After 30 min at 20 °C, 81% of the coenzyme was reduced. The enzyme was removed, the coenzyme was purified, and the samples for ^1H NMR measurement were prepared as described with *Leuconostoc* ADH. We found 84 ± 1% [4(*R*)-^1H]- and 16 ± 1% [4(*S*)-^1H]NADH. Four percent of the nonspecific hydrogen transfer is due to the impurity of [4-^2H]-NAD^+; the rest possibly stems from nonenzymatic hydrogen transfer between NADH and NAD.[42]

(42) Ludowieg, J.; Levy, A. *Biochemistry* **1964**, *3*, 373-378.

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Registry No. NAD^+, 53-84-9; $NADP^+$, 53-59-8; [4-^2H]NAD^+, 60797-91-3; [4(*S*)-^2H]NADH, 10021-11-1; EC 1.1.1.1, 9031-72-5; EC 1.1.1.2, 9028-12-0; EC 1.1.1.6, 9028-14-2; EC 1.1.1.156, 39342-20-6; EC 1.1.1.14, 9028-21-1; EC 1.1.1.56, 9014-23-7; dehydrogenase, 9035-82-9.

Vibrational Circular Dichroism of Polypeptides. 8. Poly(lysine) Conformations as a Function of pH in Aqueous Solution†

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Abstract: The conformational transitions of poly(L-lysine) in D_2O solution over the range of pH 7-12 have been studied by using vibrational circular dichroism (VCD). Results in the amide I region show that gradual changes in VCD sign patterns qualitatively reflect the expected transitions from random-coil through right-handed α-helical to antiparallel β-sheet structures. The data indicate that, under our high-concentration measurement conditions, intermediate structures may occur during the pH titration and that at high pH the α-helix is conformationally unstable with respect to β-sheet formation. Additional data on the poly(lysine) conformation in methanol-water solution are presented to illustrate the VCD of the stable α-helix and to study the coil-helix transition in this solvent. This is the first report of solution-phase β-sheet VCD and the first report of VCD for all three major secondary structural types in the same polypeptide. Additionally, these data conclusively demonstrate that VCD is a viable technique for measurements on aqueous solutions and that information regarding secondary structure is available from such measurements.

Recently, we and others have demonstrated that vibrational circular dichroism (VCD) can be used to differentiate among the characteristic secondary structures of polypeptides.[1-10] These studies have shown that protonated α-helical structures yield bisignate amide I and amide A bands[1,2] and a monosignate amide II band[3] whose signs are dependent on the screw sense of the helix and not on the chirality of the α-carbon atom. For right-handed helices, the amide I line shape is modified to a three-peak pattern upon deuteration.[3] Other structural types such as the random coil[6] and 3_{10}-helix[7] have been shown to give solution-phase VCD that is distinguishable from that of the α-helix. In films, β-sheet structures gave frequency-shifted and lower amplitude VCD as compared to α-helices.[4] Up to this time, solution-phase polypeptide β-sheet VCD has not been reported. Polypeptide VCD in aqueous solution, with reasonable signal-to-noise ratio (S/N), has also proven to be elusive. This paper contains the first examples of both.

Poly(lysine) can provide a good test of the characteristic nature of the above VCD measurements in that it is reported to undergo transitions from random-coil through α-helix to β-sheet structures with variation of pH and temperature.[11-17] This variety of structures is facilitated by its polyionic nature due to the lysine side chain. These three structural types have been used by Greenfield and Fasman[16] and others[18-20] as the basis for methods of deconvoluting the electronic CD of globular proteins into component parts. Such procedures have subsequently led to assignment of the fraction of various secondary structural units present in a variety of proteins.

Here, we will present the VCD of these same structural types for poly(lysine), show that it is distinctive for each, and suggest that it could provide the basis of a new method for conformational analysis of polypeptides and proteins. Second, we will demonstrate that the structural transitions proposed for this system are not as simple as was previously thought, that the α-helical state is

† Part 7: Narayanan, U.; Keiderling, T. A.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1986**, *108*, 2431-2437.

(1) Singh, R. D.; Keiderling, T. A. *Biopolymers* **1981**, *20*, 237-240.
(2) Lal, B.; Nafie, L. A. *Biopolymers* **1982**, *21*, 2161-2183.
(3) Sen, A. C.; Keiderling, T. A. *Biopolymers* **1984**, *23*, 1519-1532.
(4) Sen, A. C.; Keiderling, T. A. *Biopolymers* **1984**, *23*, 1533-1545.
(5) Narayanan, U.; Keiderling, T. A.; Bonora, G. M.; Toniolo, C. *Biopolymers* **1985**, *24*, 1257-1263.
(6) Yasui, S. C.; Keiderling, T. A. *Biopolymers* **1986**, *25*, 5-15.
(7) Yasui, S. C.; Keiderling, T. A.; Bonora, G. M.; Toniolo, C. *Biopolymers* **1986**, *25*, 79-89.
(8) Narayanan, U.; Keiderling, T. A.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1986**, *108*, 2431-2437.
(9) Yasui, S. C.; Sen, A. C.; Keiderling, T. A.; Toniolo, C.; Bonora, G. M. *Proceedings of the 9th American Peptide Symposium* In *Peptides: Structure and Function*; Deber, C. M., Kopple, K. D.; Hruby, V. J., Eds.; Pierce Chemical: Rockford, IL, 1985; pp 167-172.
(10) Lipp, E. D.; Nafie, L. A. *Biopolymers* **1985**, *24*, 799-812.
(11) Rosenheck, K.; Doty, P. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 1775-1785.
(12) Sarkar, P. K.; Doty, P. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 981-989.
(13) Davidson, B.; Tooney, N.; Fasman, G. D. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 156-162.
(14) Townend, R.; Kumosinski, T. F.; Timasheff, S. N.; Fasman, G. D.; Davidson, B. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 163-169.
(15) Davidson, B.; Fasman, G. D. *Biochemistry* **1967**, *6*, 1616-1629.
(16) Greenfield, N.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4108-4116.
(17) Painter, P. C.; Koenig, J. L. *Biopolymers* **1976**, *15*, 229-240.
(18) Susi, H.; Timasheff, S. N.; Stevens, L. *J. Biol. Chem.* **1967**, *242*, 5460-5466.
(19) Warashina, A.; Kegani, A. I. *Biopolymers* **1972**, *11*, 529-547.
(20) Lippert, J. L.; Tyminski, D.; Desmeules, P. J. *J. Am. Chem. Soc.* **1976**, *98*, 7075-7080.

