Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 133–141

BIOORGANIC & MEDICINAL CHEMISTRY

Vibrational Circular Dichroism Spectroscopy of Selected Oligopeptide Conformations

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Received 5 June 1998; accepted 8 October 1998

Abstract—Vibrational circular dichroism (VCD) has been shown to be a useful technique for characterization of the qualitative secondary structure type for linear polypeptides and oligopeptides. A brief review of characteristic spectral responses and applications is given. Since VCD is dependent on relatively short range interactions, it detects residual structure in such oligomers even if long range order is lost. VCD studies presented here for Lys oligomers as well as Lys and Glu polymers as a function of length, salt added and temperature, confirm residual local order in these 'random coils'. Comparison to results with Pro oligomers, supports an interpretation that these extended structures have a left handed twist conformation. The 'coil' VCD is shown to be significantly reduced in intensity by temperature increase and by decrease in peptide length. By contrast, for partially α-helical Ac-$(AAKAA)_3$GY-NH_2 oligomers, the spectrum changes to the high temperature Lys_n shape on heating, first losing then gaining intensity, indicating an equilibrium shift between structured states, from helix to coil (locally ordered) forms. VCD is shown to be a useful technique for monitoring local order in otherwise random coil structures.

Introduction

Most optical spectroscopic secondary structure studies of peptides involve use of electronic circular dichroism (ECD) measured in the far UV for the n–π* and π–π* transitions of the amide linkage.[1] Coupling of the involved electronic transition dipoles leads to the extended chirality, which is characteristic of the chain conformation and results in an observed ECD spectrum that has a sign/frequency profile that is characteristic of different secondary structures. Since these transitions are broad and fully overlapped, their individual frequencies are not very important for such spectra–structure correlation. However, ECD bandshapes and intensities for polypeptide chains are length dependent, which can adversely impact application to oligomers and to chains containing aromatic groups.

Before the development of ECD methods, IR based analyses[2] were a prime means of characterizing polypeptide conformations. Due to the natural resolution of the vibrational region of the spectrum, these IR methods were based initially on assignment of component band frequencies to various secondary structural types. Most effort focused on the amide I (C=O stretch) band with additional use of the amide II (N–H deformation plus C–N stretch) in the IR. More recently, the very weak and dispersed amide III (oppositely phased N–H deformation plus C–N stretch, which is actually intense in Raman spectra) has been used. Since both IR and Raman give rise to single signed spectral bandshapes that are effectively just the dispersed sum of the contributions from all the component transitions, the bandshapes for polypeptides that have diverse structural units, such as would be characteristic of globular proteins, are very similar. Due to the high signal to noise ratio (S/N) of Fourier transform IR (FTIR), this disadvantage can be somewhat avoided by use of resolution enhancement employing second derivative or Fourier self deconvolution (FSD) techniques.[3] These latter methods partially compensate for the relatively low level of bandshape variation available in the IR and Raman spectra, but can be misused by the unwary and consequently subject the analysis to several assumptions that may well be unwarranted.[4] One major problem in these methods is that the frequencies assigned to specific secondary structural types are not unique and shift significantly for different residues and due to varying solvation of the peptide.[4,5] Additionally, end and non-uniformity effects for segments of a given structural type will affect the frequencies, resulting in some dispersion of the contribution of a given secondary structure segment over the spectrum. Finally most methods assume that the (amide I) dipole strengths (extinction coefficients) of all the residues, regardless of their conformation, are the same. While a useful simplifying assumption, this is known to be incorrect in detail.[4,6]

Key words: Circular dichroism; infrared; secondary structure; oligopeptide; random coil.

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0968-0896/99/$ - see front matter
PII: S0968-0896(98)00217-X

Nonetheless such FTIR methods have proven very useful for sorting out differences between proteins with similar IR bandshapes. Bandshape based analyses such as those normally used to interpret ECD spectra in terms of secondary structure[1] have also been applied to FTIR and Raman spectra with similar success.[7]

Vibrational circular dichroism (VCD) can be viewed as a combination of these two spectral approaches, having the bandshape variability of CD with the frequency resolution of IR.[8–10] For qualitative analyses one can utilize the bandshape and its frequency position to predict the dominant secondary structural type in a peptide or protein.[8,11–14] Quantitative secondary structure predictions for proteins are possible with bandshape analyses of VCD[15,16] and, in combination with ECD and FTIR, much more detailed insight into secondary structure aspects can be obtained.[16,18] Qualitative bandshape analyses of the dominant structural type have become the standard approach for most peptide VCD studies[8,19] since in such smaller molecules the frequencies are problematic due to solvent effects and the inhomogeneity of the peptide secondary structure (as well as end fraying).

