Proc. Natl. Acad. Sci. USA
Vol. 74, No. 8, pp. 3208–3212, August 1977
Biochemistry

Identification of β,β-turns and unordered conformations in polypeptide chains by vacuum ultraviolet circular dichroism

(polypeptide conformation/β-pleated sheets/reverse turns/protein structure)

S. BRAHMS*, J. BRAHMS*, G. SPACH†, AND A. BRACK†

* Institut de Recherche en Biologie Moléculaire (C.N.R.S.), Université Paris VII, 2 Place Jussieu, 75005 Paris, France; and † Centre de Biophysique Moléculaire (C.N.R.S.), 45045 Orleans, France

Communicated by H. A. Scheraga, May 16, 1977

ABSTRACT **Different conformations of polypeptides were characterized by measurements of the circular dichroism (CD) extended into the vacuum ultraviolet region. (*i*) The linear β-pleated sheet structure was characterized in a broad ultraviolet region down to 165 nm by examination of copolypeptides composed of alternating hydrophobic and hydrophilic amino-acid residues, e.g., poly(Lys-Leu-Lys-Leu). A short-wavelength intense band was found at about 169 nm, which is characteristic of β-pleated sheet conformation. (*ii*) The β-turns were experimentally measured using poly(Ala_2-Gly_2) in a broad spectral region down to 165 nm with accuracy. The observed CD spectrum is in excellent qualitative agreement with the theoretical curve calculated by Woody for the β-turns of type II and/or I of Venkatachalam. The similarity in shape between the theoretical curve and the observed CD spectra suggests a dominance of β-turn segments in the poly(Ala_2-Gly_2) structure. The presence of β-turns in poly(Ala_2-Gly_2) is also in agreement with the characterization of this polypeptide by solid state methods (electron microscopy and x-ray diffraction). The CD spectrum of β-turns is characterized by a very intense band at 207.5 nm and strong negative bands at 191 and 169 nm. Copolypeptides such as poly(Ala_2-Gly_3) and poly(Ala_3-Gly_3) yielded a similar type of CD spectrum, analysis of which indicates that a large fraction of their residues is contained in β-turn regions. (*iii*) The CD spectrum of the unordered chain of these alternating copolypeptides in salt-free solution is observed in the vacuum ultraviolet region.**

The knowledge of optical parameters, particularly from circular dichroism (CD), of different conformations encountered in polypeptides is of primary importance since it may represent a basis for structural investigation of proteins in solution. In contrast to a relatively good agreement between theoretical and experimental results concerning the α-helical conformation in polypeptides and proteins (1–4), the understanding of the optical properties of other structural forms, such as the β-forms and the unordered conformation, is far from satisfactory.

The β-pleated sheet structure has been investigated in synthetic polypeptides such as poly(L-lysine) under various conditions (5–7), e.g., poly(L-serine) (8), poly(L-threonine), poly(L-valine), and poly(L-isoleucine) (9), and in proteins such as silk fibroin (10). As pointed out by Kubota and Fasman (9), large differences exist in the CD spectra of these compounds, although each was considered to be that of a β-structure. Most probably the causes of these discrepancies reside in the rich variety of β-structures which may consist not only of antiparallel and parallel β-chains, but also of various β-bends (or reverse β-turns), not yet described in the characterization of CD spectra, and of twisted β-pleated sheets found in globular proteins (11). Another difficulty is related to the more or less expressed turbidity of β-pleated sheet structures in solution.

The form of the CD spectrum associated with an unordered polypeptide chain varies greatly whether one considers representative charged homopolypeptides at neutral pH such as poly(L-lysine) (5) and poly(L-glutamic acid) (12), collagen (13), or the optical parameters extracted from some proteins by the procedure of Chen *et al.* (14).

