Vacuum Ultraviolet Circular Dichroism of Protected Homooligomers Derived from L-Leucine

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Abstract: The vacuum ultraviolet circular dichroism is reported for films of BOC(L-Leu)$_n$OMe, where n = 2–7. The hexamer and heptamer take up a β conformation in which both parallel and antiparallel chains are present. The results are compared with an earlier study on alanine, valine, and norvaline oligomers.

The vacuum ultraviolet circular dichroism (VUCD)[2] of films of oligopeptides having the general formula BOC(L-X)$_n$OMe, where X = Ala, Val, or Nva and n = 2–7, has been reported previously.[3,4] The β conformation was found to develop in a significant amount in all three series in films cast from TFE, at the trimer in the alanine series, the hexamer in norvaline, and the heptamer in valine. Based on a comparison of the observed CD from 170 to 200 nm with calculated spectra[5-8] for parallel and antiparallel sheets, it was concluded that the β conformation which develops in the case of alanine contains antiparallel chains, whereas parallel chains predominate in the valine series. The norvaline heptamer conformation was assigned to a mixture of parallel and antiparallel chains in approximately equal amounts. Those conclusions are also in agreement with published infrared data for the same series of peptides.[9]

It was also suggested in the earlier work[3] that the type of β sheet that is favored, either parallel or antiparallel, is determined by two structural features of the side chain, its overall bulkiness and the presence of branching at the β-carbon position. Bulkiness and β branching were both suggested to favor the parallel sheet, as in the case of valine. If these two features are in fact the determinants, then it is expected that the behavior of leucine oligomers should resemble that of the norvaline series, since the side chains of both are moderately bulky, but β branching is absent. We report here experimental VUCD measurements for the leucine series which indicate that the β conformation which is formed is indeed similar to that formed by norvaline oligomers, namely one which contains both antiparallel and parallel chains.

Experimental Section

The details of the synthesis and the chemical and optical characterizations of BOC(L-Leu)$_n$OMe where n = 2–7 are described in ref 10. TFE solutions (2 mg/mL) were allowed to stand for 48 h. Films were cast on 1-mm thick CaF_2 disks by evaporation of the solution to dryness in a nitrogen-filled glove bag at 23 °C. The operation of the spectrometer was described previously.[3,11] Tracings of actual recorded spectra are presented in Figures 1 and 2.

We scaled our ellipticity data to an approximately uniform absorbance of unity by calibrating the photomultiplier gain at the wavelength of maximum absorption. Such a procedure corresponds to calibrating the dynode voltage on commercial instruments. This estimate agreed with the absorption measured on a Cary 14 absorption spectrometer assuming a uniform film thickness. Our results are therefore reported in Figures 1 and 2 in terms of total ellipticity, θ, in units of degree, scaled to an (approximately) uniform maximum absorbance of unity.

Results and Discussion

Figure 1 shows the film CD of BOC(L-Leu)$_2$OMe and BOC(L-Leu)$_3$OMe from 150 to 230 nm. The trimer spectrum above 190 nm resembles the contribution to CD from an internal Leu–Leu peptide chromophore within a randomly coiled chain.[12] Throughout the region from 150 to 230 nm the trimer spectrum is very similar to the one reported previously[3] for BOC(L-Nva)$_2$OMe. The spectrum of BOC(L-Leu)$_2$OMe shown in Figure 1 is rather unusual. Since the CD of BOC(L-Ala)$_2$OMe, BOC(L-Val)$_2$OMe, and BOC(L-Nva)$_2$OMe previously reported[3] resembled the contribution to CD from an internal peptide chromophore within a randomly coiled chain,[12] the CD of BOC(L-Leu)$_2$OMe probably reflects a specific ordered structure in the film of the dimer. The significant features in the CD of the dimer are (a) a positive band near 218 nm, (b) a pair of oppositely signed bands centered around 194 nm (the absorption maximum), and (c) a negative band near 173 nm. It is worth noting that this difference between the film CD of the leucine dimer and the other dimers does not appear in TFE solution.

