnitude and contribute to the mixing of $C{=}O_1/C{=}O_2$ and the $C{=}O_1/C{=}O_3$. Inspection of Figure 1 reveals that in the standard type I geometry, $C{=}O_1$ and $C{=}O_2$ are close to each other, and both point below the plane of the ring. This near parallel geometry will produce a large coupling energy, but little optical activity. $C{=}O_2$ and $C{=}O_3$ interact with an energy of about 6 cm^{-1}, which appears the optimum value for generating VCD couplets.

Based on these arguments, the observed VCD spectra of the three tetrapeptides can be categorized by a combination of $C{=}O_1/C{=}O_2$ and $C{=}O_1/C{=}O_3$ interactions (low-frequency couplet) and a $C{=}O_2/C{=}O_3$ interaction (high-frequency couplet). The eigenvectors of the coupling energy matrix provide a description of the modes resulting from the coupling of the transitions of the individual amide transitions. Inspection of Tables 4 and 6 reveals that the resulting modes are similar for the type I and type II β-turns, although their coupling energies and VCD intensities are quite different. The eigenvectors demonstrate that the low-frequency vibration at *ca.* 1642 cm^{-1} is largely due to the $C{=}O_1$ vibration. In both cases, the highest frequency vibrations (*ca.* 1692 cm^{-1}) may be attributed largely to an in-phase combination of the $C{=}O_2/C{=}O_3$ groups; however, the contribution of the $C{=}O_1$ coordinate to this vibration differs in sign between the type I and type II turns. The 1680-cm^{-1} vibrations in both types of turns are due to the out-of-phase combination of the $C{=}O_2/C{=}O_3$ groups.

Table 6. Eigenvectors of the V_{ij} Matrix for the Type II β-Turn

	1692.0	1679.5	1643.4
$C{=}O_1$	0.179	−0.014	0.984
$C{=}O_2$	−0.690	−0.715	0.115
$C{=}O_3$	−0.702	0.699	0.138

*c*CPFC in DMSO exists mostly in the type I conformation (Figure 3, short dashes). In the less polar solvent DMSO/$CDBr_3$, the spectrum resembles that of the type II conformation, but still contains some positive intensity just below the zero crossing frequency. We interpret this to be due to a mixture of type I and type II conformation.

In contrast, *c*CPdFC exhibits nearly pure type II conformation in the low-polarity solvent DMSO/$CDBr_3$ and a mixture of type I and type II structures in pure DMSO. Based on these facts, we conclude that the higher polarity solvent will expose the carbonyl groups to the solvent, so that the $C{=}O_2$ prefers to stay in a position similar to that found in the type I turn and, secondly, that the disulfide bond certainly makes some contribution to the β-turn conformation. It can either limit the formation of an intramolecular hydrogen bond or constrain the distance

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Table 7. Distribution of Conformations in the Cyclic Tetrapeptides

	DMSO	DMSO/$CDBr_3$
C(CPGC)	II, I(III)*	II & I(III)
C(CPFC)	I(III)	II & I(III)
C(CPdFC)	II & I(III)	II

between C_1 and C_4. Similar results of interconversion between turn conformations were found using other techniques in cyclic peptides containing Aib at the $i + 2$ position.[21]

We expect the VCD spectrum of *c*CPGC in DMSO/$CDBr_3$ (*cf.* Figure 2) to be a mixture of both type I (or III) and type II turns, with the former dominating. With increasing solvent polarity, the fraction of type I conformation becomes more pronounced which is manifested by an increase in the $C{=}O_2$/$C{=}O_3$ couplet. A listing of the distribution of the conformations found in the cyclic tetrapeptides in different solvents is presented in Table 7.