In the present work three improvements were introduced. First, since almost all previous experimental investigations were confined to limited spectral region in the near ultraviolet down to 190 nm, we have extended the investigation down to 165 nm with the help of a specially constructed vacuum CD apparatus. This is particularly important for the characterization of the β-forms of polypeptide chains because they have never been characterized in such a large spectral region in solution. CD spectra in the vacuum ultraviolet of some short oligopeptides of $(alanine)_n$ and $(valine)_n$ (where $n = 2–7$) were reported, containing apparently substantial amounts of β-structure (15). The measurements were made on oligopeptide samples in the solid state (films) and thus the molar ellipticities are not obtained (15). Second, we have used various copolypeptides composed of alternating hydrophobic (L-leucine) and hydrophilic (L-lysine or L-glutamic acid) residues which, in the presence of salts, form a stable water-soluble β-sheet structure of a bilayer type with all hydrophobic residues on the interior side and all hydrophilic residues on the other side (16, ‡). This β-structure is optically clear. In addition, such polypeptides, in the absence of salt, may exist in an unordered conformation, which should be favored if the alternation of the sequence is broken, for instance, by insertion of extra glycyl residues. Third, in addition to identification of linear antiparallel β-structures, we attempted experimentally to characterize β-turns. These β-turn conformations were only recently analyzed by Venkatachalam (17) and their optical activity was theoretically investigated by Woody (18) but no precise experimental CD data are presently available (14, 19). CD spectra were reported for cyclo(Val-Pro-Gly-Gly)$_n$ ($n = 3, 4$) and for the corresponding polytetrapeptide ($n = 40$), which presumably contain a high proportion of β-turns (20). According to the authors, the polymer is characterized by a segmental motion of repeating units which decreases the contribution of adjustments to the optical properties of a single unit. Only the results for the polymer agree qualitatively in the near ultraviolet with those given in the present paper, but the amplitude is one order of magnitude smaller, most probably reflecting a smaller fraction of β-turns than was assumed by the authors. X-ray analysis of 12 globular proteins

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: CD, circular dichroism.

‡ A. Brack and A. Caille, unpublished data.

has revealed that the proportion of β-bends can include as much as 33% of total amino-acid residues (21, 22). In previous CD investigation of proteins and polypeptides, the β-bends were not taken into account as such and were included in the characterization of unordered chains and/or in β-pleated sheets (14, 19).

MATERIALS AND METHODS

Materials. Optically pure samples of poly(Lys-Leu-Lys-Leu), poly(Leu-Glu-Leu-Glu), poly(Gly-Lys-Leu-Lys-Leu), and poly(Pro-Lys-Leu-Lys-Leu) were obtained by condensation of the corresponding tetra- or pentapeptide 2-hydroxyphenyl esters. Synthesis and characterization of the samples will be described elsewhere.‡ Poly(Ala_2-Gly_2), poly(Ala_2-Gly_3), and poly(Ala_3-Gly_3) samples were synthesized by condensation of the peptide pentachlorophenyl esters (23).

Lysine-containing polymers isolated as hydrochlorides are usually readily soluble in water. Some samples of poly(Lys-Leu-Lys-Leu) could only be dissolved in water after they had been recovered from a trifluoroacetic acid solution by drying under reduced pressure. Glutamic acid-containing polypeptides isolated as sodium salts are not soluble in water. However, the free acid form, obtained by precipitation with water from a trifluoroacetic acid solution, goes slowly into solution in the presence of an equivalent amount of sodium hydroxide. An increase of ionic strength was obtained by adding concentrated aqueous salt solution to the polymer solution.

Copolymers of alanine and glycine are insoluble in water. Translucent gels are obtained by diluting trifluoroacetic acid solutions with a large excess of water followed by centrifugation and repeated washings of the gel with water. A transparent thin layer is obtained by pressing the gel between two quartz windows.

CD Measurements. Vacuum ultraviolet CD apparatus was constructed in the I.R.B.M. laboratory and will be fully described elsewhere (S. Brahms and J. Brahms, unpublished data). Briefly, the radiation from a deuterium light source is dispersed by a single vacuum monochromator (Pouey) type ASM.5 (Licence ANVAR-C.N.R.S.) equipped with a concave holographic grating. The entire system is characterized by high luminosity, and the holographic grating has the advantage of reducing very strongly the amount of stray light to a value lower than 10^{-6} at 1700 Å. The Rochon polarizer and the photoelastic modulator (see refs. 24 and 25) were made from magnesium fluoride and calcium fluoride, respectively. The dichrograph works on line with a minicomputer "Plurimat S" (Intertechnique). The demodulated signal from the lockin amplifier after an analog-to-digital conversion is calculated and analyzed. The dichrograph was calibrated with a nondeliquescent *n*-propylammonium salt of d-10 camphorsulfonic acid, which improves the accuracy of calibration (26). This aqueous salt solution has an ellipticity $[\theta]$ of 8180 deg·cm^2 dmol^{-1} at 295 nm. Our measurements give an ellipticity $[\theta]$ of 17,100 at 192 nm.

