Circular Dichroism of β Turns in Peptides and Proteins†

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ABSTRACT: Circular dichroism (CD) spectra are reported for two groups of cyclic hexapeptides having β turns whose geometry can be firmly established by X-ray crystallography and by NMR spectroscopy. One series contains the sequence L-Pro-D-Phe in the geometry of the classical type II β turn, while the second group has the sequence D-Phe-L-Pro in the closely related geometry of the gramicidin S turn. CD data on the hydrogenated peptides show that in neither series do Cotton effects due to the aromatic phenylalanyl chromophore make a significant contribution to the spectra in the 195–240-nm region. In spite of the close geometric similarity of the β turns of these two groups of peptides, their CD spectra are quite distinct. Furthermore, comparison of our data with the CD spectra of published models for β-turn structures suggests that it may not be possible to characterize the contribution of all β turns to the CD spectra of proteins by a single model curve. The CD spectra of model β turns will be more useful in characterizing the folding of oligopeptides and sequence polypeptides, where a single type of turn is present.

A CD spectrum characteristic of the α-helical polypeptide chain is observed for polypeptide model systems as well as for proteins known from X-ray crystallography to have a high proportion of α-helical structure. The CD spectrum of the β-pleated-sheet structure is reasonably characteristic but somewhat more variable than that of the α helix. There are at least two different types of β structure, the parallel and the antiparallel sheets, which could make distinct contributions to the CD spectrum. In fact, Balcerski et al. (1976) have proposed that, although these two structures have CD spectra similar in the 195- to 220-nm region, the antiparallel sheet is characterized by a positive CD band near 190 nm, while the parallel structure has a negative contribution at that wavelength. A third type of characteristic peptide chain folding is now recognized. The point at which a peptide chain reverses direction often involves just two residues joining two antiparallel chains which may in some cases be hydrogen bonded in an antiparallel β pleated sheet. However, the detailed geometry of this turn appears to be more variable than that of either α helices or various types of β sheet in proteins. A survey of eight proteins whose structures have been determined by X-ray crystallography (Lewis et al., 1973) revealed 135 examples of turns, most of which could be classified into ten rather broad categories. This survey has now been extended to cover 421 turns in 26 proteins by Chou and Fasman (1977).

One might ask then whether, in spite of the variability in the detailed geometry of the β loop, there might be a characteristic CD spectrum associated with it. Several research groups have addressed this question, each arriving at slightly different results. On the basis of NMR data, Urry et al. (1974) have proposed that the elastin polytetrapeptide is composed mainly of β turns and that its CD spectrum, therefore, should be typical of a β loop. On the other hand, Brahms et al. (1977) using arguments based on electron micrographs have proposed that poly(Ala_2-Gly_2) forms β turns and that its CD spectrum is the proper representative of the β turn. The shapes of the spectra reported by Brahms et al. (1977) and by Urry et al. (1974) are similar but the magnitudes differ greatly, the peak at 208 nm being 20-fold higher in the model of Brahms et al. (1977). Both groups present circumstantial arguments indicating that the β turn involved should be the type II turn in the notation of Venkatachalam (1968). Kawai and Fasman (1978) have recently proposed on the basis of infrared spectra in films that an open-chain tetrapeptide designed to fold in the form of a type I β loop gives a CD spectrum in dilute cyclohexane solution which is characteristic of that conformation. The CD spectrum of Cbz[1]-Gly-L-Ser(O-Bu[t])-L-Ser-Gly-*O*-stearate is closely related to the spectra of other β-turn models, as will be discussed below. Woody (1974) has made theoretical calculations of the CD spectra expected for various β-turn structures, exploring a range of conformations comparable to that found in proteins by Lewis et al. (1973). Although his calculated CD spectra for many of the possible conformations gave results consistent with those proposed in the experimental work cited above, the spectra of some of the conformations calculated by Woody (1974) differed substantially from the models of Urry and of Brahms.

