zek (Shell Development) in providing helpful suggestions on molecular weight characterization methods.

Registry No. PLGA, 59199-59-6; hexafluoro-2-propanol, 920-66-1; tetrahydrofuran, 109-99-9.

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Vibrational Circular Dichroism of Polypeptides. 11. Conformation of Poly(L-Z-lysine-L-Z-lysine-L-1-pyrenylalanine) and Poly(L-Z-lysine-L-Z-lysine-L-1-naphthylalanine) in Solution

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ABSTRACT: The vibrational circular dichroism of the title compounds in DMSO has been measured in the amide A, I, and II regions. The data are shown in both cases to be consistent with a right-handed α-helical structure being the dominant conformation. VCD avoids the interference from transitions on the aromatic substituent that makes such determinations very difficult to realize with electronic CD data alone. The measured $\Delta A/A$ values in the VCD are somewhat lower than have been seen for other known right-handed α-helices which may be attributable in part to solvent effects.

Introduction

Recently it has become possible to use vibrational circular dichroism (VCD) as an additional spectroscopic probe of polypeptide secondary structure.[1] In particular, a clear pattern has been derived for the VCD of the right-handed α-helix[2] which has been shown to be distinguishable from that of the random coil (with or without local order) and the antiparallel β-sheet.[3] Additionally, Yasui et al. have shown the 3_{10}-helix to have a VCD spectrum distinguishable from that of the α-helix.[4] This latter result implies a greater specificity in VCD for slightly differing conformations than is available with the more

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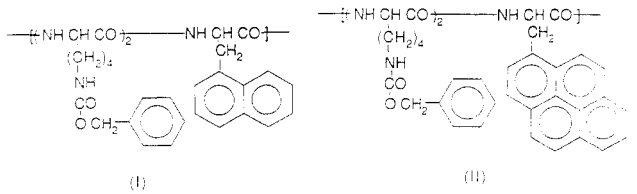
commonly utilized electronic CD.

This specificity also extends to vibrational modes. Due to the increased resolution naturally occurring in the vibrational region of the spectrum, as compared to the UV region, it is possible to study the amide vibrations without significant interference from those of the α-carbon substituents. When these substituent groups are aromatic in nature, this capability allows determination of secondary structure in situations where interpretation of electronic CD is quite difficult at best. Yasui and Keiderling have recently reported such a study of poly(L-tyrosine) in DMSO and related mixed solvent systems which resulted in a clear determination of the character and handedness of the secondary structure transformations accompanying solvent modification.[5]

Sisido and co-workers have synthesized a series of aromatically substituted polypeptides in an effort to obtain model systems for one-dimensional molecular electronic conductors.[6] In order to determine the conformation of several of these polypeptides, extensive conformational energy minimizations and theoretical (exciton model) analyses of the CD, CPL, or fluorescence detected CD were required due to the spectral overlap of the aromatic π–π^* transitions with those of the amide group.[6,7]

In this paper, using the title compounds, poly([L-Z-Lys]$_2$-L-1-pyrAla) (I) and poly([L-Z-Lys]$_2$-L-1-napAla] (II),



(I) (II)

where pyrAla = pyrenylalanine, napAla = naphthylalanine, and Z = benzyloxycarbonyl, we show that the conformation of the *polypeptide chain* for such a system can be derived in a much quicker, more straightforward, and more conclusive manner by using VCD. In addition, we demonstrate for the first time that solution-phase VCD can be obtained on submilligram quantities of a suitable chiral sample.

Experimental Section

The preparation of poly([L-Z-Lys]$_2$-L-1-napAla) was previously discussed.[8] Higher molecular weight fractions than the elution limit of Sephadex LH-20 gel ($\sim 10^4$) were collected and used in this study. Poly([L-Z-Lys]$_2$-L-1-pyrAla) was prepared by a similar procedure, using L-1-pyrenylalanine.[6b] The details of this synthesis are reported elsewhere.[9]

VCD and absorption spectra were measured on the UIC dispersive instrument that has been previously discussed in detail.[10] Samples were dissolved in DMSO or DMSO-d_6 and studied in a fixed path CaF_2 cell. No racemic samples of these compounds were available for determination of the VCD base line, so sample spectra were corrected with identical scans of either just the DMSO solvent or DMSO-d_6 solutions of poly(DL-tryptophane) (DL-Trp). The solvent base line was relatively flat but somewhat shifted from that of the sample at the modest sensitivity levels required for this spectrum. The DL-Trp base line was virtually identical with that of the solvent but was unshifted from the sample spectrum. Hence, we expect that the dispersive spectra we have measured are, at the very least, qualitatively accurate and provide a good representation of the VCD. As a further check of these results, the amide I and II VCD of the poly([Z-Lys]$_2$napAla) sample were rerun as above in TMP and additionally on a new FTIR-VCD instrument that we have constructed and described elsewhere.[11]

