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# Reassessment of the electronic circular dichroism criteria for random coil conformations of poly(L-lysine) and the implications for protein folding and denaturation studies

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The circular dichroism (CD) spectra of poly(L-lysine) in water and ethanediol/water (2:1) solutions in the temperature range −110 to 85 °C are presented. The results combined with vibrational CD data are interpreted in terms of a two-state conformational equilibrium with a left-handed *trans* polyproline II conformation being preferred at low temperatures. The relevance of these studies to the CD criteria for random-coil conformations, the study of helix-coil transitions and protein/peptide folding is pointed out.

## 1. Introduction

Electronic circular dichroism (CD) in the far and vacuum ultraviolet has been used extensively to characterise [1–3] the secondary structure content of proteins and peptides. A consensus has arisen concerning the general CD patterns associated with the various forms of the $\alpha$-helix, $\beta$-sheet and $\beta$-turn conformations; the absolute magnitude of these CD contributions is less clear. A fourth 'conformational state' with an associated CD spectrum is generally designated 'random coil'. The electronic CD spectrum of poly(L-lysine) in $H_2O$ at pH 7.6 has often been considered to derive from and has been cited [2] as a standard of a random-coil conformation. However, it has always been a debatable point [4] why some proteins said to be in a random-coil conformation lack the small positive CD at approx. 215 nm observed in this poly(L-lysine) spectrum. An excellent review of the CD spectra of polypeptides has been published [4] by Woody.

Vibrational CD spectroscopy of the amide I band at approx. 1600 $cm^{-1}$ led [5,6] two groups to question the attribution of a random-coil conformation to aqueous poly(L-lysine) (pH 7.6); the alternative possibility of a left-handed helix was mentioned [5] in these papers. The combination of vibrational and electronic CD data, including the use of cryogenic solvents and solvent perturbations, confirms that the electronic CD spectrum of polylysine arises from a two-state conformational equilibrium. This necessitates a careful reassessment of the CD criteria for the random-coil state of poly(L-lysine) and other peptides. The implications for CD studies of helix-coil transitions and other protein/peptide folding studies are discussed.

## 2. Results and discussion

Presented here are the variable-temperature electronic CD spectra of poly(L-lysine) in the range

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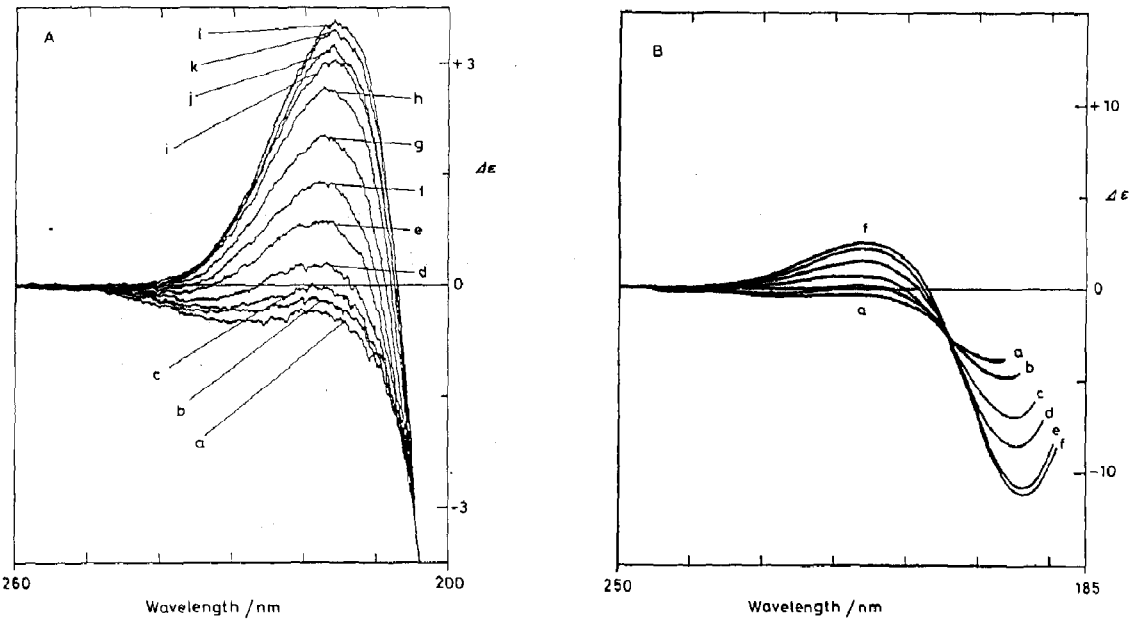