0002-7863/86/1508-5576$01.50/0

conformationally unstable at concentrations needed for VCD,[21] and that a new, stable conformation, which is an intermediate in the coil–helix transformation, can be observed at pH ~10.5. Finally, we will present VCD for poly(lysine) in methanol–water solutions that show the expected coil–helix transition for this solvent system as the fraction of methanol is increased and confirm the character of the amide I VCD for a stable α-helical phase in both protonated and deuterated forms.[21-23]

Experimental Section

Poly(L-lysine) (PLL) and poly(D-lysine) (PDL), MW(av) 225 000, were purchased from Sigma. Both samples were lyophilyzed from D_2O twice prior to use. The sampling techniques[6] and the dispersive-design UIC-VCD instrument[24] have been described in detail elsewhere. In addition, infrared absorption spectra were measured on an IBM-32 FTIR spectrometer to obtain higher resolution for the frequency data. In brief, samples were held between CaF_2 windows separated by various Teflon spacers (0.015–0.025 mm) at room temperature. VCD spectra were corrected for instrumental base-line variation by subtracting the concentration-adjusted PDL results from the respective PLL ones and then dividing by two. Such corrections are necessary because the instrumental base line, at the sensitivity needed for some of these spectra, is not flat and is sensitive to sample absorbance.[24] Solutions of poly(lysine) were prepared in D_2O at a concentration of about 25–50 mg/mL. NaOD solution, freshly prepared from sodium metal and D_2O, was used to adjust the pH from about 7 to 12. While initial concentrations were known, we were unable to obtain accurate concentrations after pH adjustment due to the associated unknown dilution factors. Hence, our data are presented in terms of IR absorbance (A) and VCD [ΔA ($=A_L - A_R$)]. VCD intensities were calibrated by using a CdS birefringent plate and polarizer, and the sign was determined by comparison to 3-methylcyclohexanone VCD.[24] The measurement of pH was made by a microcombination electrode (Ingold) and Corning 145 pH meter calibrated against standard buffers. The pH values are not corrected for the isotope effect.

The experimental conditions for obtaining unordered, α-helical and β-sheet poly(L-lysine) in aqueous solution have been often detailed.[11-18] Difficulty was encountered in obtaining a stable α-helical conformation at the high concentrations needed for VCD measurement. This has been noted previously[21] as has spontaneous partial β-sheet formation under high pH conditions.[25] In our experiments, partial formation of the β-sheet structure was experienced when pH 11 samples were allowed to stand at room temperature for more than 2 h. In order to counteract such problems, the samples were kept in an ice bath prior to the pH adjustment and VCD measurement. Even then, our data indicated some changes to have occurred by the end of two VCD scans. A stable β-sheet form of PLL was obtained by heating the mixture, at pH 11–12, to 65 °C for 45 min and then cooling to room temperature before the VCD and IR measurements. No reversion to a different (e.g., α-helical) structure was noted after cooling and scanning of the spectra. The β-sheet form appears to have developed irreversibly.[15]

To illustrate the VCD of fully α-helical PLL, samples were made up in methanol–water (96:4) and CD_3OD–D_2O solutions. Additionally, the relative D_2O concentration in the latter samples was varied from 50% to 5% to observe the VCD of PLL undergoing a coil–helix transition.[22,23] No measurement or adjustment of pH was made on these samples. To avoid methanol evaporation, these latter spectra were run in sealed sample cells consisting of BaF_2 windows separated by a 0.025-mm path as obtained from Foxboro-Wilks.

Results

Figure 1 shows VCD and absorption spectra of random-coil PLL in D_2O solution at neutral pH in the amide I and amide II regions. At neutral pH poly(L-lysine) is expected to evidence a random-coil configuration.[15,18,26,27] The VCD spectrum in the amide I region is virtually identical with that of poly(L-tyrosine) in dimethyl sulfoxide, which is also established to be in a random-coil conformation.[6,28] Due to deuteration, the amide II absorption band is shifted to 1457 cm^{-1} and gives rise to a weak bisignate VCD with a large positive bias. An additional small absorption at 1365 cm^{-1} corresponds to a negative VCD centered at the same wavelength. As can be seen from the figure, the amide II VCD intensity is quite small compared to that of the amide I in this deuterated compound.