Results

The absorption and VCD spectra of poly[(Z-Lys]$_2$pyrAla) in DMSO over the amide I and II regions

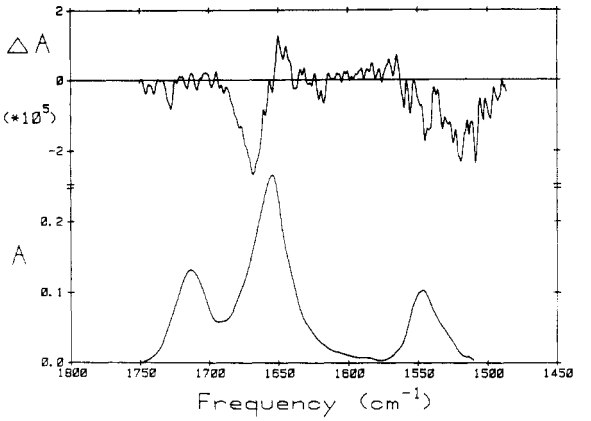


Figure 1. Absorption and VCD spectra in the amide I and II bands of poly([Z-L-Lys]$_2$-L-1-pyrAla) in DMSO solution against solvent as a base line. Time constant, 3 s; four scans averaged; concentration, ~0.5 mg in 0.1 mL. Final plot was smoothed over a 4-cm^{-1} interval, and the base line was adjusted by $\Delta A = -0.5 \times 10^{-6}$.

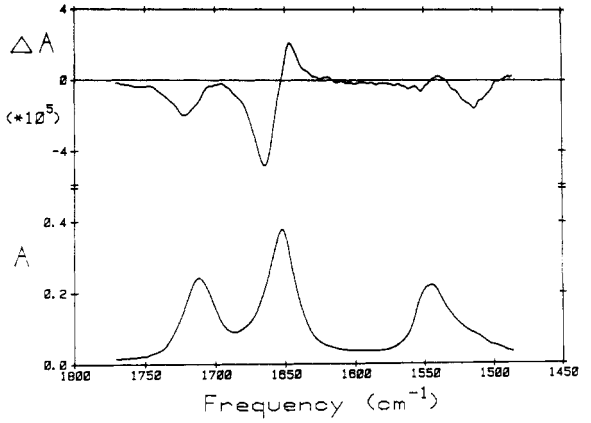


Figure 2. Absorption and VCD spectra in the amide I and II bands of poly([Z-L-Lys]$_2$-L-1-napAla) in DMSO-d_6 solution against poly(DL-Trp) in DMSO-d_6 as a base line. Time constant, 10 s; four scans averaged.

(1750–1500 cm^{-1}) are presented in Figure 1. A clear positive couplet (i.e., negative to high energy, positive to low) is found at ~1655 cm^{-1} correlated to the amide I band. Also quite noisy, negative monosignate VCD is seen, at ~1520 cm^{-1}, lower in energy than the amide II absorption maximum. This spectrum was detectable using ~500 μg of sample dissolved in ~0.1 mL of solvent with the entire sample in the light beam using our sealed cell. The exact concentration with this small amount of sample was not possible for us to determine, but the measured $\Delta A/A$ values and the general sign and band-shape patterns of the VCD provide sufficient data to distinguish among the various common polypeptide secondary structures. The lack of a racemic compound for base-line determination may account for the "floating" nature of the VCD that was initially obtained. In Figure 1 we have adjusted the base line by $\Delta A = -0.5 \times 10^{-6}$ to account for the experimental mismatch of sample and base-line VCD. Insufficient sample was available for determination of amide A VCD or for study of this VCD in a different solvent.

Similar data for the poly([Z-Lys]$_2$napAla) compound are shown in Figure 2. Here, more sample (~2 mg) was available, and DMSO-d_6 was used as a solvent, both factors of which help to improve the resultant signal-to-noise ratio (S/N). The amide I and II absorption and VCD spectra are qualitatively the same as that in Figure 1. The urethane C=O stretch of the Z group appears to have significant VCD in the latter (nap) compound, while it is

Table I
Summary of $\Delta A/A$ Values

polypeptide/solvent[a]	$\Delta A/A^b$ ($\times 10^{-4}$) amide I	amide A	ref
poly([Z-L-Lys]$_2$-L-1-pyrAla)/DMSO	1.5		this work
poly([Z-L-Lys]$_2$-L-1-napAla)/DMSO-d_6	1.8	0.72	this work
poly([Z-L-Lys]$_2$-L-1-napAla)/TMP	1.5		this work
poly(L-tyrosine)/DMSO·DCA (80:20 by vol)	2.1		5
poly(L-tyrosine)/DMSO·TMP (50:50 by vol)	1.5	0.78	5
poly(γ-benzyl L-glutamate)/$CHCl_3$	2.6	1.0	2

[a] DMSO = dimethyl sulfoxide; DCA = dichloroacetic acid; TMP = trimethyl phosphate. [b] Peak-to-peak VCD amplitude divided by peak absorbance amplitude.

