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VACUUM ULTRAVIOLET CIRCULAR DICHROISM SPECTRUM OF β-TURN IN SOLUTION

Samir K. BRAHMACHARI[+II], V.S. ANANTHANARAYANAN[+*]
S. BRAHMS[II], J. BRAHMS[II], Rao S. RAPAKA[§] and
Rajendra S. BHATNAGAR[§]

+ Molecular Biophysics Unit, Indian Institute of Science,
Bangalore 560 012, India

II Institut de Recherche en Biologie Moléculaire (C.N.R.S.)
University of Paris VII, 75005 Paris, France

§ School of Dentistry, University of California,
San Francisco, 94143, U.S.A.

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SUMMARY

The vacuum ultraviolet circular dichroism spectrum of an isolated 4 → 1 hydrogen bonded β-turn is reported. The observed spectrum of N-acetyl-Pro-Gly-Leu-OH at - 40°C in trifluoroethanol is in good agreement with the theoretically calculated CD spectrum of the β-turn conformation. This spectrum, particularly the presence of a strong negative band around 180 nm and a large ratio $[\theta]_{201}/[\theta]_{225}$, can be taken as a characteristic feature of the isolated β-turn conformation. These CD spectral features can thus be used to distinguish the β-turn conformation from the β-structure in solution.

INTRODUCTION

The ubiquitous presence of the β-turn as an important conformational feature in globular proteins has been well recognized in recent years (1,2). While a good deal of effort has been made on the delineation of the various forms of the β-turn from theoretical considerations (3,4), experimental data on the specific characteristics of the β-turn that will distinguish it from the other secondary structures like the β-structure, are few.

* To whom to address correspondence.

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High resolution nuclear magnetic resonance (NMR) and circular dichroism (CD) spectral studies have been reported for some of the β-turn peptides in solution (5-7). The CD spectrum of the β-turn is seen to have a general resemblance to that of the β-structure (8) with regard to the band position and shape in the ultraviolet wavelength region, 190-250 nm, although the magnitudes of the bands, appearing near 200 nm and 220 nm in both these two conformations, are found to be different. It is not possible to arrive at an unequivocal conclusion about the presence of the β-turn conformation in peptides and proteins from the CD spectral data in the 250-200 nm region. Recently, Brahms et al. (9) have reported the CD spectral characteristics of the β-turn and the β-structure in the polypeptides in the vacuum ultraviolet region, extending down to 165 nm. In this communication, we present the vacuum ultraviolet CD spectrum of the tripeptide, N-acetyl-Pro-Gly-Leu-OH in the 4 → 1 hydrogen bonded β-turn conformation (Fig.1). To our knowledge, this is the first report of the vacuum ultraviolet CD of a small linear peptide containg an isolated β-turn.

EXPERIMENTAL

The synthesis of the tripeptide N-acetyl-Pro-Gly-Leu-OH is reported elsewhere (11). Its purity was checked by microanalysis and it showed a single spot on precoated silica gel G plates. Trifluoroethanol (TFE) from Merck was distilled before use. The vacuum ultraviolet CD spectra reported here were recorded down to 164 nm in the apparatus constructed in the I.R.B.M. Laboratory (Paris). The details of the instrumentation and the calibration procedure are given elsewhere (9). The spectra were recorded in TFE using from 0.005 cm to 2.0 cm pathlength cells. The concentration used was from 2 mg/ml to 0.03 mg/ml. The ellipticity values are expressed in deg cm^2 $dmole^{-1}$ per residue.

