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Phenylalanine Oligopeptides
Circular Dichroism Studies in Solution

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The conformations of a series of L-phenylalanine oligomers having the general formula BOC-(Phe)$_n$-OMe ($n = 1$–9) were investigated by circular dichroism in a number of solvent systems. These studies indicate that in trifluoroethanol and in hexafluoroisopropanol these oligomers probably form β-associated conformations beginning at the hexamer.

INTRODUCTION

In the preceding paper (*1*) the synthesis, polarimetric, and ultraviolet absorption (uv) properties of a series of protected L-phenylalanine oligomers were reported. It was found that, using dimethylformamide (DMF) as a solvent, the total molar rotation ϕ_D is linearly dependent on the number of residues in the chain up to $n = 7$. Unfortunately, the octamer and nonamer are not soluble in DMF, and, consequently, it was not possible to establish whether an ordered structure forms for $n > 7$. In trifluoroethanol (TFE), deviations from linearity of ϕ_D were observed beginning at the hexamer, thereby suggesting the onset of an ordered structure for oligomers of six units or more. In order to confirm these preliminary results as well as to attempt to elucidate the type of ordered structure which is formed, an extensive examination of the circular dichroism (CD) of these oligomers in a number of solvent systems has been carried out. The results of these experiments are presented in this paper.

EXPERIMENTAL

Circular dichroism studies were carried out using a Cary 60 spectropolarimeter modified with a Model 6001 circular dichroism attachment. The experimental solutions were prepared by adding solvent to a weighed oligomer sample in a volumetric flask. Concentration studies were then carried out by diluting the above oligomer solutions to the desired concentration. In the studies performed in TFE–sulfuric acid, the oligomers were first dissolved in sulfuric acid, and the TFE was subsequently added. The resulting solutions were then stirred for 30 min.

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The spectra were obtained using 0.5-mm-, 1-mm-, and 1-cm-path-length cells. Dry prepurified nitrogen was employed to purge the instrument before and during the experiments. All spectra were recorded at ambient temperature.[2] Trifluoroethanol and *tert*-amyl alcohol were purchased from Fluka A.G., Basle; sulfuric acid from Merck A.G., Darmstadt. Hexafluoroisopropanol (HFIP) was obtained from Du Pont and Co., Wilmington, DL. All solvents were of the highest purity commercially available and were used without further purification.

Concentrated sulfuric acid was used in the preparation of all TFE–sulfuric acid mixtures. In such solvent mixtures the oligomers have their N-terminal amino groups in the ammonium form. No changes occurred other than the removal of the *t*-butyloxycarbonyl group, as demonstrated for all oligomers by thin layer chromatography (SiO_2, Merck) in chloroform–ethanol (9:1). Furthermore, the pentapeptide methyl ester free amine was isolated and characterized as its hydrochloride, mp 227–228°C, R_f (methanol–benzene, 1:2) = 0.80, single ninhydrin and chlorine-positive spot. This latter compound was identical to that obtained by treatment of the *t*-butyloxycarbonyl-tetra-(L-phenylalanyl)-L-phenylalanine methyl ester with 6 *N* HCl in anhydrous dioxane (*1*).

RESULTS AND DISCUSSION

The results of CD measurements on phenylalanine oligomers ($n = 1$–6) in TFE are shown in Fig. 1. All spectra exhibit a strong positive Cotton effect near 215 nm, followed by a weaker negative Cotton effect located near 240 nm. On going from the monomer to the hexamer, the positive band shifts gradually toward the red, the final position being at 219 nm. Conversely, the negative band shifts gradually toward the blue, being located at 236.5 nm at the hexamer. In the near-uv region CD spectra exhibit a vibrational structure characteristic of phenyl derivatives (*1–5*). Also, in this spectral region the CD pattern depends on the number of residues in the peptide chain. In fact, the well distinguished negative bands at 248 nm and 254.5 nm of the monomer become shoulders at the trimer and pentamer, respectively; at the tetramer the band at 248 nm is absent. These findings are probably due to the parallel increase of the much stronger negative band near 240 nm, which partly overlaps the vibrational bands at 248 nm and 254 nm.