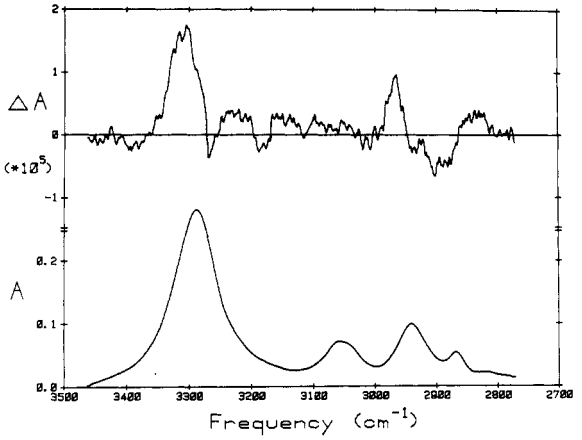


Figure 3. Absorpotion and VCD spectra of poly([Z-L-Lys]$_2$-L-1-napAla) in DMSO-d_6 solution in the amide A and C stretching region. Time constant, 3 s; otherwise as in Figure 2.

hardly distinguishable from the base line in the former (pyr), presumably due to the reduced S/N. The FTIR-VCD result is qualitatively the same but was obtained at higher resolution with somewhat lower S/N. The TMP results were virtually the same and differed only slightly, quantitatively, as summarized in Table I.

As illustrated in Figure 3, it was also possible to measure VCD in the amide A (NH stretch) and CH stretching regions of the spectrum for poly([Z-Lys]$_2$napAla) due to the increased sample size. As expected, we see a net positive VCD correlated to the large amide A absorption band centered at ~3290 cm^{-1}. This band has weak negative and then positive features to lower energy. The largest CH stretching VCD occurs near 2970 cm^{-1} and is uncorrelated to an absorption maximum. This frequency position implies an origin in an aliphatic CH_2 stretch but with possibly some contribution from the C*–H stretch at the α-position. These latter modes, however, are often found at a lower frequency.[12] By comparison, the aromatic CH's appear to give only small VCD signals.

Discussion

In the amide I region, a single band centered at 1655 cm^{-1} is seen in the absorption spectra of both compounds which correlates well to the positive couplet seen in VCD. The shape and sign of these bands directly reflect that expected for a right-handed α-helix.[2,5] The amide II band peaks at ~1545 cm^{-1} in absorbance but correlates to a broad negative band in VCD whose maximum occurs at ~1515 cm^{-1} in both polymers. This amide II VCD sign and frequency shift from absorption again reflect the right-handed α-helical results we have reported earlier.[2c,5] The fact that the amide II VCD is somewhat weaker in intensity than that of the amide I also is consistent with the previous results for right-handed α-helical structures. By way of contrast, the 3_{10}-helical VCD that we have reported[4] had a positive couplet VCD for the amide I that was weaker (in terms of peak-to-peak ΔA) than the negative VCD of the amide II.

The amide A results for poly([Z-Lys]$_2$napAla) are also consistent with those expected for a right-handed α-helix. In the previous studies of α-helical VCD, this band was shown to be strongly positively biased and to have three distinct components of which the highest energy one was dominant.[2,5] The shape and position of the amide A absorption and VCD indicate little, if any, interaction with the lower energy aromatic CH stretches.

In terms of conformational delineation, these results in conjunction with our previous experience indicate that the dominant conformation of both poly([Z-Lys]$_2$pyrAla) and poly([Z-Lys]$_2$napAla) is a right-handed α-helix. It is interesting to note that the most likely conformation of poly(L-1-pyrAla) has been proposed to be a left-handed helix.[7] It is very clear from our data that such is not the case in the copolymer of pyrAla with (Z-Lys)$_2$. On the other hand, initial analyses of the CD of poly(L-1-napAla) favored a right-handed δ-helical conformation,[6] but subsequent extended analysis altered that hypothesis to favor the right-handed α-helix for all of the poly([Z-Lys]$_m$napAla), m = 0–4, variants.[8] Our data for the m = 2 species confirm the final conclusion in at least that case.

However, the magnitude of the VCD in the amide I band for these polymers, particularly in the pyrAla case, is somewhat lower than is typically seen with α-helical polypeptides in $CDCl_3$ solution (see Table I). This is consistent with the variation in $\Delta A/A$ values seen for poly-tyrosine) in various DMSO containing mixed solvents[5] and for poly(L-lysine) in CD_3OD·D_2O solution.[3] Some variations in band shape were also noted in these latter cases (Tyr and Lys) as compared to the $CHCl_3$ results. However, the two polymers studied here do have the same amide I band shape as did the $CHCl_3$-soluble polypeptides.[2] In the presence of strong hydrogen bonding solvents (helix breakers), the VCD of the amide I typically seems to be weaker than in less polar solvents such as $CHCl_3$. In our studies, the absorption bands in hydrogen bonding solvents also had a different shape[5] from those in low polarity solvents, which could indicate a difference in the splitting of the amide I components or a coupling to other vibrational modes.

