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Poly(β-amino acid) Helices. Theoretical $\pi-\pi^*$ Absorption and Circular Dichroic Spectra

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ABSTRACT: Absorption and circular dichroic (CD) spectra of the $\pi-\pi^*$ transition near 190 nm are predicted for helical structures proposed for poly(β-amino acids) in accordance with the hydrogen-bonding schemes of Fernández-Santín *et al.* We consider four helical structures which are sterically unhindered when alkyl substituents on C^{α} and C^{β} are in *R* configurations, including two right-handed forms and two left-handed forms. Calculations are made for polymers of β-alanine, (*R*)-3-amino-2-methylpropanoic acid, (*R*)-3-aminobutanoic acid, (2*R*,3*R*)-3-amino-2-methylbutanoic acid, and (1*R*,2*R*)-2-aminocyclohexanecarboxylic acid. The predicted absorption and CD spectra are qualitatively similar for the four helices, showing some sensitivity to backbone structure and relatively little sensitivity to side-chain substitutions. The most notable features of the spectra are (i) CD band couplets centered near 200 nm with a +/− pattern, (ii) large hyperchromicities of 48−115% in the absorption spectra, and (iii) linear dichroic spectra dominated by a strong parallel dichroism in one of the right-handed helices and strong perpendicular dichroism in the left-handed helices. It is suggested that poly[(1*R*,2*R*)-2-aminocyclohexanecarboxylic acid] would be a good candidate for experimental study because one of the helices should be particularly stable for this case. Available experimental absorption and CD spectra are qualitatively consistent with the predictions for any of the four helices, though the observed CD spectra are less intense.

Introduction

Poly(β-amino acids) are expected to show some structural similarities to the biologically important poly(α-amino acids), since the main difference is the presence of an additional backbone carbon atom in the β-amino acid residue. Several studies on the synthesis and characterization of these materials over the past 30 years have been aimed at discovering ordered molecular conformations.[1−16] A recent series of interesting papers from the laboratories at Barcelona[9−16] have given new evidence that novel helical structures occur in these materials.

The purpose of this paper is to present predicted ultraviolet absorption and circular dichroic (CD) spectra of several hypothetical helical structures that have been proposed for poly(β-amino acids), using the dipole interaction theory which we have applied to a number of poly(α-amino acid) structures. These predictions offer additional clues to the structures of molecules for which CD spectra are available. We hope they will eventually serve as new tests of the theoretical model by suggesting new experimental tests and molecular systems for study.

We will use the following notation to specify helical structures: $R_{\pm n}$ denotes a right-handed helix in which NH_i is hydrogen bonded to $CO_{i\pm n}$, using residue numbering from N terminus to C terminus. $L_{\pm n}$ denotes a left-handed helix with the same hydrogen-bonding interval.

A brief survey of the past search for ordered poly(β-amino acid) conformations is in order. Kovacs *et al.*[1] found that poly(β-L-aspartic acid) [poly(βAsp)] in aqueous solution underwent changes in UV absorption intensity, optical rotation, and deuterium exchange rate upon titration of the carboxyl groups; they interpreted this as evidence of a conformational change and proposed an R_{+2} helical structure for the nonionized form. Subsequent work by Hardy *et al.*[17] and Saudek *et al.*[18] raised doubt about this interpretation because of the tendency for poly(Asp) samples to occur as mixtures of α and β linkages. Bestian[2] and Schmidt[4] reported that poly[(*S*)-β-aminobutyric acid] [poly(*S*-βAbu)] in films and fibers cast from organic acid solutions had antiparallel sheet structures analogous to the β-sheets of poly(α-amino acids), based on X-ray diffraction and IR dichroism evidence. Glickson and Applequist[3,5] studied poly(β-alanine) [poly(βAla)] in aqueous solution by proton NMR and deuterium exchange kinetics, demonstrating a disordered structure without extensive intramolecular hydrogen bonding. Chen *et al.*[6] studied optical properties of poly(*S*-βAbu) in organic solvents and concluded that this material exists in a β-associated form in hexafluoro-2-propanol (HFIP) from the similarity of UV absorption, CD, and IR spectra to those of poly(α-amino acids) in β-sheet conformations. Yuki *et al.*[7,8] studied poly(α-isobutyl-L-aspartate) [poly(*S*-βAspOiBu)], an ester of poly(βAsp), by optical methods and concluded that it exists as a β-sheet structure in trifluoroethanol (TFE) solution and in solid films cast from TFE solution. Fernández-Santín *et al.*[9,10] studied poly(*S*-βAspOiBu) by X-ray diffraction of fibers and powders cast from chloroform solution; they found patterns consistent with two helical forms in hexagonal and tetragonal crystal structures. From model studies, they proposed four different hydrogen-bonded helices for the poly(β-amino acid) backbone. They favored an L_{-4} helix (1L in their notation) for the hexagonal form and an R_{-5} helix (3R in their notation) for the tetragonal form. Bella *et al.*[11] further refined the helical models to satisfy both the X-ray data and energy minimization; they supported both the L_{-4} helix and the R_{+2} (2R) helix in the hexagonal form but preferred the R_{+3} (4R) helix in the tetragonal form. In a refinement of the energy minimization to include intermolecular interactions, Alemán *et al.*[12] concluded that the R_{+2} (2R) helix would be more stable than the L_{-4} helix in the hexagonal crystal. Experimental support for this conclusion was found by López-Carrasquero *et al.*,[13] who did IR dichroism studies on films of poly(*S*-βAspOiBu) and other esters of poly(βAsp) cast from TFE solution, concluding that the R_{+2}

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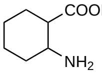
Table 1. β-Amino Acid Names and Structures

name	structure	abbrev
3-aminopropanoic acid (β-alanine)	$H_2N\text{-}CH_2\text{-}CH_2\text{-}COOH$	βAla
(*R*)-3-amino-2-methylpropanoic acid	$H_2N\text{-}CH_2\text{-}CHCH_3\text{-}COOH$	βAib
(*R*)-3-aminobutanoic acid	$H_2N\text{-}CHCH_3\text{-}CH_2\text{-}COOH$	βAbu
(2*R*,3*R*)-3-amino-2-methylbutanoic acid	$H_2N\text{-}CHCH_3\text{-}CHCH_3\text{-}COOH$	βAmb
(1*R*,2*R*)-2-aminocyclohexanecarboxylic acid		βAcc

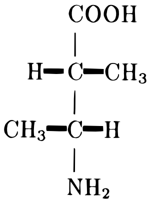
helix is consistent with the observed dichroism, while the L_{-4} helix is not. A refinement of the structures of esters of poly(βAsp) to fit X-ray data and energy minimization by Navas *et al.*[15] likewise favored the R_{+2} helix in the hexagonal crystal and the R_{+3} helix in the tetragonal form.

