
FOR THE RECORD

Structural composition of β_I - and β_{II} -proteins

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Abstract

Circular dichroism spectra of proteins are sensitive to protein secondary structure. The CD spectra of α -rich proteins are similar to those of model α -helices, but β -rich proteins exhibit CD spectra that are reminiscent of CD spectra of either model β -sheets or unordered polypeptides. The existence of these two types of CD spectra for β -rich proteins form the basis for their classification as β_I - and β_{II} -proteins. Although the conformation of β -sheets is largely responsible for the CD spectra of β_I -proteins, the source of β_{II} -protein CD, which resembles that of unordered polypeptides, is not completely understood. The CD spectra of unordered polypeptides are similar to that of the poly(Pro)II helix, and the poly(Pro)II-type (P_2) structure forms a significant fraction of the unordered conformation in globular proteins. We have compared the β -sheet and P_2 structure contents in β -rich proteins to understand the origin of β_{II} -protein CD. We find that β_{II} -proteins have a ratio of P_2 to β -sheet content greater than 0.4, whereas for β_I -proteins this ratio is less than 0.4. The β -sheet content in β_I -proteins is generally higher than that in β_{II} -proteins. The origin of two classes of CD spectra for β -rich proteins appears to lie in their relative β -sheet and P_2 structure contents.

Keywords: Protein secondary structure; β -rich proteins; protein CD; P_2 structure

Polypeptide conformations that determine protein secondary structures give rise to characteristic circular dichroism (CD) spectra, resulting in the remarkable sensitivity of protein CD spectrum to its secondary structure content (Yang et al. 1986; Johnson 1988; Sreerama and Woody 2000a). Proteins are classified into different tertiary structure classes (Levitt and Chothia 1976) based on the secondary structure topology. Proteins that have predominantly α -helical structures are grouped under α -rich proteins (also called $\alpha\alpha$ and all- α), those with predominantly β -sheets under β -rich proteins (also called $\beta\beta$ and all- β), and those with separate or intermixed α -helical and β -sheet regions are called $\alpha + \beta$ and α/β proteins, respectively. It is rather difficult to distinguish the $\alpha + \beta$ and α/β proteins based on their CD spectra, and they are combined to form the $\alpha\beta$ class for the purpose of CD analysis (Sreerama et al. 2001).

The CD spectra of α -rich proteins have the characteristics of CD spectra of model polypeptides in α -helical conformation. The CD spectra of $\alpha\beta$ proteins also have features of α -helix CD, which dominates protein CD, but with reduced amplitudes. β -Rich proteins exhibit a variety of CD spectra, and they form two distinct sets (Manavalan and Johnson 1983). Wu et al. (1992) classified the β -rich proteins into β_I - and β_{II} -proteins based on the two types of CD spectra: β_I -proteins have CD spectra that resemble those of model β -sheets, and the CD spectra of β_{II} -proteins resemble those of unfolded proteins. Although the dominating effect of β -structure explains the β_I -protein CD, distorted and/or short β -strands have been suggested (Manavalan and Johnson 1983) to be responsible for β_{II} -protein CD.

Experimental and theoretical considerations are not compatible with attributing the CD spectra of β_{II} -proteins to highly twisted β -sheets. Toniolo et al. have measured the CD spectra of β -sheets formed by the association of heptameric homo-oligopeptides with side chains ranging from Ala (Toniolo and Bonora 1975), Val and Ile (Toniolo et al. 1974). These are expected to vary in the degree of twisting, with small linear and γ -branched peptides having little

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twist, and β -branched peptides forming strongly twisted sheets (Chou and Scheraga 1982; Chou et al. 1982). In all cases, the spectra showed a strong positive band in the 190–220 nm region, with the sheets expected to be more strongly twisted having stronger bands. Theoretical calculations (Manning et al. 1988) are consistent with these observations. Earlier theoretical calculations (Woody 1969) on planar β -sheets as small as two strands of two residues predicted a CD pattern much like that of extensive β -sheets. This argues against attributing the CD pattern of β_{II} -proteins to short-stranded β -sheets.