Anticipating that variations among the experimental CD spectra might result from differences in the various β-turn conformations of the β-turn models, we have studied the CD of two groups of cyclic hexapeptides having β turns whose conformations can be firmly established by X-ray crystallographic data and NMR spectroscopy. We are able to define two distinct types of CD spectra associated with two of the many possible turn geometries.

Experimental Section

The chemical syntheses of the cyclic hexapeptides whose CD is reported here have been published elsewhere (Kopple et al., 1973, 1974; Pease, 1975). Hydrogenations of the phenylalanyl aromatic rings to cyclohexyl were done using Adams catalyst in acetic acid. The hydrogenated peptides were recrystallized from ethanol to thin-layer chromatographic homogeneity and were free of starting material and aromatic proton resonances.

Concentrations of solutions for CD spectroscopy were determined by weighing, and the absorption spectrum of each sample was determined on a Cary 15 spectrophotometer with nitrogen purging. CD measurements were made on a Cary 60

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[1] Abbreviations used: Cbz, carbobenzoxy; Cha, cyclohexylalanine; *O*-Bu[t], *O*-*tert*-butyl; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; TFE, trifluoroethanol.

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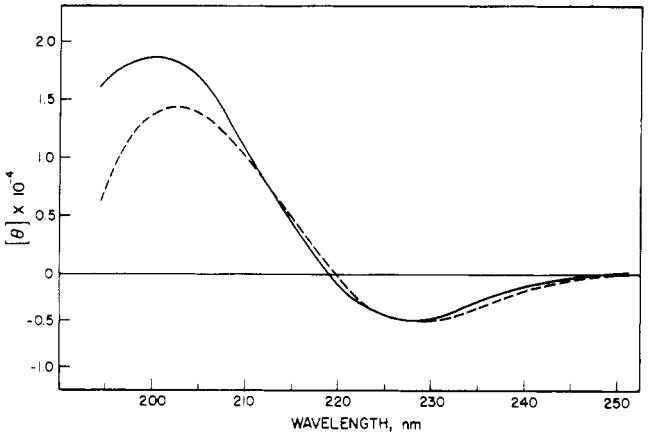

FIGURE 1: Circular dichroism per peptide residue for cyclo(L-Orn-L-Pro-D-Phe)$_2$ (- - -) and for cyclo(L-Orn-L-Pro-D-Cha)$_2$ (—) in hexafluoro-2-propanol.

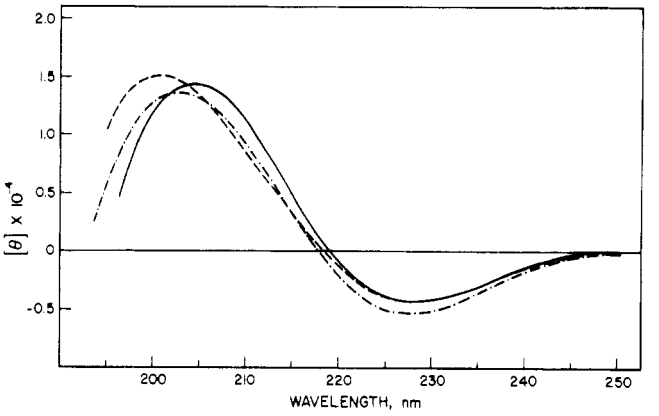

FIGURE 2: Circular dichroism per peptide residue for cyclo(Gly-L-Pro-D-Phe)$_2$ (—), for cyclo(Gly-L-Pro-D-Cha)$_2$ (- - -), and for cyclo(L-Ala-L-Pro-D-Phe)$_2$ (- • -) in hexafluoro-2-propanol.

spectropolarimeter equipped with a Cary 6002 CD accessory. The spectra were multiply scanned, signal averaged, and digitally filtered using the Fourier digital smoothing method (Bush, 1974). HFIP and TFE were used as solvents because of the conformational data available from NMR spectra of the cyclic hexapeptides in these solvents and because of their good UV transmittance properties.

