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Circular dichroism studies of distorted α-helices, twisted β-sheets, and β-turns

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Theoretical models for calculating the circular dichroism (CD) of biopolymers have been constructed which allow the evaluation of the effects of geometric distortions within regular secondary structures. Outward tilting of the carbonyl group within α-helical structures yields calculated CD spectra with diminished intensity and a red-shifted maximum near 190 nm. The α_{II}-helix provides an extreme example of this type of α-helix distortion. It is predicted that a mixture of α and α_{II} structures in bacteriorhodopsin can account for its anomalous CD spectrum. The minimum length of α-helix required to produce an α-helix-like CD spectrum is calculated to be two to three turns (seven to eleven residues), while helices greater than 30 residues should provide adequate models of an infinite helix. Twisting of β-sheets is predicted to lead to an increase in CD intensity and significant shifts in band position. Calculated CD spectra for β-turn models are accurate for types II and II′, but appear to be inadequate for type I turns.

1. Introduction

Circular dichroism (CD) spectroscopy is one of the most widely used techniques for studying peptide conformation in solution [1]. While it does not provide as detailed information as NMR, particularly the recently developed two-dimensional NMR techniques, it remains an extremely useful method because it is sensitive to different aspects of peptide conformation and can be measured at much lower concentrations. Thus, sample requirements are reduced and problems of aggregation and limited solubility are avoided. Much of the development of the CD technique has depended on empirical correlations. However, the theory of CD has been successful in interpreting the experimental results in a qualitative and frequently semiquantitative fashion. In a few cases, e.g., with homopolymers of aromatic amino acids, theoretical calculations have been indispensable in making sound conformational assignments from CD measurements [2,3].

The following is a discussion of some recent theoretical work which we have performed to shed light upon two questions which are difficult to answer experimentally because of a dearth of model systems and/or uncertainty in the exact conformation of available model systems. One question has to do with the effects of distortions and end effects on the CD of α-helices. The other concerns how the well-known deviations from Pauling and Corey's β-sheet [4] affect the CD of these structural elements. We also briefly review the status of the CD of β-turns, although this is not an area in which we have recently worked.

First, we describe very briefly the theoretical approach we have taken in our calculations. The methods we use were developed by Tinoco [5],

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Schellman and co-workers [6] and Woody [7], but are based upon the earlier work of Kirkwood [8] and Moffitt [9]. The molecule is divided into a set of chromophoric groups, each of which has one or more distinct and well-characterized electronic transitions. For example, the amide group has an $n\pi^*$ transition at approx. 220 nm and a $\pi\pi^*$ transition at approx. 190 nm. From spectroscopic studies on small molecules and from MO theory, we can obtain information about the electric and magnetic dipole transition moments of these transitions in the isolated groups. We need to determine the elements of a matrix [6] in which the energies of the excited states in the isolated groups are the diagonal elements, the coupling between different groups being the off-diagonal elements. This matrix, upon diagonalization, yields the excited-state energies for the composite molecule as eigenvalues. The coefficients which permit us to calculate the rotational strength and dipole strengths and thence the CD and absorption spectra are obtained as eigenvectors.

This theoretical approach has been successful in broad outline, but has not attained total reliability or qualitative accuracy [1,10,11]. While explaining the overall appearance of α-helix and β-sheet CD spectra in the 180–250 nm region, there have been problems. Incorrect spectra have been predicted for type I β-turns [12] and the poly(Pro)II helix [10]. Quantitatively correct CD spectra for many other structures are obtained, including β-sheets, type II β-turns, poly(Pro)I helices, and the α-helix. However, even for the thoroughly studied α-helix, two problems still occur. A strong, negative 'helix' band near 180 nm is predicted [10,13] but is not observed experimentally [14,15], and the $n\pi^*$ rotational strength is generally underestimated [13].