Another example of a β-turn that was studied by VCD is *c*GPGdAP in bromoform. The observed VCD and absorption spectra are shown in Figure 5. The absorption spectrum consists of two major peaks with frequencies of 1640 and 1690 cm^{-1}. The VCD spectrum shows two couplets, a strong negative couplet at low frequency (1630−1650 cm^{-1}) and a positive couplet at high frequency (1685−1700 cm^{-1}). NMR[23] and X-ray[24] studies indicated that this pentapeptide forms a type II β-turn stabilized by a hydrogen bond between $C{=}O_{Gly(1)}$ and $HN_{D\text{-}Ala}$ and a γ-turn between $C{=}O_{D\text{-}Ala}$ and $HN_{Gly(1)}$. The carbonyls involved in the two turns are both tertiary amide I′ groups that form intramolecular hydrogen bonds and are consequently observed at low frequency. Furthermore, the two tertiary C=O groups of *c*GPGdAP are located below the ring of the peptide and are very close to each other. The three secondary C=O groups are above the peptide ring, but only $C{=}O_{Pro(2)}$ and $C{=}O_{Gly(3)}$ are relatively close to each other, and $C{=}O_{Pro(5)}$ is far away from any other carbonyl group. Thus, one would expect two separate couplets: a low-frequency couplet between the tertiary amide vibrations, and a high-frequency couplet for the secondary amides. The observed VCD spectrum exhibits this pattern.

This coupling pattern can be confirmed by NECO computations. Large values of V_{ij} are found to occur between the tertiary amide I′ vibrations of $C{=}O_1$ and $C{=}O_4$ and the secondary amide I′ vibrations of $C{=}O_2$ and $C{=}O_3$. The weakest coupling occurs with $C{=}O_5$. Computed VCD spectra, based on the NMR solution structure, are shown in Figure 5. The frequency and shapes of the calculated VCD patterns agree reasonably well with the observed VCD results: the couplet at low frequency corresponds to the two tertiary carbonyls of the fused turns, and the positive couplet at higher frequency represents a partial spectrum characteristic of β-turns. The computed VCD spectrum based on the X-ray data shows less agreement with the observed VCD spectrum. This is understandable in terms of a conformational change of peptide experiences due to the forces in the crystal. Our data demonstrate how sensitive VCD is to conformational changes of peptides, even if this change is relatively minor. The above results can also be used to draw the same conclusion about the structure of the peptide, namely the large distance and unfavorable coupling geometry of $C{=}O_5$. Furthermore, since the VCD pattern of *c*GPGdAP is determined by fused β- and γ-turns, we cannot use it for the interpretation of an individual or single β-turn. Finally, the interpretation of the VCD results of *c*GPGdAP suggests that the NECO model is an adequate method for the qualitative interpretation of the observed VCD spectra.

Conclusion

After the discussion in the previous section, the question arises of whether or not a VCD pattern for "prototypical turn structures" exists, and what VCD spectrum can be expected for each of the β-turn conformations. In larger peptides and proteins, "prototypical" β-turns may not exist at all, since any turn structure may be influenced significantly by hydrogen bond patterns and hydrophobicity of the pocket surrounding the turn. Therefore, the conclusions reached in the discussion above may only hold for the model systems selected.

When we published the VCD spectrum of *c*GPGdAP, we had hoped that it would exhibit a "typical" β-turn VCD. We conclude after this more detailed study that *c*GPGdAP gives the VCD pattern for fused turn structures, and not that of a single β-turn. However, it appears that the VCD spectra of *c*CPFC in DMSO and that of *c*CPdFC in DMSO/$CDBr_3$ represent the VCD patterns of standard type I and type II β-turn structures, respectively. We arrive at this conclusion by a comparison of previously derived structures and observed VCD results, and a comparison of the observed VCD patterns with those calculated for standard β-turn geometries. These calculations are based on the ϕ and φ angles of residues $i + 1$ and $i + 2$ in standard type I (−60, −30; −90, 0) and type II (−60, 120; 80, 0) β-turns. These four angles determine uniquely the geometry of the three carbonyl groups involved in β-turns.

Experimentally and computationally, we obtain a positive couplet for both type I and type II turns. For the type I turn, an additional positive VCD signal is observed at about 1635 cm^{-1}, followed by the couplet at about 1675/1690 cm^{-1}. For the type II turn, a broad couplet between 1635 and 1690 cm^{-1} is observed.

Acknowledgment. Support of this research by the National Institute of General Medical Sciences (GM 28619), several NSF grants for instrumental development, and a number of PSC-CUNY Faculty Research awards are gratefully acknowledged.

JA950988A

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