Essentially analogous features are observed in the CD spectra obtained in HFIP, where the whole oligopeptide series is soluble (Fig. 2). The intensities and positions of CD bands compare well with those observed in TFE.

In a less polar solvent, such as *tert*-amyl alcohol (TAA) (Fig. 3), the negative CD band near 240 nm is greatly enhanced with respect to each of those observed in TFE or HFIP. In the near-uv region the vibronic structure of CD patterns of dimer and higher oligomers is red-shifted by about 1 nm with respect to the fluoroalcohols; but three additional positive Cotton effects are apparent at 265, 258, and 252 nm in the monomer. Moreover, in both solvents, the vibrational CD band near 248 nm is absent starting from the dimer, whereas the CD band at 255 nm becomes a shoulder at the tetramer. Also in this case there is probably partial overlapping of the strong negative band near 240 nm with the vibrational bands at 248 nm and 255 nm.

As far as the near-uv region is concerned, detailed analyses of the vibrational structure

[2] The data are expressed in terms of total molar ellipticity [θ].

of the CD spectra of phenylalanine and its derivatives have been carried out independently by several workers (*2–5*). Our results are totally consistent with the data of these authors; in particular the effects of solvent polarity and of substituted amino and carboxyl groups on location, number, and sign of CD bands parallel those previously reported.

In the far-uv, strong positive Cotton effects between 215 and 220 nm have been found in a number of phenylalanine derivatives including poly-L-phenylalanine in the

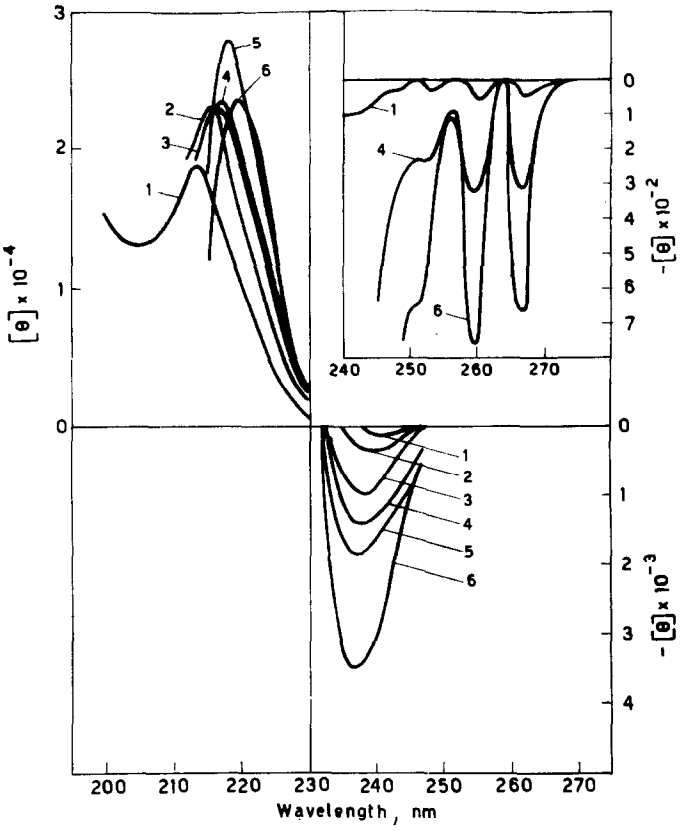

FIG. 1. CD spectra of BOC-(L-Phe)$_n$-OMe in trifluoroethanol.

"random coil" conformation (*3–10*). It is presently well established that this CD band contains contributions from both the peptide and phenyl chromophores.