The conditions of polypeptide concentrations and pathlength were chosen in order to ensure reliable measurements to 165 nm. The use of D_2O as a solvent instead of H_2O allows us to extend the measurements to this wavelength region. The polypeptide concentrations were of the order of 5–10 mg/ml and the cell thicknesses were varied from a few micrometers to 10 μm. Each polypeptide was measured at several concentrations and pathlengths, from 165 to 260 nm. Control measurements were made in D_2O dilute solution in 0.5 and 1 mm pathlength cells in the 180–260 nm spectral range. In most cases, the concentration of polypeptides was determined from two independent measurements of the absorbance and by weight.

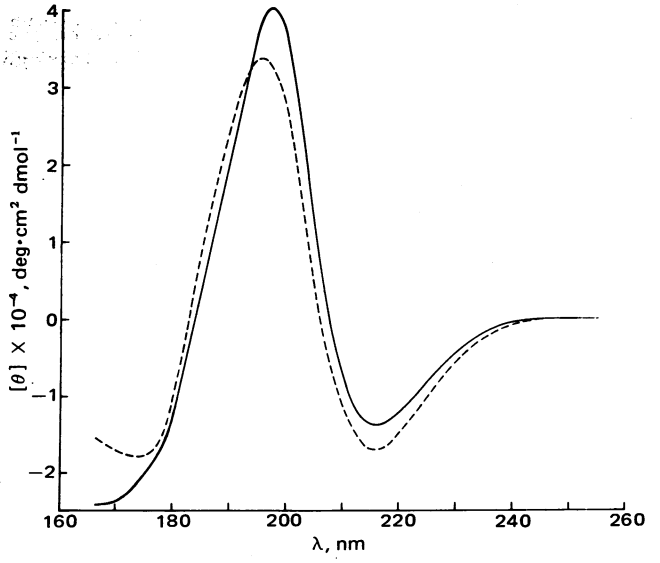


FIG. 1. CD spectra of poly(Lys-Leu-Lys-Leu) in 0.1 M NaF at pH 7 (—) and of poly(L-lysine) at pH 11 (- - -).

The absorbance of samples did not exceed 0.1 at 205 nm.

RESULTS AND DISCUSSION

β-Pleated Sheet Structures. Poly(Leu-Glu-Leu-Glu) and poly(Lys-Leu-Lys-Leu), in the presence of salt, yield optically clear solutions and qualitatively similar CD spectra of the linear β-pleated sheet type. The spectra exhibit the following bands: 216 nm, 196 nm, and a band at about 169 nm (Fig. 1) of rather appreciable intensity and characteristic of the β-form. This band is not present in the α-form.

These spectra of alternating copolypeptides essentially indicate very similar features with respect to the band position of poly(L-lysine) (Table 1). Quantitative differences, however, exist in the intensity of the bands. In particular, the value of the band at 216 nm seems to be lower in poly(Lys-Leu-Lys-Leu), whereas that at 196 nm is of lower intensity for poly(L-lysine). Correspondingly, the ratio of $[\theta]_{196}/[\theta]_{215}$ varies from 2.9 to 1.74 for these polymers. The higher ratio found for the alternating polypeptides may be explained by their good solubility in the β-form and by the absence of light scattering, which very often accompanies the formation of β-forms and perturbs optical measurements. Also, the β-pleated sheet structures formed by these alternating copolypeptides composed of hydrophobic and hydrophilic residues are probably of a regular bilayer type without bends (16). They may represent a situation similar to that of globular proteins in which such a type of asymmetric interface exists and can be considered to be appropriate models of β-pleated sheet structure.