An alternative model has been developed by Applequist [16,17], who has applied it to all of the structures mentioned above, focussing on the $\pi\pi^*$ transition. In principle, the method takes into account all higher energy transitions which are electrically allowed, since its central feature is the coupling of the $\pi\pi^*$ transition with atomic polarizabilities centered at each atom, but it neglects the $n\pi^*$ and other electrically forbidden transitions. This method gives agreement comparable to the previously discussed calculations for the $\pi\pi^*$ region of the α-helix [18] and also avoids the helix band. It gives reasonable predictions for both poly(Pro) [19] helices and for type I turns as well as type II [20]. However, it predicts some features for β-sheets [21] which are contrary to experiment, especially a strong negative band near 200 nm in the antiparallel case. Moreover, the results are very sensitive to side-chain identity and conformation, predictions for which there is little experimental support. This acute sensitivity to side-chain features probably reflects some inherent weaknesses in Applequist's model, which involves a dipole-dipole interaction between the spherical polarizabilities at each center. A point dipole-dipole model is used, even for neighboring atoms at bonding distances. The validity of this aspect of the model has been questioned by Thole [22].

2. Distorted α-helices

Many previous studies employing the matrix method [6,7] suffered from a number of limitations. First, the parameters were derived from various empirical and semi-empirical sources and were not internally consistent. Second, the localization of the $n\pi^*$ transition to the carbonyl oxygen is not an accurate description of the excited state. Third, only the $n\pi^*$ and $\pi\pi^*$ transitions were included. In reality, other transitions should be considered, such as discrete higher energy amide transitions, very high energy $\sigma\sigma^*$ type transitions, and charge-transfer transitions. All of these factors are currently being evaluated by our group.

To address these deficiencies, a model has been developed based upon CNDO/S calculations [23–25] on the amide chromophore. The details will be presented elsewhere, but some points are worth noting. It is internally consistent and presents a more accurate description of the $n\pi^*$ transition.

The effects of including other discrete amide transitions, such as the $n'\pi^*$ and $\pi_\perp\pi^*$ excited states, has been studied, but our discussion here will be restricted to a model containing only the