Results

In Figure 1, the CD spectrum for cyclo(L-Orn-L-Pro-D-Phe)$_2$ is compared with that of the hydrogenated analogue cyclo(L-Orn-L-Pro-D-Cha)$_2$. Except for the absence of the weak bands at 258 to 270 nm due to phenylalanyl absorption (not shown in Figure 1) in the spectrum of the first peptide, the spectra are quite similar over a range of 200–240 nm. In Figure 2, spectra of cyclo(Gly-L-Pro-D-Phe)$_2$ and its hydrogenated analogue are compared. As in the case of the ornithine peptide, the spectra in the range of 195–240 nm are quite similar. Also included in Figure 2 is the CD spectrum of cyclo(L-Ala-L-Pro-D-Phe)$_2$; the similarity of its spectrum to those of the other four peptides will be noted.

In Figure 3, we show the CD spectrum of cyclo(L-Orn-D-Phe-L-Pro)$_2$ and its hydrogenated analogue. These CD spectra are qualitatively different from those of the retro isomer (Figure 1), but the cyclohexylalanyl and phenylalanyl forms have spectra which are quite similar to each other in the range of 195–240 nm. In Table I are summarized the maxima and minima for the curves of Figure 1–3 along with relevant CD

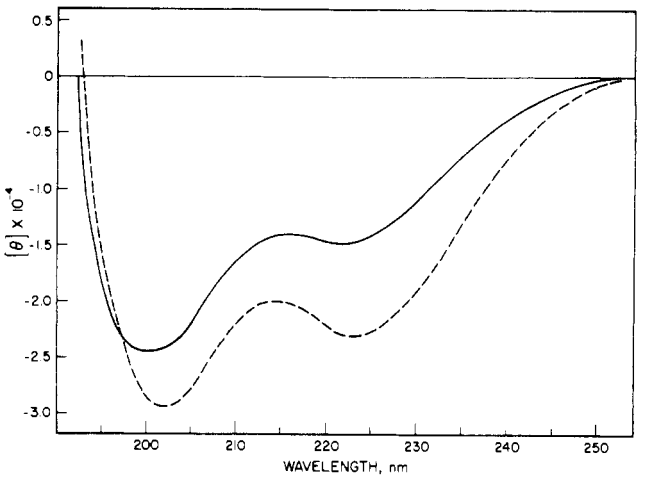

FIGURE 3: Circular dichroism per peptide residue for cyclo(L-Orn-D-Phe-L-Pro)$_2$ (—) and for cyclo(L-Orn-D-Cha-L-Pro)$_2$ (- - -) in hexafluoro-2-propanol.

TABLE I: CD Spectral Parameters for Cyclic Peptides.

type II β loop	λ_1 (nm)	$[\theta]_1$ ($\times 10^{-3}$)	λ_2 (nm)	$[\theta]_2$ ($\times 10^{-3}$)
cyclo(L-Orn-L-Pro-D-Phe)$_2$[a]	227	−4.2	202	14.4
cyclo(L-Orn-L-Pro-D-Cha)$_2$[a]	227	−4.0	200	18.7
cyclo(Gly-L-Pro-D-Phe)$_2$[a]	227	−4.5	205	14.6
cyclo(Gly-L-Pro-D-Cha)$_2$[a]	227	−4.1	200	15.8
cyclo(L-Ala-L-Pro-D-Phe)$_2$[a]	227	−5.7	202	13.7
cyclo(Gly-L-Pro-Gly)$_2$[b]	225	−3.0	199	15.8
cyclo(Gly-L-Pro-D-Ala)$_2$[b]	226	−4.4	200	27.0
cyclo(L-Ala-L-Pro-Gly)$_2$[b]	223	−5.2	196	24.4
cyclo(L-Val-L-Pro-Gly)$_2$[b]	222	−6.0	196	17.0
cyclo(L-Ala-L-Pro-D-Ala)$_2$[b] (H_2O)	227	−4.0	201	31.0
gramicidin S loop				
cyclo(L-Orn-D-Phe-L-Pro)$_2$[a]	222	−14.9	200	−25.0
cyclo(L-Orn-D-Cha-L-Pro)$_2$[a]	222	−23.2	202	−29.4
gramicidin S[c] (H_2O)	218	−30.0	206	−32.0
gramicidin S hydrogenated[c] (H_2O)	218	−29.0	206	−32.0
cyclo(L-Ala-Gly-L-Pro)$_2$[b]	218	−6.0	202	−6.0
cyclo(L-Val-Gly-L-Pro)$_2$[b]	222 (sh)	−9.0	201	−15.0
cyclo(Gly-D-Val-L-Leu-Gly-D-Orn-L-Orn)[d]	221	8.2	212 (sh)	4.8