β-Turns. β-Turns in polypeptide chains consist of four amino acids with hydrogen bonding between the CO group of residue 1 and the NH group of residue 4 (17, 27). These β-turns can be classified into 10 groups on the basis of the conformational angles φ and ψ of the second and third residues, the two crucial positions at the corner of the bend. According to Scheraga and coworkers, the most populated groups found in eight proteins are groups I, II, III, and IV (27). Tabulation of the overall distribution of the amino acids in these reverse turns indicates a high frequency of Pro in position 2 and of Gly and Asn in position 3. However, analysis of the distribution of the amino acids within the different types of β-turns (Table 2) shows that Pro has a high frequency in position 2 of type I only, whereas Gly

Table 1. Spectral characteristics of β-turns, β-pleated sheet, and unordered structure

	λ_1, nm	$[\theta]_1$	$\lambda_{\theta=0}$, nm	λ_2, nm	$[\theta]_2$	$\lambda_{\theta=0}$, nm	λ_3, nm	$[\theta]_3$	λ_4, nm	$[\theta]_4$	$[\theta]_2/[\theta]_1$
β-Turns											
$(Ala_2\text{-}Gly_2)_n$	227	−5,200	221	207.5	+63,200	199	191	−48,100	169	−39,000	
$(Ala_2\text{-}Gly_3)_n$	223	−4,700	216.5	206	+28,700	194.5	182	−23,000	173	−27,600	
$(Ala_3\text{-}Gly_3)_n$	222	−9,800	214.5	205	+25,800	192	182	−16,000	173	−22,800	
β-Pleated sheet											
$(Lys^+\text{-Leu-}Lys^+\text{-Leu})_n$*	216	−13,600	207.5	196.5	+40,500	184	169	−24,600			2.98
$(Leu\text{-}Glu^-\text{-Leu-}Glu^-)_n$†	216	−16,500	206	196	+41,200						2.50
$(Lys)_n$‡§	216.5	−16,800	206	195	+33,000	182.5	172	−18,000			1.96
	217	−18,400		195	+32,000						1.74
Unordered chains											
$(Leu\text{-}Glu^-\text{-Leu-}Glu^-)_n$	232	−2,000	221	218	+1,000	213.5	197.5	−40,000	172	−11,000	
$(Gly\text{-}Lys^+Leu\text{-}Lys^+Leu)_n$	228	−2,500	—	217	+1,000	—	196.5	−41,000	172	−10,000	
$(Pro\text{-}Lys^+Leu\text{-}Lys^+Leu)_n$	227	−1,600	—	—	—	—	197	−37,900	172	−11,600	
$(Lys^+)_n$	238	−500	234	217.5	+5,000	211	196	−43,800	170	−10,800	

* 0.1 M KF, pH 5.
† 0.1 M $(NH_4)_2SO_4$, pH 5.
‡ pH 11.2.
§ Greenfield and Fasman (5).

dominates in position 3 of type II. Serine also appears favorable in any position in many types of β-turn, and especially in type I. The sequence X-Pro-Gly-Y is scattered between many groups and does not appear in group I, the most populated type of chain reversal in proteins. The sequence X-Pro-Ser-Y should be a good candidate for type I β-turns, whereas X-Ala-Gly-Y may be representative of type II.

Poly(Ala_2-Gly_2), poly(Ala_2-Gly_3), and poly(Ala_3-Gly_3) were previously studied as models for silks. Electron microscopy showed that the powders precipitated by dialysis of LiBr solution consisted of small aggregates of β-structure sometimes elongated into long and narrow ribbons, the minimum thickness of which could be estimated to be less than 100 Å (28). Because the average length of the molecules that span the thickness of the ribbons is about 1000 Å, the chains must be bent. The abundance of the bends may depend on the procedure used to prepare the samples.

CD characteristics of poly(Ala_2-Gly_2) are reported in Fig. 2 and Table 1. The spectrum is at variance with the linear β-pleated sheet, but shows great qualitative similarities with the theoretical spectrum calculated by Woody (18) for β-turns of type I or II (Fig. 2). The ratio $[\theta]_{205}/[\theta]_{225}$ is of the order of 12.