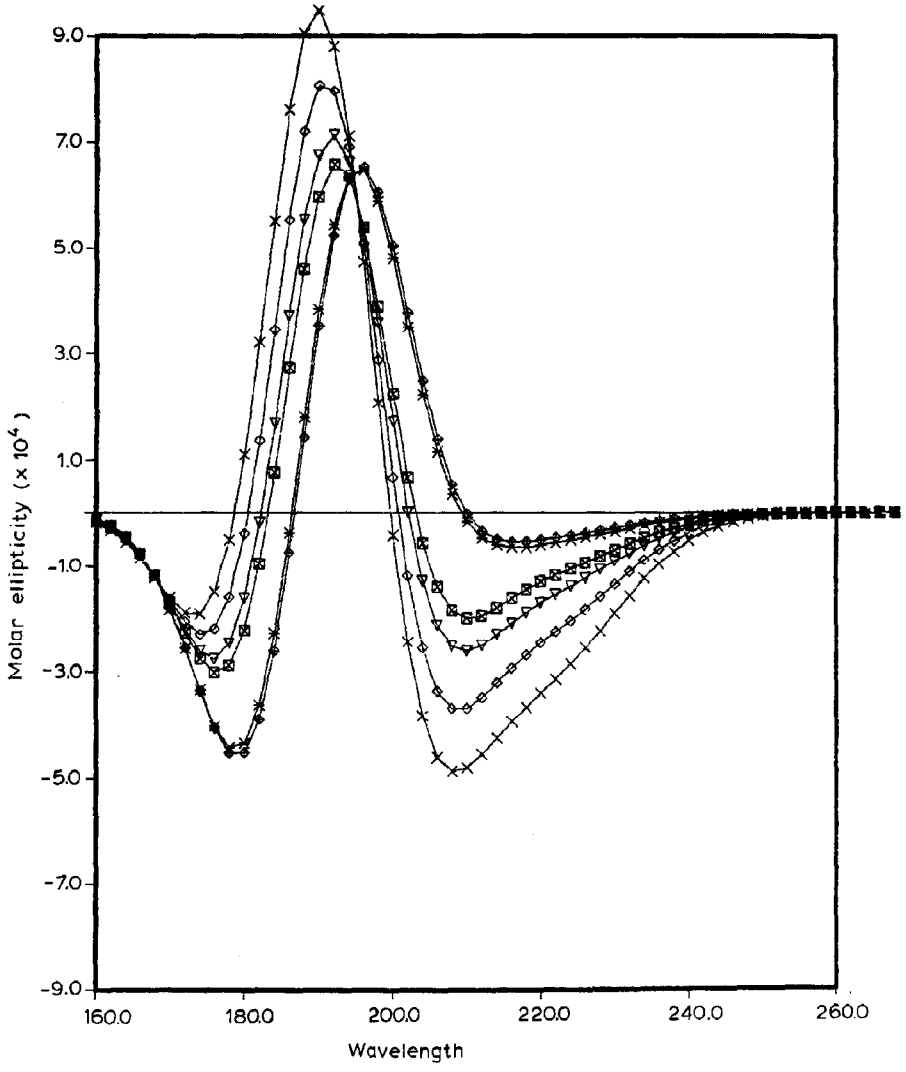


Fig. 1. Calculated CD spectra for 50-residue α-helices with $\phi = -48°$, $\psi = -57°$ (×); $\phi = -53°$, $\psi = -52°$ (◇); $\phi = -57°$, $\psi = -47°$ (▽); $\phi = -59°$, $\psi = -44°$ (⊠); $\phi = -66°$, $\psi = -41°$ (★); and $\phi = -67°$, $\psi = -44°$ (⊕).

$n\pi^*$ and $\pi\pi^*$ transitions. Once this model was established, the effects of systematic distortion of the α-helix were examined. Systematic distortion is defined as a perturbation that affects each residue in the same fashion. Nonsystematic distortion would include the insertion of 'helix-breaking' residues, such as proline, into the helix, or the dynamic fluctuation of the α-helical conformation. Although the α-helical region of Ramachandran space is quite restricted for L-α-amino acids [26], some variance is tolerated. In general, the sum of the ϕ and ψ angles is reasonably constant. As ϕ becomes more negative, ψ becomes less negative and the carbonyl group tilts outward, making it more accessible to solvent. Such helices are termed hydrophilic [27]. These helices sacrifice internal hydrogen-bonding efficiency, but gain increased opportunity to hydrogen bond to the solvent or side chains.

Calculations were performed on six distinct,

50-residue α-helices, ranging from hydrophobic (such as the structure of Pauling and Corey [28]) to hydrophilic structures (see fig. 1). Two trends are evident. First, the strong positive band near 190 nm red-shifts and decreases in intensity with increased hydrophilicity. Second, the rotational strength of the $n\pi^*$ transition near 220 nm also decreases.

An extreme example of a hydrophilic helix has been proposed and termed the α_{II}-helix [29]. This structure, while possessing quite different ϕ and ψ angles, retains the rise and twist of the standard α-helix (3.6 residues per turn, 1.5 Å rise per residue). Krimm and Dwivedi [30,31] have suggested that the α_{II} structure occurs in bacteriorhodopsin, which has an anomalously high amide I stretching frequency for a protein with a high helix content. Outward tilting of the carbonyl would decrease internal hydrogen bonding, strengthen the carbonyl bond, and raise its stretching frequency, pro-