Background for peptide VCD studies

In order to build a data base for qualitative interpretation of VCD data, it is necessary to obtain spectra for known peptide structures of a uniform conformational type. The most straightforward method of doing this is to use polypeptides for which structural parameters have been established over several decades of work. Some α-helical polypeptides are soluble in nonaqueous environments, which permits measurement of VCD for several amide transitions.[12] For β-sheets such extensive solution-phase studies are not possible due to solubility problems.[20] On the other hand, there are polypeptides that are soluble in aqueous solution that undergo transitions to helical or sheet-like forms under perturbations of pH or salt effects.[21]

Aqueous solutions, while more relevant to biochemical problems, do restrict the ease of obtaining VCD for different bands due to strong solvent absorption interference. H_2O has a major absorption band at $1650\,cm^{-1}$, directly overlapping the amide I region of prime interest for peptide and protein studies and completely wiping out consideration of the region above $3000\,cm^{-1}$. As a consequence, many VCD studies have been carried out in D_2O, resulting in exchange of most of the amide protons, and focusing on the amide I (denoted amide I′ for the N–D exchanged peptide), which is the most accessible mode, being only minimally shifted in frequency (and presumably, character) from its H_2O value. By contrast, in D_2O the amide II′ is strongly shifted and altered in character, and the amide A′ (N–D stretch) and III′ are not detectable due to solvent interference. Both the amide II and III transitions can be routinely studied for molecules in H_2O solution, thus avoiding problems of deuterium exchange.[13] However the amide A is only accessible in nonaqueous environments.[12,22] Measurement of amide I VCD in H_2O is possible[14] using very short path length cells and relatively high concentrations. While such studies have proven useful for globular proteins, in general they are not compatible with most peptides due to solubility limitations and aggregation problems.

Strong VCD features are found for right-handed α-helices that consist of a negative couplet in the amide A ($\sim 3300\,cm^{-1}$), positive couplet (broad, weak $+\Delta A$ then intense $-\Delta A$, with increasing frequency) in the amide I ($\sim 1655\,cm^{-1}$), a negative band lower in frequency than the absorption maximum in the amide II ($\sim 1550\,cm^{-1}$), and net positive through the general amide III region ($1400–1200\,cm^{-1}$).[12,13,22,23] These bandshape patterns are generally characteristic of right handed α-helices except that deuteration of the amide N–H was shown to change the shape of the right handed α-helical amide I VCD to a three peaked (−, +, −) pattern (amide I′)[12,14,21,24] and to shift the amide II′ to $1450\,cm^{-1}$ with retention of its negative sign but significant loss of intensity. Both of these latter patterns are maintained in D_2O solutions for peptides that have sufficient solubility and a stable α-helical conformation.[21,24] For helices, these patterns are remarkably stable, clearly persisting even in proteins (compare Figure 1(a) with (d)).

The β-sheet and coil forms have been shown to have distinctly different amide I′ VCD spectra from that of the α-helix, but these are also consistent for a variety of polypeptides.[21,24] The VCD spectra observed for β-sheet polypeptides in D_2O have weak negative amide I′ VCD corresponding to the two widely split absorbance features at $\sim 1615\,cm^{-1}$ and $1690\,cm^{-1}$, which appear to be characteristic of forming antiparallel β-strands due (most probably) to aggregation (Figure 1(e)). The solubility of

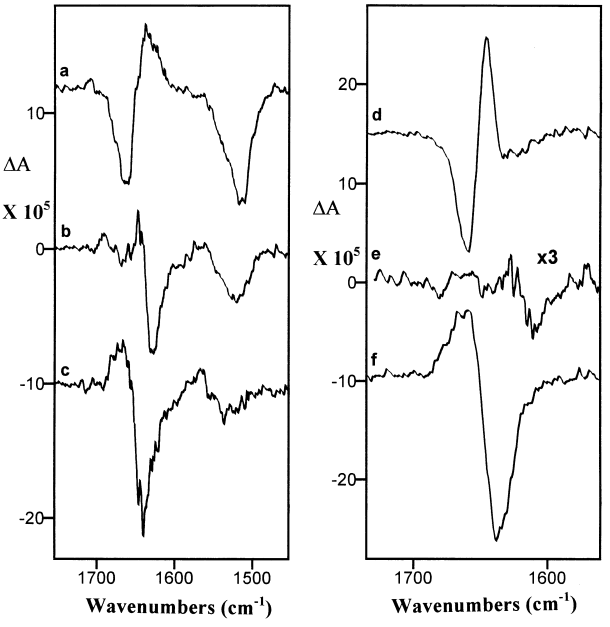

Figure 1. Comparison of VCD spectra of (a) albumin (α-helical), (b) chymotrypsin (β-sheet), and (c) poly-L-lysine (pL-K) at pH = 7 (random coil) measured in water with (d) poly LKKL in added salt (α-helical), (e) poly KL in added salt (β-sheet), and (f) pL-K (random coil) measured in D_2O. Spectra were rescaled to an absorbance of 1.0 for the amide I or I′.

these peptides at IR compatible concentrations is marginal, which additionally mitigates against obtaining data for them in H_2O where even higher concentrations are required. Hence these widely split, negative VCD patterns relate more to denatured aggregated proteins than to the short sheet segments seen in globular proteins, which give rise to negative couplets centered around 1630 cm^{-1} (Figure 1(b)).[11,14]