Fig. 1. CD spectra of poly(L-lysine)·HBr in ethanediol/water (2:1), pH 7.6. (A) 260–200 nm region: (a) 82, (b) 69, (c) 60, (d) 45, (e) 21, (f) 2, (g) −26, (h) −55, (i) −75, (j) −85, (k) −95, (l) −105° C. (B) 250–185 nm region: (a) 84, (b) 60, (c) 22, (d) −28, (e) −80, (f) −102° C.

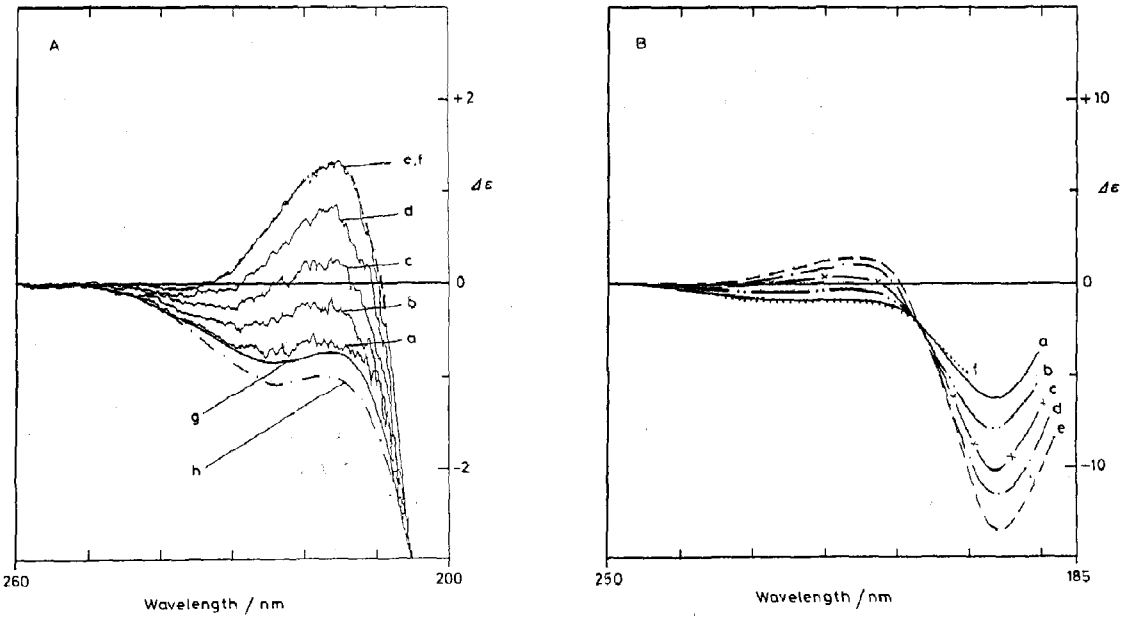


Fig. 2. CD spectra of poly(L-lysine)·HBr in water, pH 7.6. (A) 260–200 nm region: (a) 85, (b) 62, (c) 43, (d) 23, (e) 6° C, (f) 23° C with 4 M urea, (g) 7° C with 5 M NaCl, (h) 87° C with 5 M NaCl. (B) 250–185 nm region: (a) 87, (b) 63, (c) 40, (d) 19, (e) 6° C (f) 22° C with 5 M NaCl.