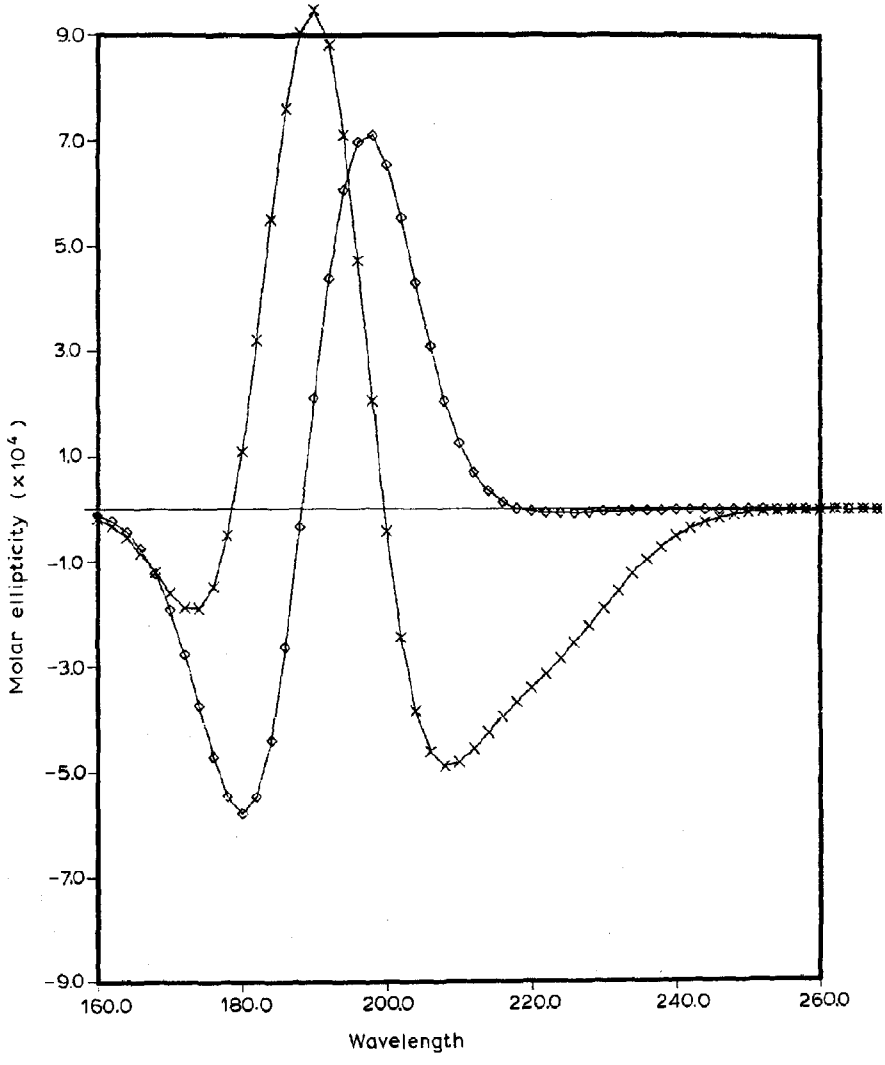


Fig. 2. Calculated CD spectra for a 50-residue α-helix ($\phi = -48°$, $\psi = -57°$) (×) and its α_{II} counterpart ($\phi = -71°$, $\psi = -36°$) (◇).

vided that any external hydrogen bonds formed are weak. The driving force for the $\alpha \rightarrow \alpha_{II}$ conformational change is thought to be the frequent occurrence of serine and threonine residues in the membrane-spanning regions [31]. Formation of hydrogen bonds to the side chains would be facilitated by the α_{II} structure, and allow accommodation of these somewhat polar residues within the hydrophobic portion of the lipid bilayer. Recent Raman and infrared studies support the existence of a significant amount of a distorted α-helical structure in bacteriorhodopsin [32,33].

Bacteriorhodopsin displays an anomalous CD spectrum [34–36]. The intensity corresponds to approx. 50% helix, as compared with the 80% inferred from electron diffraction [37]. To determine whether the presence of α_{II}-helices could account for the anomalous CD observed for bacteriorhodopsin, calculations were conducted on two α_{II}-helical structures. The first ($\phi = -71°$,