Thus far the CD spectrum has played little definitive role in assigning the conformations of poly(β-amino acids), due to the lack of either empirical correlations which apply directly to these materials or theoretical calculations which would relate CD spectra to structure. The only report of previous theoretical work we find is the abstract of Manning *et al.*,[19] who indicate that the observed CD spectrum of poly(*S*-βAspOiBu) in chloroform is consistent with calculated spectra for two helices with 3.25 residues per turn (presumably L_{-4} and R_{+2}) but inconsistent with the calculated spectrum for helices with 4 residues per turn (presumably R_{+3}).

These findings prompted us to undertake the present study, in which we apply a theory of absorption and CD spectra whose parameters have been developed in applications to poly(α-amino acids).[20] The unique features of the theory are (i) it is based on classical electromagnetic theory using empirical polarizabilities of atoms and chromophores as input data and (ii) it includes all major contributions to the molecular polarizability in the interaction problem, giving rise to borrowing of dipole rotational strength and the consequent nonconservative behavior of the ultraviolet absorption and CD bands. The theory has been shown to reproduce the major features of the experimental absorption and CD spectra of the π−π* band near 200 nm in various poly(α-amino acid) helices.[20] The present calculations predict spectral features that could not have been expected from known spectra of poly(α-amino acids), including large hyperchromic effects, strong couplet CD band patterns, and, in some of the hypothetical structures, strong linear dichroic effects that could serve to distinguish among the structures.

Stereochemical Considerations

The β-amino acids are potentially chiral at both the α and β carbon atoms. For a single alkyl substituent at either atom the absolute configurations are defined by the Fischer projection for (2*R*,3*R*)-3-amino-2-methylbutanoic acid:



$$\begin{array}{c} COOH \\ | \\ H-C-CH_3 \\ | \\ CH_3-C-H \\ | \\ NH_2 \end{array}$$

In the present study we treat the β-amino acids listed in Table 1, which contain substituents (methyl or bridging tetramethylene) in the configurations shown above. The properties of the *S* enantiomers can be deduced by symmetry from those of the *R* enantiomers.

Table 2. Possible Helices for Homopolymers with *R* Configuration at All Chiral Carbon Atoms

polymer	helices
$(\beta Ala)_n$	R_{+2}, L_{+2}, R_{+3}, L_{+3}, R_{-4}, L_{-4}, R_{-5}, L_{-5}
$(\beta Aib)_n$	R_{+2}, R_{+3}, L_{-4}, L_{-5}
$(\beta Abu)_n$	R_{+2}, R_{+3}, L_{-4}, L_{-5}
$(\beta Amb)_n$	R_{+2}, R_{+3}, L_{-4}, L_{-5}
$(\beta Acc)_n$	R_{+2}

Diastereomers containing mixed *R*,*S* configurations are not considered, as none of the proposed helices is able to accommodate such forms. The abbreviations in the table are taken to imply the *R* configurations unless specifically stated to be otherwise.

The four possible hydrogen bonding schemes for poly(β-amino acid) helices proposed by Fernández-Santín *et al.*[10] could each exist in either right- or left-handed forms if not prohibited by steric interactions involving the side chains. They numbered their schemes 1, 2, 3, 4, corresponding to the hydrogen bond intervals −4, +2, −5, +3, respectively, in our notation. We have used molecular models to examine the possible helices for various possible side chains. Table 2 shows those helices belonging to the Fernández-Santín schemes which are sterically unhindered for each of the β-amino acid residues of Table 1. For $(\beta Ala)_n$ all of the 8 helices are unhindered. In any of those forms the C−H bonds are oriented in either axial or equatorial directions; in the axial positions substitution of an alkyl group for hydrogen is not sterically allowed, while the equatorial positions do not appear to offer hindrance to fairly large substituents. Thus, the residues with methyl side chains are restricted to a single helix sense in each hydrogen bond scheme. The case of $(\beta Acc)_n$ is especially interesting because only one of the helical forms (R_{+2}) is consistent with the chair form of the cyclohexane ring.

Theory

The dipole interaction model provides a means of calculating optical properties of molecules based on the polarizabilities of atoms and chromophores which interact solely by way of induced dipole fields when in the presence of an electric field.[21−23] Details of the theory and its application to the prediction of absorption and circular dichroic spectra and other spectral properties are provided elsewhere.[20] In short, chromophores, such as the NC′O group in peptides, have anisotropic polarizabilities consisting of a contribution from the π−π* transition and a contribution from higher energy transitions, while atoms behave as isotropic units. All atoms and chromophoric groups mutually interact via induced dipole moments, and the response of the entire molecule to light is expressed in terms of normal mode electric dipole moments, $\boldsymbol{\mu}^{(k)}$, normal mode magnetic dipole moments, $\mathbf{m}^{(k)}$, and normal mode wavenumbers of oscillation, $\bar{\nu}_k$. In our calculation of spectral properties we define both a normal mode dipole strength and a normal mode rotational strength by $D_k = [\mu^{(k)}]^2$ and $R_k = \mathbf{m}^{(k)} \cdot \boldsymbol{\mu}^{(k)}$, respectively. The molecular spectral properties calculated are maximum absorption wavelength