On the other hand, the amide II VCD in the pyrAla case has a $\Delta A/A$ (comparing the 1550-cm^{-1} absorbance to the 1520-cm^{-1} VCD) that is comparable to the values seen for other polypeptides in other solvents. But clearly the uncertainty due to low S/N is a problem for this band. In the napAla case, the amide II VCD is again substantially weaker than we have reported for other α-helices,[2,5] and the $\Delta A/A$ value for the amide A band is equivalent to that reported for other α-helices (Table I).

We feel that this apparent difference in amide I magnitudes with solvent change may be more a measure of band width than of structural integrity of the helix. As the amide I is broadened, due to its bisignate nature, the VCD will necessarily fall off. This is simply the effect of having overlapping, oppositely signed bands. This band is after all composed of a large number of components whose splitting and relative intensity will depend on small deviations from the ideal α-helical conformation. Since the $\Delta A/A$ values seen here, ~1.5 × 10^{-4} pyrAla and 1.8 × 10^{-4} for napAla, are within the range seen (1.5–2.1 × 10^{-4}) for poly-L-tyrosine in various solvents, one can reasonably

assume that the poly([Z-Lys]$_2$pyrAla) and poly([Z-Lys]$_2$napAla) helices are at least as uniform as those of poly(L-tyrosine) in the conditions studied.[5] The details of solvent vs. structural integrity effects on VCD have yet to be worked out; thus it is possible that a new interpretation of these magnitude variations may emerge with increased experience. However, at this time, there is no reason to attempt to conclude that the somewhat reduced relative strength of the amide I VCD for these heteroaromatic polypeptides has a reliable conformational interpretation.

Finally we should note that only quite low VCD is found corresponding to the aromatic CH stretches. This is in spite of the fact that the electronic CD indicated a regular, helical arrangement of the aromatic groups, especially in poly([Z-Lys]$_2$napAla).[8] We feel that due to the planarity of these side groups, the dominant mechanism through which these bands can gain VCD intensity is that of dipolar coupling.[13] The strength of such coupling will depend on the dipolar strength of the modes involved (proportional to their infrared absorbance intensity) and spatial separation, upon which the VCD should vary roughly as the inverse square. This separation has been estimated to be 6.7 Å for the center-to-center distance between nearest-pair naphthyl groups in poly([Z-Lys]$_2$napAla).[8] Both factors work against there being significant VCD due to the nap or pyr CH stretch modes. Alternatively, the absence of aromatic CH stretch VCD may be due to the overlap of several vibrational modes having different CD's and resulting in a net cancellation. In fact, the broad, nondescript VCD seen in this region (3000–3100 cm^{-1}), if real, may come from the Z group amine blockers on the Lys. This conclusion follows from the significant VCD found at 1720 cm^{-1} which is attributable to the urethane CO stretch on the Z group.

On the other hand, the aliphatic CH's give rise to substantial VCD. This could be due to their more substantial local asymmetry, especially for modes having a major contribution from hydrogens on the α- and β-carbons. This may be an indication of restricted conformational flexibility of these aliphatic chains when bound to an α-helix. Earlier studies have shown that CH_2 stretches can couple to yield significant VCD when conformationally restricted, such as in a six-membered ring.[14] This kind of coupling would explain the underlying bisignate nature of the 2935–2970-cm^{-1} VCD and its nonalignment with the 2945-cm^{-1} absorption band.

Conclusion

In summary, we have used VCD to determine the secondary structure of two heteropolypeptides with aromatic substitution. Both poly([Z-Lys]$_2$napAla) and poly([Z-Lys]$_2$pyrAla) are right-handed α-helical in DMSO solution. Furthermore we have demonstrated that acceptable VCD can be measured under favorable circumstances on submilligram quantities. These results provide an alternative method of determining polypeptide secondary structures when interference by side-group electronic transitions compromise more established methods.

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Registry No. Poly(L-Lys(Z)-L-Lys(Z)-L-(1-pyrenyl)Ala), 109720-17-4; poly(L-Lys(Z)-L-Lys(Z)-L-(1-pyrenyl)Ala) (SRU), 109720-14-1; poly(L-Lys(Z)-L-Lys(Z)-L-(1-naphthyl)Ala), 102633-76-1; poly(L-Lys(Z)-L-Lys(Z)-L-(1-naphthyl)Ala) (SRU), 102633-66-9.

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