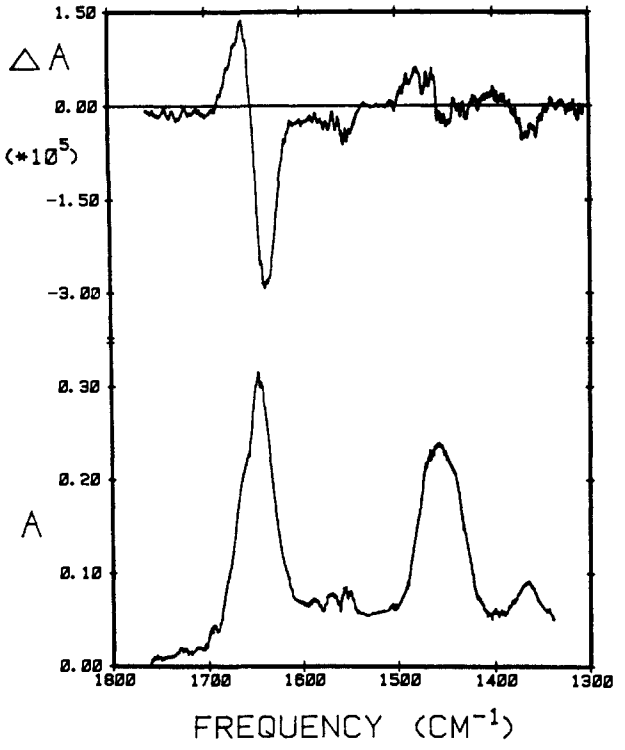

Figure 1. VCD and IR absorption spectra in the amide I and amide II regions of PLL in D_2O at pH 7.3, path length ~ 0.015 mm, four scans averaged, 10-s time constant, resoltuion ~11 cm^{-1} in the amide I region and 8 cm^{-1} in the amide II region.

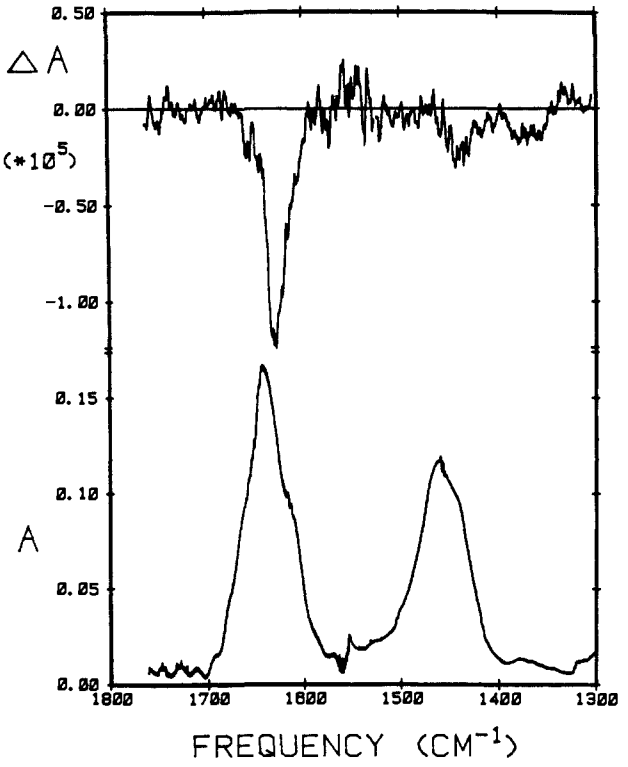

Figure 2. VCD and IR absorption spectra in the amide I and amide II regions of PLL in D_2O at pH 10.5, as in Figure 1.

(21) Hanssum, H.; Rûtherjans, H. *Biopolymers* **1980**, *19*, 1571–1585.
(22) Epand, R. F.; Scheraga, H. A. *Biopolymers* **1968**, *6*, 1383–1386.
(23) Liem, R. K. H.; Poland, D.; Scheraga, H. A. *J. Am. Chem. Soc.* **1970**, *92*, 5717–5724.
(24) Keiderling, T. A. *Appl. Spectrosc. Rev.* **1981**, *17*, 189–226.
(25) Pederson, D.; Gabriel, D.; Herman, J. *Biopolymers* **1971**, *10*, 2133–2145.
(26) Yu, T. J.; Lippert, J. L.; Peticolas, W. L. *Biopolymers* **1973**, *12*, 2161–2176.
(27) Chen, M. C.; Lord, R. C. *J. Am. Chem. Soc.* **1974**, *96*, 4750–4752.
(28) Wen, K.-J.; Woody, R. W. *Biopolymers* **1975**, *14*, 1827–1840.

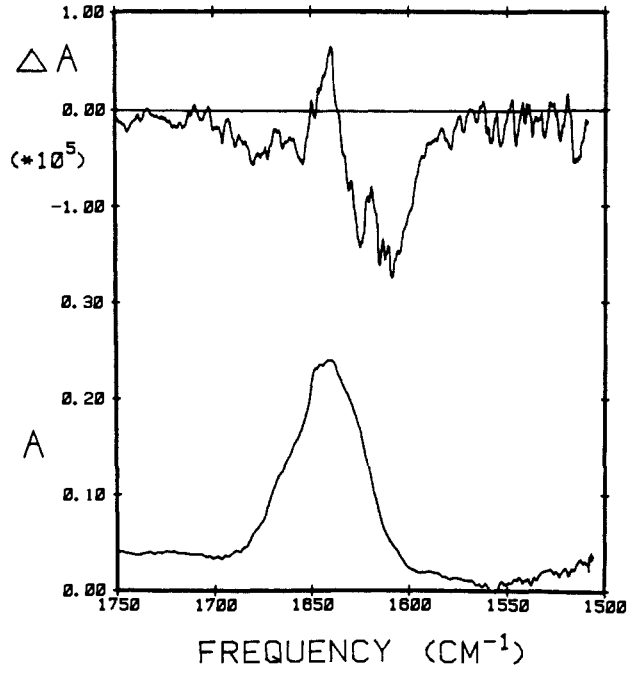

Figure 3. VCD and IR absorption spectra in the amide I region of PLL in D_2O at pH 11.5, as in Figure 1.

When this sample was adjusted to about pH 10.5, it became more viscous and gave the spectra shown in Figure 2. Only a negative monosignate VCD at 1630 cm^{-1} is observed. The amide I IR absorption is broader than at low pH, and the shoulder at 1660 cm^{-1}, which corresponds to the positive VCD of Figure 1, has disappeared while a new shoulder has grown in near 1610 cm^{-1}. The amide II region shows changes as well. The positive bias of the VCD at 1475 cm^{-1} has disappeared, leaving only a negative band at about 1440 cm^{-1}. The other small absorption shifted to 1380 cm^{-1} and gave the same negative VCD. This structure appears to be stable in our measurements and is reproducibly formed at this pH.