Table 2. Distribution of major residues in positions 2 and 3 among various β-turn types and of some dipeptide sequences in positions 2-3*

	Total	I	II	III	Others
β-Turns	129	48	20	18	43
Position 2					
Pro	16	11	2	2	1
Ser	17	7	2	1	7
Gly	13	1	1	2	9
Position 3					
Gly	26	2	13	2	9
Ser	17	9	1	1	6
Sequence 2-3					
Pro-Gly	5	0	2	2	1
Pro-Ser	4	4	0	0	0
Ala-Gly	4	0	3	0	1

* Tabulated from ref. 27.

How much linear β-structure is still present in that spectrum is difficult to assess with precision. However, the good agreement with the theoretical spectrum indicates a high proportion of β-turns. Therefore, the main contribution of this structure is to induce a red shift of all bands of the linear structure and an increase of the intensity of the positive band at 207.5 nm. Our results bring experimental support to the hypothesis of Woody (18), who attributed the peculiar spectra of poly(L-serine) or its derivatives and of derivatives of poly(L-cysteine) observed previously by Fasman and coworkers (29) to the occurrence of β-turns.

In Fig. 3 are reported the CD spectra calculated for mixtures of linear β-structures and of poly(Ala_2-Gly_2) of varying amounts. Two isodichroic points are observed located at 202 and 226 nm, the ellipticities of which are 23,600 and 5200 deg·cm^2 dmol^{-1}, respectively.

The spectra of poly(Ala_2-Gly_3) and poly(Ala_3-Gly_3), the characteristics of which are given in Table 1 and in Fig. 4, differ

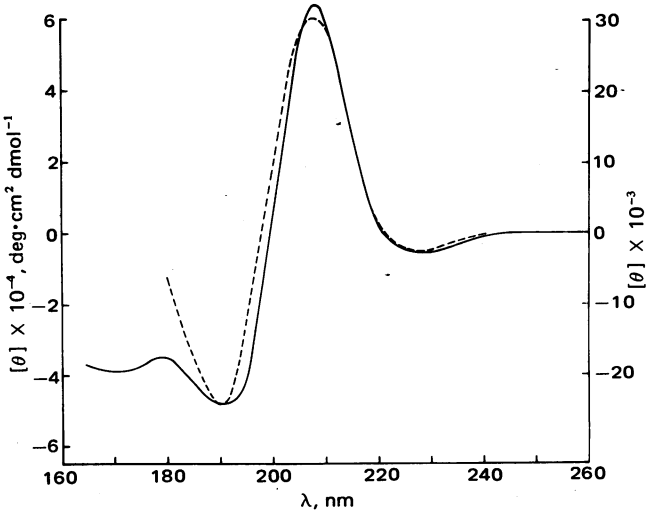
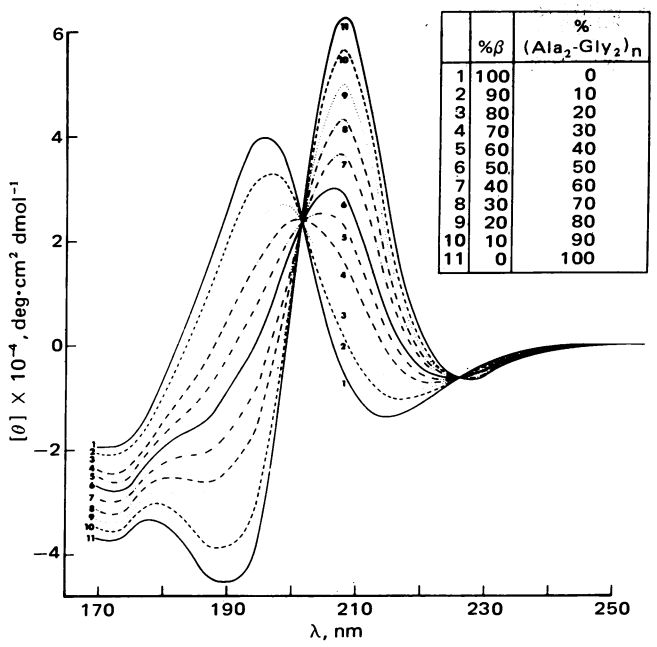


FIG. 2. CD spectra of poly(Ala_2-Gly_2) (—, left coordinate) and of type II β-turn calculated by Woody (18) (- - -, right coordinate). The agreement in shape with the theoretical curve is remarkably good, especially in the long-wave region. However, the ellipticity of the calculated CD is about half that of the experimental data.



