Conformational Characterization of Terminally Blocked L-(αMe)Val Homopeptides Using Vibrational and Electronic Circular Dichroism. 3_{10}-Helical Stabilization by Peptide−Peptide Interaction

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Abstract: Vibrational and electronic circular dichroism (VCD and ECD) and Fourier transform infrared (FTIR) spectra of the homo-oligopeptide series Z-[L-(αMe)Val]$_n$-OtBu (n = 3−8) and selected Ac-[L-(αMe)Val]$_n$-OtBu oligomers (n = 4, 6, 8) are presented. This is the first VCD study of a complete homopeptide series formed exclusively by C$^\alpha$-methylated amino acids. VCD spectra were measured for the oligomers in 2,2,2-trifluoroethanol (TFE) and $CDCl_3$ over the amide I and amide II spectral regions (1750−1475 cm^{-1}). These oligopeptides, irrespective of the N-terminal group, were found to indicate formation of at least a partially 3_{10}-helical conformation for main-chain lengths as short as n = 4 and a fully developed 3_{10}-helix by n = 6 at high peptide concentrations. A 3_{10}-helical conformation for the octamer is consistent with previous spectroscopic studies and crystallographic results. The ECD spectra were measured for the oligomer series in TFE and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) over the 260−190 nm region. The ECD spectra, again for both N$^\alpha$-blocking groups, indicate a helical structure for the octamer, a mixed ordered/unordered structure at n = 6, and a predominantly coil form for n = 4. The octamer ECD band shape and FTIR absorption maximum are concentration dependent. At higher concentrations, the ECD mimics that which has been associated with a 3_{10}-helical conformation, while at lower concentrations the ECD is more typical of an α-helix. A study of the octamer in HFIP indicates a gradual transition from the 3_{10}-like to α-helical-like ECD spectra with time. While indicating the need for further study, these data are the first evidence of the possibility of a 3_{10}-helix to α-helix equilibrium shift induced by a change in peptide−peptide interactions, with aggregation favoring the 3_{10}-helical form.

Introduction

It has been shown that homo-oligomers consisting of C$^\alpha$-methylated α-amino acids exhibit a preference toward the formation of stable 3_{10}-helical structures.[1] X-ray and vibrational circular dichroism (VCD) studies of these compounds reveal that C$^\alpha$-methylated L-amino acids with linear and β-branched side chains tend to induce mainly a right-handed helical form, while there is a tendency for the opposite result (left-handed helical form) to occur with those compounds containing C$^\alpha$-methylated L-amino acids with γ-branched side chains.[1,2] In earlier studies of oligomers containing a high fraction of α-aminoisobutyric acid (Aib), VCD has additionally proven to have a unique ability to characterize and discriminate between α-helical and 3_{10}-helical secondary structural types.[3−6]

Conventional electronic CD (ECD), measured in the UV, is very sensitive to the sense of the helix, but can be affected by the presence of certain blocking groups, in particular, aromatic chromophores. In this regard, the contribution of the N-terminal *p*-bromobenzamido chromophore (*p*BrBz) in ECD has been exploited as a probe for the assignment of the screw sense of helical peptides.[7,8] VCD, on the other hand, is largely unaffected by the nature of the blocking group. Its most diagnostic bands are amide vibrational modes whose resultant band shapes arise from interamide coupling within the chain.[4,9,10] In an earlier study,[11] Toniolo and co-workers demonstrated experimental realization of an ECD band shape discrimination between

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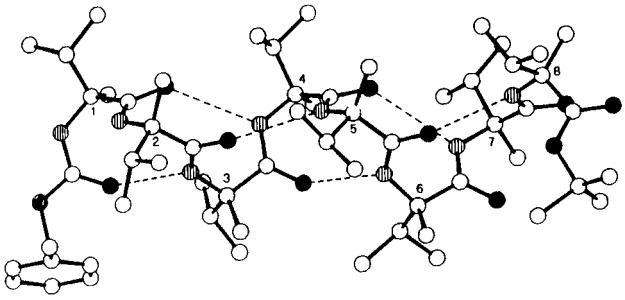

Figure 1. X-ray structure for Z-[L-(αMe)Val]$_8$-OtBu sequence (refs 11 and 15).

α-helical and 3_{10}-helical conformations that was originally suggested by theoretical calculations of Manning and Woody.[12] This test correlates these two structures with the ratio of the CD intensity of the peptide $n \rightarrow \pi^*$ transition at 222 nm to that of the parallel component of the exciton-split peptide $\pi \rightarrow \pi^*$ transition occurring at 207 nm ($R = [\theta]_{222}/[\theta]_{207}$). Its success stands in contrast to earlier ECD analyses by Balaram and co-workers[13] who interpreted their experimental results, which in fact qualitatively follow the Toniolo et al. model,[11] as failing to distinguish between the two conformations by ECD. A recent report by Andersen and co-workers[14] urges caution in using $[\Theta]_{222}/[\Theta]_{207}$ values smaller than unity as diagnostic of 3_{10}-helices and raises helix length effects as another potential source of ECD distortions. It was evident that a more detailed study reconciling these observations was needed. The sensitivity of VCD to 3_{10}-helix formation and its relatively short-range length dependence suggested a possible resolution of some of these issues. Furthermore, we propose an expansion of the criteria for stable 3_{10}-helix formation to encompass concentration effects.

Herein we describe the VCD and ECD properties of the homopeptide series Z-[L-(αMe)Val]$_n$-OtBu ($n = 3-8$) along with selected Ac-[L-(αMe)Val]$_n$-OtBu oligomers ($n = 4, 6, 8$) [Z = benzyloxycarbonyl; Ac = acetyl; OtBu = *tert*-butoxy; (αMe)Val = C^α-methylvaline] based on the C^α-methylated, β-branched amino acid (αMe)Val. Both X-ray crystal structures as well as ^{1}H NMR and FTIR absorption solution studies indicate that the terminally blocked Z-[L-(αMe)Val]$_8$-OtBu peptide maintains a stable right-handed 3_{10}-helical conformation under various conditions.[1,11,15] Its crystal structure is depicted in Figure 1. Right-handed 3_{10}-helices are characterized by 1 ← 4 intramolecular C═O···H−N hydrogen bonds and mean φ,ψ backbone torsion angles of −57°, −30°.[16,17] The backbone torsion angles for Z-[L-(αMe)Val]$_8$-OtBu, based on the crystal structure, were found to be very close to these values.[1,11,15] Utilizing VCD, FTIR, and ECD spectroscopic techniques, we correlate ECD patterns with an independent determination of 3_{10}-helical formation, effectively decreasing concentration from the well-established structure under the crystal conditions. The effect of peptide−peptide interactions is analyzed via concentration dependent ECD and FTIR studies of the octapeptide.