As mentioned above, the solution was cooled in an ice bath prior to the adjustment to pH 11–12 in order to slow down the formation of the β-sheet structure. The VCD and absorption spectra of poly(L-lysine) undergoing these dynamic changes are given in Figures 3–5.

Figure 3 shows the first stage of PLL transition after adjusting to pH 11–12. The absorption bandwidth is narrower than at pH 10.5, and a small positive VCD peak centered at 1640 cm^{-1} and negative band at ~1670 cm^{-1} grow into the VCD. The resultant VCD line shape is similar to but much more asymmetrical than that of the amide I band of a deuterated right-handed α-helix.[3,6,9] The relative size of the 1620-cm^{-1} negative VCD is reflective of the pH 10.5 intermediate spectrum (Figure 2). We were unable to measure the VCD in the amide II region since, after 3–4 h, the spectra have changed to that described below.

In Figure 4 is shown VCD of the partially converted state between the α-helical and the antiparallel β-sheet conformation.[15,18,26,27] The small absorption band at 1680 cm^{-1} and a large one at 1610 cm^{-1} are consistent with gradual formation of an antiparallel β-sheet conformation.[15,18,21,25] The VCD spectrum also shows changes in the intensity and sign pattern that appear to be the result of the overlap of α-helical and β-sheet spectra (see below). The amide II band at 1450 cm^{-1} shows virtually no VCD, but the small absorption band at 1380 cm^{-1} still gives a very weak, broad negative VCD.

The result of final conversion to the β-sheet conformation by heating of the sample at pH 11–12 in D_2O[15,18] is seen in Figure 5. The PLL absorption spectrum yields the expected, strong perpendicularly polarized band at about 1610 cm^{-1} and the weaker parallel band at 1680 cm^{-1}.[29] Both absorption bands give rise

(29) Miyazawa, T.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, *83*, 712–719.

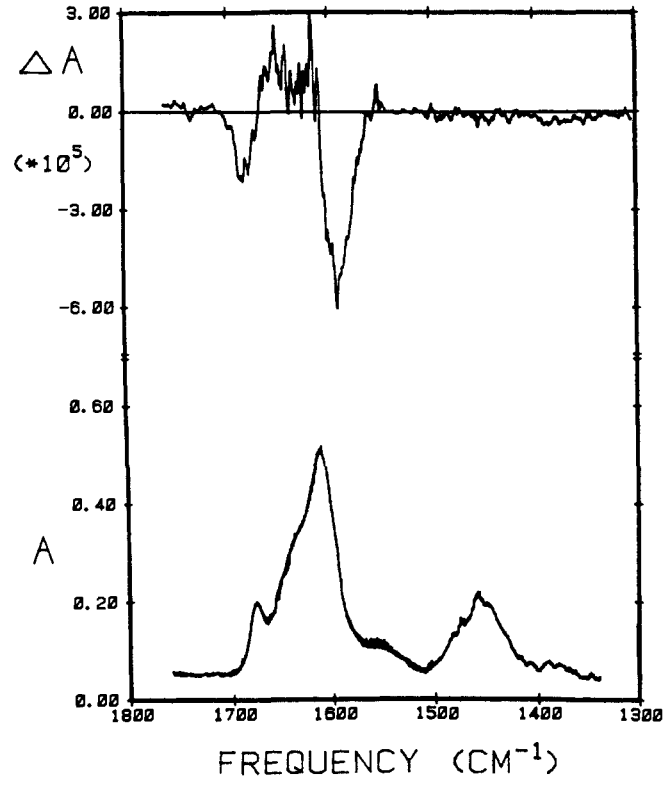

Figure 4. VCD and IR absorption spectra in the amide I region of PLL in D_2O at pH 11.5, 3–4 h after the pH adjustment, as in Figure 3.

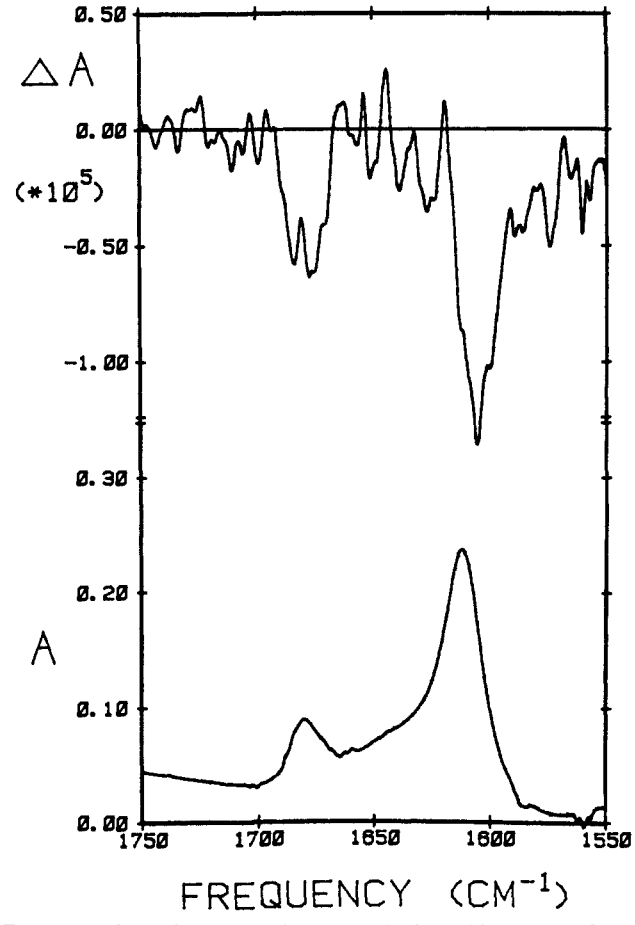

Figure 5. VCD and IR absorption spectra in the amide I region of PLL in D_2O at pH 11.5 and heated at 65 °C for 45 min, path length 0.025 mm, as in Figure 1.