$\lambda_{\max}$, band splitting Δ, mean oscillator strength $\bar{f}$, and polarized oscillator strengths $f_{\|}$, $f_{\perp}$. These are related to the calculated normal mode properties as follows:

$$\lambda_{\max} = [\sum_{k=1}^{q} \bar{\nu}_k{}^2 D_k / \sum_{k=1}^{q} D_k]^{-1/2} \tag{1}$$

$$\begin{aligned}\Delta &= \langle \bar{\nu}_{\perp}{}^2 \rangle^{1/2} - \langle \bar{\nu}_{\|}{}^2 \rangle^{1/2} \\ &= [\sum_{k=1}^{q} \bar{\nu}_k{}^2 D_{k\perp} / \sum_{k=1}^{q} D_{k\perp}]^{1/2} - [\sum_{k=1}^{q} \bar{\nu}_k{}^2 D_{k\|} / \sum_{k=1}^{q} D_{k\|}]^{1/2}\end{aligned} \tag{2}$$

$$\bar{f} = (4 m_e \pi^2 c^2 / 3 e^2 q) \sum_{k=1}^{q} D_k \tag{3}$$

$$f_{\perp} = (4 m_e \pi^2 c^2 / 3 e^2 q) \sum_{k=1}^{q} D_{k\perp} \tag{4}$$

$$f_{\|} = (4 m_e \pi^2 c^2 / 3 e^2 q) \sum_{k=1}^{q} D_{k\|} \tag{5}$$

where q is the number of normal modes. The subscripts $\|$ and $\perp$ refer to values for light polarized parallel and perpendicular to the helix axis. The conversion from dipole strength to oscillator strength contains the factor[24] $(4 m_e \pi^2 c^2 / 3 e^2)$, where m_e, c, and e are the electron mass, speed of light, and electron charge, respectively.

Absorption and CD spectra are calculated in terms of the residue-molar absorption coefficient ϵ from the normal mode properties[24] assuming a medium with refractive index of unity:

$$\epsilon = (8 \pi^2 \bar{\nu}^2 N_A \Gamma / 6909 p) \sum_{k=1}^{q} D_k / [(\bar{\nu}_k{}^2 - \bar{\nu}^2)^2 + \Gamma^2 \bar{\nu}^2] \tag{6}$$

$$\Delta\epsilon = (32 \pi^3 \bar{\nu}^3 N_A \Gamma / 6909 p) \sum_{k=1}^{q} R_k / [(\bar{\nu}_k{}^2 - \bar{\nu}^2)^2 + \Gamma^2 \bar{\nu}^2] \tag{7}$$

where N_A is Avogadro's number, $\bar{\nu}$ is the wavenumber of the light, Γ is the half-peak bandwidth of a Lorentzian band, and p is the number of residues. The oscillator strengths of eqs 3–5 are "per residue", and the rotational strengths reported here are given in debye-bohr-magnetons (DBM) per residue.[24] We set $\Gamma = 4000$ cm^{-1} in all calculations reported here.

Methods

Coordinates of atoms in the various helices were generated from the bond lengths, bond angles, and torsion angles, as described previously.[25] The $-C^{\alpha}HX-C'O-NH-$ fragment was generated as for poly(α-amino acids). The $-C^{\beta}HX'-$ group, with tetrahedral bonding about C^{β}, was attached to the N terminus such that the NC^{β} bond length was 1.475 Å [identical to the NC^{α} bond length in the poly(α-amino acid) helix] and the $C^{\alpha}C^{\beta}N$ bond angle was taken as 110°, the value for the $C'C^{\alpha}N$ angle in poly(α-amino acids). In poly(β-alanine) the X and X′ groups are hydrogen atoms. Alkyl side chains were incorporated by replacing equatorial hydrogen atoms with tetrahedral methyl groups arranged in staggered conformations [$\theta(C'C^{\alpha}CH) = \theta(NC^{\beta}CH) = -60°$]. For βAcc the group $-CH_2CH_2CH_2CH_2-$ was substituted for equatorial hydrogens on C^{α} and C^{β}, forming a cyclohexane ring in a chair conformation with 60° torsion angles alternating in sign around the ring.

Table 3. Optimized Geometry of Poly(βAla)$_{12}$ Helices

	R_{+2}	R_{+3}	L_{-4}	L_{-5}
ϕ (deg)	134.3	141.5	119.9	132.7
ξ (deg)	−60.0[a]	−86.3	−106.0	−117.9
ψ (deg)	139.9	150.3	129.8	145.3
ω (deg)	180.0[a]	180.0[a]	180.0[a]	180.0[a]
unit twist (deg)	110.9	90.0	−102.5	−80.5
axial translation (Å)	1.56	1.22	1.44	1.09
residues per turn	3.25	4.00	3.51	4.47
NO distance (Å)	2.79	2.79	2.79	2.79
HNC angle (deg)	7.5	1.0	7.0	4.6
OCN angle (deg)	9.6	1.0	7.4	4.8

[a] Parameter which remained fixed during optimization.

Bella *et al.*[11] gave torsion angles for three of the four helix types from their energy minimizations. We find we cannot use their torsion angles with the bond lengths and bond angles adopted here, as they produce highly nonlinear N−H···O=C groups and unacceptably short NO distances (e.g., 2.40 Å). To obtain suitable values for the torsion angles ϕ, ξ, and ψ (defined by the atoms $C^{\alpha}C^{\beta}NC'$, $C'C^{\alpha}C^{\beta}N$, and $NC'C^{\alpha}C^{\beta}$, respectively) we carried out a search for values that would minimize the deviation of the N−H···O=C group from linearity (by minimizing ∠HNC and ∠NCO) and the deviation of the NO distance from 2.79 Å.[26] A nonlinear optimization method was used,[27] taking as starting torsion angles the values of Bella *et al.* or values estimated from scale models. We took the amide bond torsion angle ω to be fixed at 180°. For the R_{+2} helix ξ was found to be near the staggered conformation −60° both in our optimization and in the energy minimizations of Bella *et al.*[11] We therefore chose to fix $\xi = -60°$ in the optimization for the R_{+2} helix in order to simplify the incorporation of the cyclohexane ring in (βAcc)$_n$. The resulting torsion angles and other geometric quantities for the four helix types are shown in Table 3. The torsion angles are in the vicinity of those of Bella *et al.* for the three cases they reported, but discrepancies of as much as 26° occur. The discrepancies do not necessarily reflect on the validity of our optimization, since the energy optimizations themselves are subject to uncertainties of several degrees.[12] The helices described here are consistent with the bulk of geometric data on related small molecules and should be reasonably close to minimum-energy forms.