Figure 2 shows the CD of the tetramer through heptamer. The tetramer and pentamer are largely disordered; their CD is similar to that of the trimer (note the difference in the scales of Figures 1 and 2). There is a large difference in CD on going from the pentamer to the hexamer. The large positive CD band at 199 nm indicates that there is a substantial amount of β conformation in the film of the hexamer. In the heptamer the positive band is even larger, so that the CD spectrum above 190 nm is that characteristic of a well-developed β conformation. With respect to the positions of extrema and crossovers, the spectrum of BOC(L-Leu)$_7$OMe closely resembles that of BOC(L-Nva)$_7$OMe. There is a positive band near 152 nm, a crossover near 163 nm, and a negative band near 178 nm in the spectra of both heptamers. Another crossover appears at 188 nm in the leucine heptamer; in the heptamer of norvaline a crossover occurs at 186 nm.[3] The intensity of the heptamer band near 200 nm is twice that of the hexamer for both leucine (Figure 2) and norvaline.[3] The 200-nm band of the norvaline heptamer is somewhat larger than that of the leucine band, a difference which shows up in TFE/H_2O solutions as well.[13] Intensities are largely determined by the polarizabilities of the side chains, rather than the relative orientation of the backbone chains. In terms of the positions of the extrema and crossovers, which are most reflective of the conformation, the spectra of BOC(L-Leu)$_7$OMe and BOC(L-Nva)$_7$OMe are virtually identical.

In previous work[3] it has been shown that the norvaline heptamer spectrum can be closely reproduced by a composite spectrum made up of the CD of alanine and valine heptamers, equally weighted. The similarity of the leucine heptamer CD to that of the norvaline heptamer indicates that it too is intermediate between the CD of the alanine and valine heptamers. If the assignments of the alanine and valine heptamer conformations are made to the antiparallel and parallel sheets, respectively, the intermediate nature of the leucine and norvaline spectra makes it very likely that the leucine and norva-

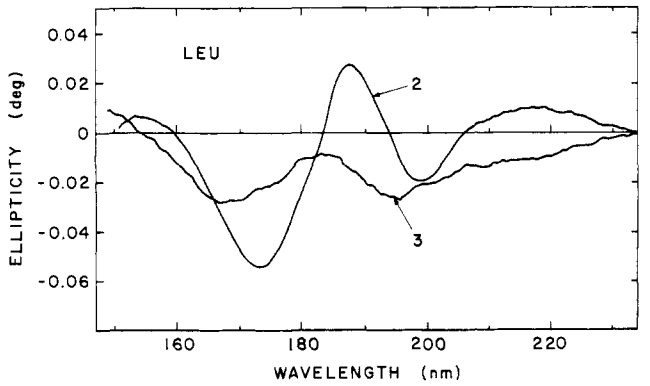

Figure 1. Circular dichroism of BOC(L-Leu)$_2$OMe and BOC(L-Leu)$_3$OMe.

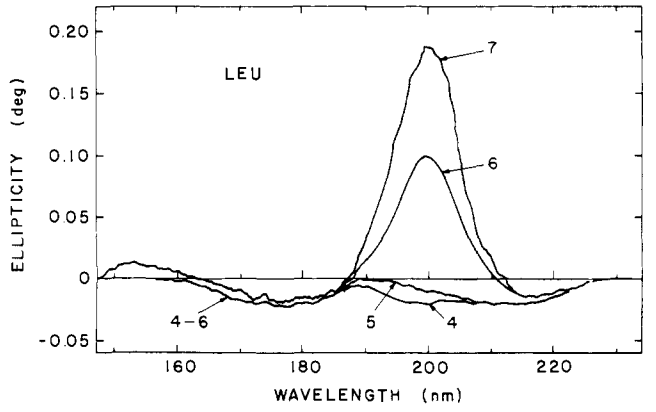

Figure 2. Circular dichroism of BOC(L-Leu)$_n$OMe, n = 4-7.

line heptamer conformations contain both parallel and antiparallel chains. That is, with respect to any given chain, the probabilities that a neighboring chain is oriented parallel or antiparallel to it are both large.