to negative VCD, although the lower energy VCD band is shifted down in energy to ~1605 cm^{-1}. Also, the values of $\Delta A/A$ have

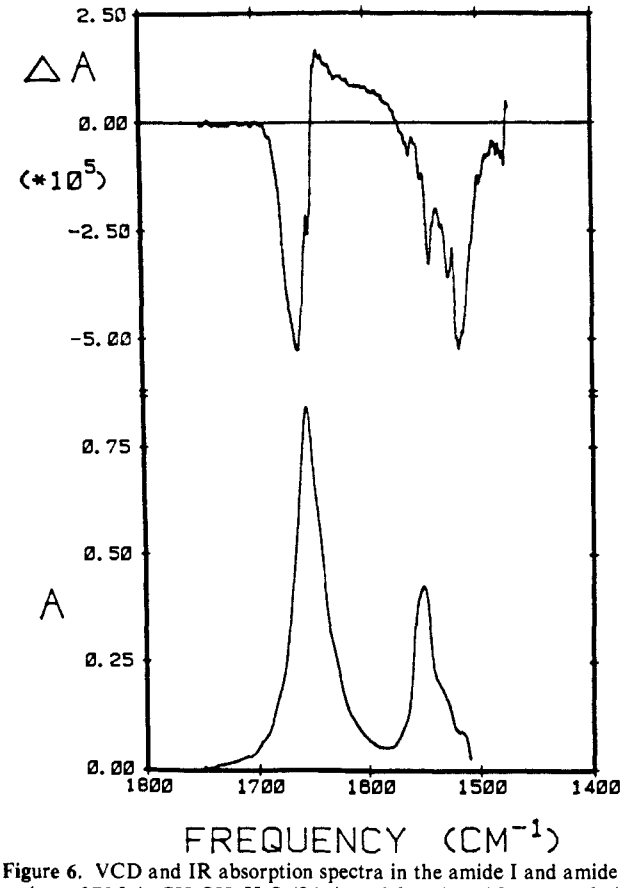

Figure 6. VCD and IR absorption spectra in the amide I and amide II regions of PLL in CH_3OH–H_2O (96:4), path length 0.025 mm, resolution ~12 cm^{-1} with 10-s time constant.

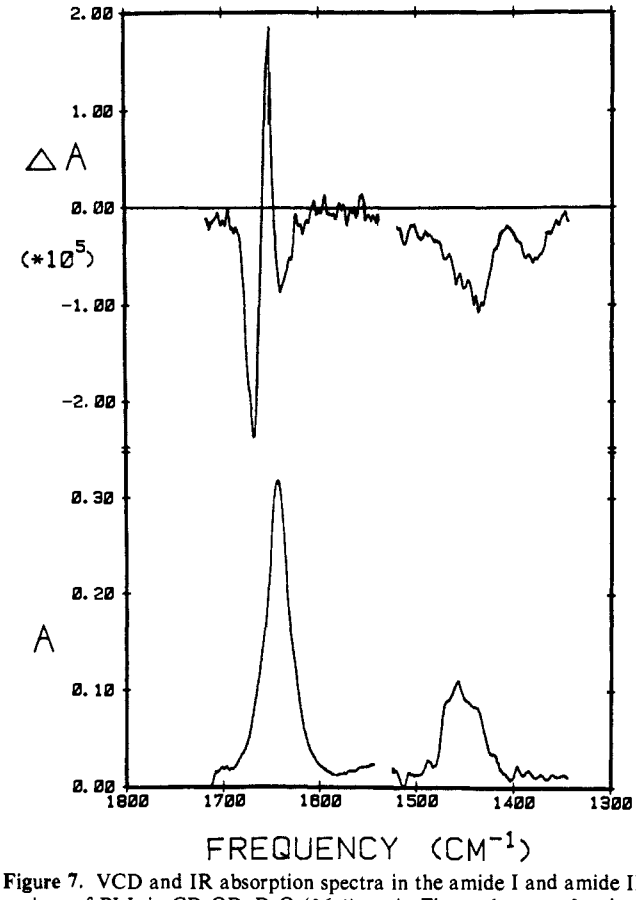

Figure 7. VCD and IR absorption spectra in the amide I and amide II regions of PLL in CD_3OD–D_2O (96:4), as in Figure 6 except 3-s time constant in the amide I region and 10-s time constant in the amide II region.

fallen from those of the intermediate state (Figure 4). We were unable to detect VCD in the amide II region for this conformation.

To demonstrate the amplitude and line shape for the VCD of poly(L-lysine) in a stable α-helical conformation, we made measurements in methanol–water mixtures.[21-23] In Figures 6 and 7 are shown the VCD and absorption of PLL in 96:4 methanol–water and in 96:4 CD_3OD–D_2O, respectively. In the former case, solvent absorption is quite severe, even at the short path lengths used, especially in the vicinity of the amide I band. Hence, base-line artifacts resulting from noise overload of our amplifier circuitry are possible. This may explain the distorted amide I band shapes in both absorption and VCD as well as the excess noise seen in the amide II band as compared to that found for other protonated right-handed α-helices.[1-3]