The negative CD band near 240 nm has been assigned to an overlapping of a narrower positive band near 215 nm with the long-wavelength tail of a broader negative amide band near 225 nm ($n \rightarrow \pi^*$ transition). Support for this explanation is given by our CD results in TFE containing 10% sulfuric acid. In such a solvent mixture the negative Cotton effect at 240 nm is absent. This finding is consistent with protonation of the amide groups by the strong acid with the consequent disappearance of the $n \rightarrow \pi^*$ band (*4, 11–14*).

In order to investigate whether an ordered structure in the oligomers is formed on increasing the number of amino acid residues in the chain, we have reported the total

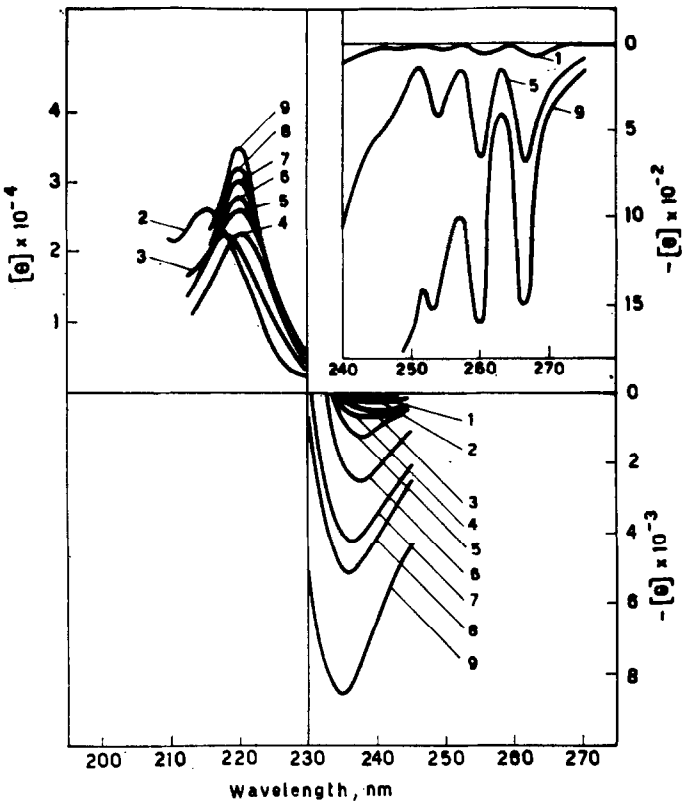

FIG. 2. CD spectra of BOC-(L-Phe)$_n$-OMe in hexafluoroisopropanol.

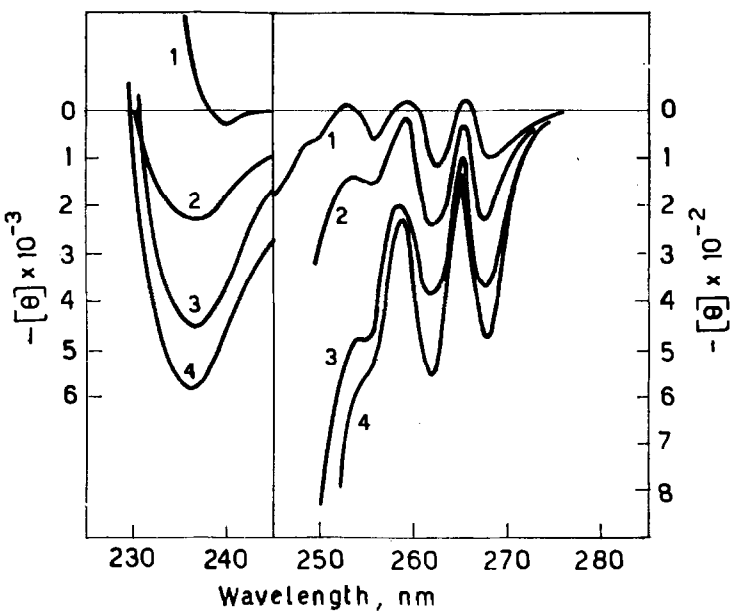

FIG. 3. CD spectra of BOC-(L-Phe)$_n$-OMe in *tert*-amyl alcohol in the 230–275 nm region.

molar ellipticity values at 220 nm and 240 nm in all the solvents examined as a function of n.