RESULTS AND DISCUSSION

It is well known from the statistical analysis of the crystal structure data on globular proteins (2,10) that Pro and Gly are among the most favourable residues to occur in the 2nd and 3rd

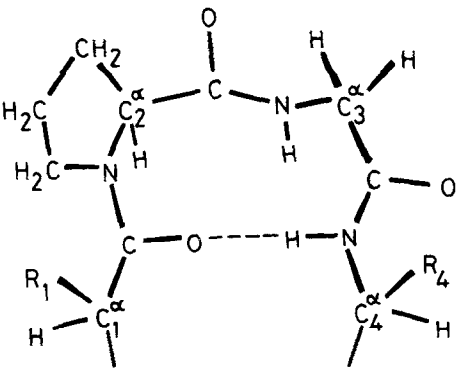

Figure 1 Schematic diagram of a 4 → 1 hydrogen-bonded β-turn in a tetrapeptide sequence containing Pro in the 2nd position and Gly in the 3rd position.

position of the β-turn, respectively (as shown schematically in Fig.1). In addition, as we have demonstrated elsewhere (10), the nature of the residue in the 4th position (i.e., the C-terminal end) of the β-turn plays an important role in governing the extent of β-turn conformation in a given tetrapeptide sequence of the type Z-Pro-Y-X or Z-Pro-Gly-X. Our NMR and CD studies (11,12) on a series of peptides of the type N-acetyl -Pro-Gly-X-OH, where X = Gly, Ala, Leu, Ile and Phe, reveal that Leu in the 4th position is by far the best in stabilizing the 4 → 1 hydrogen-bonded β-turn conformation. This is substantiated by the statistical analysis of the globular protein data (10). Our choice of N-acetyl-Pro-Gly-Leu-OH for the characterization of the vacuum ultraviolet CD spectral features of the β-turn was dictated by the above considerations.

The vacuum ultraviolet CD spectra of N-acetyl-Pro-Gly-Leu-OH in TFE in the wavelength region 164-250 nm, taken at three temperatures, at the peptide concentration of 2 mg/ml, is shown in Fig.2. An important feature of these spectra is the presence of a relatively large negative band arond 180 nm, besides a positive band around 199 nm and a weak negative band near 225 nm. All these bands are

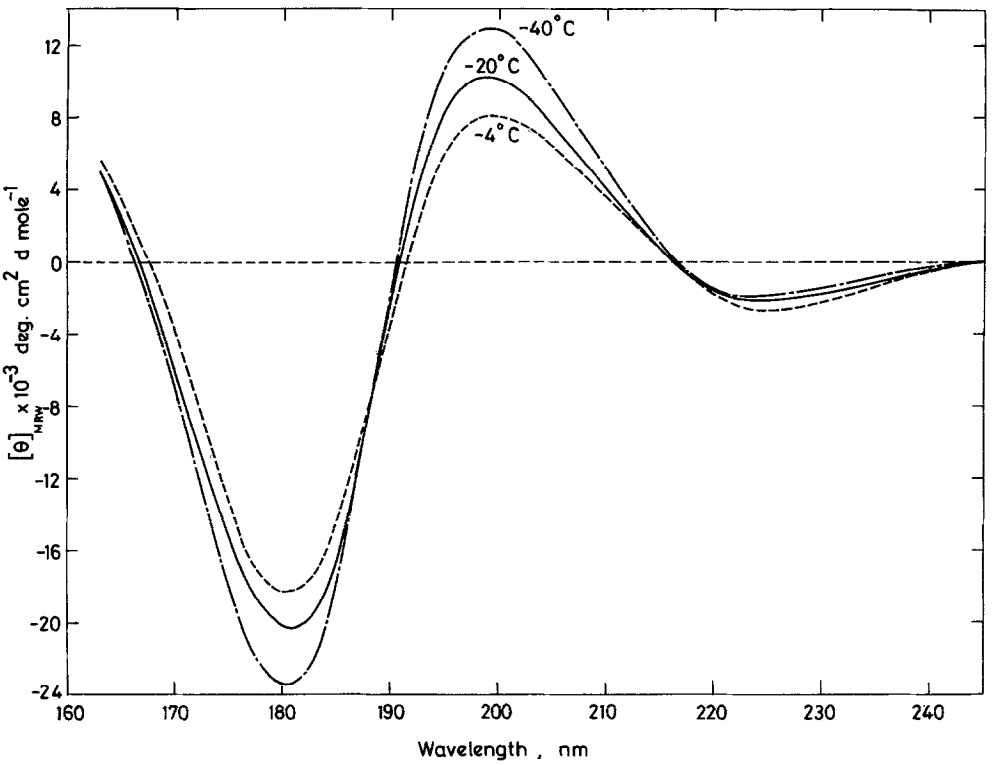

Figure 2 Vacuum ultraviolet CD spectra of N-acetyl-Pro-Gly-Leu-OH in TFE, (---) at - 4°C ; (——) at - 20°C and (-·-·-·) at - 40°C ; concentration 2 mg/ml and pathlength used 0.005 cm.