Fernández-Santín *et al.*[10] gave torsion angles for an R_{-5} helix (3R in their notation) which are not simply related to our angles for the L_{-5} helix. Their helix is an alternative solution to the same hydrogen-bonding scheme. When we construct a helix with their torsion angles and our bond lengths and bond angles, we obtain a structure with a very long N−H···O=C hydrogen bond (NO distance 2.99 Å) and very short contacts between C=O and both C^{α}−H and C^{β}−H (OH distances 1.58–1.60 Å). We do not consider this helix further here. It should also be noted that, in building molecular models, one finds that each of the hydrogen-bonding schemes can be satisfied by more than one backbone conformation. For present purposes we favor the choices given here, as they are relatively free of steric overlaps, high-energy C^{α}−C^{β} torsion angles, and bent hydrogen bonds.

Parameters used for the optical calculations are given in Table 4. NC′O polarizability parameter set H_y is the set that gave the best overall agreement with experimental π−π^* absorption and CD spectra of poly(α-amino acid) helices in our recent study.[20] For comparison our parameter set from a 1979 study[24] is also shown; this

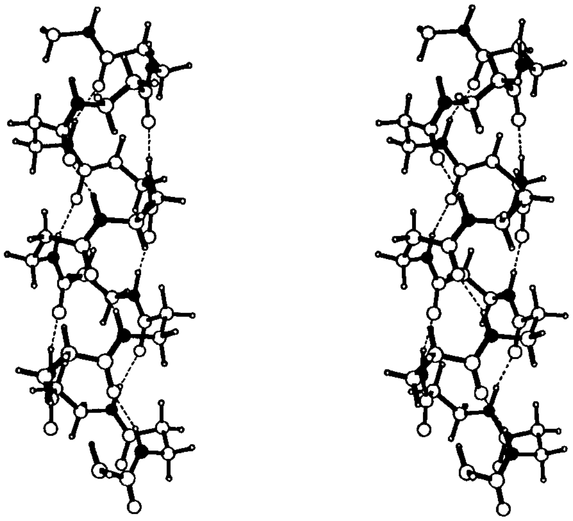


Figure 1. Stereo image of R_{+2} (βAla)$_{12}$. N atoms are black in all figures.

Table 4. NC′O Parameters and Atom Polarizabilities Used in Calculations of Helix Optical Properties

parameter	set H_y	1979
(Δx, Δy) (Å)	(0.0, 0.1)	(0.0, 0.0)
$\hat{\alpha}_{A,1}$ (Å^3)	2.744	1.94
$\hat{\alpha}_{A,2}$ (Å^3)	1.137	0.76
$\hat{\alpha}_{A,3}$ (Å^3)	3.592	2.58
$\hat{\phi}$ (deg)	54.2	52.3
f_0	0.149	0.196
λ_0 (nm)	173.6	170.3
θ_0 (deg)	9.3	21.5
α_C, alkane (Å^3)	0.777	0.878
α_H, alkane (Å^3)	0.172	0.135
α_H, amide (Å^3)	0.149	0.161

set is used in some of the present calculations to show the sensitivity of predictions to the parameters. The NC′O group is placed in a local coordinate system with the atoms in the xy plane, the NC′ bond on the x axis centered at the origin, and the O atom in the first quadrant. The NC′O tensor center is at (Δx,Δy) relative to the NC′ bond center. The nondispersive core polarizability of NC′O has components $\hat{\alpha}_{A,1}$ and $\hat{\alpha}_{A,2}$ in the NC′O plane with $\hat{\alpha}_{A,1}$ oriented at polar angle $\hat{\phi}$. The $\pi-\pi^*$ transition of the isolated NC′O group is characterized by oscillator strength f_0, wavelength λ_0, and transition moment polar angle θ_0. Isotropic polarizabilities of nonchromophoric atoms are denoted α_H and α_C.

Computations were performed on a DEC 3000/300L workstation using double-precision Fortran programs.

Experimental spectra shown in the figures were obtained by scanning the published graphs using Arc software in the GIS Support and Research Facility, Iowa State University.

Results

Figures 1−4 show stereopairs of the major helical structures of (βAla)$_{12}$. The helix senses shown are those that will accommodate larger side chains in the R configurations for alkyl substituents. Figure 5 shows (βAcc)$_{12}$ in the R_{+2} helix, where it can be seen that the side chains are attached at the equatorial positions on the backbone carbons. All of the four helices show equatorial positions that are accessible to large side chains, though only in R_{+2} does the ξ angle accommodate the cyclohexane ring of βAcc in an unstrained chair form.

It should be noted that the much-studied poly(S-βAspOiBu) is sterically related to poly(R-βAbu) by replacement of the CH_3 group in the latter with the COOiBu group of the former. Thus the allowed helices for poly(R-βAbu) are also allowed for poly(S-βAspOiBu).

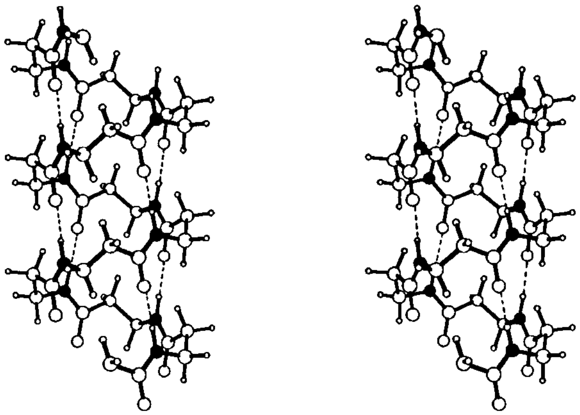


Figure 2. Stereo image of R_{+3} (βAla)$_{12}$.

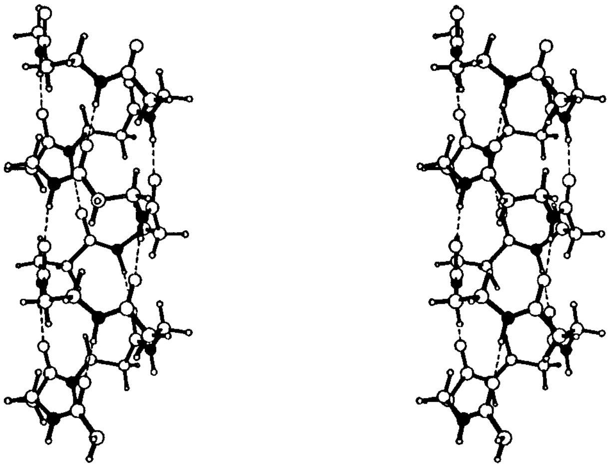


Figure 3. Stereo image of L_{-4} (βAla)$_{12}$.