In TFE it appears that at both wavelengths there are negative deviations from linearity starting from the hexamer (Fig. 4). This result seems to confirm (*1*) the onset of an ordered structure in this solvent at this stage.

In HFIP, where all the oligomers are soluble, we observe a rather peculiar behavior (Fig. 5). There is a linear dependence on chain length of the dichroic absorption at

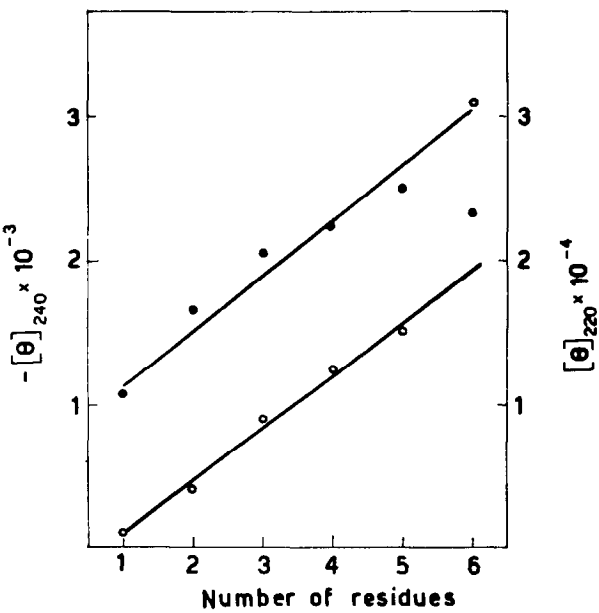

FIG. 4. Plot of total molar ellipticity values of BOC-(L-Phe)$_n$-OMe in trifluoroethanol at 240nm (—○—) and at 220 nm (—●—).

220 nm, while negative deviations from linearity at 240 nm are apparent starting from the hexamer. If our hypothesis is correct, i.e., that in this solvent a secondary structure similar to that present in TFE begins to form at the hexamer, we are forced to conclude that the negative CD maximum present near 240 nm in the oligophenylalanines is in general more sensitive to conformational changes of the peptide backbone than the positive CD maximum at 220 nm.

Finally, in TAA where only the first four oligomers are soluble, the total molar ellipticity values at 240 nm are linear function of chain length.

From the results presented in this paper we conclude that an ordered structure might be formed by oligophenylalanines in TFE and HFIP ($n \geq 6$). It is interesting to note that in both solvents the CD patterns of the structured higher members of the oligopeptide series do not correspond to any of the CD spectra typical of ordered conformations of nonaromatic polypeptides (*15*), nor to the spectrum of ordered poly-L-phenylalanine (*4*, *16*). However, in light of these data (*4*, *16*), our results seem to indicate that the ordered structure which begins to form at $n = 6$ in TFE or HFIP is not a right-handed α-helix. From the theoretical and experimental data available (*4*, *8*, *9*, *16–23*), it appears

that one can also clearly exclude the left-handed helical conformation for the structured oligophenylalanines. Hence, we suggest that phenylalanine oligomers tend to assume an associated β-structure in these fluoroalcohols. Our CD concentration studies, although not sufficient to justify a definite conclusion, seem to confirm our conformational analysis (*24*). This hypothesis is in agreement with previous investigations carried out by different authors on homo-oligopeptide systems. In fact, oligo-L-alanines (*25*), oligo-L-isoleucines (*26*, *27*), and oligo-γ-benzyl-L-glutamates (*28*, *29*) have heretofore been observed to exist in associated β-conformations in solution.