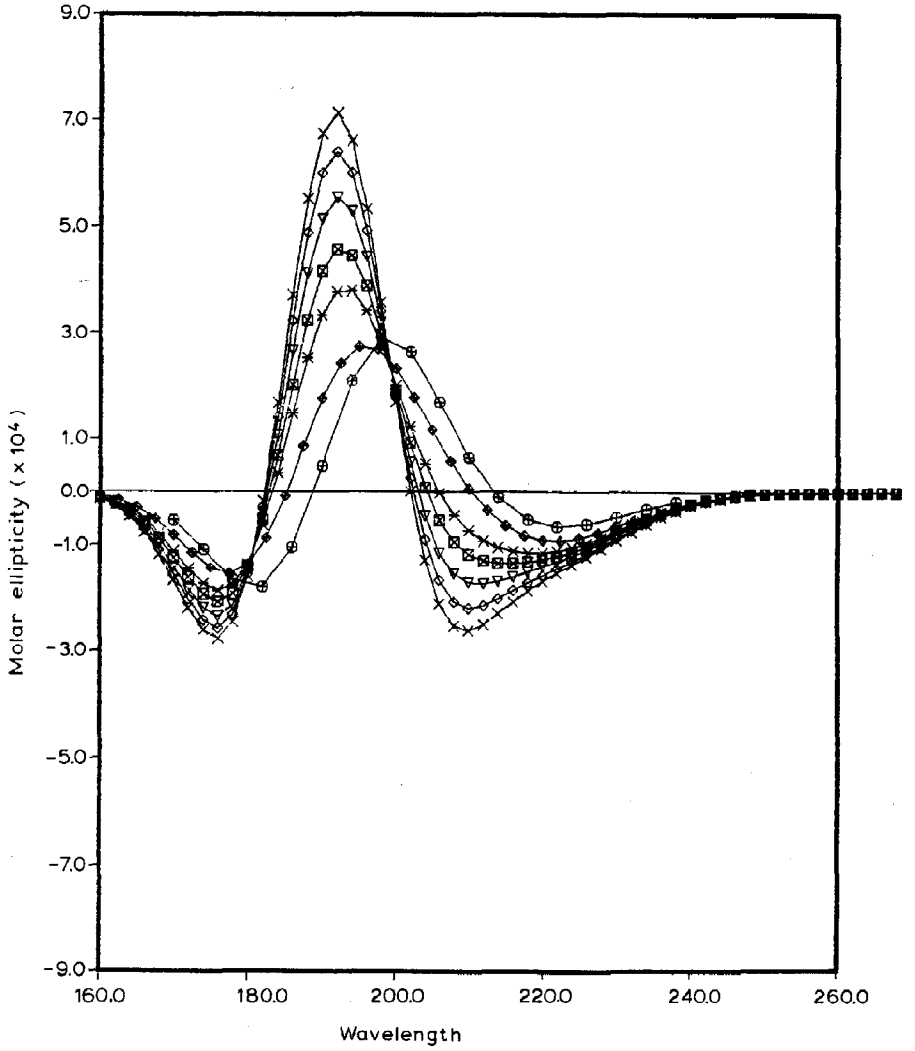


Fig. 3. Calculated CD spectra for α helices ($\phi = -57°$, $\psi = -47°$) of 50 (×), 30 (◇), 20 (▽), 14 (⊠), 11 (★), 7 (⊕), and 4 (⊕) residues in length.

$\psi = -36°$) is the α_{II} variation of the Pauling and Corey helix ($\phi = -48°$, $\psi = -57°$). The second ($\phi = -93°$, $\psi = -18°$) is the variation of the standard α-helix ($\phi = -53°$, $\psi = -52°$). It is clear that the $\phi = -93°$, $\psi = -18°$ helix is so distorted and gives rise to such an unusual CD spectrum that its occurrence in bacteriorhodopsin would be easily detected. In contrast, the $\phi = -71°$, $\psi = -36°$ α_{II}-helix yields a CD spectrum resembling that of the hydrophilic helices, with a small negative rotational strength for the $n\pi^*$ transition and only about half of the intensity seen for the positive 190 nm band of the α-helix (fig. 2). A combination of the α- and α_{II}-helix CD spectra could easily result in the spectrum observed experimentally. Therefore, our calculations are consistent with the presence of significant amounts of both the α_{II}- and α-helix structures.

Globular proteins can accommodate α-helical segments ranging from one turn (3–4 residues) to many turns (upward of 40 residues) in length. It is important to determine the minimum length of an α-helix required to produce a CD spectrum indicative of that structure. Also, the number of residues required to approximate an infinite helix is of interest. Calculations were performed on α-helical structures ranging from four to 50 residues in length (fig. 3). While the $n\pi^*$ rotational strength does not decrease precipitously until the helix is less than ten residues, the appearance of distinct parallel and perpendicularly polarized CD bands requires 11–15 residues (3–4 turns). The rotational strength of both the 190 and 220 nm bands (on a per residue basis) approaches a limiting value beyond 30 residues. An α-helix of this length can then be thought of as an adequate model of an infinite helix. These findings are similar to those predicted previously [10,13], but contrast with the results of Applequist, who found little variation in helices beyond three to four residues in length [18].