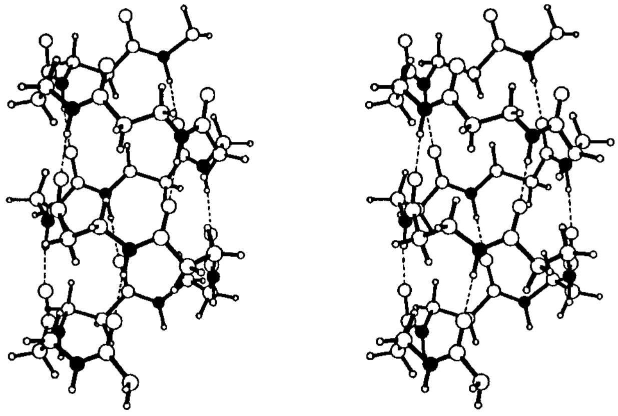


Figure 4. Stereo image of L_{-5} (βAla)$_{12}$.

The axial hydrogens on adjacent turns of the helices are separated from their nearest-neighbor axial hydrogens by distances ranging from 2.03 Å in R_{+3} to 2.40 Å in R_{+2}. These distances are sterically permissible, considering the hydrogen van der Waals radius of 1.2 Å.[26] However, any substituents much larger than hydrogen would not be permitted in the axial positions.

The effects of the helix structure on the predicted properties of the $\pi-\pi^*$ transition with parameter set H_y can be seen Table 5 and Figure 6, where results are given for (βAla)$_{12}$ in each of the four forms under consideration. In Table 5 and subsequent tables the CD spectrum is described by the wavelengths of the major extrema λ_{CD1}, λ_{CD2} and by the rotational strengths R_1,

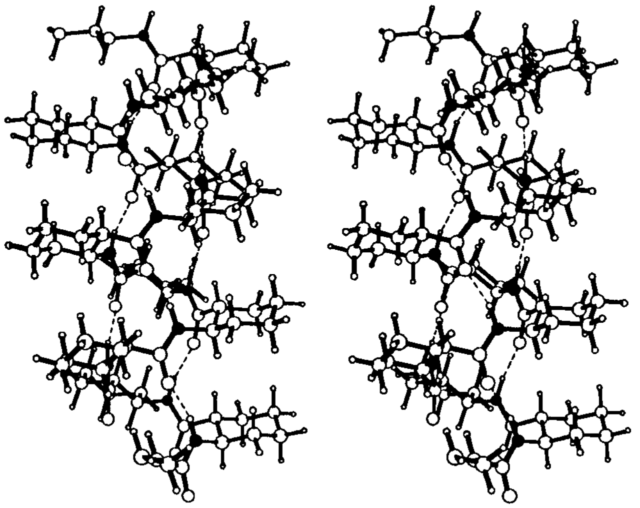


Figure 5. Stereo image of R_{+2} (βAcc)$_{12}$.

Table 5. Spectral Properties of the $\pi-\pi^*$ Transition in (βAla)$_{12}$ Helices Calculated with Set H_y

property	R_{+2}	R_{+3}	L_{-4}	L_{-5}
λ_{max} (nm)	198	197	191	192
Δ (cm^{-1})	2260	1659	3232	2680
$\bar{f}$	0.32	0.30	0.22	0.27
$f_{\parallel}$	0.20	0.14	0.01	0.01
$f_{\perp}$	0.12	0.16	0.21	0.26
R_1 (DBM)	3.33	3.90	1.15	1.78
R_2 (DBM)	−2.90	−3.55	−0.87	−1.50
λ_{CD1} (nm)	204	204	204	204
λ_{CD2} (nm)	192	194	192	194

R_2 of the corresponding bands, found by summation of rotational strengths of normal modes above and below 200 nm, respectively. The following are notable features of the calculations:

1. Remarkably, the CD spectra are qualitatively similar for the four helices, showing a nearly conservative +/− couplet centered near 198 nm. The experimental spectra shown for poly(βAbu) and poly(S-βAspOiBu) show a similar pattern, though weaker in intensity. In both cases the polymers were believed by the investigators to be in β-sheet forms rather than any of the proposed helical forms. We return to this point in the discussion section.

2. The absorption spectra are predicted to be exceptionally intense. The hyperchromicity, defined by $(\bar{f} - f_0)/f_0$, varies from 48% for the L_{-4} helix to 115% for the R_{+2} helix. The experimental spectrum for (S-βAspOiBu)$_n$ shows an intensity comparable to the larger of these predictions.

3. The behavior of the parallel- and perpendicular-polarized components of the absorption spectrum, seen in the data of Table 5, may offer the best means of distinguishing among the various helices. The splittings Δ are moderately large and positive, indicating that parallel and perpendicular bands should be resolvable in a polarized spectrum, with the parallel band occurring at the longer wavelength. The $f_{\parallel}$ and $f_{\perp}$ values indicate that the parallel band should be dominant in R_{+2} while the perpendicular band should be strongly dominant in L_{-4} and L_{-5}. In R_{+3} the predicted bands are almost equal in intensity. (It must be noted that these distinctions are related primarily to the hydrogen-bonding interval in the helices; the same results in the absorption spectra would be obtained for the opposite helix senses with the same hydrogen-bonding intervals.)