Experimental Section

Materials. The synthesis and characterization of members of the homopeptide series Z-[L-(αMe)Val]$_n$-OtBu ($n = 3-8$) and Ac-[L-(αMe)Val]$_n$-OtBu ($n = 4, 6, 8$) are described in reference 15. The solvents used for spectroscopic study include 2,2,2-trifluoroethanol (TFE), deuteriochloroform ($CDCl_3$), and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), which were obtained from Aldrich and used without further purification.

Vibrational CD. VCD spectra were recorded using a newly constructed dispersive VCD instrument whose design reflects several concepts put forth for a compact spectrometer by Diem and co-workers.[18] The instrument utilizes a 0.3 m focal length monochromator (McPherson 218) operated with 2 mm slit widths. Fast f/5.2 light collection is used to improve throughput, and collimation of the beam, through the polarizing optics and sample, is used to reduce spectral artifacts. This instrument incorporates a sensitive MCT detector and high-temperature carbon rod light source to improve signal-to-noise ratio (S/N).[19]

Samples for the homo-oligopeptide series Z-[L-(αMe)Val]$_n$-OtBu ($n = 3-8$) were prepared in $CDCl_3$, while those for selected Ac-[L-(αMe)-Val]$_n$-OtBu oligomers ($n = 4, 6, 8$) were prepared in TFE, both having a solution concentration of ∼5 mg/100 μL. The need to measure each series in a different solvent was a direct consequence of the impact of these two N^α-blocking groups on the peptide solubility. After the samples were prepared, they were immediately transferred to a rectangular demountable cell composed of two CaF_2 windows separated by a 25 μM Mylar spacer (Graseby Specac). VCD spectra were collected using a 10 s time constant as the average of four scans. An identical number of scans of just the solvent were collected and averaged for the purpose of base line correction and subsequently subtracted from the averaged sample spectrum. Calibration of the VCD spectra was achieved utilizing our standard methodology.[20,21] Dispersive absorption spectra were also obtained under identical conditions for purpose of normalization.

IR Absorption. IR absorption spectra were also recorded over the fully accessible mid-IR at 4 cm^{-1} nominal resolution with a BioRad-Digilab FTS-40 (or the equivalent) Fourier transform IR (FTIR) spectrometer by averaging 1024 scans. Spectra were measured from the same samples used for VCD studies. Additional concentration-dependent FTIR spectra were obtained by sequential dilution of selected oligopeptides in TFE or $CDCl_3$ and by placement of the samples in cells with progressively thicker spacers (up to 1mm).

Electronic CD. The ECD spectra for both the Z-[L-(αMe)Val]$_n$-OtBu ($n = 3-8$) and Ac-[L-(αMe)Val]$_n$-OtBu ($n = 4, 6, 8$) peptide series were measured in TFE and HFIP using a Jasco model J-600 spectropolarimeter (Chicago) routinely at concentrations of ∼0.1 mM (1 mm cell) and ∼1.0 mM (0.1 mm cell) over the 260−190 nm region. The ECD concentration studies used various cells ranging from 6 μm to 1 mm in path length, the former using the same construction as for the IR experiments. The TFE concentration dependence results were confirmed over a wider concentration range and extended in terms of further studies of the effects of time from dissolution in HFIP using a Jasco J-720 instrument (Padova). The data are expressed in terms of [Θ], the molar ellipticity (deg cm^2 dmol^{-1}) per residue.

Results

Vibrational CD and FTIR Absorption. The IR absorption and VCD spectra for the Z-[L-(αMe)Val]$_n$-OtBu ($n = 3-8$) and selected Ac-[L-(αMe)Val]$_n$-OtBu oligomers ($n = 4$, 6, and 8) series in $CDCl_3$ and TFE solutions are shown in Figures 2 and 3, respectively. For the sake of comparison, all VCD spectra were normalized to have a peak absorbance of 1.0 for the amide I. Interference from absorbance bands of the C-terminal ester and the N-terminal urethane blocking groups (1710−1720 cm^{-1}) can lead to some error in this absorbance-based effective concentration correction, which is a larger problem for the shorter oligomers. Thus, the plot reads $\Delta A/A$ directly on the amide I absorbance peak and offers an approximate normaliza-

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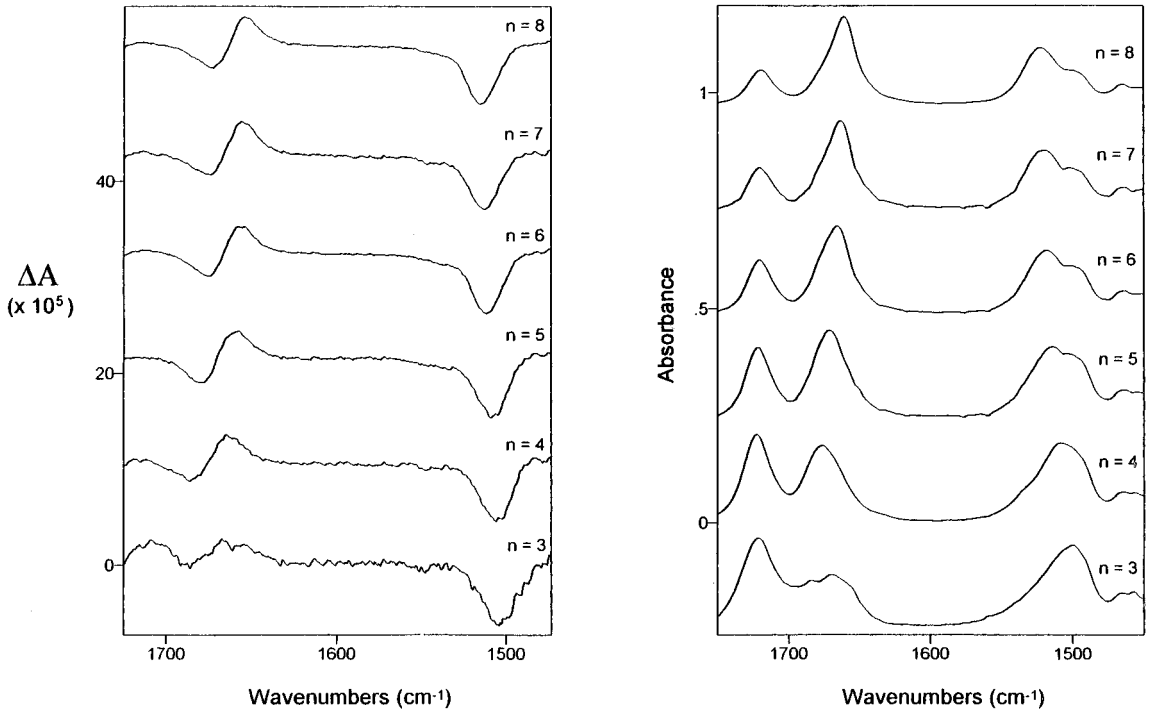