In deuterated methanol, solvent-absorption interference is not so severe, and the expected, clear, three-featured (−+−) amide I VCD results. While the result is again asymmetrical, the distortion is opposite that of Figure 3 in that the high-energy negative peak dominates. This is consistent with our finding a strong negatively biased VCD (also to high energy) for the protonated PLL in methanol (Figure 6). For these CD_3OD solutions, peak-to-peak $\Delta A/A$ for the amide I is $\sim 1.3 \times 10^{-4}$, which is somewhat smaller than that which would be expected for a deuterated α-helix from our previous studies[6] and is about twice as large as that seen in our D_2O titration result (Figure 3). The very sharp, intense amide I absorption seen in Figure 6 may be somewhat exaggerated due to the strong overlapping solvent bands that could be inadequately compensated by our single-beam instrument.[24]

The amide II result in Figure 6 is consistent in magnitude, line shape, and frequency with what we have observed for other right-handed α-helices, being negative and lower in energy (~1515 cm^{-1}) than the intense, perpendicularly polarized absorption band at ~1550 cm^{-1}.[29] The deuteration-shifted amide II VCD is again negative and is also lower in frequency than the corresponding absorption maximum. The amide II VCD intensity is comparable to that of the amide I in both deuterated and protonated species, contrary to what is seen for the other deuterated secondary structures discussed above.

In a 50:50 CD_3OD–D_2O mixed solvent,[22] PLL gave a broad amide I absorption band and a VCD consistent with that shown in Figure 1, i.e., bisignate, positive to higher energy with a modest negative bias and peak-to-peak $\Delta A/A \sim 10^{-4}$. Thus, a VCD consistent with the established random-coil conformation is seen.[15,18] Upon change of the solvent ratio, the amplitude of the measured signal somewhat decreased but still maintained a broad absorption and the general random-coil VCD sign pattern[6] to <90% CD_3OD. However, by 90% the positive lobe (Figure 1) had virtually disappeared, and a spectrum analogous to that in Figure 2 resulted, indicative of an intermediate state in the helix-coil transition. At 95% the absorption became much sharper, and the VCD, at least partially, flipped in sign to the pattern illustrated in Figure 7. This is consistent with previous reports of a coil-helix transition[22] where the resultant state in 95% methanol is a right-handed α-helix.[30]

The frequencies corresponding to the absorption and VCD of the amide I transition for poly(lysine) under all the above solution conditions are summarized in Table I.

Discussion

As is evident from the figures, we can easily distinguish three major secondary structure types—random coil, right-handed α-helix, and antiparallel β-sheet—via their VCD in the amide I region. The spectral changes are at least as, if not more, dramatic as that seen with conventional UV-CD. Their distinguishability is enhanced by the narrow bandwidths seen in the IR as compared with that seen in the UV. Hence, the established frequency shift of the amide I between α-helix and β-sheet is enhanced by the

(30) Peggion, E.; Cosani, A.; Terbojevich, M.; Scoffone, E. *Macromolecules* **1971**, *4*, 725–731.

Table I. Poly(L-lysine) Infrared and Vibrational Circular Dichroism Frequencies

condition[a]	conformation	amide I freq, cm^{-1} IR[b]	amide I freq, cm^{-1} VCD[c]
D_2O, pH 7.3	random coil	1660 (sh) 1646	(+) 1660 (-) 1635
D_2O, NaOD pH 10.5	unordered (?)[d]	1642 1610 (sh)	(-) 1630
D_2O, NaOD pH 11.5	mixture of α-helix, random coil, and β-sheet	1681 1636 (sh) 1610	(-) 1685 (+) 1650 (+) 1615 (-) 1590
D_2O, NaOD pH 11.5, heated[e]	antiparallel β-sheet	1681 1610	(-) 1678 (-) 1605
CH_3OH–H_2O (96:4)	right-handed α-helix	1651	(-) 1660 (+) 1640
CD_3OD–D_2O (96:4)	right-handed α-helix	1642	(-) 1666 (+) 1650 (-) 1636

[a] The concentration of PLL is about 3–5% by weight; pH values are not corrected for the isotope effect. [b] Frequencies are measured by the IBM-32 FTIR spectrometer with 2-cm^{-1} resolution. [c] Frequencies are obtained from UIC-VCD instrument with 11-cm^{-1} resolution. The + and - signs represent the positive and negative features of the VCD absorption bands. [d] Intermediate structure during the conformational transition from random coil to α-helix and antiparallel β-sheet at high concentration (3–5% by weight). [e] See text for procedure.

concomitant VCD change from a tightly overlapped bisignate (or a triple peak in D_2O) band shape to two separated negative bands. On the other hand, the very small frequency change between α-helix and random coil is made dramatic by the virtual sign reversal in their VCD spectra.

One possible note of confusion does remain. As previously noted in the case of poly(L-tyrosine),[6] our measured random-coil amide I VCD is qualitatively similar to that found for left-handed α-helices of L-amino acids.[2,3] For poly(tyrosine), it was possible to use the amide A bands to distinguish between the helical and coil structures.[6] In aqueous solution, the amide A is not accessible due to solvent interference, and, as for the only currently accessible alternate band, the amide II S/N in D_2O solution is too poor for reliable structural differentiation. However, amide I band widths, as seen in our CD_3OD experiments, might be used to differentiate the left-handed α-helix from the random coil. The large band width of random-coil amide I IR absorption in comparison to that of β-sheets has been previously discussed.[31] By comparison of results presented here, the differences in amide I bandwidth for an α-helix (Figure 7) and random coil (Figure 1) are noticeable but not dramatic. From our previous results,[3] it appears that the left-handed α-helical poly(β-benzyl-L-asparate) has at least as narrow an amide I band as do various right-handed α-helices we have studied. Thus, it may prove possible to couple IR absorption band shape with VCD to distinguish random coils and left-handed α-helices; but, at present, the data set is too limited to be conclusive. Hence, characterization of the left-handed, L-amino acid polypeptide at the VCD level remains a question open to debate, which may be best settled by coupling our data to other spectral (e.g., UV-CD) or physical results.