Assignment of the leucine conformation to a mixture of parallel and antiparallel chains is also consistent with infrared data. The N-deblocked leucine heptamer[9] shows a weak infrared shoulder near 1690 cm^{-1}. In the corresponding alanine heptamer a discrete band occurs at 1694 cm^{-1}, and in the valine heptamer neither a band nor a shoulder appears in that region. Komoto et al.[14] also observed a weak band near 1685 cm^{-1} during the early stages of polymerization of leucyl N-carboxyanhydride initiated by butylamine in acetonitrile.

In the four β sheets studied to date a CD crossover has been observed between 178 and 192 nm. The location of this CD crossover may provide the most sensitive criterion for distinguishing between the two types of β sheet. A crossover near 178 nm occurs when the chains are predominantly antiparallel, as in alanine. Crossovers at 186 (norvaline), 188 (leucine), and 192 nm (valine) indicate increasing amounts of parallel chains. Parallel β chains are probably predominant in the unblocked valine heptamer,[9] since the infrared band near 1690 cm^{-1} characteristic of antiparallel β chains is absent.

In terms of a linear scale the β conformation of the norvaline heptamer would be described as having equal amounts of antiparallel and parallel chains, and that of leucine heptamers as having somewhat more than half its pairs of chains in a parallel orientation.

Theoretical calculations of the CD of β sheets carried out by several groups[5-8] indicate that there are good grounds for expecting such a relationship between the position of the crossover and the relative amounts of parallel and antiparallel chains. Figure 3 indicates in a very schematic way the overall results of the theoretical CD calculations.[5-8] In both types of sheet a negative CD band is expected near 220 nm originating

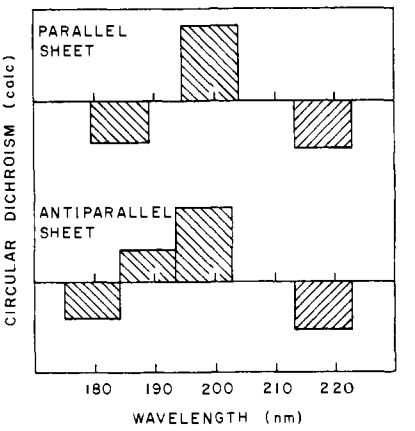

Figure 3. Schematic representation of the calculated CD of antiparallel and parallel β conformations, extracted from the results published in ref 5-8.

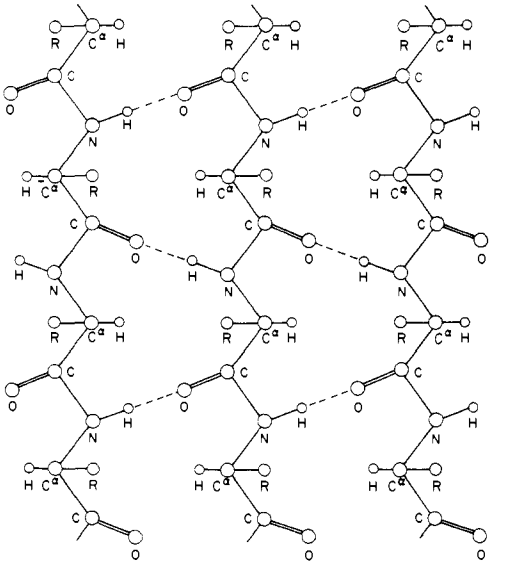

Figure 4. Schematic representation of the parallel β sheet.