Figure 2. Amide I and amide II VCD and FTIR data in $CDCl_3$ for the Z-[L-(αMe)Val]$_n$-OtBu series ($n = 3-8$).

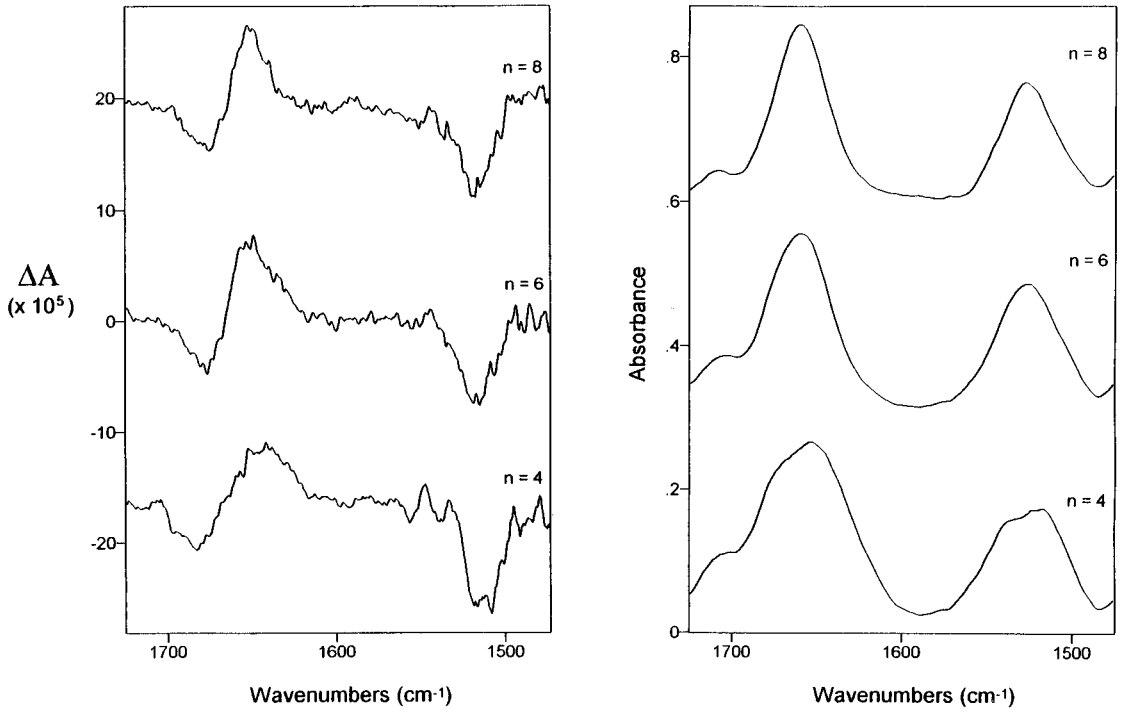

Figure 3. Amide I and amide II VCD and FTIR data in TFE for the Ac-[L-(αMe)Val]$_n$-OtBu series ($n = 4, 6, 8$).

tion of the VCD for peptide content of oligomers of different lengths. This is comparable to the typical method of plotting ECD in molar ellipticity per residue. For all but the shortest peptide ($n = 3$) in the Z-blocked oligomer series in $CDCl_3$, the amide I VCD band is a nearly conservative positive couplet, negative to higher energy (Figure 2). The zero-crossing point steadily shifts from about 1675 to 1665 cm^{-1} with increasing peptide main-chain length. This finding parallels previous observations (as can also be seen explicitly in the FTIR spectra reported here) for the absorbance maximum of the amide I.[6,15] This parallel of absorbance with VCD suggests that the interference effects of the blocking groups on the absorbance are minimal. As is characteristic of a 3_{10}-helix,[2,3,5,6] the amide II VCD is more intense than the amide I VCD, yielding a strong negative band with its maximum between 1504 and 1514 cm^{-1} and with its frequency increasing (moving closer to the amide I) with increasing main-chain length.

VCD spectra were also measured for the series Ac-[L-(αMe)-Val]$_n$-OtBu ($n = 4, 6, 8$), but with TFE as the solvent, to determine what effect, if any, the N^{α}-blocking group might have on the conformation and resulting VCD spectra (see Figure 3). The characteristic VCD spectral profiles for the Ac-blocked series were generally comparable in shape and intensity to those of the Z-blocked oligomer series with some exceptions in detail, while the Ac-blocked peptides have broader IR absorption bands. In general, the amide I VCD zero-crossing shift is minor,

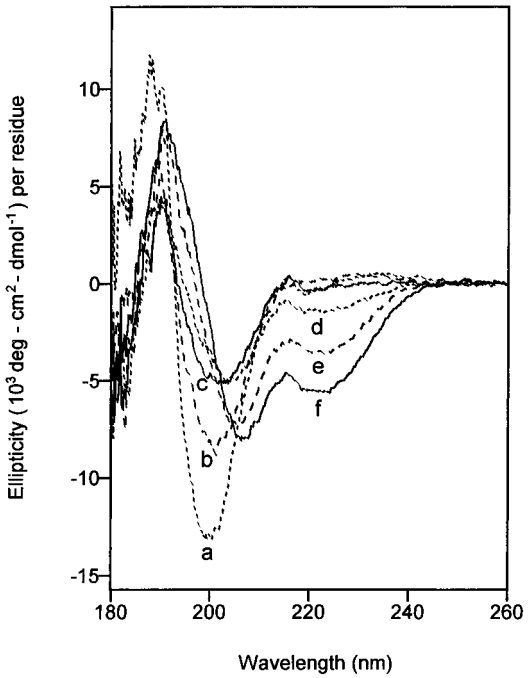

Figure 4. ECD spectra for the Z-[L-(αMe)Val]$_n$-OtBu series (n = 3−8; a−f, respectively) in TFE (∼0.1 mM) over the spectral range of 260−190 nm.