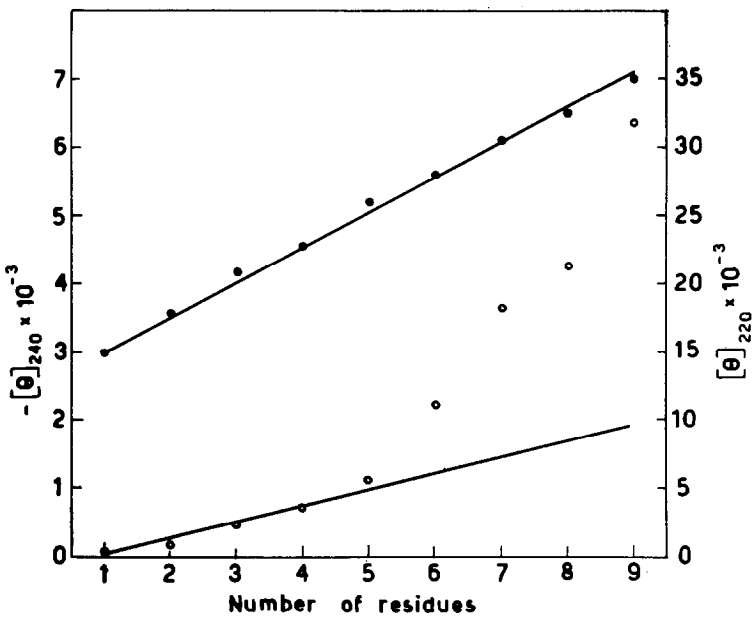

FIG. 5. Plot of total molar ellipticity values of BOC-(L-Phe)$_n$-OMe in hexafluoroisopropanol at 240 nm (—○—) and at 220 nm (—●—).

By contrast, Engel *et al.* (*30*) suggest that a randomly coiled conformation is assumed by protected L-tyrosine oligopeptides ($n = 1$–12) in 1,2-propanediol, where the homopolymer exists as a right-handed helical structure. This conclusion has been drawn particularly from the continuous and gradual variation of residue molar ellipticity values with n. However, it is impossible to reconcile these data with an ordered conformation for hexa-L-tyrosine, which was proposed on the basis of fluorescence polarization spectra (*31*). In this context, our data suggest that conformational assignments are much more difficult in the case of oligotyrosines than in the case of oligophenylalanines; in fact, the negative CD band near 235 nm present in the latter, which is extremely sensitive to structural changes, is totally absent in the former. This behavior results from the fact that in tyrosine peptides, in contrast to phenylalanine peptides, the aromatic ^1L_a band is lower in energy than the $n \rightarrow \pi^*$ peptide transition (*17*).

In addition to the intrinsic interest of phenylalanine oligopeptides, a further reason for careful consideration of their CD properties as related to conformation is the current strong interest in Cotton effects, both in the near- and far-uv regions, associated with

this amino acid residue. Phenylalanine is the only aromatic amino acid in a number of peptide hormones, antibiotics and protein fragments such as the cyclic peptides Gramicidin-S (*32–35*) and antanamide (*36, 37*), and linear peptides, bradykinin (*38–43*) and S-peptide of RNase A (*40, 44–48*). In particular, the linear nonapeptide hormone bradykinin also exhibits a substantial negative CD band near 235 nm accompanied by a positive band near 220 nm (*38–43*). Brady et al. (*38, 39*) interpreted these data as indicating that bradykinin exists in aqueous solution as a "nearly random coil"; in fact, these authors considered as conclusive that the spectrum of the hormone is similar, as far as position and sign of Cotton effects are concerned, to the spectra of ionized poly-L-lysine and poly-L-glutamic acid (*15*). Phenylalanine homo-oligopeptides in partially ordered conformation (Figs. 1 and 2) exhibit CD patterns in the 210–250 nm region similar to that of ionized polypeptides. Thus, a conformational assignment for bradykinin based only on position, sign, and magnitude of dichroic bands in this spectral region is not completely reliable. This point of view is confirmed by recent calculations carried out by Cann (*41*). In conclusion, our results represent a clear warning in assigning uncritically the conformations of polypeptides containing phenylalanine residues merely on the basis of a single CD curve.

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