[a] This work. [b] Pease (1975). [c] Urry et al. (1968). [d] Kopple et al. (1978).

data gathered from the literature. They are grouped according to type of CD curve, and we propose that they are grouped according to the type of β-turn geometry as well.

Discussion

Although aromatic side chains have been observed in some cases to make substantial contributions to the CD spectra of cyclic hexapeptides (Bush and Gibbs, 1972; Blaha et al., 1969), the close similarity of the CD of the phenylalanine-containing cyclic hexapeptides and those of the hydrogenated analogues argues that these aromatic side chains do not contribute to the spectra of any of the peptides shown in Figures 1–3 in the 200–240-nm range.

The conformations of cyclo(L-Ala-L-Pro-D-Phe)$_2$ and of cyclo(Gly-L-Pro-D-Phe)$_2$ have been determined in the solid-state by X-ray crystallography (Brown and Teller, 1976; Yang

and Brown, unpublished results). The fact that in both structures the phenylalanyl side chains are directed away from the backbone helps to rationalize the absence of strong Cotton effects due to the perturbation of the aromatic chromophore by the peptide chain. The β turn in these compounds comprises the L-Pro-D-Phe sequence. The peptide dihedral angles summarized in Table II are close to those of the type II β turn as defined by Venkatachalam (1968). The NMR spectra of cyclo(L-Ala-L-Pro-D-Phe)$_2$ and of cyclo(L-Orn-L-Pro-D-Phe)$_2$ in HFIP indicate a conformation completely consistent with the X-ray crystallographic structure (Kopple et al., 1974). Since the phenylalanyl side-chain chromophores make a negligible contribution to the CD spectra shown in Figures 1 and 2, the CD bands observed are characteristic of the peptide backbone folded in a type II β turn. Since the spectra of Figures 1 and 2 represent peptides with three different amino acids as the extended residue, all probably with slightly different average conformational angles in solution, the similar CD curves imply a single spectrum type which is characteristic of the type II β turn. In Table I, the data of Figures 1 and 2 are grouped with CD data for other cyclic hexapeptides having similar sequences, each of which shows an NMR spectrum suggesting that each contains a β turn composed of L-Pro followed by a D residue (Pease, 1975). The CD spectrum are rather similar to our data of Figures 1 and 2. A long-wavelength minimum occurs near 225 nm having an ellipticity of approximately −5000 per peptide residue, accompanied by a maximum near 200 nm having an ellipticity of between 15 000 and 20 000. The variations for various peptides having L-Pro-D-XXX turns are certainly outside experimental error and reflect real differences in conformational averages, but the similarities in magnitude and curve shape imply a CD spectrum characteristic of a type II β turn having dihedral angles near those reported for cyclo(L-Ala-L-Pro-D-Phe)$_2$ and cyclo(Gly-L-Pro-D-Phe)$_2$ (see Table II). This result is consistent with the calculations of Woody (1974) which showed that modest variations in the dihedral angles in the region of the type II β-turn conformation cause quantitative changes in the calculated CD spectrum but that the general pattern remains similar to that of the β-sheet CD spectrum with the maxima shifted somewhat to the red.