Various polypeptides (e.g. poly-L-lysine, pL-K) thought to form coils turn out to have a surprisingly intense, negative couplet amide I VCD typically centered near 1645 cm^{-1} (intense negative followed by a broad weak positive to increasing frequency, which is opposite in sign pattern to that of the α-helix) that is insensitive to deuteration (Figure 1(c) and (f)). This pattern is exactly the same shape but smaller in amplitude as that characteristic of polyproline II (pL-P II),[25] a left-handed 3_1 helix of *trans* peptides. This coincidence of bandshape coupled with extensive oligomer studies has implied that this VCD pattern is characteristic of a local left-handed twist, which may arise from an extended strand form or from a high concentration of left-hand turn conformations in an otherwise long-range disordered or coil molecule.[25] This is consistent with much older proposals by Tiffany and Krimm[26] that these structures can best be viewed as an 'extended-helix' conformation that, at least locally, has similarities to that of left-hand helical pL-P II. All of these observations are consistent with the 'random coil' having locally ordered regions of a left-handed helical twist sense. VCD data cannot distinguish between the degree of twist in either the turns or strands due to the expected non-uniformity of any long-range structure in such 'coils'. Thus the random coil polypeptide form is seen in VCD to lack long range order but maintain some short range structure. This paper will give examples demonstrating that the VCD signal resulting from such order is not intrinsic to the residues (local chirality) nor to the peptides themselves (unique sequence) but does depend on the stability of this structure, which in turn is a function of sequence and environment.

Other structural types can be characterized with VCD, especially if magnitude as well as bandshape were analyzed and if additional bands were studied. A number of oligopeptides have been studied[27] establishing that the 3_{10} helix (Fig. 2(e)) has VCD of the same sign pattern as the α-helix (Fig. 2(a)), but gains distinguishability since its amide I VCD has a conservative couplet shape that is much weaker than that of the amide II, while the opposite is true for the α-helix.[23] This provides a means of differentiating these two related helical structures and identifying mixed structures such as occur in longer, blocked Aib-Ala (Aib = amino-isobutyric acid) alternate oligomers.[28] The tripeptide of this series (Fig. 2(d)) evidences little if any secondary structure and little if any VCD, while the hexapeptide (Fig. 2(c)) is dominantly 3_{10} helical and the dodecapeptide (Fig. 2(b)) is of mixed structure. Similar characterizations of the VCD for β-bend ribbons, alternate *cis–trans* DL proline oligomers, parallel versus antiparallel strands and various turns have been attempted, but are less well established.[29,30]

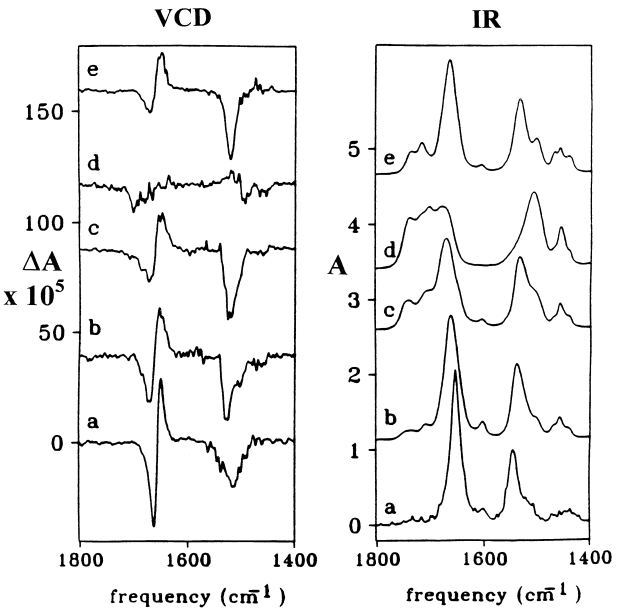

Figure 2. Comparison of VCD for α-helical (a), mixed (b) and 3_{10} helical (c,e) oligomers. (a) $(Met_2\text{-}Leu)_6$, (b) $(Aib\text{-}Ala)_6$, (c) Ala-$(Aib\text{-}Ala)_3$, (d) Ala-Aib-Ala, and (e) Aib_5-Leu-Aib_2.

A very important property of peptide VCD, that was established via the oligomer studies described above, is the general length dependence of the characteristic spectral features. Intense VCD signals can be found for oligomers such as Z-$(Aib)_2$(L-Leu)$(Aib)_{n\text{-}3}$-OMe, $n = 3$ to 8, Z-$(\alpha\text{-Me-L-Val})_n$-OBu, $n = 2$-8, Ac-$(Aib\text{-L-Pro})_n$-OMe, and 3_1 helical $(L\text{-Pro})_n$, $n = 3$ to 12,[25,27,29] that at short lengths have VCD patterns that closely parallel those of the spectra for longer chains. Importantly, these VCD spectra are only somewhat reduced in intensity (judged on the basis of ΔA/A, which partially normalizes for residue concentration), thus providing evidence that VCD arises from relatively short range interactions. For example, for a series of 3_{10}-helical oligomers, it was found that the VCD band shape for all three amide bands studied, I, II, and A, was established at least by the $n = 4$ length.[27] Furthermore, the magnitude of the VCD reached a nearly constant value per subunit value by $n = 5$. This corresponds to less than two turns of the 3_{10} helix or, alternatively, the coupling of two type III β-turns. Similarly, comparison of the ECD and VCD of Pro_n oligomers showed that ECD increased as $1/n$ while the VCD had a $1/n^3$ dependence,[8,25] indicating that a shorter range interaction is dominant in VCD. This arises from the oligopeptide ECD being primarily due to electric dipole coupling but the VCD having, in addition to the through-space dipolar coupling, a through-bond mechanical coupling encompassing mixing of vibrational modes on adjacent subunits, an interaction that is intrinsically short range in nature.