slightly blue-shifted compared to the theoretically expected band positions for the type-II β-turn (13). In order to avoid any intramolecular interaction, we have also recorded the vacuum ultraviolet CD spectra at low concentration (0.03 mg/ml) using a larger pathlength. These spectra measured down to 174 nm are shown in Fig.3. The magnitude of the weak negative band at 225 nm decreases and the positive band shifts to 201. The exact positions and magnitude of these extrema at - 4°, - 20° and - 40°C are given in Table I. The agreement between our experimental CD spectrum at low concentration at the lowest temperature studied (Fig.3) and the theoretical spectrum for an isolated type-II β-turn (13) can thus be considered as quite good. However, the experimental spectrum is displaced by

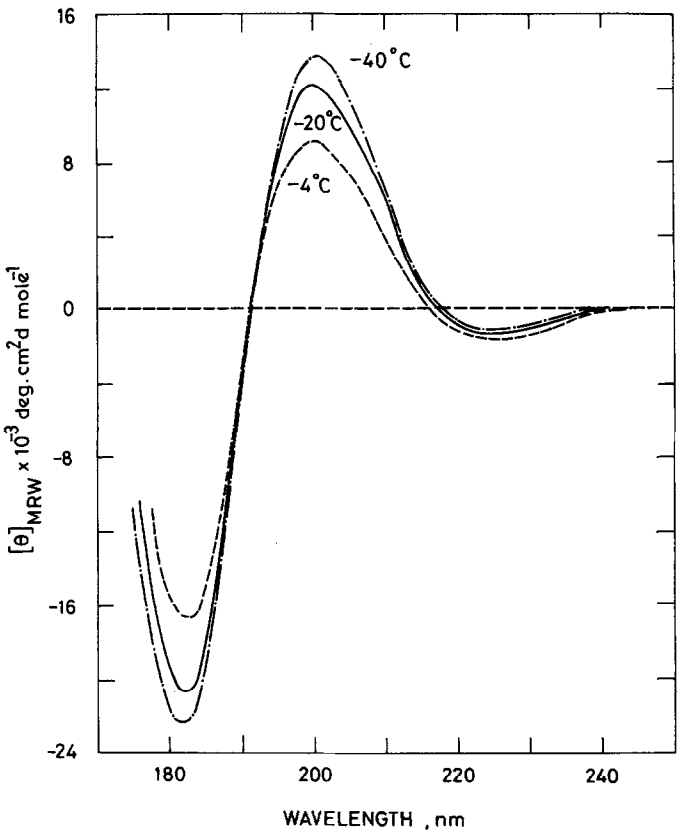

Figure 3 Vacuum ultraviolet CD spectra of N-acetyl-Pro-Gly-Leu-OH in TFE, (---) at - 4°C ; (——) at - 20°C and (-·-·-·) at - 40°C ; concentration 0.03 mg/ml and pathlength used 0.5 cm.

TABLE I

VACUUM ULTRAVIOLET CD DATA OF N-acetyl-Pro-Gly-Leu-OH IN TFE AT DIFFERENT TEMPERATURES*

Temperature °C	λ_1 nm	$[\theta_1]$ a	λ_2 nm	$[\theta_2]$ a	λ_3 nm	$[\theta_3]$ a	$R = \frac{[\theta_2]}{[\theta_1]}$
- 4	225.0	-1,650	201.0	+ 9,200	182.5	- 16,600	- 5.6
- 20	225.0	-1,300	201.0	+12,300	182.5	- 20,600	- 9.5
- 40	225.0	-1,200	201.0	+13,800	182.0	- 22,400	-11.5

* concentration of peptide 0.03 mg/ml.

a expressed in deg cm^2 dmole^{-1}.

about 5 nm to the blue, which could arise from solvent effect on the transitions. Also, the observed magnitude of the 201 nm band is smaller than that of the theoretical value (13).