Unordered polypeptide CD spectra are similar to the CD of poly(Pro)II (Woody 1992; Shi et al. 2002). The poly(Pro)II helix is a left-handed helix with three residues per turn with repeating backbone dihedral angles (ϕ , ψ) \approx (-70° , $+150^\circ$). The similarity of CD spectra led Tiffany and Krimm (1968) to suggest that short stretches of poly(Pro)II-like (P_2) conformation form a significant fraction of unordered polypeptides. Analyses of crystal structures have shown that the P_2 conformation can constitute an appreciable fraction of secondary structure in globular proteins (Adzhubei and Sternberg 1993; Sreerama and Woody 1994; Stapley and Creamer 1999).

We have analyzed the crystal structures of β_I - and β_{II} -proteins to find a structural basis for the two different classes of CD spectra of β -rich proteins. The two secondary structures that give rise to the basic features of the CD spectra of β_I - and β_{II} -proteins, which are β -sheet and P_2 structure, respectively, are compared to explain the origin of β -protein CD. We find that the relative compositions of β - and P_2 -structures in β -rich proteins determine the type of β -protein CD spectrum.

Results and discussion

The α -rich proteins have a large α -helical secondary structure fraction that gives rise to CD spectra that are reminiscent of the CD spectra of model α -helices. Proteins that have a large β -sheet fraction, in contrast to α -rich proteins, give rise to two types of CD spectra that are classified as β_I - and β_{II} -CD (Wu et al. 1992). The CD spectra of 16 β -rich proteins are shown in Figure 1. Characteristic CD spectra of β_{II} -proteins, shown in Figure 1A, have a negative band around 200 nm. Some of the β_{II} -protein spectra have a small positive band around 190 nm, and some have a positive band or a negative shoulder around 220 nm. On the other hand, CD spectra of β_I -proteins (Fig. 1B) have a significantly stronger positive band around 190 nm and a comparable negative band in the 210–220 nm region. CD bands above 225 nm are observed in both β_I - and β_{II} -proteins, presumably due to aromatic and disulfide groups.

The CD spectra of individual secondary structures, α -helix, β -sheet, and P_2 -conformation, were deconvoluted from a reference protein set of 37 globular proteins used in the

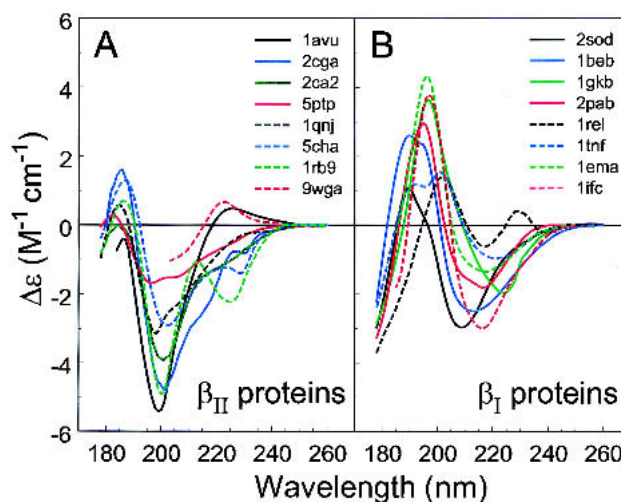


Figure 1. CD spectra of β_I - (B) and β_{II} - (A) proteins. The proteins are identified by the PDB code for the structure used in this study and the corresponding names of proteins are given in Materials and Methods. The sources of the CD spectra are also listed in Materials and Methods.

CDPro secondary structure analysis programs (Sreerama and Woody 2000b). These are shown in Figure 2A. The CD spectra of an α -helical polypeptide (poly[Glu]; Toumadje et al. 1992), a β -sheet polypeptide (poly[Leu-Lys] in 0.1 M NaF, pH 7; Brahms et al. 1977), and of poly(Pro)II in trifluoroethanol at room temperature (Jenness et al. 1976) are shown in Figure 2B. The CD spectrum of α -helical structure in globular proteins has the characteristic positive band around 192 nm and two negative bands around 208 and 222