As a consequence, short peptide fragments that have a stable conformation can give rise to a substantial intensity in the observed VCD in a mixed structure, so that, for example, the VCD of β-turns may turn out to have a distinct contribution. This aspect of VCD conformational analysis is less well developed, but some examples

exist such as the type III β-turn arising in short Aib peptides[27] and the type I and II turns that have been studied by Diem and co-workers using cyclic peptides.[30]

Before turning to the empirical studies prepared for this paper, it is useful to present some theoretical results that represent the state of the art in terms of theoretical analyses of peptide VCD. As has been well established, the magnetic field perturbation (MFP) method of calculating VCD with ab initio wave functions as implemented by Stephens[10,31] is fairly reliable for the simulation of the VCD of small molecules. We have extended this calculational approach to a model system containing two peptide bonds whose relative ϕ,ψ torsional angles were varied to replicate those in the α-helix, β-sheet, 3_{10} helix, and Pro II (left-handed 3_1) helix polypeptide conformations.[32] The computed results for the amide I and II bands of dipeptides constrained to those ϕ,ψ angles qualitatively reflect the experimental H_2O VCD patterns found for proteins or peptides in those dominant forms. That this method is successful is consistent with the observations that short range effects strongly influence VCD.

Model calculations for peptides of any size comparable to those that yield definitive experimental VCD band shapes present a formidable challenge for quantum chemical approaches to vibrational spectral simulation. However, recent results by Bour and co-workers[33] provide a method of extracting the force field and atomic polar and axial tensors obtained via ab initio MFP calculations for a small molecule, such as the dipeptide, and using them to approximate the same properties of a larger peptide constrained to a given test geometry. This approach yields a reasonable theoretical estimate of the larger molecule's spectral properties because it preserves the important short range properties that dominate VCD. It is this short range sensitivity coupled with the intrinsically fast time scale of an infrared measurement that permits VCD to probe the structural nature of an unfolding or fluctuating peptide. These spectra represent a dynamic equilibrium between multiple conformations, but differentiations among these conformations may require consideration of only a limited number of local residue coupling geometries. Determining the most dominant local geometries is the topic of interest that underlies the study presented in this paper.

Results and Discussion

We have previously demonstrated that typical random coil polypeptides, such as poly-L-Lys (pL-K) or poly-L-Glu (pL-E) in D_2O and even poly-L-tyrosine in DMSO give VCD with an identical band shape to that of poly-L-Pro II (pL-P II), but with about half the intensity.[12,24,25,34] In addition we showed that the pL-P II VCD bandshape persisted for oligomers down to very short lengths, indicating it to be a measure of local conformation. From this we concluded that the 'coil' structures indeed had significant residual structure and hypothesized that this structural form might consist of local left hand turns or left handed twists of extended structure, being essentially loops having sections with a conformation analogous to the twisted strands one might find in a β-sheet. If such structure exists in a random coil, one expects to be able to observe a transition to a less ordered state under various perturbations. Our previous report,[25] which was centered on Pro_n oligomers, demonstrated that salt would alter the observed VCD bandshape for model pL-P II peptides but temperature would not change it. Here we consider some other peptides and seek a comparison with these model studies. Varying molecular weight samples of pL-K in D_2O at pH 7 yield VCD of decreasing magnitude and increasing bandwidth for shorter chain lengths as shown in Figure 3 (top). These shapes are all consistent with the pL-P II shape and suggest a left handed twist interaction as previously discussed,[25] but the loss of intensity for lower molecular weights implies that this remnant of extended structure is stabilized by the presence of longer chains, which presumably permit less hydration in such a locally structured segment than is present in fully solvated shorter chains. At high pH, and low temperature, the longer oligomers form a mixed helix–coil–sheet structure, consistent with earlier studies of poly-Lys,[24] as seen in Figure 3 (bottom). (The sheet fraction is confirmed by sharp features rising at ~1610 and 1680 cm^{-1} in the IR absorbance, not shown, but does not become dominant unless the sample is heated. The helical contribution is evident due to the negative features above 1660 cm^{-1} and the large positive at 1640 cm^{-1} and is independently confirmed by ECD studies.) However the shorter oligomer, n~8, maintains its negative couplet

