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CIRCULAR DICHROISM OF TYPE 13 β-TURN IN LINEAR TRIPEPTIDES CONTAINING L-PROLINE AND D-ALANINE

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SUMMARY

The linear tripeptides tBoc-L-Prolyl-D-alanyl-L-leucine and tBoc-L-prolyl-D-alanyl-L-valine have been shown, from circular dichroism (CD) and infrared spectral data, to take up the 4 → 1 hydrogen bonded β-turn conformation in organic solvents. The CD spectra of these tripeptides in trifluoroethanol exhibit a positive n → π* band around 220 nm contrary to the usual negative band observed for the type II β-turn. The observed CD spectra of the tripeptides provide the first examples of those predicted theoretically by Woody for peptides containing L,D sequences and adopting the Venkatachalam type 13 β-turn. This conformation is seen to revert to the type II β-turn when the N-terminal protecting group is acetyl or when the C-terminal residue is glycine. These data are shown to have a direct bearing on the interpretation of the CD spectra of globular proteins.

Much attention has recently been paid towards understanding the conformational features of the various types of β-turns because of their importance in the structure of peptides and proteins. Venkatachalam [1] analyzed the expected values of the dihedral angles (ϕ_2, ψ_2 and ϕ_3, ψ_3) of the residues in a tripeptide unit involved in 30 types of β-turn. Analysis of β-turns in peptides and glubular proteins [2,3] has revealed many of these β-turn types occurring with varying frequencies in these molecules. It should therefore be of interest to determine the experimental parameters that characterize the different types of β-turn in solution. Circular dichroism (CD) has been extensively used as a relatively simple tool for detecting and estimating secondary structures in general. The presence of distinct types of β-turn would require that we know the CD spectral features of each of tyese types. We report here the CD spectrum of what appears to be a Venkatachalam type 13 β-turn taken up by the peptides tert-

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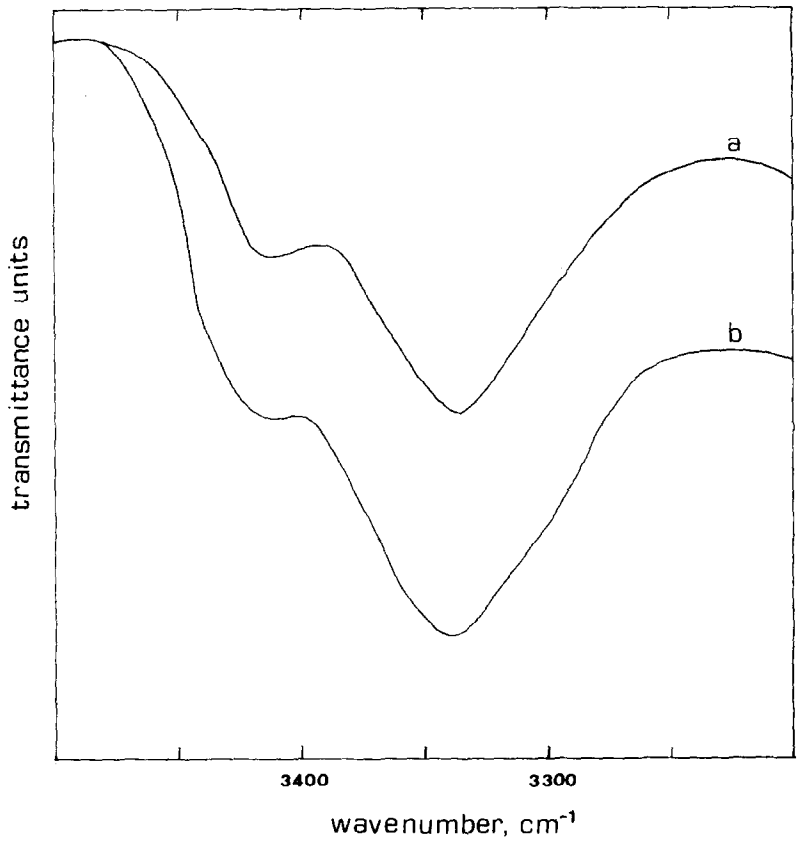

Fig. 1: Infrared spectra in the amide NH region of (a) BocPDAV and (b) BocPDAL in $CHCl_3$.

butyloxycarbonyl-L-prolyl-D-alanyl-L-leucine (BocPDAL) [4] and tert-butyloxy-carbonyl-L-prolyl-D-alanyl-L-valine (BocPDAV).