3. Twisted β-sheets

Two types of models for β-sheets have been used in CD studies: polypeptides of high molecular weight and oligomers of defined length. Early studies utilized homopolypeptides such as poly(Lys) heated at high pH [38,39], or esters and ethers of poly(Ser) and poly(Cys) [40]. More recently, alternating copolymers such as poly(Leu-Lys), which are readily soluble in water, in contrast to heated poly(Lys), have proven to be useful models [41,42]. Fig. 4 shows the CD spectra of three β-forming polymer models in aqueous solution. The amplitudes of the two observable CD bands vary by more than a factor of two, but the wavelengths of the negative $n\pi^*$ band (~216 nm) and the positive band (195–200 nm) show only small variations.

Toniolo, Stevens and co-workers have studied the CD spectra of blocked oligomers of the type Boc-$(X)_n$-OMe where X = Ala [43,44], Val [44,45], Leu [46], Ile [45,47], Met [48], with $n = 1$–7. In trifluoroethanol or trifluoroethanol/water mixtures and in films, the CD spectra of these systems indicate unordered conformations for $n < 6$ and

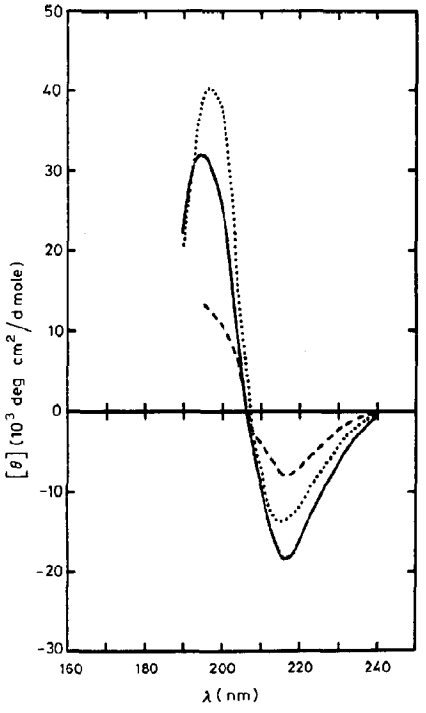


Fig. 4. The circular dichroism of polypeptides in the β-sheet conformation. Poly(Lys), pH 11.1 after heating at 52° C for 15 min, followed by cooling to 22° C (———) [72]; poly(Lys) in 1% SDS (------) [73]; poly(Leu-Lys) in 0.1 M NaF, pH 7 [41].

Table 1

CD parameters for Boc-(X)$_n$-OMe (in trifluoroethanol solution)

X	λ_{max} (nm)	$[\theta]_{max}$ (degree cm^2 dmol^{-1})
Ala	197	64000
	217	−10000
Val	202	108000
	222	−13000

β-conformations for $n = 7$ and, in some cases, for $n = 6$. The data in table 1 are taken from two representative examples, the blocked heptamers of Ala [43] and Val [45] in trifluoroethanol. For the Ala heptamer, the band positions agree well with those shown in fig. 4, although the amplitude of the positive band is roughly 50% larger than that of poly(Leu-Lys) shown in fig. 4. The CD spectrum of the Val heptamer is substantially different, with the maxima of both bands shifted by approx. 5 nm to the red and about twice the amplitude of the Ala heptamer.

In general, the CD spectra of model β-sheets show much greater variability than those of model α-helices. There are several possible sources of such variability. Oligopeptides and polypeptides can form antiparallel, parallel, or mixed sheets. The number of strands in the sheet and the length of the strands can vary. The degree of twist [49] of the β-sheet is a potentially important variable.

Although the model systems have generally been characterized as either antiparallel or parallel, and the strand length of the oligomer models is well-defined, the remaining parameters are unknown for any of the model systems. Conformational energy calculations carried out by Scheraga and co-workers [50–52] indicate that, for homopolymers, increasing bulk of the side chains leads to increasing degrees of twisting in the sheets, and to a preference for parallel over antiparallel sheets. The latter conclusion agrees with the experimental results of Toniolo and co-workers who, on the basis of X-ray diffraction [53], infrared [54,55] and CD [44] evidence, have concluded that, in the case of blocked heptamers, alanine residues form antiparallel sheets whereas valine and isoleucine residues form parallel sheets.