On the other hand, the random-coil conformation itself is well established for poly(L-lysine) at neutral pH[15] and poly(L-tyrosine) in Me_2SO,[28] and both polypeptides give virtually identical amide I VCD.[6,9] For poly(L-tyrosine), the random-coil amide I band shape has been shown to be relatively unaffected by deuteration while the α-helical VCD is not.[6] The same appears to be true in PLL. For the random coil, the peak-to-peak $\Delta A/A$ for the random coil ($\sim 1.3 \times 10^{-4}$) is quite large in both polypeptides. From our point of view, this is probably due to the relatively local nature of the dipolar coupling that presumably is a prime source of the observed amide I VCD.[32] Even in a true random coil, a limited region of (ϕ,ψ) space will be the most heavily populated. This could lead to many nearly equivalent dimer-type couplings that would contribute additively to the VCD. Since vibrational dipoles are small, the contribution of the extended polymeric structure to the VCD is expected to be more limited than is the case for electronic CD.

The α-helical phase of poly(L-lysine) is not conformationally stable under the conditions of our experiment as it tends to form a β-sheet structure with time. This explains the unusual breadth of the absorption band in Figure 3 and the asymmetry seen in the VCD as compared to both our earlier published deuterated α-helical VCD[3,6] and the CD_3OD–D_2O results in Figure 7. The increased negative VCD in Figure 3 at ~ 1620 cm^{-1} is most likely due to overlap with a contribution from the growing in of the β-sheet conformation. This becomes clear, considering the time-dependent VCD changes shown in Figure 4. In addition, in Figure 3, a mixed α–β structure is implicated by the relatively low value of $\Delta A/A$ seen (~ 5–8×10^{-5}) as compared to that previously measured ($\sim 2 \times 10^{-4}$) for α-helices.[3,6]

Previous studies of the PLL conformation in MeOH–H_2O have reported 100% formation of an α-helical structure.[22,30] Our data (Figure 7) for PLL in CD_3OD–D_2O has $\Delta A/A$ somewhat below that seen for other α-helices in $CHCl_3$ and Me_2SO.[3] While this difference may be due to the solvent, no previous data exist on the solvent dependence of $\Delta A/A$ to confirm this. In small molecules such an effect has *not* yet been seen. An alternate explanation for this difference in VCD magnitude would be that a fully α-helical form is not attained in CD_3OD. Our data cannot, at present, substantiate or refute this possibility. A referee has suggested that small changes in (ϕ,ψ) caused by a change of side chain could also explain the reduced VCD in the poly(lysine) α-helix. Our experiments varying the CD_3OD:D_2O ratio indicate that a change from random coil to α-helix results above 90% CD_3OD and that this VCD change is accompanied by significantly narrowed absorption. Furthermore, the VCD goes through an intermediate state at $\sim$90% methanol that parallels the pH 10.5 state (Figure 2) found in the aqueous titration result. This result tends to substantiate that a new conformation for PLL has been found (vide infra). Both the coil and helix VCD have in common band shapes that share an overlapping, low-energy negative VCD that, in principle, could mask some residual random-coil structure in the α-helix. Furthermore, it should be noted that previous polypeptide VCD studies[3] dealt with polypeptides having more polarizable side groups. It is possible that relative polarizabilities could lead to a variation in intensity among polypeptides of the same secondary structure.[33]

A final comment about the α-helical band shape can be made. In the previously published VCD of deuterated α-helices in $CHCl_3$, the three-peaked amide I VCD pattern was relatively symmetrical. In poly(L-tyrosine) in Me_2SO–TMP and here for PLL in CD_3OD–D_2O, the lower energy negative VCD band is significantly weaker. If, as has been proposed,[10] this pattern must be a result of splitting of the various exciton bands, that splitting must be solvent dependent to explain this band-shape variation. In particular, the limited data set available suggests that hydrogen-bonding solvents may contribute in the deuterated α-helical pattern shown in Figure 7 as opposed to the symmetrical one found in $CHCl_3$.

The final conformationally stable structure seen at high pH after heating, an antiparallel β-sheet,[15,18] gives an unexpected, negative amide I VCD of relatively low magnitude ($\Delta A/A \sim 5 \times 10^{-5}$). From dipolar coupling considerations, Schellman has predicted that the parallel and perpendicular polarized bands in the β-sheet should each have VCD with approximately equal

(31) Chirgadze, Y. N.; Shestopalov, B. V.; Venyaminov, S. Y. *Biopolymers* **1973**, *12*, 1337–1351.

(32) Schellman, J. A. In *Peptides, Polypeptides and Proteins*; Blout, E. R., Bovey, F. A., Goodman, M., Lotan, N., Eds.; Wiley: New York, 1974; pp 320–337. Snir, J.; Frankel, R. A.; Schellman, J. R. *Biopolymers* **1975**, *14*, 173–196.

(33) Barnett, C. J.; Drake, A. F.; Kuroda, R.; Mason, S. F. *Mol. Phys.* **1980**, *41*, 455–468.

magnitudes but be opposite in sign.[32] Clearly, such a result is not observed. This negative VCD must then not result from interpeptide dipolar coupling of degenerate local modes but must arise from intermode coupling either within each peptide subunit or with other modes of the polymer (i.e., all the vibrational modes). While the amide II might be a likely mixing mode, we see no detectable VCD ($\Delta A/A < 10^{-5}$) there in the β-sheet.