Materials and Methods

Amino acids and their derivatives, dicyclohexylcarbodiimide (DCCI), isobutyl chloroformate and N-hydroxysuccinimide (OHNSu) were obtained from Sigma Chemical Co., U.S.A. All the solvents were Fisher certified reagents except trifluro-ethanol (TFE) which was obtained from Aldrich Chemical Inc., Montreal. The tripeptides were synthesized by coupling tBoc-L-ProONSu with D-alanine to get the dipeptide which was then coupled to L-leucine (or L-valine) methyl ester hydrochloride by the mixed anhydride method and subjected to alkaline hydrolysis. H-Pro-D-Ala-L-Leu-OH and N-acetyl-L-Pro-D-Ala-L-Leu-OH were obtained by removal of the tBoc group with formic acid and subsequent treatment with acetic anhydride in acetic acid. The purity of the peptides was determined by amino acid and elemental analyses.

CD spectra were recorded with a Jasco J-20 spectropolarimeter using 0.1 to 1.0 mm cells. The sample concentration ranged from 0.2 to 1.0 mg/ml. The molar ellipticities [θ] were calculated based on the molecular weights of the respective

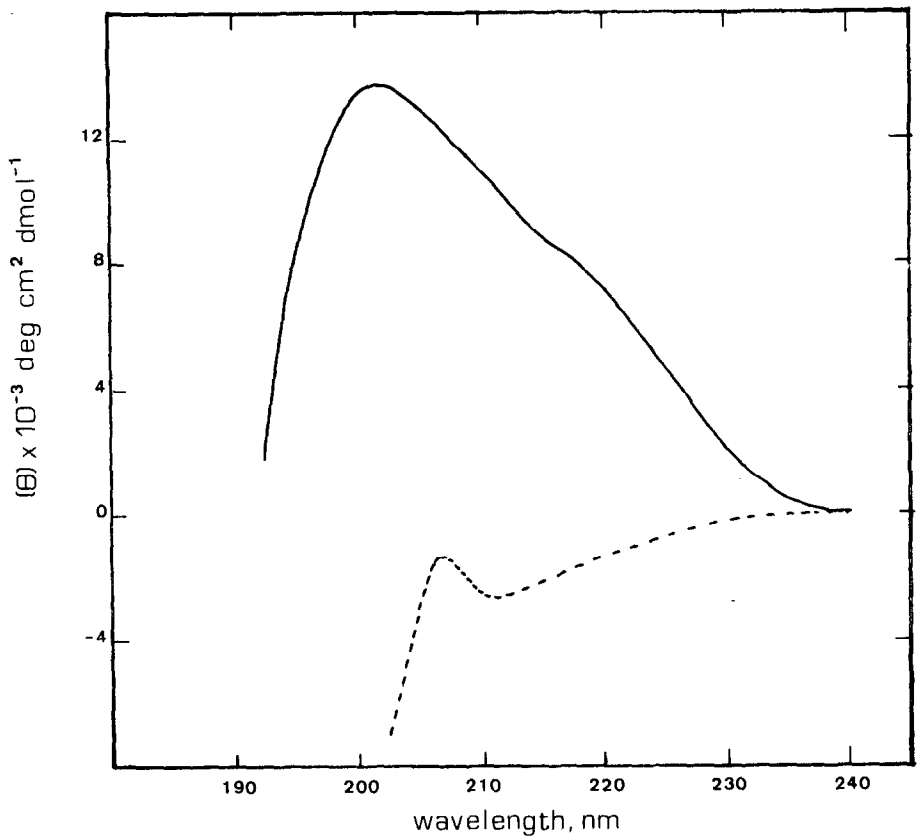

Fig. 2: CD spectra of BocPDAL in TFE at 0°C (———) and in 0.1M NH_4HCO_3 at 25°C (- - - -).

tripeptides. Infrared (IR) measurements were made using a Perkin-Elmer model 283 spectrophotometer on 1.5 to 10.0 mg/ml concentrations of the samples in chloroform in a 1.0 mm cell against the solvent.