The dihedral angles (Table II) for the D-Phe-L-Pro residues of the gramicidin S turn, which have been proposed by Ovchinnikov et al. (1970) on the basis of NMR studies, define a turn which would be classified as type II′ by the schemes of Lewis et al. (1973) and of Chou and Fasman (1977) which allow up to 40° variation from the true type II′ turn of Venkatachalam (1968). This same gramicidin S type turn has been found in cyclo(L-Orn-D-Phe-L-Pro)$_2$ by Kopple et al. (1973). In Table II, we highlight the differences between the classic type II turn, number 14 in Venkatachalam's list, and the gramicidin S turn, which is the mirror image of the closely related turn numbered 13 by Venkatachalam (1968). In spite of the geometric similarity of the type 13 and type 14 turn, the CD spectra of the type II and gramicidin S turns do not bear a mirror-image relationship (see Table I). The CD spectrum of the cyclic hexapeptide cyclo(L-Orn-D-Phe-L-Pro)$_2$ (Figure 3) is quite similar to that of gramicidin S and both bear a fortuitous resemblance to that of the α-helical polypeptide (Urry et al., 1968). However, theoretical calculations by Pysh (1968) and by Woody (1974) of the spectrum to be expected for the gramicidin S turn of Table II are in agreement with the observed spectrum.

The data of Figure 3 as well as those of Urry et al. (1968) on the hydrogenated analogue of gramicidin S indicate that the phenylalanyl side-chain chromophore makes a negligible contribution to the CD in the 195–240-nm region for both

TABLE II: Dihedral Angles for Proposed β-Turn Types.

	type II			gramicidin S type		
	cyclo(Ala-Pro-D-Phe)$_2$[a]	cyclo(Gly-Pro-D-Phe)$_2$[b]	type 14[c]	cyclo(Orn-gramicidin S[d]	D-Phe-Pro)$_2$[e]	type 13′[c]
ϕ_2	−60	−62	−60	55	70	60
ψ_2	122	132	120	−110	−120	−120
ϕ_3	78	105	90	−60	−60	−60
ψ_3	9	12	0	−40	−60	−30

[a] Brown and Teller (1976). [b] Yang and Brown (1978). [c] Venkatachalam (1968). [d] Ovchinnikov et al. (1970). [e] Kopple et al. (1973).

cyclo(L-Orn-D-Phe-L-Pro)$_2$ and for gramicidin S. Both peptides retain the same CD spectrum in a variety of solvents, indicating a common, stable β-turn structure for them; NMR data support this.

Grouped in Table I along with the CD data taken from Figure 3 and those for gramicidin S are data for cyclo(L-Ala-Gly-L-Pro)$_2$ and for cyclo(L-Val-L-Gly-L-Pro)$_2$ (Pease, 1975), compounds that could have a Gly-L-Pro β turn similar to that of gramicidin S. The CD spectra have a shape similar to that exhibited by the model peptides cyclo(L-Orn-D-Phe-L-Pro)$_2$ and gramicidin S, but the magnitudes of the ellipticities are substantially reduced. This result suggests that cyclo(L-Ala-Gly-L-Pro)$_2$ and cyclo(L-Val-Gly-L-Pro)$_2$ are rather flexible structures exhibiting an equilibrium among conformers, a major contributor to which is a β loop having the Gly-L-Pro turn in a conformation similar to that of gramicidin S.

In a recent paper, Kopple et al. (1978) have discussed the conformation of the comparatively flexible cyclic hexapeptides cyclo(Gly-D-Leu-L-Leu)$_2$, cyclo(Gly-D-Val-L-Leu)$_2$, and cyclo(Gly-D-Val-L-Leu-Gly-D-Orn-L-Orn) and concluded that among the conformers contributing to the average structure the predominant conformation has the I -Leu-Gly turn. Such a turn may by viewed as the mirror image of that of gramicidin S (D-Phe-L-Pro), and, indeed, the CD curve for cyclo(Gly-D-Val-L-Leu-Gly-D-Orn-L-Orn)$_2$ (Table I) is similar in shape to that of an inverted α-helix curve (Kopple et al., 1978). The magnitude of the ellipticity is substantially lower than that of gramicidin S (Table I), probably because of the conformational flexibility of these compounds. Nevertheless, the CD data may be interpreted as indicating that cyclic hexapeptide structures with a L-Orn-Gly or a L-Leu-Gly sequence in the inverted gramicidin S turn are preferred in these compounds over a type II or II′ turn which would be expected for a Gly-D-XXX turn or a D-XXX-L-YYY turn sequence.