It should be also noted that this is the first report of β-sheet, polypeptide VCD in solution and that these are VCD of deuterated polypeptides. For protonated *oligopeptides*, VCD has been observed for β forms with various band shapes that are, in fact, different from that shown in Figure 5.[8] In addition, our film VCD of polypeptides in a β-sheet conformation yielded bisignate band shapes, but this probably is due to the effects of β film formation.[4,5,8] It is gratifying to note, however, that α-chymotrypsin and carbonic anhydrase, both globular proteins that are known from crystal structure data to have significant β-sheet components,[34,35] give amide I VCD at $\sim$1630 cm^{-1}, which evidences a strong negative bias.[9] A systematic study of β-sheet containing proteins is under way in our laboratory to determine the generality of these results.

An additional piece of evidence for the importance of intermode mixing is the relatively low intensity of the amide II VCD seen in these and other deuterated polypeptide spectra.[3,6] Mixing of the amide I and II modes could explain the sharp change in their respective VCD spectra with an increase in their splitting from $\sim$100 to $\sim$200 cm^{-1} and the consequent change in the character of their vibrational coordinates.[29] That such deuteration effects are more pronounced in the α-helix than the coil should not be so surprising upon consideration of their relative structures. The 5→1 hydrogen bond of the α-helix should serve to couple the C=O stretch and CNH deformation in a coherent manner throughout the polymer. This coherence will tend to give rise to a larger VCD effect, irrespective of source, and the effective decoupling attendant upon deuteration will thus have a larger effect.

The new aspect for poly(lysine) conformational analysis that becomes evident from our data is highlighted in Figure 2. The transition at high pH from coil to helix apparently involves an intermediate structure that is conformationally stable at pH 10.5 and gives a decidedly different VCD spectrum from either the coil or the helix. The IR absorption has a new shoulder grown in at $\sim$1620 cm^{-1}. The VCD, on the other hand, has a strong negative band at $\sim$1630 cm^{-1} that appears to be equivalent to the negative component of the bisignate spectrum seen for the random coil (Figure 1). While a parallel VCD is found in the CD_3OD–D_2O solvent variation study, the absorption band shape is less clear due to solvent interference. The change of the absorption and VCD from Figures 1 to 2 can be correlated if it is assumed that the positive band in the random-coil spectrum has shifted down in energy and, thus, has become cancelled by the negative VCD band that does not move. This would explain the asymmetric negative VCD band shape (Figure 2) and the shift in energy of the IR absorption shoulder from higher to lower than the main 1650-cm^{-1} peak. In line with this correlation, the VCD intensity for this new conformer is substantially weaker than that of the negative band for the random coil. Such a scenario would be consistent with the random-coil bisignate VCD (Figure 1) being the result of the overlap of two independent monosignate VCD bands as opposed to its being of a dipolar coupling origin. This argues for the source of such VCD as being local in origin or, at least, not being due to the long-range extended structure. Such a local character would be, however, apparently independent of the nature of the side chain.

The above view of the pH-dependent (and solvent-dependent) coil–helix transition would then lead us to postulate that two different "random-coil" structures exist for poly(L-lysine), one at low pH and one at high pH. The low-pH one apparently has the more typical structure judged from its similarity to the poly(tyrosine) result. With regard to the new, high-pH "random-coil" structure for PLL, one might be tempted, from the frequency and sign of the VCD (Figure 2), to hypothesize a contribution from a β structure. Unfortunately, nothing in our data can confirm such a hypothesis. Presumably, our data imply that the relative population of accessible parts of (ϕ,ψ) space are significantly varied with pH change without inducing any long-range order that would affect the previously studied electronic CD. Hence, the short-range sensitivity of VCD has let us distinguish two "random-coil" PLL conformations. A reviewer has proposed an alternative interpretation of this high-pH "random-coil" PLL as being a mixture of coil and α-helical conformations. We have not been able to synthesize the Figure 2 result from a combination of our typical coil and helix results, which should be possible if this new state of PLL is indeed a mixture. Our parallel findings in the CD_3OD–D_2O experiment let us clearly eliminate such a posibility in that case since both forms—α-helix and random coil—can be studied as nearly pure conformers in this system.

Conclusion

We have shown in this paper that VCD is indeed measurable for polypeptides in aqueous solution with good S/N and that it can yield spectra of structural interpretability. In one polypeptide, poly(L-lysine), we have shown what appears to be characteristic right-handed α-helical, antiparallel β-sheet, and random-coil VCD for the amide I band. Corresponding amide II data were less easily measured under these conditions. In addition to these characteristic spectra we have identified a new "random-coil" PLL conformation that appears to be stable at these concentrations at pH $\sim$10.5 or in 90% methanol. This occurs in our experiments as an intermediate conformation in the well-known PLL coil–helix transition with increasing pH or increasing methanol concentration.

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Note Added in Proof. After this paper was accepted for publication, we became aware that the work of Paterlini, Freedman, and Nafie on polylysine VCD had been submitted for publication.[36] They have obtained similar spectroscopic results but have interpreted the random coil results as being due to an extended helix. If such a structure is present, it must be ordered on only a local level to be consistent with all of our data. These authors also propose vibration-induced electronic currents to explain the β-sheet VCD.

Registry No. L-Lysine homopolymer, 25104-18-1; poly(L-lysine), SRU, 38000-06-5.

(34) Liljas, A.; Kannan, K. K.; Bergsten, P. C.; Waara, I.; Fridborg, K.; Strandber, B.; Carlbom, U.; Järup, L.; Lövgren, S.; Petef, M. *Nature (London), New. Biol.* **1972**, *235*, 131.

(35) Saxena, V. P.; Wetlaufer, D. B. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 969–72. Birktoft, J. J.; Blow, D. M.; Henderson, R.; Steitz, T. A. *Philos. Trans. R. Soc. London, B* **1970**, *257*, 67.

(36) Paterlini, M. G.; Freedman, T. B.; Nafie, L. A. *Proc. Soc. Photo. Inst. Eng.* **1985**, *553*, 274–75. Paterlini, M. G.; Freedman, T. B.; Nafie, L. A. *Biopolymers*, in press.