RESULTS

The IR spectra in the amide NH region of BocPDAL and BocPDAV are shown in Fig. 1. Two bands can be seen, one at 3420 cm^{-1} appearing as a shoulder and corresponding to the free NH group and the other as the major band at 3340 cm^{-1} arising from the NH group involved in hydrogen-bonding. The ratio of the band intensities remained the same over the concentration range 4.0 x 10^{-3}M to 2.5 x 10^{-3}M, indicating the absence of significant contributions from intermolecular hydrogen-bonding.

The CD spectra of BocPDAL and BocPDAV in TFE and 0.1 M NH_4HCO_3 solutions are shown in Figs. 2 and 3 respectively. In TFE, the spectra of both the

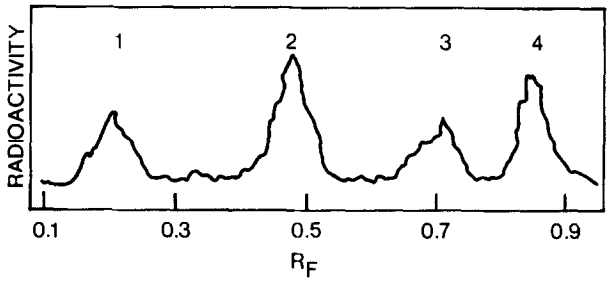

Figure 1. Radiochromatogram scan of the products formed after incubation of 5-methylthio[U-^{14}C] ribose with cell-free extract of E. aerogenes. A very similar distribution of radioactivity was observed when 5-[methyl-^{14}C]methylthioribose was used as substrate. Experimental details are described under Materials and Methods.

crystallization of the radioactive product, isolated from incubation mixtures with 5-methylthio-[U-^{14}C]ribose, with unlabeled methionine to constant specific radioactivity. After four sequential crystallizations, the values were 9.4 x 10^4, 9.3 x 10^4, 9.2 x 10^4, and 9.4 x 10^4 cpm/mmol. To corroborate the identification, a sample was treated with hydrogen peroxide; the product comigrated with methionine sulfoxide in both solvent systems (R_f 0.20 and 0.15). Incubation of another sample with L-amino acid oxidase yielded a product migrating with 2-keto-4-methiolbutyric acid in both solvent systems (R_f 0.72 and 0.49).

Compounds 3 and 4 were identified as 2-keto-4-methiolbutyric acid and 2-hydroxy-4-methiolbutyric acid by co-chromatography with reference material in solvent systems A and B (Table I). The compounds did not contain an amino group, but the methiol group was present as indicated by a positive platinum iodide test (17) and recovery of radioactivity when $^{14}CH_3$-labeled substrate was used.

Using the same chromatographic analysis, radioactive methionine was identified as a reaction product in incubation mixtures which contained either 5-[methyl-^{14}C]methylthioribose-1-phosphate or 5-methylthio[U-^{14}C]ribose-1-phosphate as the substrate.

which adopt the 4 → 1 hydrogen-bonded type II β-turn conformation. The dipeptide t-Boc-L-Pro-D-Ala-OH (BocPDA), on the other hand, does not exhibit the band at 3340 cm^{-1}. The intramolecular hydrogen bond in BocPDAL and BocPDAV is therefore taken to be of the 4 → 1 type involving the urethane carbonyl group and the secondary amide group of L-Leu and L-Val respectively. This is further confirmed by the 15 cm^{-1} shift in ν_{CO} (not shown) for the urethane carbonyl on going from BocPDA to BocPDAL or BocPDAV. The possibility of a 4 → 1 hydrogen-bonding giving rise to the β-turn conformation in our tripeptides is augmented by the theoretical calculations of Chandrasekharan et al. [7] on peptides containing L and D residues at the 2nd and 3rd position of the β-turn and by the observation of a type-II β-turn by Aubry et al. [8] in N-isobutyl-L-Pro-D-Ala-isopropylamide.

