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Theoretical and Experimental Circular Dichroic Spectra of the Novel Helical Foldamer Poly[(1*R*,2*R*)-*trans*-2-aminocyclopentanecarboxylic acid]

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Circular dichroism (CD) spectroscopy is often the first structural method applied to new peptides and proteins, because the measurement is simple to perform and data from the far-UV region provide insight into secondary structures in solution. Measurements that provide higher resolution structural information, such as X-ray crystallography and NMR spectroscopy, require much more time and material for data acquisition and analysis. Current efforts in many laboratories are directed toward the identification of unnatural oligomers and polymers that adopt compact and well-defined folding patterns ("foldamers").[1−14] Short oligomers of β-amino acids (β-peptides), for example, have recently been shown by X-ray crystallography and/or NMR spectroscopy to adopt a variety of helical or sheet secondary structures.[1−7] Earlier studies of β -amino acid homopolymers led to proposals of helical and sheet conformations;[15−17] however, the conformations of these high polymers in solution have never been fully elucidated.

Rapid progress in foldamer development requires simple and convenient assays for the formation of well-defined conformations. While the extensive experimental CD spectra available for poly(α-amino acids) provide a basis for interpreting spectra in terms of secondary structures of these polymers,[18,19] there is no reason to expect the same empirical correlations to apply to homologous molecules such as the β-peptides. In principle, a physical theory that relates the CD spectrum to known properties of the atoms and chromophores should serve as a basis for interpreting observed spectra in terms of structure. Here we describe theoretical and experimental studies that establish the CD signature of a novel β-peptide helix and provide a test of the validity of one current theory for this purpose.

Various research groups, including ours, have independently reported theoretical[17] and experimental[1,2,4−6] circular dichroic spectra of particular observed or hypothetical poly(β-amino acid) helices, though until now no comparison has been made between the observed CD spectrum and the theoretical spectrum for a known structure. Such a comparison is made here for the helix found by Appella *et al.*[4] for poly[(1*R*,2*R*)-*trans*-2-aminocyclopentanecarboxylic acid], here abbreviated (β Cpc)$_n$ (see Figure 1). The helix found for the hexamer in methanol solution by NMR spectroscopy is a novel left-handed structure designated as a 12-helix in the notation of Appella *et al.*[3] (indicating a 12-atom hydrogen-bonded ring) or an L_{-3} helix in the notation of Bode and Applequist[17] [indicating a left-handed helix with hydrogen bonds between $(NH)_i$ and $(CO)_{i-3}$]. The L_{-3} helix is a very compact structure, having only 2.6 residues per turn and virtually no void volume in the interior. Bode and Applequist[17] gave spectral predictions for poly(β-amino acids) in the less compact R_{+2}, R_{+3}, L_{-4}, and L_{-5} helices but not the L_{-3} helix.

The calculations reported here are based on the idealized L_{-3} helix illustrated in Figure 2. The first step was to generate the structure of Ac−(β Cpc)$_{10}$−OMe by simulated annealing calculations.[20] The average backbone torsion angles ϕ, ξ, ψ, ω, side chain torsion angles χ^1, χ^2, χ^3, χ^4, and side chain bond angles were found from the simulation as listed in Table 1. A regular helix was generated using these average quantities for all residues combined with a set of covalent bond lengths and bond angles used in previous optical calculations.[17] The repeating unit was $-C^{\alpha}H-C'O-NH-C^{\beta}HX-$, where X represents the portion of the cyclopentane ring connecting C^{α} and C^{β}. No additional end groups were attached. Because the bond geometries differ slightly from those of the annealed structure, a final optimization of the backbone torsion angles, as described previously,[17] was necessary to achieve realistic hydrogen bond geometries. The final optimized geometry is given in Table 1 and is seen to be close to the annealed structure. The rms difference in non-hydrogen atom positions in the two structures is 0.162 Å.