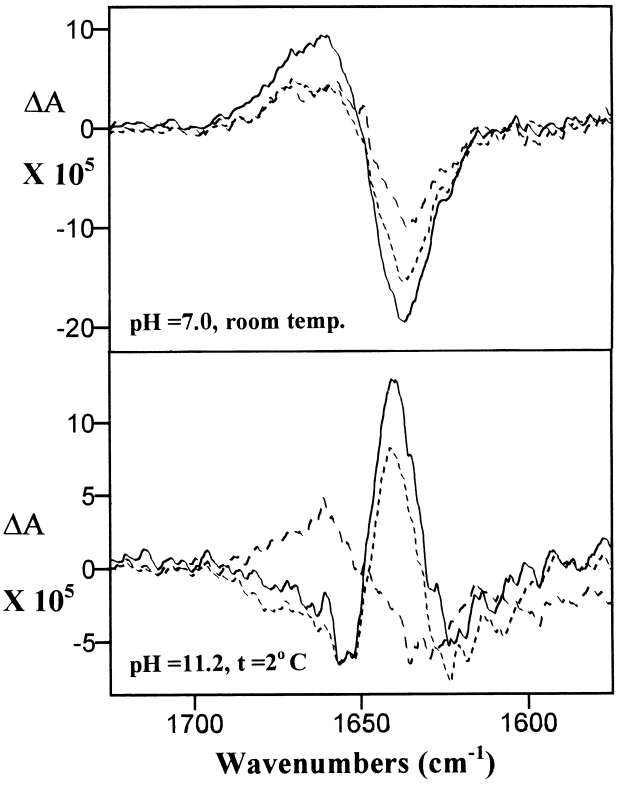

Figure 3. Poly L-Lys VCD spectra at pH 7 and room temperature (top) and at pH 11.2 and 2 °C (bottom) for MW ~1000 (long dashed), ~25,000 (short dashed) and ~250,000 (solid) samples.

shape with some broadening despite the rise in pH. In other words, the local conformation attributed to the 'coil' form is a residual structural unit in the short oligomers and, for these short lengths, is the only one stable at high pH. This is consistent with the overall length-induced stability that is well known for α-helices and is also consistent with substantial fractions of the β-form arising from intra-chain cross-strand hydrogen bonding in the longer strands.

VCD of a similar bandshape to that of the very low molecular weight pL-K ($n\sim8$) but even weaker intensity is also seen for Glu_3, Lys_3 and Lys_4 (Fig. 4). This negative couplet pattern is by contrast distinctly different from the signals seen for Glu_2 and Lys_2, which seem to have a net weak positive VCD for the amide I′ band at $\sim$1650 cm^{-1}. This shift in the dipeptide bandshape indicates that the characteristic coil VCD pattern is due to coupling of amides and not to any intrinsic chirality of the amino acid peptide link. Furthermore such a coupling-dependent signal changes with conformation, confirming that it is not intrinsic but is dependent on geometry. It is interesting to note that the Pro_2 has a negative amide I′ VCD[35] and that this linkage is susceptible to being in a *cis* rather than the standard *trans* conformation.

These signals, even when very well developed in longer chain pL-E or pL-K polypeptides are subject to substantial loss of intensity upon heating (Fig. 5) or addition of salt (Fig. 6) further confirming that the signal seen in these 'random coil' peptides is due to residual structure. Pro oligomers were the model for understanding the residual structure in 'random coils'. Yet when Pro_{12} is heated to 75 °C only negligible loss in VCD intensity occurs in its signal, which is fully three times more intense than for pL-E.[25,35] To study this further we measured VCD for the more weakly structured, low molecular weight pL-K with $n\sim8$. Again substantial VCD signal is lost on heating, even though the initial intensity is only half that of the pL-E (Fig. 7). In addition the IR bandshape broadens and shifts up in frequency, paralleling changes seen in higher molecular weight pL-K and pL-E (Figs 5 and 6). These changes are reversible as evidenced by recovery of the VCD signal on cooling. The reversible nature of this spectroscopic transition confirms the locally ordered structure hypothesis we previously put forward[25] based only on data for the Pro oligomers. If there were no structure, just a disordered chain, the signal would not systematically change with the increased disorder that will accompany increase in temperature (due to the entropy