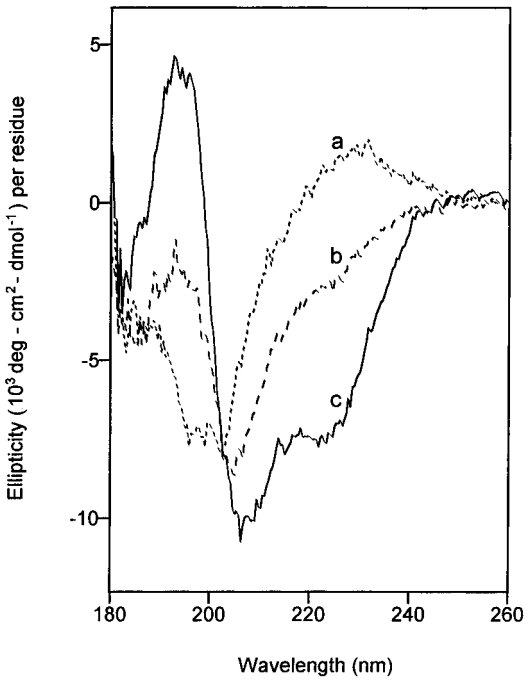

Figure 5. ECD spectra for the Ac-[L-(αMe)Val]$_n$-OtBu series (n = 4, 6, 8; a, b, c, respectively) in TFE (∼0.1 mM) over the spectral range of 260−190 nm.

being most evident in comparison of the n = 6 and 8 oligomer spectra with that of the n = 4 oligomer, which yields a much broader and weaker couplet. In addition, the amide II VCD intensity is not as dominant, being more similar to that of the amide I, and accompanied by a reduction in S/N. However, the S/N reduction can be attributed to the interference of the TFE absorbance, as compared to $CDCl_3$, which becomes more significant in the amide II region. On the basis of this observation, it appears that the nature of the N^{α}-blocking group has little discernible effect on the VCD spectra of these L-(αMe)-Val homo-oligopeptides and that both Ac- and Z-blocking result in oligomers with virtually the same degree of well-developed structure in these two solvents at this concentration (∼50 mM), at least for $n \geq 5$.

Electronic CD. ECD spectra were first measured for the Z-[L-(αMe)Val]$_n$-OtBu (n = 3−8) oligomer series under dilute (∼0.1 mM) conditions in TFE (Figure 4). The spectra of the longest oligopeptides show a negative maximum at ∼225 nm (peptide $n \rightarrow \pi^*$ transition) with a somewhat larger negative maximum occurring at ∼208 nm (parallel component of the exciton-split peptide $\pi \rightarrow \pi^*$ transition). This is followed by a positive maximum of similar magnitude at ∼190 nm (perpendicular component of the exciton-split peptide $\pi \rightarrow \pi^*$ transition) which tends to fall beyond the range of reliable ECD measurement. The absolute magnitude of the molar ellipticity changes nonlinearly with decreasing peptide main-chain length, which is an indication of decreasing structural stability for the shortest oligopeptides. The extent and direction of this change for the 225 nm band are very different from those of the 208 nm band. As compared to the shorter oligomers (n = 5, 6), the n = 7 and 8 oligomers have a more intense parallel $\pi \rightarrow \pi^*$ band whose position is shifted to longer wavelengths. This may indicate a continuing conformational transition at oligomer lengths longer than where a stable structure formation was suggested by the VCD spectral results, or it may be a manifestation of the longer-range length dependence of ECD.[10] Certainly, the negative ECD intensity having a maximal excursion below 200 nm for the shorter oligomers (n = 3−5) would be consistent with there being significant contribution from disordered components in those structures under these dilute conditions.[14,22]

Although the ECD spectra for the longest Z-blocked oligomers are indicative of peptide species having highly helical secondary structural character, they are somewhat different from those previously published for the Ac-[L-(αMe)Val]$_8$-OtBu peptide.[11,15] In order to test the origin of this deviation, the ECD of the Ac-blocked (n = 4, 6, 8) peptide series were remeasured under the same dilute TFE solution conditions (∼0.1 mM) as used for the Z-blocked oligomers. The pattern of band shape change in the Ac-blocked oligomers with increasing chain length (Figure 5) is qualitatively similar to that of the Z-blocked peptides. Importantly, however, the dilute longest oligopeptide (n = 8) yields an ECD pattern (particularly in the region above 200 nm) more typical of a partial α-helical structure than that proposed for the 3_{10}-helix.[11,15] However, the 195 nm band is still quite weak. The parallel behavior of the ECD for the Ac- and Z-blocked series in dilute TFE solution eliminates the aromatic blocking group (Z) as the major cause for the apparent spectral discrepancy (Figure 4) from the previous results,[11,15] but nevertheless it does imply that the Z-group has a minor contribution in this region, somewhat diminishing the negative ellipticity amplitude overall.