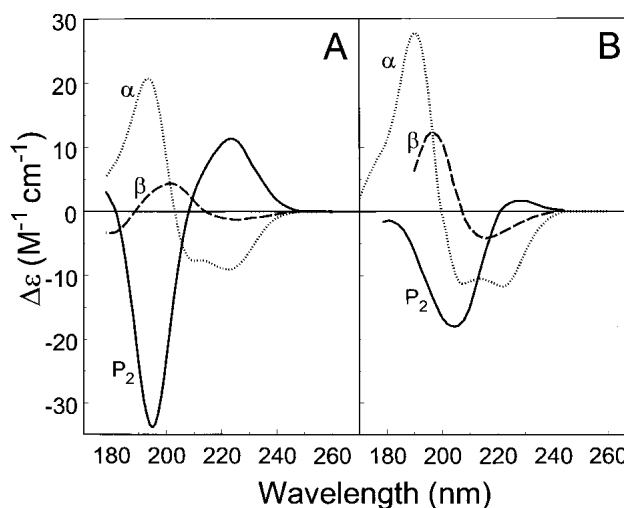


Figure 2. (A) CD spectra of α -helix, β -sheet, and P_2 structure deconvoluted from a reference-protein set of 37 proteins. The method for deconvolution of CD spectra has been described in Sreerama and Woody (1994). (B) CD spectra of model polypeptides in α -helical (Toumadje et al. 1992), β -sheet (Brahms et al. 1977), and poly(Pro)II helix (Jenness et al. 1976) conformations.

nm, similar to that of model α -helical polypeptides. The CD spectrum of β -sheet structure extracted from globular protein CD spectra has the typical positive and negative bands around 195 and 218 nm, respectively, of the CD spectra of model β -sheets. The CD spectrum of the P_2 structure is similar to that of poly(Pro)II. The position of the negative band in the model poly(Pro)II helix, which has tertiary amides, is red-shifted in comparison with that of globular proteins, in which secondary amides predominate. The amplitude of the negative CD band in P_2 structure is larger than that of the positive band in β -sheets in both model systems and globular proteins.

Comparison of CD spectra of β -rich proteins (Fig. 1) with model polypeptide CD spectra (Fig. 2B) indicates that β_I -proteins have CD features that are seen in the spectra of model β -sheets, and β_{II} -proteins have CD features seen in the model P_2 helix. It is clear that the presence of β -sheets in β_I -proteins is largely responsible for the CD spectra of β_I -proteins. However, β_{II} -proteins exhibit P_2 -like CD despite the presence of a significant β -sheet content. The P_2 conformation can form a significant fraction of secondary structure in globular proteins (Adzhubei and Sternberg 1993; Sreerama and Woody 1994; Stapley and Creamer 1999). Although they lack the inter- or intra-strand hydrogen bonds that define α -helices and β -sheets, they are generally identified by the regular geometric features of the backbone structure. The crystal structures of the β -rich proteins considered in this work were analyzed and the number of residues in α -helix, β -sheet, and P_2 structures were determined (see Materials and Methods). We have considered both the single residues in P_2 -conformation and clusters of

two or more P_2 -residues in determining the P_2 structure. Even in an isolated P_2 -residue the orientation of two successive peptide groups are such that the interpeptide interactions expected in a P_2 helix are possible.

The number of residues in α -helix, β -sheet, and P_2 structure for β_I - and β_{II} -protein crystal structures are given in Table 1. The first eight proteins are β_{II} -proteins and the last eight are β_I -proteins. Two numbers are given for the number of residues in α -helices; the number in parenthesis corresponds to the number of residues of 3_{10} helix. The β_{II} -proteins have a larger fraction of residues in P_2 structure (f_{P_2} , which can be obtained by dividing the number of residues in P_2 structure by the total number of residues, is greater than 15%) than the β_I -proteins (f_{P_2} less than 13%), with the exception of Bence-Jones protein ($f_{P_2} = 18\%$). The β -sheet content in the β_I -proteins are generally larger ($f_{\beta} > 40\%$) than that in β_{II} -proteins ($f_{\beta} < 40\%$).

The ratio of the number of residues in P_2 - and β -structures (f_{P_2}/f_{β}) is given in the last column of Table 1. Although β_{II} -proteins have the ratio $f_{P_2}/f_{\beta} > 0.4$, for β_I -proteins, this ratio is < 0.3 , except for Bence-Jones protein ($f_{P_2}/f_{\beta} = 0.38$), which has a large β -sheet content ($\sim 50\%$). Two proteins, rubredoxin and wheat germ agglutinin, have a very high f_{P_2}/f_{β} ratio (> 1.0) but they also have a smaller β -sheet content than the rest and a comparable α -helix content. They can be classified as $\alpha\beta$ proteins, but we have included them here as β_{II} -proteins because their CD spectra are similar to those of β_{II} -proteins. These two proteins also have P_2 content in excess of 20%.