We have explored the relationship between CD and the degree of twisting of β-sheets by calculating the CD of twisted sheets [50–52]. These calculations, unlike those described above, were performed using the parameters previously applied to the α-helix [7], β-sheet [56], and β-turns [57], except that the center of the $\pi\pi^*$ transition was located at the carbonyl carbon [58].

Fig. 5 shows the results for antiparallel and parallel sheets of alanine and valine, representing slightly twisted and strongly twisted sheets, respectively. For the alanine sheets, the CD amplitudes are predicted to be relatively low, with the

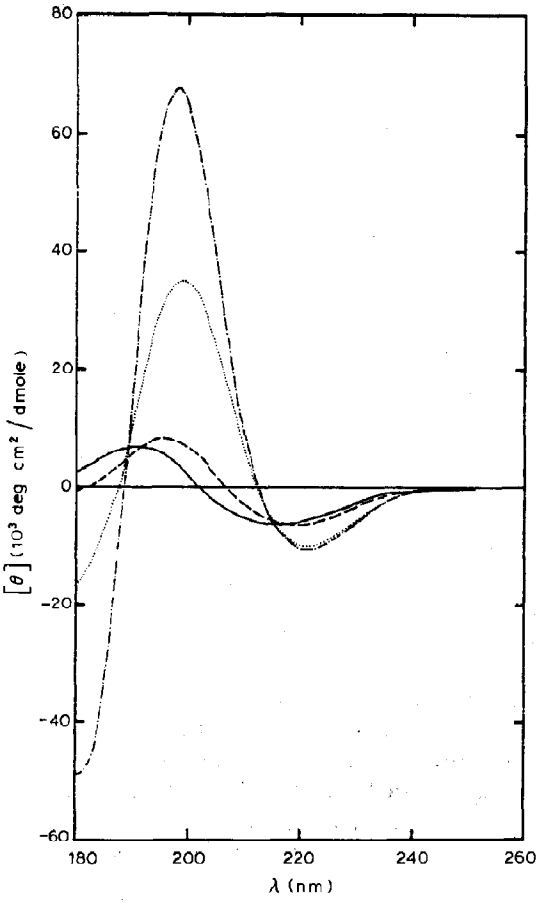


Fig. 5. Calculated CD spectra of four twisted β-sheets. Antiparallel 3×8 poly(Ala) (— — —); parallel 3×8 poly(Ala) (———); antiparallel 3×6 poly(Val) (· · · · · ·); parallel 3×6 poly(Val) (· – · – ·). Geometries from Scheraga and co-workers [50–52]. The sizes of the sheets are specified by $n \times m$, where n is the number of strands and m denotes the number of residues per strand.

positive and negative bands comparable in intensity. The spectra are predicted to be similar for those of flat β-sheets (data not shown). There is little difference in the CD predicted for antiparallel and parallel sheets, although the wavelengths of extrema and crossovers are shifted slightly to the red for the antiparallel sheet. Although the amplitudes are somewhat low, the predicted CD spectrum for the antiparallel alanine sheet resembles that observed experimentally for poly(Lys) (fig. 4). Poly(Lys) generally forms antiparallel sheets [38], and the absence of branching at the β-carbon is consistent with sheets of low twist.

The strongly twisted sheets of valine are predicted to give CD spectra which differ markedly from those of flat or slightly twisted sheets. The $n\pi^*$ rotational strength increases significantly, but the most striking feature is the strong positive couplet which develops in the $\pi\pi^*$ region. Thus, the positive maximum near 195 nm in the spectrum of the flat or slightly twisted sheet red-shifts to wavelengths above 200 nm and increases in intensity to approx. 30 000 degree cm^2 $dmol^{-1}$ in the antiparallel sheets and 60 000–70 000 degree cm^2 $dmol^{-1}$ in the parallel sheets.

The results for the highly twisted sheets agree qualitatively with the experimental spectra [45] for the blocked heptamer of valine. The red shift of the maxima relative to the spectra for the alanine heptamer [43] and the large amplitude are reproduced, although the amplitude of the positive band is underestimated. The spectra reported for poly(Leu-Lys) [41] and the blocked heptamer of alanine have wavelengths of extrema which are characteristic of slightly twisted sheets, while the intensity of the positive $\pi\pi^*$ band and the ratio of this intensity to that of the negative $n\pi^*$ band agree better with the predicted results for the strongly twisted antiparallel sheets. These systems may be twisted to an extent which is intermediate between the nearly flat and the strongly twisted sheets.