Consequently, the ECD of the Ac-blocked peptides were again remeasured under higher concentration conditions more closely mimicking those of the previously published results (0.6−1.0 mM).[11,15] While the spectra of the n = 4, and 6 oligomers change only slightly, the spectrum of the octamer changes substantially, particularly for the $n \rightarrow \pi^*$ band. This high-concentration result now agrees favorably with the previous reports[11,15] in both shape and intensity. On the basis of this observation, a detailed concentration study of the ECD spectra of both the Ac-[L-(αMe)Val)]$_8$-OtBu and Z-[L-(αMe)Val)]$_8$-OtBu peptides in TFE was performed (Figure 6 illustrates the Ac results). For the Ac-blocked octamers at high concentrations (0.5−5 mM), which encompass those conditions used for the

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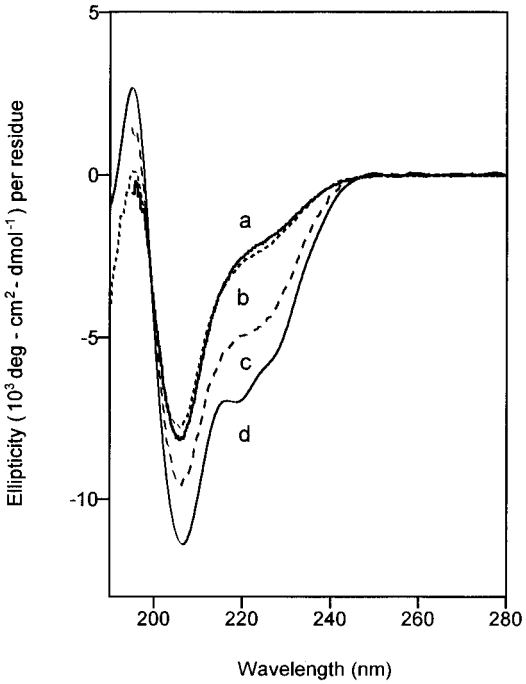

Figure 6. Concentration-dependent ECD spectra [(a) 6.15 mM; (b) 0.543 mM; (c) 0.0543 mM; (d) 0.0109 mM] for Ac-[L-(αMe)Val]$_8$-OtBu in TFE over the spectral range of 260−190 nm.

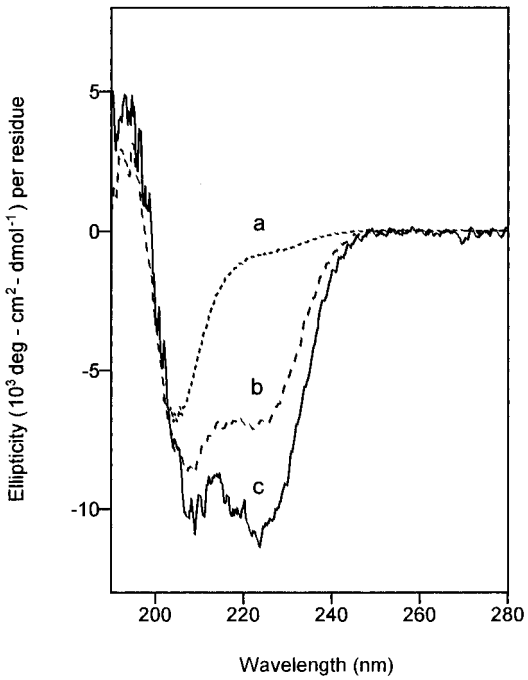

Figure 7. Concentration-dependent ECD spectra [(a) 1.17 mM; (b) 0.167mM; (c) 0.0197 mM] for Ac-[L-(αMe)Val]$_8$-OtBu in HFIP over the spectral range of 280−185 nm.

previously published work,[11,15] considerable agreement was observed with literature data, resulting in a relatively weak 222 nm band ($R \ll 1$) and very weak 190 nm band, two features which have been associated with 3_{10}-helix formation.[11,12,15] However, when the peptide concentration was reduced, the 222 and 190 nm bands increased in intensity. At the lowest concentrations studied, the ECD spectral band shapes obtained for this octamer changes to a more intermediate pattern in the near-UV, encompassing features characteristic of an α-helix with end fraying where both the 207 and 222 nm bands have significant intensity, although in all cases $R < 1$. In addition, the spectra in Figure 6 reflect an approximate isodichroic point centered at about 200 nm. Including spectra (not shown) measured with smaller concentration increments, the transition from one spectral type to another is seen to occur at ∼0.6 mM in octamer. However, the 190 nm feature remains very weak compared to model α-helical ECD spectra[12,23] even at the lower concentrations studied. This may indicate that the 190 nm feature could be a diagnostic for helical type, or it may just be symptomatic of increased end fraying at low concentration because the ECD of the coil component tends to counteract the helical contribution below 200 nm. Some aspects of the 190 nm ECD response are probably indicative of the difficulty in making reliable far-UV ECD measurements on more dilute samples, which we are forced to study using longer path length cells. A strictly comparable concentration effect on the ECD pattern was found for the Z-octamer in a parallel test experiment with the exception that the 225 nm $n \rightarrow \pi^*$ band had a somewhat different shape, which presumably is evidence for the residual effects of the Z-blocking group contributions. Thus, the nature of the blocking group did not have any appreciable consequences on the concentration variation of the resultant ECD spectra and presumably not on the variations in the underlying conformations leading to it.

To further study this ECD concentration effect on peptide conformation, the Ac-blocked octamer was studied in HFIP between ∼1 and 0.02 mM. The same change from a concentrated ECD spectrum with virtually no $n \rightarrow \pi^*$ (222 nm) intensity to one more like an α-helix is seen (Figure 7). In the latter case, however, $R \approx 1$ was obtained for the dilute species. The samples studied in Figure 7 were measured on fresh solutions, immediately after dissolution. However, when they were remeasured after standing, the spectra at all these concentrations had the general shape of the most dilute one in Figure 7. Done more systematically, on letting the solution stand at 4 °C, the spectrum of the concentrated sample gradually changed from having no $n \rightarrow \pi^*$ ECD to having a strong $n \rightarrow \pi^*$ ECD within a week (Figure 8) and to having $R > 1$ within a month after preparation. Such a remarkable time dependence of the ECD band shape has not been found with other solvent systems, although minor changes do occur in TFE solutions.