	%β	% $(Ala_2\text{-}Gly_2)_n$
1	100	0
2	90	10
3	80	20
4	70	30
5	60	40
6	50	50
7	40	60
8	30	70
9	20	80
10	10	90
11	0	100

FIG. 3. Calculated CD spectra containing various percentages of linear β-sheet and poly(Ala_2-Gly_2). The numbers indicate calculated spectra corresponding to different proportions of β-sheet and of poly(Ala_2-Gly_2). The numbers indicate calculated spectra corresponding to different proportions of β-sheet and of poly(Ala_2-Gly_2) (see *Inset*).

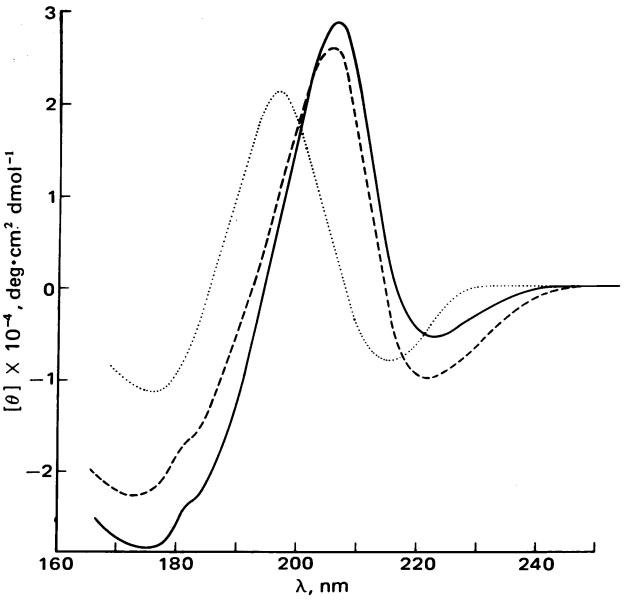


FIG. 4. CD spectra of poly(Ala_2-Gly_3) (—) and poly(Ala_3-Gly_3) (- - -). The dotted curve represents the resulting linear β-pleated sheet contribution to the CD spectrum of poly(Ala_2-Gly_3) after subtraction of 46% of poly(Ala_2-Gly_2) CD.

from the spectrum of poly(Ala_2-Gly_2) but can be interpreted as mixtures of linear β-pleated sheet, β-bend structures, by comparison with the spectra of Fig. 3. The isodichroic point is located at 202 nm and the value of θ is 23,000 deg·cm² dmol⁻¹; poly(Ala_2-Gly_3) and poly(Ala_3-Gly_3) contain 46 and 42% of poly(Ala_2-Gly_2), respectively. This lends further support to the idea that the experimental CD spectrum of poly(Ala_2-Gly_2) represents a valuable model of a β-turn structure. After subtraction of the poly(Ala_2-Gly_2) contribution from poly(Ala_2-Gly_3), the resulting curve is in good agreement with the spectrum of a β-pleated sheet (Fig. 4). However, the poly(Ala_3-Gly_3) spectrum differs slightly from the poly(Ala_2-Gly_3) CD spectrum in the long-wavelength region by the presence of a weak additional negative contribution.

Unordered Polypeptide Chain. When dissolved in aqueous solution in the absence of salts, poly(Leu-Glu-Leu-Glu), poly(Gly-Lys-Leu-Lys-Leu), and poly(Pro-Lys-Leu-Lys-Leu) exhibit spectra similar to that of charged poly(L-lysine) (Fig. 5 and Table 1). The extension of the spectral region to about 165 nm indicates the presence of two bands at about 197 and 170 nm. These results obtained in the vacuum ultraviolet region are in agreement with those obtained with charged poly(L-glutamic acid) (12) and qualitatively with denatured collagen (13).