The band around 190 nm in the CD spectrum of P_2 structure is of opposite sign and has greater amplitude than the

Table 1. Residues in α -helix, β -sheet, and P_2 conformations in β_{II} and β_I proteins

Class	Protein	PDB code	N_{chain}	N_{res}	N_{α}	N_{β}	N_{P_2}	f_{P_2}/f_{β}
β_{II}	Soybean trypsin inhibitor	1avu	1	181	3 (3)	71	29	0.41
	Elastase	1qnj	1	240	27 (13)	90	43	0.48
	Chymotrypsin	5cha	1	245	33 (6)	80	40	0.50
	Trypsin	5ptp	1	223	23 (7)	71	36	0.51
	Carbonic anhydrase	2ca2	1	259	40 (19)	77	39	0.51
	Chymotrypsinogen	2cga	2	490	66 (30)	167	88	0.53
	Rubredoxin	1rb9	1	53	9 (9)	12	13	1.08
	Wheat germ agglutinin	9wga	2	342	72 (40)	60	69	1.15
β_I	Fattyacid binding protein	1lfc	1	132	15 (0)	77	5	0.06
	Prealbumin	2pab	2	252	16 (0)	114	15	0.13
	β -lactoglobulin	1beb	2	324	54 (23)	133	22	0.17
	Tumor necrosis factor	1tnf	3	471	9 (9)	206	40	0.19
	Concanavalin A	1gkb	2	474	18 (18)	219	62	0.28
	Superoxide dismutase	2sod	4	604	11 (7)	230	63	0.27
	Green fluorescent protein	1ema	1	235	16 (8)	105	31	0.30
	Bence-Jones protein	1rei	2	214	6 (6)	105	40	0.38

The PDB code of the protein structure used in the analysis is given. N_{chain} corresponds to the number of polypeptide chains and N_{res} to the total number of residues in the protein structure. N_{α} gives the total number of residues in α - and 3_{10} -helical structures (H and G, respectively, according to DSSP assignments); the number of residues in 3_{10} -helix are given in parenthesis. N_{β} and N_{P_2} give the number of residues in β and P_2 structures, respectively. The ratio of P_2 to β structure is given as f_{P_2}/f_{β} . The first eight proteins have β_{II} -CD spectra and the last eight have β_I -CD spectra (Fig. 1).

corresponding band in β -sheet CD spectrum (Fig. 2). This difference is more pronounced in globular proteins, which may partly be a result of the restrictive nature of the P_2 structure assignment method and partly a result of the deconvolution method. The larger contribution of the P_2 structure, in comparison to β -sheets, to the protein CD spectra is consistent with the observation that β -rich proteins with an f_{P_2}/f_{β} ratio > 0.4 have poly(Pro)II-like CD spectra.

The origin of a protein CD spectrum lies in the secondary structure content of that protein. The origin of two classes of CD spectra for β -rich proteins appears to lie in their relative β -sheet and P_2 structure contents. The unordered-like or poly(Pro)II-like CD of some β -rich proteins has its source in the P_2 structure content of β_{II} -proteins. Although β_I -proteins also have some P_2 structure, they have a relatively higher β -sheet content, which is probably responsible for the resemblance of their CD spectra to those of model β -sheets. β_{II} -proteins, on the other hand, have a smaller β -sheet content and a larger P_2 -content than that in β_I -proteins resulting in their unordered-like or poly(Pro)II-like CD. The relative compositions of β - and P_2 -structures in β -rich proteins apparently give rise to the two classes of β -rich protein CD.

Variations in the β -sheet structure in proteins may also influence the CD spectra of β -rich proteins. Two parameters that define a β -sheet structure—length and twist—are somewhat interdependent: β -sheets with longer strands generally form relatively flat sheets, and β -sheets with short strands have a tendency to form more strongly twisted sheets. The average length of β -sheets (determined as the ratio of the number of residues in β -strands to the number of β -strands) in the β_I - and β_{II} -proteins considered in this work are comparable. β_I -proteins had slightly longer β -sheets (~ 7 residues) than β_{II} -proteins (~ 5 residues). However, this difference seems unlikely to lead to a qualitative difference in CD spectra.