in the n-π^* amide transition; no significant differences are expected between parallel and antiparallel sheets in this region. However, the CD arising from the π-π^* transition dominates the region from 175 to 200 nm, and the splitting pattern of that transition is different in the two sheets below 195 nm, although similar above that region. That is, in both sheets a large positive band is expected near 200 nm originating from the component of the π-π^* transition polarized parallel to the chain. Although that band is expected to be at slightly lower energy in the parallel sheet than in an antiparallel sheet (as is in fact observed in the valine and alanine spectra), the difference is small. On the other hand, the component polarized perpendicular to the chain shows weak positive CD in the antiparallel sheet (Figure 3) and negative CD in the parallel sheet; i.e., the distinction involves a difference in the sign of the CD near 190 nm. This can explain the difference between the CD of alanine (positive near 190 nm) and valine (negative near 190 nm). The crossover will occur at higher wavelengths in a parallel sheet than in an antiparallel sheet (Figure 3).

In a qualitative sense a predominance of parallel chains appears to be favored both by an overall bulkiness of the side chain and by the presence of β branching in the side chain. To account for this behavior we propose the following picture. In both the parallel and antiparallel pleated sheets (Figures 4 and 5, respectively) the C-O ···H-N bonds are linear; in the antiparallel sheet 10- and 14-membered rings are formed and in

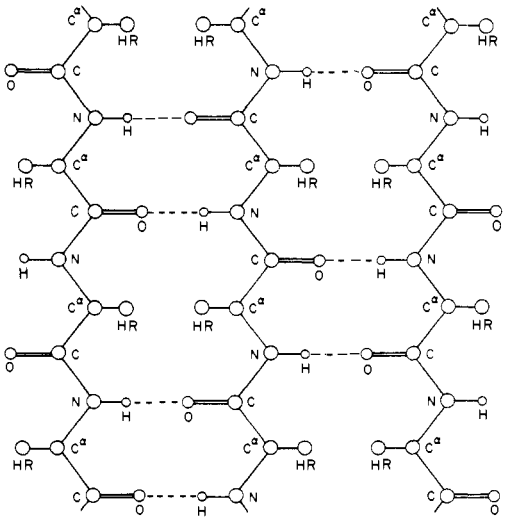

Figure 5. Schematic representation of the antiparallel β sheet. Note the side chain–side chain close contact in neighboring segments.

the parallel sheet 12-membered rings. In the antiparallel sheet these bonds are perpendicular to the axis of the peptide chain, whereas in the parallel sheet they are not. The C_α–C_β bonds are perpendicular to the mean plane of the sheet in the antiparallel arrangement, but not in the parallel sheet, and the chains are also more extended in the antiparallel sheets. In particular, in the absence of a C_β atom (poly-gly), the latter feature makes the antiparallel sheet the favored arrangement. When bulky side chains are present, however, another structural feature becomes the overriding factor; namely the unfavorable contact in the antiparallel arrangement between side chains of neighboring segments (compare Figures 4 and 5). This short distance, analogous to the 1,3-axial–axial interactions in cyclohexane derivatives, is absent in the parallel sheet. Thus, the β sheet which is favored in the case of oligopeptides containing β-branched amino acid residues (Val, Ile) is the parallel sheet, and bulkiness in general increases the tendency to form the parallel arrangement.

In the present case of leucine oligopeptides, there is a substantial number of parallel chains in the β sheet which is formed. This increased tendency for parallel chain alignment when side chains are bulky has also recently been observed in a study of the stereoselective dimerization of model dipeptide molecules carried out by Cung et al.[15] They find intermolecular association of a model alanine dipeptide in which the associated chains are antiparallel; in the case of the association of leucine dipeptides they observe parallel alignment, and cite the same steric factor to account for their results as we invoke here.

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References and Notes

(1) (a) Brown University; (b) University of Padova.
(2) The following abbreviations are used: CD (circular dichroism), VUCD (vacuum ultraviolet circular dichroism), BOC (*tert*-butyloxycarbonyl), OMe (methoxy), Ala (alanine), Leu (leucine), Nva (norvaline), Val (valine), gly (glycine), TFE (2,2,2-trifluoroethanol).
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