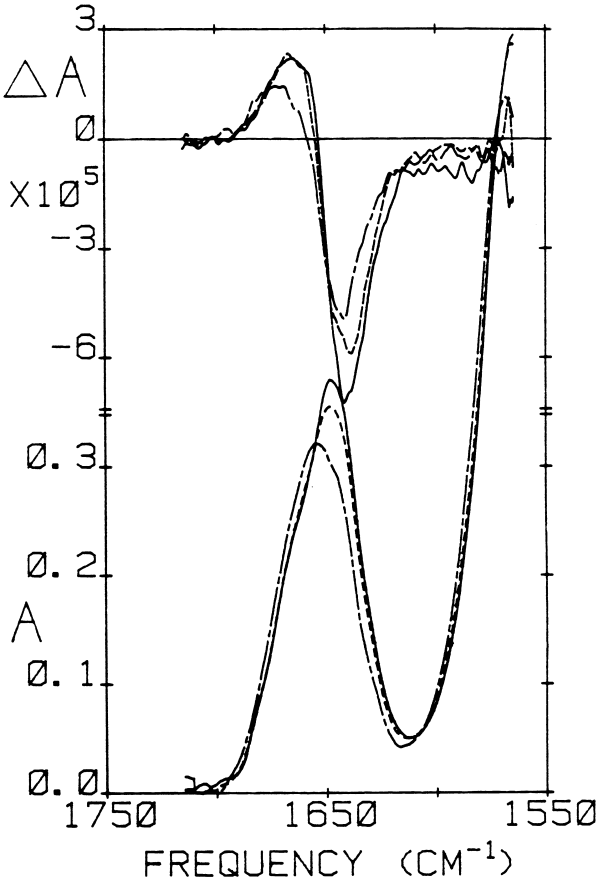

Figure 5. IR absorption and VCD spectra of poly-L-glutamic acid at neutral pH at 5 °C (connected), 25 °C (dashed) and 75 °C (dot dash).

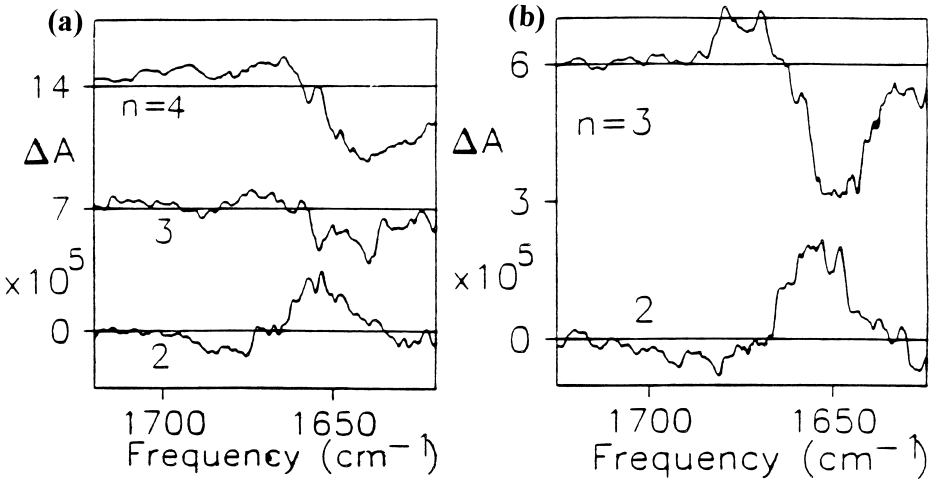

Figure 4. VCD spectra of $(Lys)_n$, $n=2$–4 in D_2O at neutral pH (left) and $(Glu)_n$, $n=2$–3 in D_2O at neutral pH (right). The spectra were rescaled to $A_{max}=1.0$ in the amide I so that VCD scale reads directly in $\Delta A/A$ at A_{max}.

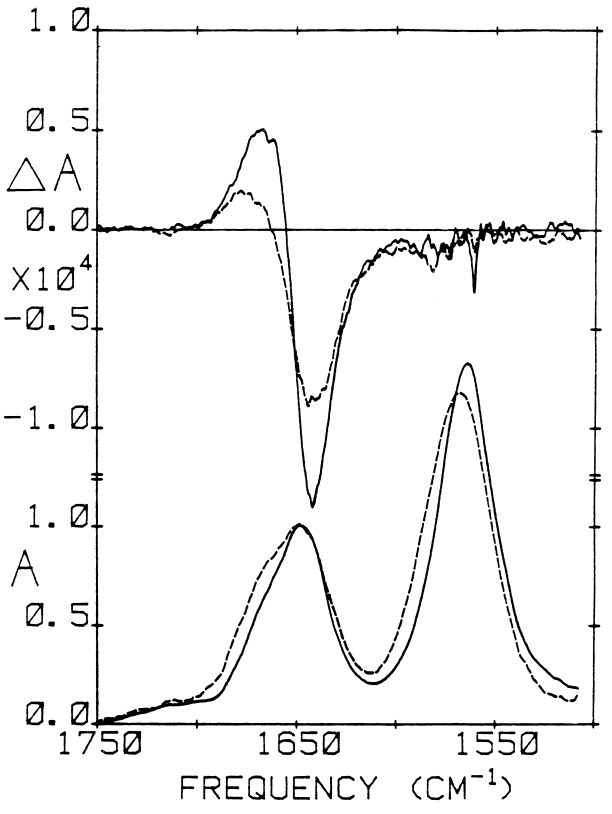

Figure 6. IR absorption and VCD spectra of poly-L-glutamic acid in D_2O (connected) and in 4.5 M $LiClO_4$ (dashed). The spectra are rescaled to have peak absorbance in the amide I of $A_{max} = 1.0$ so that the VCD scale reads directly in $\Delta A/A$ at A_{max}.