To afford a comparison of concentration effects in the ECD that imply variable structure with the well-defined, high-concentration 3_{10}-helical VCD response, we carried out a series of FTIR experiments on the Z-blocked octamer at various dilutions in $CDCl_3$ and TFE. As we have reported, in $CDCl_3$, the N−H stretch varied little over the range from 0.1 to 1.0 mM in octamer concentration.[15] Similarly, in $CDCl_3$ the amide I showed no change in frequency or width from 2 to 0.02 mM. However, in TFE the amide I band showed slight broadening to low frequency upon dilution from 4 to 0.9 mM in octamer. (TFE interference prohibited reliable solvent subtraction at higher dilution. Attempts to do similar tests in HFIP had even worse solvent subtraction problems.) The difference in TFE and $CDCl_3$ response and solvent subtractability is most likely a manifestation of the effects of hydrogen bonding of the solvent to the peptide and increased end fraying under more dilute conditions in hydrogen-bonding solvents.

Discussion

Helical Stability and Length Dependence. The L-(α-Me)-Val-based peptides studied here have been established to have uniform 3_{10}-helical structures in the crystal state.[15] All other spectral evidence indicates a maintenance of that structure in solution at least for the longer oligomers. For example, the

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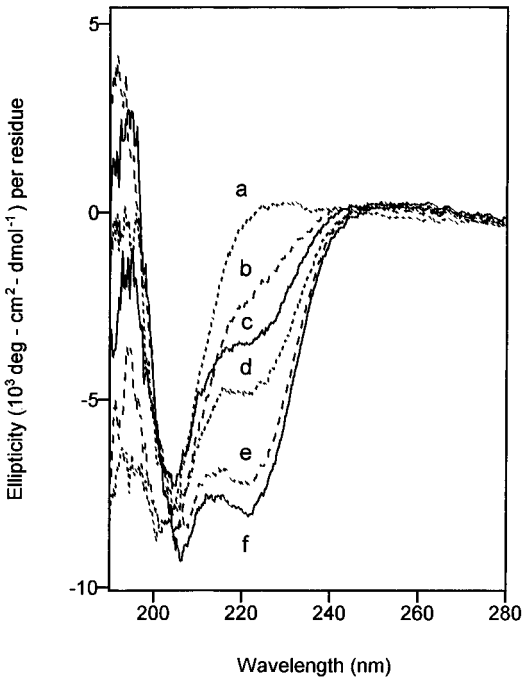

Figure 8. Time-dependent ECD spectra [(a) $t = 0$; (b) $t = 1$ day; (c) $t = 2$days; (d) $t = 3$ days; (e) $t = 6$ days; (f) $t = 7$days] for Ac-[L-(αMe)Val]$_8$-OtBu in HFIP over the spectral range of 280 −190 nm. Peptide concentration = 1.47 mM.

amide I and II bands for the octamer in the solid state are at 1654−1657 and 1522−1529 cm^{-1}, respectively, while those same bands at high concentration in various solvents ($CDCl_3$, TFE, HFIP) range from 1658 to 1662 cm^{-1} and 1522 to 1526 cm^{-1}, respectively. Such an overlap is inconsistent with any significant structural change upon solvation, at least in these relatively concentrated environments. Since we have previously shown VCD to uniquely sense 3_{10}- vs α-helical formation[3−6] and C$^\alpha$-methylated peptides to have a unique ECD pattern that may be characteristic of 3_{10}-helical formation,[11] this study expands previous work to correlate VCD and ECD spectral data for these molecules. Two variables, length of the peptide and concentration, were a prime focus here with two others, the solvent and N$^\alpha$-blocking group effects, being of additional interest.

The amide I and II VCD data presented above for the longer ($n > 4$) oligomers ($CDCl_3$ and TFE as solvents, high peptide concentration) are comparable in shape and intensity to those we have previously established for a number of other 3_{10}-helical oligopeptides[2,3,5,6,24] and are qualitatively different from those spectral features as found in the VCD of α-helical structures.[4,9,10,25−31] The 3_{10}-helix exhibits a more intense amide II and a significantly weaker amide I band than is encountered with α-helical molecules. Additionally, the 3_{10}-helical amide I VCD band has a near conservative couplet shape with a positive bias, if any, in contrast to the strongly negatively biased couplets typically encountered with α-helical molecules.[25−31]

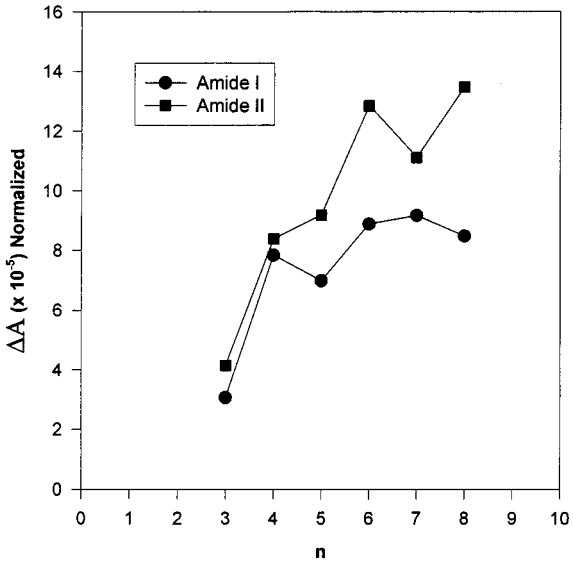

Figure 9. Δ*A*/*A* for amide I and amide II data for the Z-[L-(αMe)-Val]$_n$-OtBu ($n = 3-8$) in $CDCl_3$.

Consequently, the VCD observations confirm that in both $CDCl_3$ and TFE solvent systems and for both Z- and Ac-N$^\alpha$-blocking groups, at least the higher oligomers maintain the dominant 3_{10}-helical conformation in concentrated solution that they exhibit in the crystal state.[15]