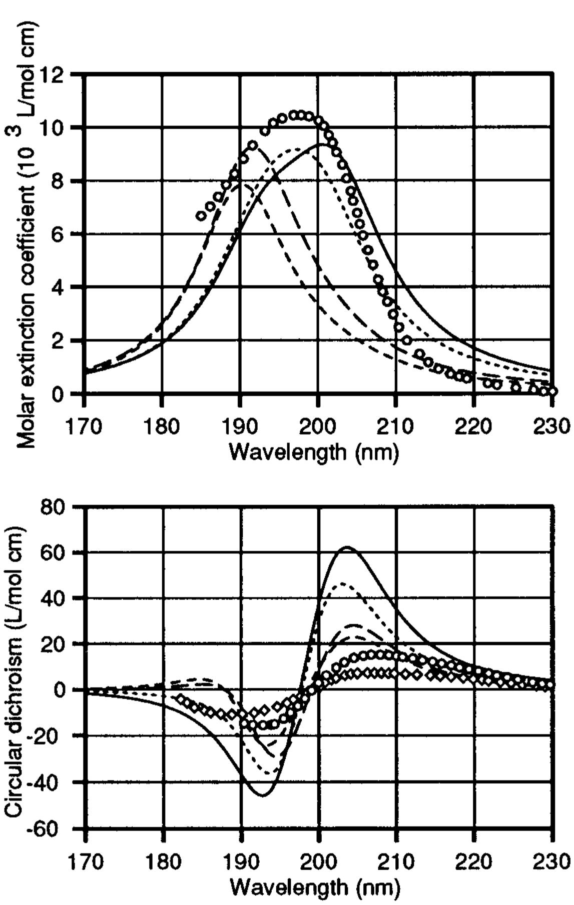


Figure 6. Absorption and CD spectra of poly(β-amino acid) helices using parameter set H_y. Theoretical curves are for (—) R_{+2}, (···) R_{+3}, (- - -) L_{-4}, and (— — —) L_{-5} (βAla)$_{12}$. Experimental data are for the following solutions: (○) poly(S-βAbu) in 95:5 HFIP/H_2O from Chen *et al.*[6] with sign reversed to correspond to the R isomer; (◇) poly(S-βAspOiBu) in TFE from Yuki *et al.*[7]

Table 6. Spectral Properties of the $\pi-\pi^*$ Transition in (βAla)$_{12}$ Helices Calculated with 1979 Parameters

property	R_{+2}	R_{+3}	L_{-4}	L_{-5}
λ_{max} (nm)	200	198	195	196
Δ (cm^{-1})	986	425	2259	1677
$\bar{f}$	0.30	0.26	0.21	0.23
$f_{\parallel}$	0.23	0.17	0.05	0.05
$f_{\perp}$	0.06	0.09	0.16	0.18
R_1 (DBM)	2.42	0.00	2.00	2.75
R_2 (DBM)	−2.18	0.13	−1.60	−2.44
λ_{CD1} (nm)	204	204	196	196
λ_{CD2} (nm)	194	190	188	188

The corresponding predictions using the 1979 parameter set are shown in Table 6 and Figure 7. The CD spectra are less intense than those with set H_y, while the absorption spectra are similar in most respects aside from the smaller splittings. The separation of the rotational strengths in Table 6 into bands above and below 200 nm does not reflect the appearance of the CD spectra well in this case, as the band patterns are more complex than with the H_y parameters. A potentially significant result in Figure 7 is that the CD spectrum for the R_{+2} helix is more nearly comparable to the experimental spectra shown than is any of the other spectra calculated with 1979 or H_y parameters.

Figure 8 shows the calculated absorption and CD spectra for the R_{+2} helix with all of the possible residues of Table 1, using parameter set H_y. Further details for this case are given in Table 7. Tables 8−10 give corresponding data for the various residues in each of the three remaining helices. The absorption spectra, including components of oscillator strength and splittings, are predicted to be insensitive to alkyl substitu-

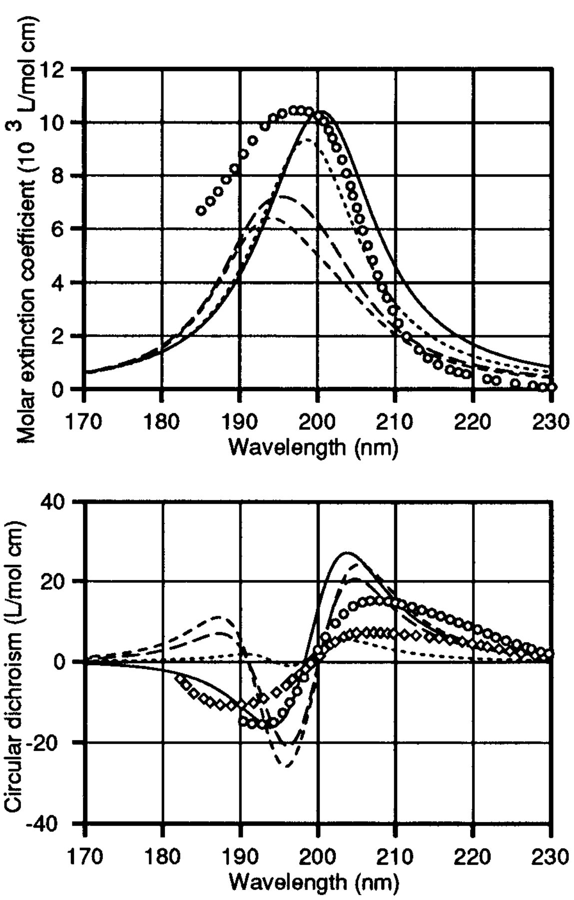


Figure 7. Absorption and CD spectra of poly(β-amino acid) helices using 1979 parameter set. Theoretical curves are for (—) R_{+2}, (···) R_{+3}, (- - -) L_{-4}, and (— — —) L_{-5} $(\beta Ala)_{12}$. Experimental data are as stated in Figure 6.

tions in all cases. Methyl substitutions produce small but systematic shifts in CD spectra, the effect being primarily a negative contribution to the band by each methyl group added. These calculations indicate that the absorption and CD spectra both reflect mainly the backbone structure and are relatively independent of the side chains.

A referee of this paper suggested that we comment on the sensitivity of the calculations to the conformational parameters for a given hydrogen-bonding scheme. To answer this briefly, we have repeated the calculations for $(\beta Ala)_{12}$ in the R_{+2} form using the most fully refined conformation angles of Bella *et al.*[11] ($\phi = 151.3°$, $\xi = -59.1°$, $\psi = 118.1°$, $\omega = 180°$) in place of those in Table 3. The calculated spectra show only very small shifts from those of the R_{+2} case in Figure 6; *i.e.*, the absorption peak is reduced by 10% and the CD peaks are reduced by 6–10%, with displacements of 1 nm or less in wavelength. For this example, at least, the spectra are insensitive to variations of several degrees in the torsion angles.