We now turn to the question of how the CD spectra of the two different types of β turns characterized in this paper might be useful in the interpretation of the CD spectra of proteins. The type II turn in the notation of Venkatachalam (1968), here epitomized by cyclo(Gly-L-Pro-D-Phe)$_2$, was originally thought to contribute to protein structures only for sequences of the type L-XXX-Gly, in which glycine could play the role of the D residue. However, under a somewhat relaxed definition of the type II turn, Lewis et al. (1973) found many examples in which an L amino acid was accommodated as the second residue of the turn in proteins whose structures had been determined crystallographically. The gramicidin S type turn, on the other hand, seems not to be common in proteins. Among the 421 β turns analyzed by Chou and Fasman (1977), 20 examples related to that proposed for gramicidin S were detected.

We have described CD spectra associated with two well-

TABLE III: Comparison of CD Spectral Parameters for Various Proposed Model β Turns.

	λ_{min} (nm)	$[\theta]_{min}$ ($\times 10^{-3}$)	λ_{min} (nm)	$[\theta]_{max}$ ($\times 10^{-3}$)
poly(L-Val-Pro-Gly-Gly)[a]	225	−2.0	202	3.0
poly(L-Ala-L-Ala-Gly-Gly)[b]	227	−5.0	207	63.0
N-Cbz-Gly-L-Ser(*O*-Bu^t)-L-Ser-Gly-OR[c]	221	−7.8	198	72.0
cyclo(Gly-L-Pro-D-Phe)$_2$[d]	227	−4.5	205	14.6

[a] Urry et al. (1974), in methanol. [b] Brahms et al. (1977), in water. [c] Kawai and Fasman (1978) (R = stearyl), in cyclohexane. [d] Typical type II β turn from this work.

defined β-turn geometries from among the numerous types that may occur in proteins. The CD spectra of the two types are quite distinct, although the geometries do not differ greatly. [For example, a nuclear Overhauser experiment showing H^{α}(Pro) and H^{N}(Gly) to be in proximity in peptides containing the Pro-Gly sequence (Abu Khaled and Urry, 1976) does not distinguish them.] We may expect that the variation of β-turn geometry in proteins will lead to such a range of CD spectra that it is not possible to define a single CD spectrum to be used to measure β-turn content in the way that helix and sheet content are estimated. Certainly the variations in β-turn geometry seen in the tabulations of Lewis et al. (1971) and of Chou and Fasman (1977) are greater than the difference between the type II turn and the gramicidin S turn.

On the other hand, by ignoring the gramicidin S turn, we can arrive at a tabulation of CD spectral parameters for one set of models of protein β turns that exhibits some consistency. The curves summarized in Table III are qualitatively similar in shape to that of the β sheet with maxima and minima significantly red shifted. This curve was called type B by Woody (1974) and was predicted by his calculations to be the curve type most commonly shown by various types of β turns. The quantitative differences in the magnitudes and wavelengths of the extrema summarized in Table III suggest possible pitfalls in the use of any one of the models to represent β turns in proteins. One possible interpretation of the quantitative differences in CD extrema is that they represent differences between types I and II β turns. Unfortunately, this hypothesis does not correlate the CD data with the β-turn geometries claimed by the various authors. Our model, for which the evidence of a classical type II turn is strong, shows spectral parameters quite different from the models of Urry et al. (1974) and of Brahms et al. (1977), both of which are claimed to be of type II. This interpretation could be tested by CD data on model β turns for which definitive geometric data is available. Although our results raise doubts about the use of any one model β-turn CD to fit the CD spectra of proteins, the models described in this work and in Table III may well find service in characterizing the conformations of oligopeptides and of sequenced polypeptides having a fixed and repeated turn geometry.

Acknowledgments

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