The ratio, R, between the ellipticity values of the positivie peak around 205 nm and that of the negative band around 225 nm, has been utilized by Brahms et al. (9) to distinguish the β-turn conformation from the β-structure. This ratio was found to increase with the increase in the extent of β-turn conformation. The solvent dependent and temperature dependent NMR chemical shift data of the NH protons of a series of tripeptides of the type N-acetyl-Pro-Gly-X-OH, show that there is a direct correlation between the extent of hydrogen bonded β-turn conformation and the ratio R (11,12). In the case of our tripeptide N-acetyl-Pro-Gly-Leu-OH, as the temperature is lowered, the absolute value of the ratio $[\theta]_{201}/[\theta]_{225}$ is found to increase (Fig.3), indicating an increase in the extent of β-turn conformation of this tripeptide in TFE. The experimentally observed value (about 11.5) of R for N-acetyl-Pro-Gly-Leu-OH at the lowest temperature studied is in excellent agreement with the value (about 12) for the theoretically calculated spectrum of Woody (13) for the type-II β-turn conformation of Venkatachalam (3). (The R value for the type-I β-turn is found to be about 6 (13)). Our R value is also very close to the value of 12 observed for poly (Ala_2-Gly_2) (9). At lower peptide concentration, the coil $\rightleftharpoons$ β-turn equilibrium is shifted to the right, due to a lesser possibility of any intermolecular stabilization of the coil state. As evident from the Fig.3, around - 40°C, the ratio R shows saturation and attains the theoretically expected value for 100 % β-turn conformation. Further cooling to - 60°C does not change the spectrum significantly indicating complete attainment of the folded β-turn conformation of this peptide at the lowest temperature. Thus

the spectrum of N-acetyl-Pro-Gly-Leu-OH at the lowest temperature can be taken as representative of an isolated β-turn conformation in solution. Another supporting evidence comes from the fact that in an analogous compound, N-acetyl-Pro-Gly-Phe-OH, the presence of a typical type-II β-turn conformation has been demonstrated by single-crystal X-ray crystallography (12).

The presence of a distinct strong negative band around 180 nm, as predicted by Woody (13) for the isolated β-turn and observed here for N-acetyl-Pro-Gly-Leu-OH, with a slight blue shift, provides new possibilities for characterization of β-turns in solution. This spectrum and particularly the presence of 180 nm band along with a large $[\theta]_{201}/[\theta]_{225}$ ratio can thus be used to distinguish the β-turn conformation from the β-structure, and also the cross β-structure from that of the antiparallel β-structure. In addition, the availability of the CD spectrum of the β-turn would be of use in the interpretation of the CD spectra of globular proteins.

Towards the completion of this work, the CD spectrum of another tetrapeptide, Cbz-Gly-L-Ser-(O-Bu^t)-L-Ser-Gly-O-Stearyl ester, has been published by Kawai and Fasman (14). In dilute solutions in cyclohexane, the CD spectral features of this tetrapeptide in the 195-240 nm wavelength region resemble those for the β-turn calculated by Woody (13). However, the observed spectrum is blue-shifted by about 4-7 nm from the theoretical spectrum and the magnitudes of the CD bands as well as those of the ratio, R, are different from those obtained by us and by Woody (13). Our CD data presented in this paper differ from those of Kawai and Fasman (14) in (a) the nature of the tetrapeptide - the one used by the latter authors has bulky blocking and protecting groups in addition to presence of the long

hydrocarbon chain at the carboxyl end (b) the nature of the solvent used and, most importantly, (c) the range of wavelength covered, ours extending the CD spectrum to the vacuum ultraviolet region.

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