Figure 3 shows the theoretical and experimental CD spectra in the amide $\pi-\pi^*$ band near 200 nm. The theoretical spectra were obtained for the dipole interaction model using the polarizability parameters described previously for the $NC'O$ chromophores and the nonchromophoric atoms.[17,21,22] The curves for (β Cpc)$_6$ and (β Cpc)$_{12}$ show that the CD spectrum is sensitive to chain length in this range. It is particularly notable that the experimental spectrum for tBoc-(β Cpc)$_6$−OBzl in methanol agrees well with theory in the major observed band at 205 nm. The observed spectrum in trifluoroethanol confirms the presence of the predicted negative band near 190 nm, but the differences from the methanol spectrum suggest that other conformations may be present in trifluoroethanol. The presence of a weak negative band at 220 nm in both experimental spectra is likely due to the amide $n-\pi^*$ transition, which is omitted from the calculation. The curve for (β Ala)$_{12}$ is similar to that for (β Cpc)$_{12}$ except for a much weaker negative band near 190 nm. This shows a sensitivity of the spectrum of the L_{-3} helix to side chain structure that was absent from the theoretical spectra of other poly(β-amino acid) helices.[17]

Table 2 summarizes the theoretical parameters of both the absorption and CD spectra obtained in these calculations. The

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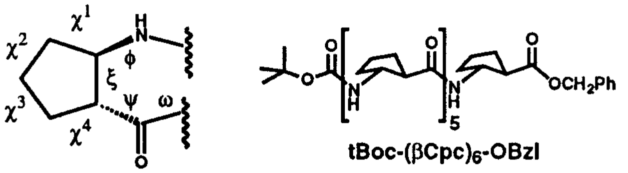

Figure 1. Structure of tBoc-(β Cpc)$_6$−OBzl with notation for torsion angles.

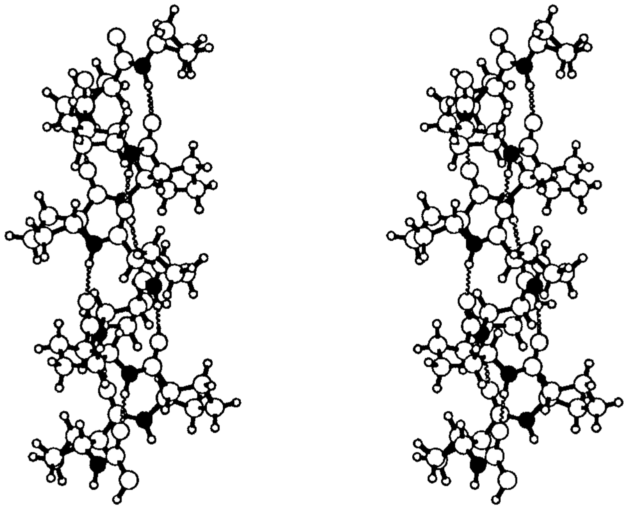

Figure 2. Stereo image of (β Cpc)$_{10}$ in L$_{-3}$ helix. Nitrogen atoms are black. Produced by MacMolplt program courtesy Brett Bode.

Table 1. Geometry of the L$_{-3}$ (βCpc)$_n$ Helices

	annealed[a]	optimized[b]
ϕ (deg)	94.7	95.0
ξ (deg)	−92.1	−94.3
ψ (deg)	101.5	103.0
ω (deg)	−178.5	180.0
unit twist (deg)	−140.7	−139.7
axial translation (Å)	2.05	2.10
residues per turn	2.56	2.58
NO distance (Å)[c]	2.79	2.78
HNC angle (deg)[c]	7.48	12.4
OCN angle (deg)[c]	13.06	13.5
($C'NC^{\beta}C^{\gamma}$)	−148.70	−148.70
χ^1 ($NC^{\beta}C^{\gamma}C^{\delta}$)	−118.82	−118.82
χ^2 ($C^{\beta}C^{\gamma}C^{\delta}C^{\epsilon}$)	−27.56	−27.56
χ^3 ($C^{\gamma}C^{\delta}C^{\epsilon}C^{\alpha}$)	43.49	43.49
χ^4 ($C^{\delta}C^{\epsilon}C^{\alpha}C'$)	−165.62	−162.19
$\angle NC^{\beta}C^{\gamma}$	110.46	110.46
$\angle C^{\beta}C^{\gamma}C^{\delta}$	105.76	105.76
$\angle C^{\gamma}C^{\delta}C^{\epsilon}$	103.12	103.12
$\angle C^{\delta}C^{\epsilon}C^{\alpha}$	101.71	100.99

[a] Average values from annealing simulation. [b] Optimization of ϕ, ξ, ψ to fit NO distance 2.79 Å and linear NH···OC group, with "standard" backbone bond lengths and bond angles and ω fixed at 180.0°. [c] Geometry of NH···OC group.