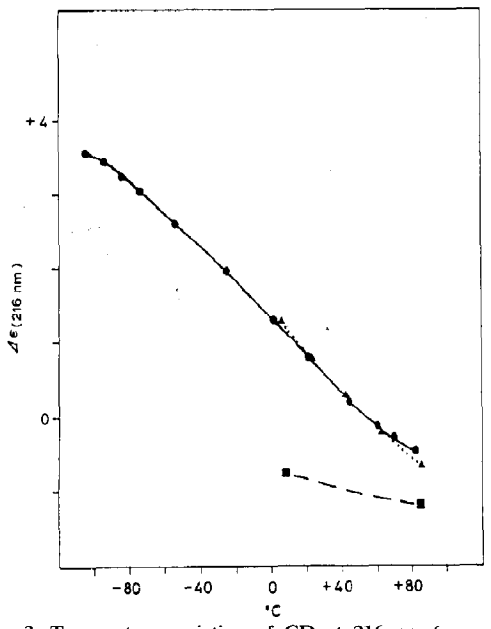


Fig. 3. Temperature variation of CD at 216 nm (———). Ethanediol/water (2:1); (·····) water, pH 7.6; (- - - - -) water, pH 7.6 with 5 M NaCl.

−110 to 85 °C in ethanediol/water (2:1) at pH 7.6 (figs. 1 and 3) and 4 to 80 °C (figs. 2 and 3) in water at pH 7.6. In the two solvents there is clearly continuity between −110 and 85 °C associated with a conformational equilibrium between two states as indicated by the observation of an isodichroic point at about 203 nm. Data are also reported for solutions in 5 M NaCl (figs. 2B and 3) and 4 M urea (fig. 2A).

The two states can be characterised as follows:

(i) A low-temperature state completely established below −90 °C (fig. 3) which is ordered and characterised at pH 7.6 by the vibrational CD [5,6] and the −100 °C electronic CD. The published [5,6] room-temperature vibrational CD spectra must correspond to about 50% of this state (see fig. 3). The electronic CD has a positive band at 218 nm ($n \rightarrow \pi^*$) with a negative band at approx. 195 nm ($\pi \rightarrow \pi^*$).

(ii) A high-temperature state which has an electronic CD spectrum similar to that observed at pH 7.6 in 5 M NaCl (figs. 1B and 2B). The relatively minor temperature dependence of the 5 M NaCl solution gives identity to this state with its lack of detectable vibrational CD [5]. The electronic CD spectrum shows two negative bands, one at 225 nm ($n \rightarrow \pi^*$), the other at approx. 195 nm ($\pi \rightarrow \pi^*$).

The wavelength specification of a CD band maximum is often misleading. This is particularly true for overlapping positive and negative CD components that occur at similar, but not equal, wavelengths. Cancellation presents 'artificial' maxima. Thus, solutions containing both of the states described here may possess a three-banded CD spectrum. Accordingly, the low-energy part of the spectrum may present 'residual' peaks (negative in sign) around 235 nm. This is demonstrated by the continuity of the CD data presented in figs. 1A and 2. In this manner, we interpret the data of Tiffany and Krimm [7] and others [3], which were discussed [3] at length by Woody. Many of the CD data that have been ascribed to random-coil polypeptide structures can be discussed in terms of the low- and high-temperature CD spectra defined here.

Two other experimental observations related to poly(L-lysine) are noteworthy. Firstly, a typical denaturing medium, 4 M urea, does not disrupt the low-temperature, aqueous, pH 7.6 state of poly(L-lysine) (fig. 2A). On the contrary, 4 M urea appears to stabilise this state by some 15 °C. Secondly, the more usual cryogenic solvent methanol/glycerol (9:1) induces a remarkable immediate conversion to an α-helix even at room temperature (fig. 4). Accordingly, we advocate the use of an ethanediol/water mixture as a good cryogenic replacement for pure water.