Discussion

The present calculations lead to several predictions that could be tested experimentally and which may serve to identify the helical structures proposed for poly(β-amino acids):

1. All of the four proposed helices for the *R* configurations of alkyl-substituted residues are predicted to have CD spectra whose major feature is a +/− couplet centered near 200 nm. This is true for both the right-handed and left-handed forms.

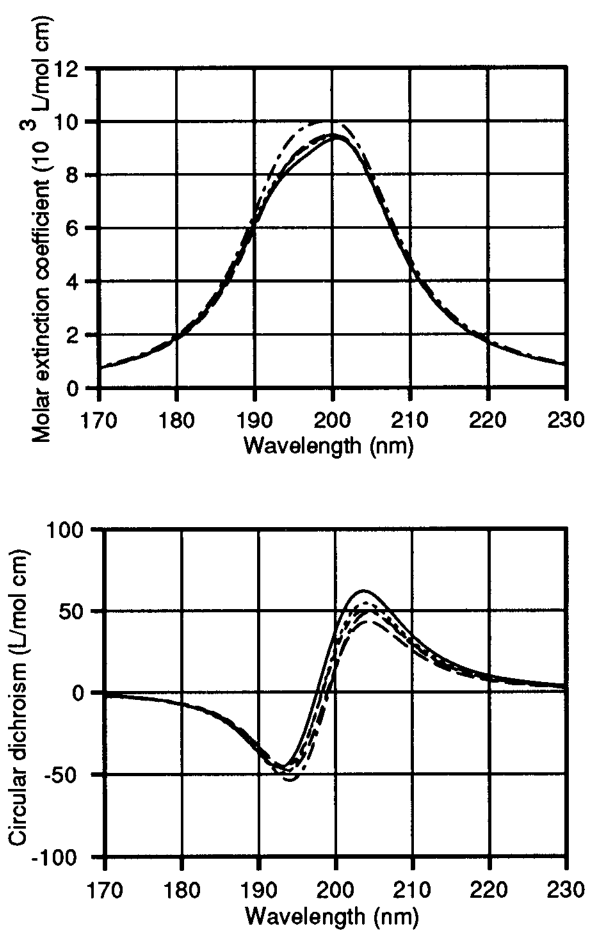


Figure 8. Theoretical absorption and CD spectra of R_{+2} poly(β-amino acid) helices using H_y parameters: (—) $(\beta Ala)_{12}$; (···) $(\beta\ Aib)_{12}$; (- - -) $(\beta Abu)_{12}$; (— — —) $(\beta\ Amb)_{12}$; (— · —) $(\beta Acc)_{12}$.

Table 7. Spectral Properties of the π–π* Transition in R_{+2} Helices Calculated with Set H_y

property	$(\beta Ala)_{12}$	$(\beta Aib)_{12}$	$(\beta Abu)_{12}$	$(\beta Amb)_{12}$	$(\beta Acc)_{12}$
λ_{max} (nm)	198	198	198	199	198
Δ (cm^{-1})	2260	2146	2104	1992	2010
f	0.32	0.33	0.32	0.32	0.34
$f_{\parallel}$	0.20	0.20	0.20	0.19	0.19
$f_{\perp}$	0.12	0.13	0.13	0.13	0.15
R_1 (DBM)	3.33	3.22	3.04	2.89	3.29
R_2 (DBM)	−2.90	−3.03	−2.85	−2.92	−3.31
λ_{CD1} (nm)	204	204	204	204	204
λ_{CD2} (nm)	192	194	194	194	194

Table 8. Spectral Properties of the π–π* Transition in R_{+3} Helices Calculated with Set H_y

property	$(\beta Ala)_{12}$	$(\beta Aib)_{12}$	$(\beta Abu)_{12}$	$(\beta Amb)_{12}$
λ_{max} (nm)	197	197	197	197
Δ (cm^{-1})	1660	1553	1512	1410
f	0.30	0.30	0.30	0.29
$f_{\parallel}$	0.14	0.13	0.14	0.13
$f_{\perp}$	0.16	0.17	0.16	0.17
R_1 (DBM)	3.90	3.64	3.24	2.39
R_2 (DBM)	−3.55	−3.57	−3.41	−2.26
λ_{CD1} (nm)	204	204	204	204
λ_{CD2} (nm)	194	194	194	194

2. All of the four helices are predicted to show large hyperchromicities in the absorption peak near 190–200 nm. The effect is somewhat greater in the right-handed helices than in the left-handed helices.

3. The absorption band for all four helices is predicted to show splitting into parallel- and perpendicular-polarized bands (relative to the helix axis), with the parallel band occurring at longer wavelength. For the left-handed forms the perpendicular band is predicted

Table 9. Spectral Properties of the $\pi-\pi^*$ Transition in L_{-4} Helices Calculated with Set H_y

property	$(\beta Ala)_{12}$	$(\beta Aib)_{12}$	$(\beta Abu)_{12}$	$(\beta Amb)_{12}$
λ_{max} (nm)	191	191	191	192
$\underline{\Delta}$ (cm^{-1})	3232	3098	3046	2935
$\bar{f}$	0.22	0.24	0.25	0.25
$f_{\parallel}$	0.01	0.01	0.01	0.01
$f_{\perp}$	0.21	0.23	0.23	0.24
R_1 (DBM)	1.15	1.11	1.21	1.16
R_2 (DBM)	−0.87	−1.07	−1.16	−1.32
λ_{CD1} (nm)	204	204	204	206
λ_{CD2} (nm)	192	194	194	194

Table 10. Spectral Properties of the $\pi-\pi^*$ Transition in L_{-5} Helices Calculated with Set H_y

property	$(\beta Ala)_{12}$	$(\beta Aib)_{12}$	$(\beta Abu)_{12}$	$(\beta Amb)_{12}$
λ_{max} (nm)	194	193	193	193
$\underline{\Delta}$ (cm^{-1})	2680	2519	2489	2351
$\bar{f}$	0.27	0.28	0.28	0.28
$f_{\parallel}$	0.01	0.01	0.01	0.01
$f_{\perp}$	0.26	0.27	0.27	0.27
R_1 (DBM)	1.78	1.62	1.72	1.55
R_2 (DBM)	−1.50	−1.62	−1.71	−1.72
λ_{CD1} (nm)	204	206	204	206
λ_{CD2} (nm)	194	196	196	196

to be much stronger than the parallel band, while the reverse is predicted for the R_{+2} form.