In contrast, the CD spectra in the near ultraviolet region, i.e., 210–240 nm, are complex and sometimes characterized by a positive contribution at about 217 nm of variable intensity. Poly(Leu-Glu-Leu-Glu), poly(Pro-Lys-Leu-Lys-Leu), and poly(Gly-Lys-Leu-Lys-Leu) have very similar CD behavior in this region. They show a negative contribution at about 230 nm accompanied by an extreme at 217–218 nm, near the base line, which in poly(L-lysine) appears as a positive contribution.

Thus, the advantage of extending the spectral region is that it allows the most characteristic features of the spectra of an unordered chain (i.e., bands below 210 nm) to be visualized, whereas the proper choice of the CD model spectrum of this conformation was previously confined to the limited near ultraviolet (30).

CONCLUSIONS

The measurements of the CD of polypeptides in aqueous solutions and gels allow one to characterize all the main conformations in a broad ultraviolet region down to 165 nm.

(*i*) The optical parameters of β-turns have been quantitatively measured. The spectrum of poly(Ala_2-Gly_2) is characterized by strong negative bands at 169 and 191 nm, a very strong positive band at 207.5 nm, and a relatively weak band at 228 nm (Fig. 1 and Table 1).

The following evidence indicates that the described spectrum of poly(Ala_2-Gly_2) is that of a structure rich in β-bends: (*a*) Electron microscopy results indicate the presence of ribbons

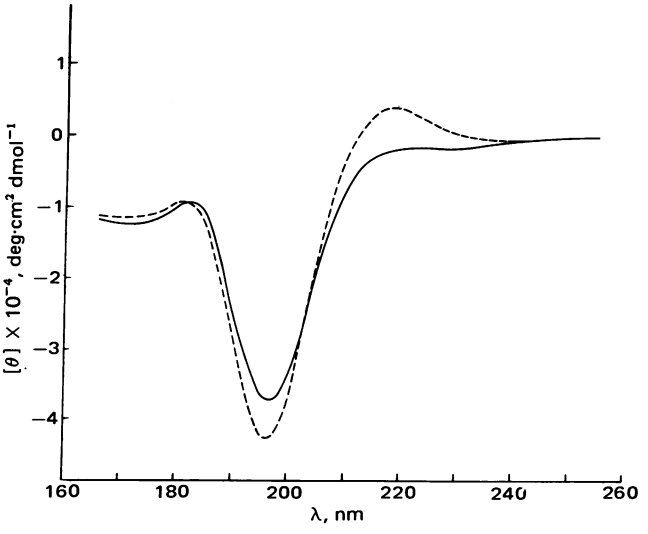


FIG. 5. CD spectra of poly(Pro-Lys-Leu-Lys-Leu) (—) in salt-free solution and of the unordered conformation of poly(L-lysine) (- - -) at pH 7.

in which the polypeptide chains must be bent (28). (*b*) The theoretical curve calculated by Woody (18) for β-turn types II and I of Venkatachalam (17) is in excellent qualitative agreement with the experimental spectrum shown in Fig. 2. (*c*) Other polypeptides investigated, poly(Ala_2-Gly_3) and poly(Ala_3-Gly_3), yield CD spectra of a similar type. The analysis of the spectra indicates that these polypeptides might contain different fractions of β-turns and of linear β-pleated sheet structure.

The extended calculations of Woody indicated that the main types of β-bends tend to yield similar CD spectra. One may thus consider the obtained spectral characterization of β-bends as being of general character.

(*ii*) The copolypeptides composed of alternating hydrophobic and hydrophilic amino-acid residues yield a stable antiparallel linear β-pleated sheet structure of a bilayer type, solutions of which are stable and optically clear. The optical characteristics of this structure have been quantitatively measured.

(*iii*) In addition, in the present work the unordered chain of these alternating polypeptides in salt-free solution is characterized in a broad spectral region. The extended spectrum of alternating polypeptides is closely related to the spectrum of charged poly(L-lysine), of poly(L-glutamic acid), and of denatured collagen (12, 13).

The extension of the spectral region below 210 nm shows the advantages of spectral measurements in the far vacuum ultraviolet region for assignment of the unordered chain structure.