The Z-blocked [L-(αMe)Val]$_n$ oligomers achieve a stable structure and yield a uniform VCD band shape pattern and intensity at relatively short main-chain lengths ($n \geq 4$, amide I). A good way to see this is to compare Δ*A*/*A* values as a function of chain length (Figure 9). The Ac-blocked L-(αMe)-Val peptides require perhaps a bit longer length for stability, but their VCD patterns as a function of increasing oligomer length are quite comparable to that of the Z-blocked peptides. In our studies of Aib-based oligomers with one L-Leu residue for chiral selection, a stable VCD response was seen for the $n > 5$ oligomers.[5] Only for the β-bend ribbon structured (L-Pro-Aib)$_n$ sequential oligopeptides was a stable conformation developed at a shorter length.[6] Proline homo-oligomers also evidence a relatively stable spectrum for short oligomers,[32,33] presumably due to their restricted N−C$^\alpha$ torsion angle caused by pyrrolidine ring formation. C$^\alpha$-Methylated residues can gain this stability only through steric influences. This solution spectral result, indicating a helical stability for relatively short L-(αMe)Val oligomers, is consistent with X-ray studies which demonstrate a more uniform regular 3_{10}-helical structure in this series of peptides than has been seen for others.[1,15] Such regularity and implied stability are consistent with this peptide series being composed exclusively from C$^\alpha$-methyl-substituted residues, known to strongly stabilize 3_{10} structures.[1] However, it is very important to realize that this 3_{10}-helical characteristic VCD band shape pattern for the amide I and II bands is not dependent on these oligomers being composed of only C$^\alpha$-methyl-substituted residues. In our earlier study of Aib-based octamers, exactly the same spectral result was found for peptides incorporating just L-Leu as for those incorporating L-Val-Gly-L-Leu.[3] Similarly, our unpublished studies of (L-Ala-Aib)$_n$ sequential oligomers alternate peptides and other Aib-containing peptides, analogous to the peptaibol antibiotic emerimicin, show the same VCD band shape patterns being developed even though a relatively higher fraction of the residues was proteinic (C$^\alpha$-H).[34]

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A more subtle feature observed in both the FTIR and VCD spectra is the steady divergence in frequency of the amide I and II bands with respect to one another as the chain length decreases. This characteristic might be correlated to the nature of the peptide bond. The frequency change with length could very well be an indication of the hydrogen-bonding state within the molecule, which in turn may be a reflection on the degree of delocalization of the π-bonding character over the amide group. On average, the frequencies are somewhat higher for the amide I band and lower for the amide II band than would normally be expected,[3,5,19] which we feel is due to a lesser degree of delocalization within the amide bond, induced in part by the tetrasubstitution at the α-carbon. The fact that the amide I shifts down in frequency and the amide II shifts up with increasing length indicates that these are a property of the increasing fraction of 3_{10}-helical structure in the oligomer, which could correlate to increased hydrogen-bond formation or to stereochemical constraints. The higher amide I frequencies may be due to partial pyramidization of the amide, which is evident in the structure of some of these oligomers.[15] It might have been thought that the longer oligomers have a larger dipole interaction that shifts the frequencies.[35] The stabilization of frequencies at $n = 6$ argues against this being very important.

It should be noted that the VCD spectra are measured under concentrated conditions, due to the need to avoid solvent interferences. As such, their conditions are more similar to previous FTIR and NMR studies than to typical ECD conditions and are not so dissimilar from the crystallographic conditions, where, by definition, the maximum inter-oligomer interaction is present. The self-consistency of all observations on these oligomers in a concentrated medium (X-ray, NMR, IR, and VCD data) confirms that they form dominant 3_{10}-helices in solution, as they do in the crystal form, and eliminates both α-helices and random coil conformations from making significant contributions to their structures, at least for the longer oligomers ($n = 6-8$).

Concentration Dependence. ECD studies on the octamers, including concentration dependence, were redone here to access a larger range of concentrations and to correlate to previous studies[11,15] indicating that there may be a characteristic 3_{10}-helical ECD spectral form. The low-concentration ECD data (Figures 4 and 5) indicated that a longer peptide length (perhaps even longer than $n = 8$) is needed to develop a stable ECD response with either blocking group, in distinct contrast to VCD- and crystal-structure-based conclusions. This implies that intermolecular interactions could stabilize the 3_{10}-helical form that clearly dominates both the VCD spectra and crystal structures of the oligomers from at least $n = 5-8$.

The ECD of the octamer is, in fact, concentration dependent. The high to low concentration variation of the ECD and FTIR spectra and the consistency of VCD spectra with previous solution studies and crystal structure results confirms that the unique ECD pattern of an intense 207 nm band and weak 222 and 190 nm bands is consistent with the known high fraction of 3_{10}-helical peptide conformation for these C^{α}-methyl-substituted peptides. In light of previous studies indicating a lack of distinguishability between α-helix and 3_{10}-helix ECD,[13,14] it is important to determine which factors lead to this band shape. C^{α}-Methyl substitution might affect the energies of the π-electronic states and could consequently distort the ECD spectrum. The fact that dilution leads to an increase in the $n \rightarrow \pi^*$ intensity for the longer oligomers demonstrates that C^{α}-methyl substitution is not the prime factor. Intermolecular interactions certainly change upon dilution, and this can lead to both conformational changes and spectral changes due to dipolar interactions between molecules.

The ambiguity presented by the band shape variation of the ECD of the octamers with concentration did not put into question whether or not the compounds were helical, but rather clouded the nature of their helicity under low concentration conditions. Andersen and co-workers[14] recently disputed interpretations of ECD presented in a report by Toniolo et al.[11] for the N^{α}-acetylated L-(αMe)Val homo-octamer and postulated by Millhauser[36] as an interpretation for the ECD of alanine-rich systems of 16−21 residue length with modest fractional helicity. The effects of inserting Aib residues into the helical domain of human pancreatic amylin was monitored by Andersen and co-workers[14] with both CD and NMR. Rather than finding Aib-induced 3_{10}-helical contributions, they concluded that short α-helical contributions were evident as characterized by the ratio $[\Theta]_{222}/[\Theta]_{207} > 1.0$ due to diminished rotational strength at the $\pi \rightarrow \pi_{\|}^*$ transition (~207 nm). We believe that these mixed Aib-protein amino-acid-based peptides[14] are too long to give any significant amounts of 3_{10}-helix and that it is incorrect to assume that mixed Aib peptides would be stronger 3_{10}-helix formers than α-helix formers in peptides of arbitrary composition and length. Actually, Aib is a strong helix former, but the nature of the helix depends mainly on the chain length, if the percentage of Aib residues does not exceed 60−65%.[37] Comparison of the Ac- and Z-blocked octamer results indicate this to be a general finding and not a function of the N^{α}-blocking group. The consistency of the VCD in two solvent systems (TFE and $CDCl_3$) implies that solvent hydrogen bonding to the peptide is not the major difference either, but it must be noted that VCD studies done here were restricted to relatively high concentrations so such analogies are limited in extrapolation.