The CD data on BocPDAL and BocPDAV in TFE presented in Figs. 2 and 3 may thus be taken to represent the β-turn conformation of these tripeptides in this solvent. (As is to be expected, the spectra of BocPDA and H-L-Pro-D-Ala-L-Leu-OH have been found by us to be different from that of the blocked tripeptide.) The β-turn is seen to be destabilized in aqueous salt solution probably due to the breaking of the intramolecular hydrogen bond (Figs. 2 and 3). While the position and magnitude of the positive CD band and the position of the cross-over point of both the tripeptides in TFE agree well with those observed earlier by us for N-acetyl-L-Pro-Gly-L-Leu-OH existing in the type II β-turn conformation [5], the longer wavelength CD band around 225 nm (arising from the n → π* transition of the peptide chromophore) is negative in the Pro-Gly analogue in contrast to its positive sign in the Pro-D-Ala tripeptides. Other model peptides that are known to exist in the β-turn types I, II, III and IV also show a negative n → π* CD band [5, 9-11]. Theoretical calculations by Woody [12] indicate that the CD spectra of β-turn types I, II and III are very similar in appearance. On the other hand, the calculated CD spectrum for a special case of the type IV β-turn that corresponds to the type 13 in the notation of Venkatachalam [1], agrees with the spectral features of our Pro-D-Ala tripeptides shown in Figs. 2 and 3. The calculated spectrum, which resembles the CD of an inverted (right-handed) α-helix and designated as belonging

to Class C, was indeed predicted by Woody [12] for peptides containing L,D sequences However, it has so far not been observed, to our knowledge, in a linear oligopeptide According to Woody, the most important factor in yielding the Class C spectrum is the conformation of the 3rd residue in the β-turn peptide, which is D-Ala in our case. The higher the values of ϕ_3 and ψ_3, the greater the probability of observing this spectrum (Woody, private communication). With the values for ϕ_2 and ψ_2 for the L-Pro residue in the vicinity of -50° and 130° respectively, the CD spectrum is expected (Woody, private communication) to be predominantly ($\sim$ 90%) of the inverted α-helix type if the ϕ_3 and ψ_3 values are about 70° and 40° for the D-Ala residue forming type 13 β-turn. However, relatively slight variations in either or both ϕ_2, ψ_2 and ϕ_3, ψ_3 are sufficient to cause the CD spectrum to assume the type II(i.e. Venkatachalam type 14)β-turn pattern (belonging to Woody's Class B spectrum) which is found in the several model peptides mentioned earlier including N-isobutyl-L-Pro-D-Ala-isopropylamide [8] which was used by Brahms and Brahms [9] as one of the models for type II β-turn. It may be noted that the type 13 β-turn spectrum found in BocPDA and BocPDAV is the mirror image of the CD spectra found in gramicidin S and several analogous cyclic peptides which have a D-amino acid in the 2nd and L-amino acid in the 3rd positions of the β-turn [11].

Further observations (to be published) on a series of tripeptides containing the Pro-D-Ala sequence show that the CD spectral features are governed by the nature of the 1st and 4th residues. Thus, while the CD spectrum tBoc-L-Pro-D-Ala-L-Ala-OH in TFE is similar to those of Leu and Val counterparts, the spectra of N-acetyl-L-Pro-D-Ala-L-Leu-OH and tert-Boc-L-Pro-D-Ala-Gly-OH exhibit a negative band at 225 nm and a positive band at 200 nm similar to that reported for N-isobutyl-L-Pro-D-Ala-isopropylamide [9]. The latter peptide was taken recently by Brahms and Brahms [9] as one of the model compounds for the type II β-turn and its CD spectrum was used by these authors in the interpretation of the CD spectra of globular proteins. The data presented here reveal that the CD spectral features of the -Pro-D-Ala- peptides are dependent on the nature of the adjoining residues. This would caution against the selection of any particular tripeptide as a model in the protein CD

spectral analysis. We have brought out elsewhere the importance of the nature of neighbouring residues in governing the stability of the β-turn conformation in Pro-Gly containing tripeptides [13]. Details of similar studies on a series of Pro-Ala tripeptides will be presented elsewhere.

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