4. The absorption and CD spectra are predicted to be dependent primarily on the backbone structure and only to a slight extent on the side-chain structure for each helix type.

5. Poly(βAcc) presents special features that warrant an experimental study. The cyclohexane ring involving the backbone and side chain greatly restricts the conformational freedom of the residue. It has been suggested[5] that internal rotation about the $C^{\alpha}-C^{\beta}$ bond is a significant destabilizing effect for ordered structures in poly(βAla); the elimination of this rotation would be expected to enhance the stability of an ordered structure. Hence, the R_{+2} helix would be a strong candidate for a structure in poly(βAcc).

None of the experimental solution spectra in Figures 6 and 7 was expected to provide a good test of the present predictions, since the investigators in each case favored β-sheet structures for these systems. In spite of this, the observed spectra show certain characteristics predicted for the helices, notably the intense absorption spectrum and the +/− CD couplet. Since the evidence for β-sheet structures was somewhat indirect, there is a reasonable possibility that the structures include helical conformations in part or in whole. This view has previously been suggested in the related study of Fernández-Santín *et al.*,[10] who interpreted NMR spectra of poly(*S*-βAspOiBu) in $CDCl_3$ as supporting a helical structure, based on broadening of proton resonances. Similarly, Bella *et al.*[11] suggested on energetic grounds that an R_{+2} helix for poly(*S*-βAspOiBu) should be the one normally found in solution. However, neither study attempted to rule out the possibility of β-sheet structures, as proposed by other workers, and further experimental work is needed to make this distinction.

López-Carrasquero *et al.*[13] reported the CD spectrum of poly(α-*n*-butyl β-L-aspartate) in $CHCl_3$ solution in the range above 220 nm, where a weak positive peak is found. They interpreted the CD data and the NMR spectrum as supporting a helical conformation. Their CD spectrum is unlike any of the experimental or theoretical spectra given above, and we do not know its significance at present.

The sensitivity of the present predictions to the optical parameters chosen indicates that some caution is necessary in interpreting observed spectra on the basis of the theory. The H_y parameter set was found to be more accurate than the 1979 parameter set in reproducing absorption and CD spectra of poly(α-amino acids),[20] and we expect the H_y set to be the more reliable set in the present case as well. The predicted spectral features listed above are qualitative features that are insensitive to the choice of parameters; hence, we feel they should be useful guides to the choice of experiments and the interpretation of spectra.

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References and Notes

(1) Kovacs, J.; Ballina, R.; Rodin, R. L.; Balasubramanian, D.; Applequist, J. *J. Am. Chem. Soc.* **1965**, *87*, 119.
(2) Bestian, H. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 278.
(3) Glickson, J. D.; Applequist, J. *Macromolecules* **1969**, *2*, 628.
(4) Schmidt, E. *Angew. Makromol. Chem.* **1970**, *14*, 185.
(5) Glickson, J. D.; Applequist, J. *J. Am. Chem. Soc.* **1971**, *93*, 3276.
(6) Chen, F.; Lepore, G.; Goodman, M. *Macromolecules* **1974**, *7*, 779.
(7) Yuki, H.; Okamoto, Y.; Taketani, Y.; Tsubota, T.; Marubayashi, Y. *J. Polym. Sci., Polym. Chem. Ed.* **1978**, *16*, 2237.
(8) Yuki, H.; Okamoto, Y.; Doi, Y. *J. Polym. Sci., Polym. Chem. Ed.* **1979**, *17*, 1911.
(9) Fernández-Santín, J. M.; Aymamí, J.; Rodríguez-Galán, A.; Muñoz-Guerra, S.; Subirana, J. A. *Nature* **1984**, *311*, 53.
(10) Fernández-Santín, J. M.; Muñoz-Guerra, S.; Rodríguez-Galán, A.; Aymami, J.; Lloveras, J.; Subirana, J. A. *Macromolecules* **1987**, *20*, 62.
(11) Bella, J.; Alemán, C.; Fernández-Santín, J. M.; Alegre, C.; Subirana, J. A. *Macromolecules* **1992**, *25*, 5225.
(12) Alemán, C.; Bella, J.; Perez, J. J. *Polymer* **1994**, *35*, 2596.
(13) López-Carrasquero, F.; Alemán, C.; Muñoz-Guerra, S. *Biopolymers* **1995**, *36*, 263.
(14) López-Carrasquero, F.; Alemán, C.; García-Alvarez, M.; de Illarduya, A. M.; Muñoz-Guerra, S. M. *Macromol. Chem. Phys.* **1995**, *196*, 253.
(15) Navas, J. J.; Alemán, C.; López-Carrasquero, F.; Muñoz-Guerra, S. M. *Macromolecules* **1995**, *28*, 4487.
(16) Navas, J. J.; Alemán, C.; Muñoz-Guerra, S. M. *Polymer* **1996**, *37*, 2589.
(17) Hardy, P. M.; Haylock, J. C.; Rydon, H. N. *J. Chem. Soc. Perkin Trans. 1* **1972**, 605.
(18) Saudek, V.; Štokrová, Š.; Schmidt, P. *Biopolymers* **1982**, *21*, 2195.
(19) Manning, M. C.; Fernández-Santín, J. M.; Puiggali, J.; Subirana, J. A.; Woody, R. W. *Biophys. J.* **1989**, *55*, 530a.
(20) Bode, K. A.; Applequist, J. *J. Phys. Chem.* **1996**, *100*, 17825.
(21) Applequist, J.; Carl, J. R.; Fung, K.-K. *J. Am. Chem. Soc.* **1972**, *94*, 2952.
(22) Applequist, J. *Acc. Chem. Res.* **1977**, *10*, 79.
(23) Applequist, J. *J. Chem. Phys.* **1979**, *71*, 4324.
(24) Applequist, J. *J. Chem. Phys.* **1979**, *71*, 4332. Erratum, *J. Chem. Phys.* **1980**, *73*, 3521.
(25) Applequist, J. *Biopolymers* **1981**, *20*, 387.
(26) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960; p 499.
(27) Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vetterling, W. T. *Numerical Recipes (Fortran Version)*; Cambridge University Press: Cambridge, U.K., 1989.

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