Very recently a paper appeared by Yokum et al.[38] that has direct bearing on the issue of 3_{10}- to α-helix equilibrium. If a peptide (in this case a decamer) is suitably amphipathic, the same peptide composition with a high proportion of C_{α}-substituted residues can be made to shift in ECD band shape from one characteristic of a predominantly 3_{10}- to one of a predominantly α-helical conformation by change in sequence. Thus, this work provides direct evidence that it is not C^{α}-substitution that favors 3_{10}- over α-helices, but rather that C^{α}-substitution stabilizes helical formation with respect to disordered structures allowing formation of very short helices. Furthermore, it becomes clear from this recent study[38] that the decrease in R seen in 3_{10}-helical peptides is not due to either residue type or to helix length, but is indeed due to helix type. Clearly, these particular amphipathic helices were optimally stabilized by interaction with SDS micelles, but this example does demonstrate that such C^{α}-methylated residues can favor either type of helix. Furthermore, these recent results suggest that the ECD patterns, at least at the level of categorizing R values, can be indicative of helix type. The use of ECD data to determine helix fraction is much less secure, as those authors recognize.[38] In this respect, the relative intensity of the amide I and amide II VCD may prove to be a more reliable indicator of the quantitative proportion of 3_{10}- *versus* α-helical formation.

Our concentration studies on the octapeptides (Figures 6 and 7) clearly show a self-aggregation effect which is evidenced by a change in ECD band shape from one partially like that of an α-helix at low concentration to one that now can safely be

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assigned to a 3_{10}-helix at high concentration. There are two possible sources for such a spectral development, conformational variation or spectral manifestation of intermolecular interactions. From the ECD alone, one might conclude the low-concentration form is partially α-helical with frayed ends. In addition, the appearance of an isodichroic point might be interpreted as arising from a transition between two different dominant conformers (3_{10}- and α-helices), the higher concentration stabilizing the 3_{10}-helical form with respect to the α-helical form. In principle, the conformational transition could be to a frayed 3_{10}-helical form, but the ECD band shape would then need to be very length dependent. The alternative explanation would be that dipole coupling between the stabilized peptides in the aggregated form leads to the 3_{10}-like ECD band shape. We unfortunately cannot do the VCD reliably at such low concentrations to detect if a 3_{10}- to α-helical conformational change is evident with that independent technique. Our FTIR studies as a function of concentration for the Z-blocked octamer in TFE solution indicate effects of some structural change. Fourier self-deconvolution of these data at high concentration indicate one major component at ~1660 cm^{-1} corresponding to the amide I band and a minor contribution to higher frequency possibly from the urethane linkage. But, at low concentration, the major peak is broadened about the residual contribution from the original amide I peak. The simplest and most reasonable model for this process is fraying of the ends at low concentration. However, conversion to a frayed α-helical form from a more highly structured 3_{10}-helical form would, in principle, lead to similar band shape changes. Differences in hydrogen bonding to the solvent or residual H_2O (from solvent impurities) as the peptide is made more dilute could cause the solvent absorption spectrum to change, become more difficult to subtract consistently, and affect the overall result. Consistent with this view, the $CDCl_3$ result shows no change on dilution, indicating the structure to be surprisingly stable in the absence of solvent hydrogen bonding. This residual stable structure in $CDCl_3$ must also be 3_{10}-helical, as determined from our VCD results. We cannot carry out a parallel ECD analysis in $CDCl_3$ due to solvent interference.

Comparison to other systems indicate that the Aib_5-Leu-Aib_2 octamer[5] shows no change in its ECD as concentration is decreased over a similar range. Similarly, the (Aib-L-Ala)$_n$ peptides yield relatively concentration-independent ECD.[34] Thus, it appears that the ECD-detected (αMe)Val octamer concentration effects are unique and would call into question whether they may be dipole-interaction induced. Using the crystal packing as a guide,[15] it is likely that at least the Z-blocked peptides have an end-to-end interaction, effectively lengthening and stabilizing the helix. Theoretical predictions[12,39,40] for the effect of α-helix length on ECD band shape patterns also indicate a loss in intensity for the 190 nm band and that the 222 nm band should be more intense than the 207 nm band.[14] While not precisely relevant, similar effects may occur for the 3_{10}-helix. With the possible exception of the HFIP results (Figures 7 and 8), none of our spectra approach the situation of $\Theta_{222} > \Theta_{207}$, even when they deviate from the formerly suggested ratios for 3_{10}-helical conformations.[11,15] Thus, we feel that for these L-(αMe)Val peptides, interpeptide interactions stabilize a 3_{10}-helical form in solution. While it is very difficult to imagine a scenario where increasing the concentration should favor a 3_{10}-helix over a α-helix conformation for a short peptide in TFE, our data do imply that it is possible this might happen for the L-(αMe)Val octamer, at least at the level of transforming to a frayed α-helix. We have not seen such concentration effects in 3_{10}-helical oligomers based on Aib residues.[34] It is possible that the longer side chain in L-(αMe)Val leads to interdigitization and stabilization interactions that would not be important with the more commonly studied Aib residue. These interdigitations would be more easily formed in a homo-oligomer having a 3_{10}-helical conformation than in an α-helical one. This is due to the $i \rightarrow i + 3$ side chain alignment along the chain and the projection of the Val side-chain group away from the axis in the 3_{10}-helix which would then create hydrophobic grooves to which other peptides might dock. If this is the case, these C^{α}-methylated species may prove to be interesting alternative structure models for interactions shown to be important for leucine-zipper proteins.[41]

Conclusion

In summary, the VCD findings described here on L-(αMe)-Val homopeptides are in agreement with previously published determinations of their 3_{10}-helix crystal structures.[1,11,15] The ECD and FTIR results for the octamer indicate a concentration-dependent solution structure, which at elevated concentrations is 3_{10}-helical and interacting. Under more dilute conditions, however, this molecule may have a propensity for a mixed 3_{10}/α-helical or frayed α-helix structure. For these peptide molecules, the nature of the N^{α}-blocking group appears to have little impact on the resultant ECD and virtually no impact for the VCD spectra.

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