4. β-Turns

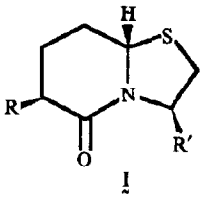
In the first theoretical study of β-turn CD, Woody [57] predicted that although β-turns could exhibit a wide range of CD patterns, the most prevalent pattern and that which was predicted for the canonical type I and type II turns [59], should resemble those of β-sheets such as poly(Lys), but have red-shifted maxima. This type of spectrum, termed class B, actually resembles those predicted in this work for strongly twisted sheets. Woody [57] also predicted that a type II′ turn, a mirror image of the type II turn, should have a CD spectrum which qualitatively resembles that of an α-helix (Class C), with a negative $n\pi^*$ band and a negative $\pi\pi^*$ couplet, although a double minimum may not be detected.

The best characterized models for β-turns are cyclic peptides for which NMR and, in some cases, X-ray diffraction data are available. For example, Bush et al. [60] have studied cyclic peptides of the type (X-L-Pro-Y)$_2$ where X is an L-amino acid or Gly and Y is a D-amino acid or Gly. For several such cyclic hexapeptides, type II β-turns at the L-Pro-Y sites have been established in the solid state [61] and in solution [62]. Bush and co-workers showed that these cyclic hexapeptides all have class B CD spectra.

Bush et al. [60] also studied cyclic hexapeptides of the type cyclo(X-Y-L-Pro)$_2$, where X is an L-amino acid and Y is a D-amino acid or Gly. Kopple et al. [63] have presented evidence from NMR for type II′ turns at Y-L-Pro in such peptides. Gramicidin S, a cyclic decapeptide, has type II′ turns at two D-Phe-L-Pro sequences, as demonstrated by NMR [64–66] and X-ray diffraction [67]. The CD spectra of the cyclo(X-Y-L-Pro)$_2$ series [60] and of gramicidin S [68] resemble those of the α-helix.

Bandekar et al. [12] have synthesized the tripeptide cyclo(L-Ala-D-Ala-Aca), where Aca denotes ε-aminocaproic acid. Conformational energy calculations and NMR provide strong evidence that this molecule is locked in a type II β-turn. The CD spectrum falls in Woody's [57] class B, as generally expected for a type II turn. However, calculations for the specific ϕ, ψ angles provided by conformational analysis predict an α-helix-like spectrum for this molecule.

Nagai et al. [69,70] have synthesized interesting models for type II′ β-turns using the bicyclic system which they call BTD (bicyclic β-turn di-



R = N-Pht
R′ = CO_2H

I

Scheme 1.

peptide) (I). They have made a cyclic tripeptide by bridging

the NH and CO termini of BTD with ε-aminocaproic acid. This model system gives an α-helix-like CD spectrum. Nagai and co-workers have used BTD units to replace the two D-Phe-L-Pro sequences of gramicidin S, yielding an analog with biological activity and CD spectrum nearly identical to that of the parent molecule.

Thus, the studies just cited provide strong support for Woody's [57] original predictions for type II and II′ β-turns. The predictions for type I turns have not fared so well, however. Two well-authenticated type I turns in cyclic peptides have been studied. Cyclic peptides of the type cyclo(Gly-L-Pro-Y)$_2$, where Y is an L-amino acid other than Val, have type I turns at the L-Pro-Y sites [71]. Similarly, there is strong evidence for a type I turn in (L-Ala-L-Ala-Aca) [12]. Both of these types of peptides have α-helix-like CD spectra, in contrast to the class B spectra predicted by Woody [57].

Further studies of β-turn models are necessary to resolve the discrepancy between theory and experiment for type I β-turns. The parameters we have developed and applied to helical systems should be used for calculations on β-turns. In addition the success of the Applequist model in dealing with β-turns [20] suggests that it may be necessary to consider higher energy transitions, approximated by polarizable groups.

Acknowledgements

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