The majority of the methods for the estimation of secondary structures from the analysis of protein CD spectra generally include α -helix, β -sheet and turns. Data sets that include P_2 structure are also available (Sreerama and Woody 1994; Johnson 1999). The performance of the CD spectral analysis, however, is not affected greatly by the introduction of P_2 structure (N. Sreerama and R.W. Woody, unpubl). Generally, the estimates of α -helix and β -sheet remain the same because the P_2 content is determined from the residues not assigned to α -helix and β -sheet. The estimates of turns and unordered structures are altered, however, because they are reassigned after P_2 structure assignment. Despite the two classes of CD spectra for β -rich proteins, the performance of the CD analysis for estimating β -sheet content is quite reasonable ($\sim 9\%$ RMS deviation between x-ray and CD estimates; Sreerama and Woody 2000b). There are two reasons for the success of CD analysis: a reference protein set that includes a diverse set of

protein CD spectra, which includes good representations of both β_I - and β_{II} -proteins; and improvements in the methods for variably selecting proteins for analysis. Variable selection allows for the creation of a protein reference set specific for the analyzed CD spectrum, for example, by always including β_{II} -proteins in the analysis of the CD spectrum of a β_{II} -protein. One could improve the reliability of the analysis of β -rich proteins by combining CD with other conformationally sensitive spectroscopic techniques (e.g., IR, VCD).

In summary, we have examined the origin of two classes of CD spectra for β -rich proteins on the basis of their secondary structure contents. The relatively higher P_2 structure content of β_{II} proteins gives rise to their unordered-like CD. Although β_I -proteins also have some P_2 structure they have a relatively higher β -sheet content, resulting in β -sheet-like CD. β_{II} -proteins, however, have a smaller β -sheet content and a larger P_2 -content than β_I -proteins. The origin of the two classes of β -rich protein CD lies in the relative compositions of β - and P_2 -structures in β -rich proteins.

Materials and methods

The following 16 β -rich proteins were used in this study: soybean trypsin inhibitor (1avu), elastase (1qnj), chymotrypsin (5cha), trypsin (5ptp), carbonic anhydrase (2ca2), chymotrypsinogen (2cga), rubredoxin (1rb9), wheat germ agglutinin (9wga), rat intestinal fatty-acid binding protein (1ifc), prealbumin (2pab), β -lactoglobulin (2beb), tumor necrosis factor (1tnf), concanavalin A (1gkb), superoxide dismutase (2sod), green fluorescent protein (1ema), and Bence-Jones protein (1rei). The PDB (Berman et al. 2000) codes of the crystal structures used are given in parenthesis. Of these proteins, the first eight (1avu–9wga) are β_{II} -proteins and the last eight (1ifc–1rei) are β_I -proteins. The CD spectra of these proteins are shown in Figure 1. Most of these protein CD spectra are from W.C. Johnson, Jr. (pers. comm.). The CD spectra of carbonic anhydrase and chymotrypsinogen are taken from Pancoska et al. (1995), and those of rat intestinal fatty-acid binding protein and green fluorescent protein are from Sreerama et al. (1999). The CD spectrum of soybean trypsin inhibitor is from Wu et al. (1992), and that of wheat germ agglutinin is from Thomas et al. (1977).

The number of residues in α -helix, 3_{10} -helix and β -sheet conformations were determined using the assignments from the DSSP method (Kabsch and Sander 1983), which uses hydrogen bonding patterns to identify these secondary structures. The number of residues in P_2 conformation was determined using the method of Sreerama and Woody (1994), which utilizes the virtual bond angle between three successive C_{α} atoms and the virtual dihedral angle between the two successive peptide-carbonyl groups and assigns secondary structures in conjunction with DSSP assignments in a hierarchical manner. For the residues not assigned to these four structures, DSSP assignments were retained. The residues in β -bridges (structure B in DSSP) were combined with β -sheets, and the α -helix and 3_{10} -helix were grouped together as α -helix (N_{α}). For proteins with more than one polypeptide chain in the structure, all chains were considered for secondary structure assignment.

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Note added in proof

The CD spectrum of clitocyprin, a cysteine proteinase inhibitor from the mushroom *Clitocybe nebularis*, was recently reported (Kidric, M., Fabian, H., Brzin, J., Popovic, T., and Pain, R.H., 2002. Folding, stability, and secondary structure of a new dimeric cysteine proteinase inhibitor. *Biochem. Biophys. Res. Commun.* **297**: 962–967), and it shows that clitocyprin is a β_{II} -protein. IR data also indicated the presence of β -structure in clitocyprin. A relatively higher content of proline residues (~10% in clitocyprin; 4–7% in β -rich proteins considered here) coupled with the β_{II} CD suggests a significant P_2 structure in clitocyprin, the confirmation of which is awaited.

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