The presented quantitative characterization of β-turns, linear β-pleated sheets, and unordered chains in a large spectral region must be of primary importance for the understanding of the conformation of polypeptides and of proteins in solution.

We are greatly indebted to Dr. Ross Williams (Ottawa) for the generous gift of *n*-propylammonium salt of d-10 camphorsulfonic acid. This research was financially supported by D.G.R.S.T.

1. Holzwarth, G. & Doty, P. (1965) *J. Am. Chem. Soc.* **87**, 218–228.
2. Woody, R. W. & Tinoco, I., Jr. (1967) *J. Chem. Phys.* **46**, 4927–4945.
3. Woody, R. W. (1968) *Biopolymers* **8**, 669–683.
4. Madison, V. & Schellman, J. (1972) *Biopolymers* **11**, 1041–1076.
5. Greenfield, N. & Fasman, G. D. (1969) *Biochemistry* **8**, 4108–4116.
6. Sarkar, P. K. & Doty, P. (1966) *Proc. Natl. Acad. Sci. USA* **55**, 981–989.
7. Townsend, R., Kumosinki, T., Timasheff, S. N., Fasman, G. D. & Davidson, B. (1966) *Biochem. Biophys. Res. Commun.* **23**, 163–169.
8. Quadrifoglio, F. & Urry, D. W. (1968) *J. Am. Chem. Soc.* **90**, 2760–2765.
9. Kubota, S. & Fasman, G. D. (1975) *Biopolymers* **14**, 605–631.
10. Iizuka, E. & Yang, J. T. (1966) *Proc. Natl. Acad. Sci. USA* **55**, 1175–1182.
11. Chothia, C. (1973) *J. Mol. Biol.* **75**, 295–302.
12. Johnson, W. C., Jr. & Tinoco, I., Jr. (1972) *J. Am. Chem. Soc.* **94**, 4389–4390.
13. Jenness, D. D., Sprecher, C. & Johnson, W. C., Jr. (1976) *Biopolymers* **15**, 513–521.
14. Chen, Y., Yang, J. T. & Chau, K. H. (1974) *Biochemistry* **13**, 3350–3359.
15. Balcerski, J. S., Pysh, E. S., Bonora, G. M. & Toniolo, C. (1976) *J. Am. Chem. Soc.* **98**, 3470–3473.
16. Brack, A. & Orgel, L. (1975) *Nature* **256**, 383–387.
17. Venkatachalam, C. M. (1968) *Biopolymers* **6**, 1425–1436.
18. Woody, R. W. (1974) in *Peptides, Polypeptides and Proteins*, eds. Blout, E. R., Bovey, F. A., Goodman, M. & Lotan, N. (John Wiley & Sons Inc., New York), pp. 338–348.
19. Baker, C. C. & Isenberg, I. (1976) *Biochemistry* **15**, 629–634.
20. Urry, D. W., Long, M. M., Ohnishi, T. & Jacobs, M. (1974) *Biochem. Biophys. Res. Commun.* **61**, 1427–1433.
21. Crawford, J. L., Lipscomb, W. N. & Schellman, C. G. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 538–542.
22. Chou, P. & Fasman, G. D. (1974) *Biochemistry* **13**, 211–222.
23. Brack, A. & Spach, G. (1972) *Biopolymers* **11**, 563–586.
24. Billardon, M. & Badoz, J. (1966) *C. R. Hebd. Seances Acad. Sci.* **262**, 1672–1676.
25. Billardon, M. & Badoz, J. (1966) *C. R. Hebd. Seances Acad. Sci.* **263**, 26–30.
26. Gillen, M. F. & Williams, R. E. (1975) *Can. J. Chem.* **53**, 2351–2353.
27. Lewis, P. N., Momany, F. A. & Scheraga, H. A. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2293–2297.
28. Lotz, B., Brack, A. & Spach, G. (1974) *J. Mol. Biol.* **87**, 193–203.
29. Fasman, G. D. & Potter, J. (1967) *Biochem. Biophys. Res. Commun.* **27**, 209–216.
30. Tiffany, M. L. & Krimm, S. (1972) *Biopolymers* **11**, 2309–2316.