parameters for the absorption spectra are maximum absorption wavelength λ_{max}, band splitting Δ between perpendicular and parallel absorption bands, mean oscillator strength f, and polarized oscillator strengths $f_{\parallel}$, $f_{\perp}$, relative to the helix axis. The CD parameters are the wavelengths of the major extrema λ_{CD1}, λ_{CD2} and the rotational strengths R_1, R_2 of the corresponding bands, found by summation of rotational strengths of normal modes above and below 200 nm, respectively. The following points are noteworthy:

1. The splitting Δ is substantially larger than values predicted for other helices,[17] which are in the range 1600−3200 cm^{-1} using the same input parameters. The value near 4000 cm^{-1} for long chains is comparable to that observed for the α-helix[23] and is probably related to the close interaction of amide chromophores in these compact helices.

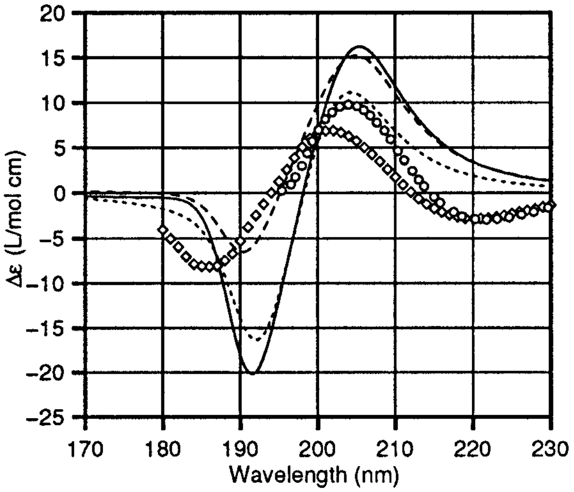

Figure 3. CD spectra of L$_{-3}$ poly(β-amino acid) helices, expressed on a residue-molar basis. Theoretical curves are for (β Cpc)$_{12}$ (—); (β Cpc)$_6$ (- -); (β Ala)$_{12}$ (− −); all using a half-peak bandwidth of 4000 cm^{-1}. Experimental data are for tBoc-(β Cpc)$_6$−OBzl at 20° in methanol (○) and in trifluoroethanol (◇) on a Jasco J-715 spectrometer.

Table 2. Spectral Properties of the $\pi-\pi^*$ Band in L$_{-3}$ Helices

property	(β Ala)$_{12}$	(β Cpc)$_{12}$	(β Cpc)$_6$
λ_{max}(nm)	189	190	190
$\underline{\Delta}$ (cm^{-1})	4058	3910	3363
$\bar{f}$	0.170	0.218	0.240
$f_{\parallel}$	0.006	0.008	0.005
$f_{\perp}$	0.164	0.210	0.235
$f_{\parallel}/f_{\perp}$	0.037	0.039	0.023
R_1 (DBM)	0.533	0.675	0.520
R_2 (DBM)	−0.249	−0.593	−0.556
λ_{CD1} (nm)	204	206	204
λ_{CD2} (nm)	190	192	192

2. The splitting is manifested by the separation of the positive and negative bands of the CD spectrum but would not be easily observed in the absorption spectrum because of the weak oscillator strength $f_{\parallel}$ of the parallel band, corresponding approximately in wavelength to the positive CD band.

3. A potentially important feature of the absorption spectrum of the L$_{-3}$ helix is the occurrence of most of the oscillator strength in the band polarized perpendicular to the helix axis. This is a characteristic of all of the poly(β-amino acid) helices examined so far in which each NH is hydrogen bonded to a CO group toward the N-terminus of the chain, while helices with hydrogen bonding in the opposite direction are predicted to show strong absorption in both parallel and perpendicular modes.[17] A test of this prediction would be possible by linear dichroism measurements on a sample of chain length sufficient to permit orientation of the molecules in a solid film.

4. The CD bands have signs and wavelengths comparable to those of the set of helices examined previously.[17] However, the rotational strengths of the bands for the L$_{-3}$ helix are substantially smaller. As noted above, the rotational strengths in this case show unusual sensitivity to side chain structure. The present findings show that the dipole interaction model for the CD calculations is consistent with observations for a structure determined independently, lending support to both the validity of the theoretical approach and the assignment of the structure. As the model has been tested primarily on poly(α-amino acids),[21] the present extension to a homologous structure gives further evidence of its utility as a structural tool.

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