What is the nature of these two states? The low-temperature poly(L-lysine) state has a CD spectrum very similar to that of collagen [8,9]. Polyproline (II) is also similar [8,9] although red-shifted by about 5 nm due to the tertiary nitrogen. In both these proteins, *secondary* structure is fully expressed as left-handed, *trans*-polyproline type II. The solution CD spectrum of collagen (ethylene glycol/water, 2:1) [7] is temperature-independent in the range 24 to −112 °C and resembles very closely, in both wavelength and intensity, the low-temperature poly(L-lysine) (pH 7.6) spectrum in fig. 1. A single left-handed helix does not seem to require hydrogen bonds for stabilisation (cf.

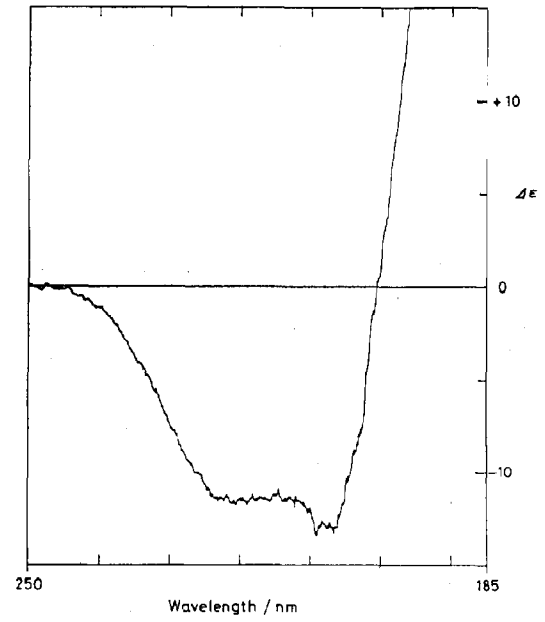


Fig. 4. CD spectrum of poly(L-lysine)·HBr in methanol/glycerol (9:1) at 22° C.

polyproline). However, urea disrupts [7] the tertiary and quaternary structure (right-handed superhelix) of collagen. This implies that the interchain hydrogen bonds are required to maintain the left-handed conformation in this case. The CD spectrum of 'denatured' collagen resembles [7] that reported here for high-temperature poly(L-lysine) (pH 7.6) (figs. 1 and 2).

The vibrational CD of poly(L-lysine) (pH 7.6) is consistent [5,6] with a left-handed helix. Indeed, recent vibrational results [10] confirm that there is a similarity between the aqueous poly(L-lysine) and polyproline II structures. These observations, coupled with the discussion in Woody's review [3], lead us to reinforce the proposal that, in solution, many oligopeptides contain a substantial fraction in a left-handed *trans*-proline type II conformation. This structure would seem to be favoured by poly(L-lysine) (pH 7.6) at −100° C.

The high-temperature state of poly(L-lysine) remains to be defined more precisely particularly with respect to its apparent lack of vibrational CD.

As a part of our extensive cryogenic studies of the CD spectra of linear oligopeptides we have on numerous occasions 'frozen out' a low-temperature conformation with electronic CD spectra similar to that found for aqueous poly(L-lysine) (pH 7.6). The CD temperature profiles of these peptides are now understandable as representing a conformational equilibrium between a structure preferred at higher temperatures and the readily defined state presented by poly(L-lysine) at pH 7.6 and −100° C. A refinement of the identity of these two states and their relevance to biologically important linear oligopeptides will be the subject of future papers.

## 3. Experimental

CD spectra were recorded with a Jasco J40CS spectropolarimeter employing techniques previously described [11]. All data are corrected for solvent contraction at low temperatures, are reversible and independent of concentration (0.15–7.0 mg/ml). Poly(L-lysine)·HBr samples were purchased from Miles-Yeda ($M_r$ 46 500: degree of polymerization (DP) 222) and Sigma ($M_r$ 20 000: DP 100).

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