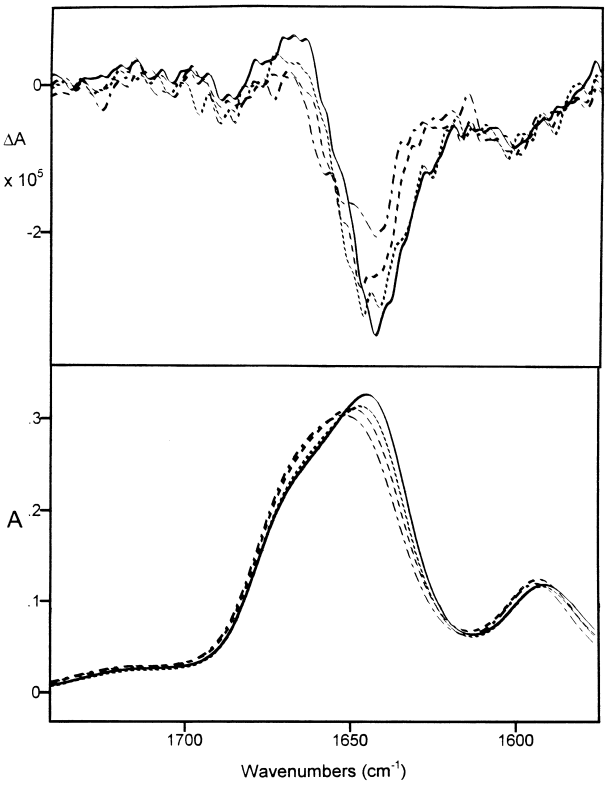

Figure 7. Oligo Lys, MW ~1000, VCD at pH 7.4 as a function of temperature. At 5 °C (connected), 25 °C (short dash), 50 °C (long dash), and 70 °C (dot-dash).

increase for such a process in a short chain). All observations are consistent in regard to loss of structure in these random 'coils'.

Turning to another structural type, Ala rich peptides, for example, Ac-$(AAKAA)_n$GY-NH_2, are known to have α-helical character at low temperatures even in water for the longer ($n = 3,4$) species.[36,37] In Figure 8 are shown the temperature dependent VCD for the $n = 3$, 17mer, which show a transition from a mixed helix and coil form to a coil form that is evidenced by a collapse of a double minimum VCD bandshape and regrowth of a negative couplet VCD bandshape characteristic of the coil form. This results at 35 °C in a spectrum that is more intense than the low molecular weight pL-K but less so than the longer chain pL-K. This series of peptide temperature dependent VCD differs from the Lys_n oligomer examples discussed above in that it actually shows a loss and then gain in signal intensity with heating. This sort of change is specifically indicative of a helix-to-coil transformation, where 'coil' implies the residual (presumably left-hand twist) structure seen in pL-K type samples. Such a spectral transition could only happen for a structure-to-structure transition where the final state, much as established above for the Lys oligomers, retains a definite local ordering. By contrast, the earlier discussed samples just systematically lost this residual structure to become more disordered and lost signal intensity with no basic change in shape, other than broadening.

This sort of behavior can be further analyzed using factor analysis techniques.[38] We have shown that the set of VCD data for Ac-$(AAKAA)_3$GY-NH_2 can be decomposed into two independent factors. The coefficient of the second factor tracks the structural equilibrium change between mixed helix–coil and coil (with local order) structures.[39] In our earlier study of the Ac-$(AAKAA)_4$GY-NH_2 21mer,[37] three factors were identified that demonstrated that an intermediate form was obtained with a substantial population in the helix to coil transformation of the longer peptide. By contrast, if the data were only monitoring structural loss of a single conformational type, just one factor would be important, and its coefficient would track the steady loss in intensity, which would represent a steady loss in structure.

The contrast of data for short homo-oligomers having only the coil structure accessible with that of these Ala-rich oligopeptides that encompass a helix–coil transition best demonstrates the necessity of interpreting the typical peptide random coil conformation as one having a characteristic short range order that can undergo a transition to an even less ordered state at high temperatures. This latter transition is presumably one of increased sampling of conformational space as the internal energy of the molecule is increased. Higher temperatures result in dynamic averaging over many structures, sampling many ϕ,ψ internal torsional conformations, which necessarily leads to reduction of the VCD signal due to cancellation. The VCD results at low temperature remain consistent with the typical peptide random coil form having a locally left-handed twist

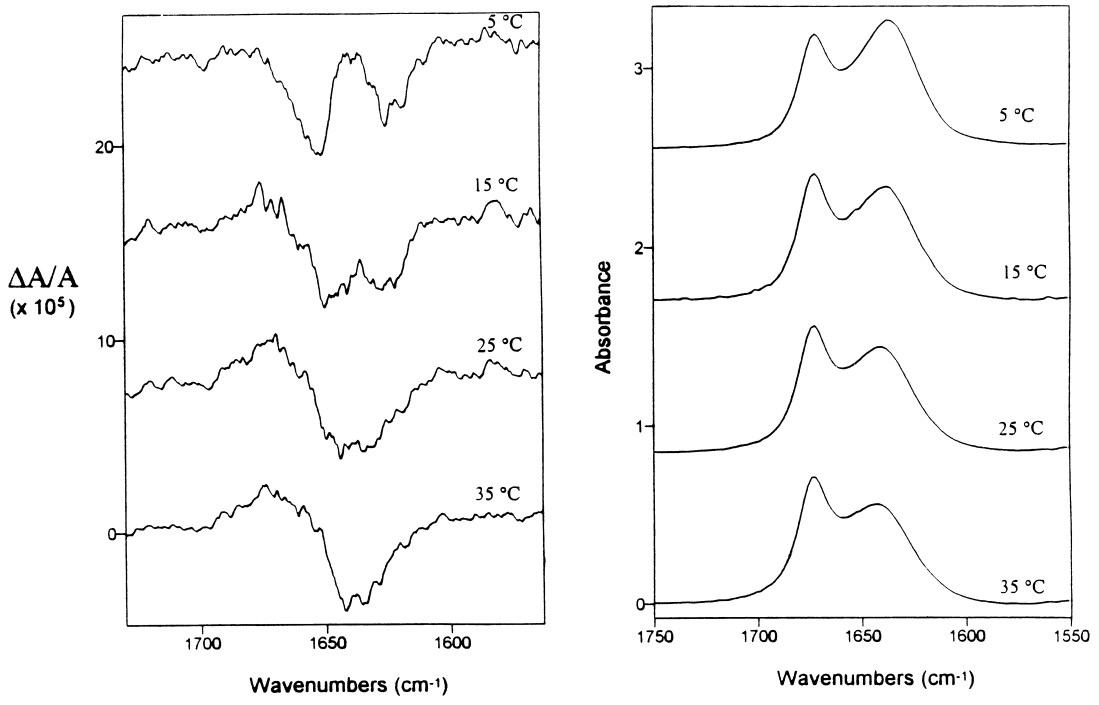

Figure 8. Amide I VCD (left) and FTIR (right) spectra for the Ac-(AAKAA)$_3$-GY-NH$_2$ in D_2O as a function of temperature from 5 to 35 °C.

extended structure. This work confirms the utility of VCD for sensing local order in peptide structures and for providing a useful means of monitoring structural change in such species that contain only such residual local order.

Experimental

Poly-L-glutamic acid (pL-E, avg. M.W. 42,000) and poly-L-lysine (pL-K, MW of 1000, ~25,000 and ~250,000), as well as lysine and glutamic acid dimers, (L-Lys)$_2$ and (L-Glu)$_2$, were purchased from Sigma. (L-Lys)$_3$, (L-Lys)$_4$, (L-Lys)$_5$ and (L-Glu)$_3$ oligomers were purchased from Bachem Bioscience, Inc. Sequential Ala-rich peptides, Ac-(AAKAA)$_3$GY-NH$_2$, were obtained from Prof. Robert Baldwin and Dr. Carol Rohl, Stanford, and used as previously described.[37] All samples were used as received with no further purification, but some samples were lyophilyzed from D_2O (Aldrich) twice to deuterate any exchangable hydrogen positions prior to measuring spectra in D_2O.

Oligopeptide and polypeptide solutions were prepared at a concentration of about 10–50 mg mL^{-1} for VCD and IR studies. All the short Lys$_n$ and Glu$_n$ oligomers and Ala-rich sample were studied in D_2O only. The temperature control experiments on pL-K (MW~1000) were done on samples in phosphate buffer at pH ~7.4. Molecular weight dependent pL-K studies were carried out in D_2O with pH adjusted using DCl or NaOD and corrected for the isotope effect.[40] The pH values were measured with a microelectrode (Ingold) and a Corning 145 pH meter calibrated against standard buffers.

VCD and absorption spectra were measured on the dispersive instrument at UIC. The techniques used and the design of the instrument have been described in detail earlier.[9] Spectra were recorded at ~10 cm^{-1} resolution, collected with a 10 s time constant and averaged over 4 scans. Baseline correction was accomplished by subtraction of the VCD spectrum of either the solvent or a solution of *N*-methylacetamide (Aldrich) in D_2O or poly-D,L-lysine provided that their amide I absorbance matched that of the sample being studied. Calibration of VCD intensities was done with a birefringent plate and a polarizer using our established technique.[9,41] Frequencies were corrected by comparison to FTIR spectra of the same samples measured using a Digilab FTS-60 FTIR spectrometer at 4 cm^{-1} resolution.

Briefly, spectroscopic samples in aqueous solutions were made by placing a small amount of solution between two CaF_2 windows separated with a 0.05 mm or 0.025 mm teflon spacers. For variable temperature experiments, a cell and holder were designed and built at UIC[42] whose temperature was controlled to ±0.2 °C by flowing water through it from a thermostatted bath (Neslab, model RTE-110) as monitored by remote sensor (Neslab, model RS-2) inserted into the jacket. The sample compartment and the monochromator were purged with dry air to reduce atmospheric absorption.

Acknowledgements

This work was primarily supported in the past by a grant from the National Institutes of Health (GM 30147), and additional support was obtained from the UIC Campus Research Board for which we are most grateful. We gratefully acknowledge Professor Robert Baldwin and Dr. Carol Rohl for the Ala-rich peptide samples, Professor Andre Brack of Orleans for the LKKL and KL sequential peptide samples as well as

Professor Claudio Toniolo, Dr. I. Lüscher and Dr. Vladimir Gut for providing some of the samples used in previous related studies upon which this work is based.

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