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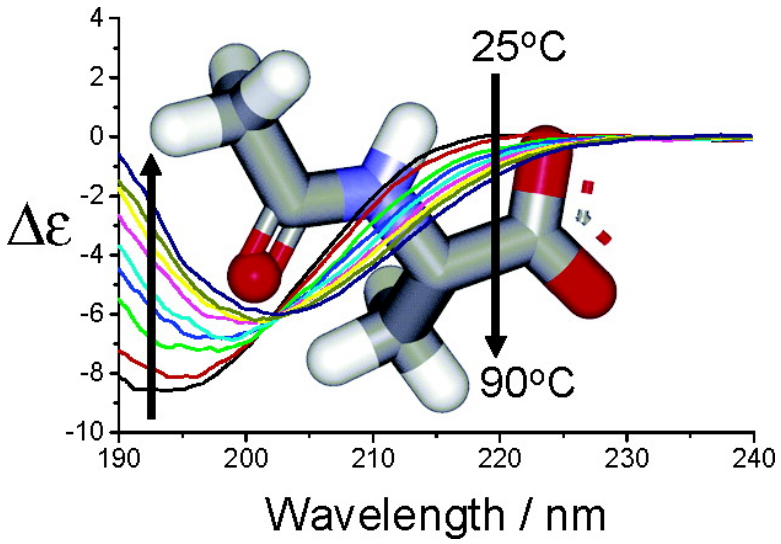
J. Am. Chem. Soc., **2005**, 127 (27), 9700-9701• DOI: 10.1021/ja052632x • Publication Date (Web): 16 June 2005



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Published on Web 06/16/2005

Single Peptide Bonds Exhibit Poly(Pro)II ("Random Coil") Circular Dichroism Spectra

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The protein "random coil" circular dichroism spectrum is strong, highly characteristic, and mysterious. It is probable that it represents a highly dynamic structure similar to the poly(Pro)II (PPII) helix, but it remains the least well-defined CD spectral form.[1] Understanding the disordered state of proteins is increasingly important to explain both protein folding and protein function. The urea-denatured polypeptide has been the starting point for measurements of protein folding, but it is now clear that even this paradigm contains residual structure. Furthermore a large number of functional proteins are now known to show features of unfolded polypeptides and have been termed "natively unfolded".[2,3]

Since the main influence of primary structure on disordered protein structures is local, it is useful to study small peptides. Here long-range effects are eliminated, and the weak effects of individual side chains are enhanced. It was assumed that such structures have no defined conformation, but this is not true. In one recent example, Eker et al.[4] present far-UV CD data using tripeptides that clearly show stable side-chain-specific conformations. This work is supported by previously published FTIR, vibrational CD, and Raman spectroscopic data.[5] It shows that alanine tripeptides adopt two conformations: a PPII-like conformation and another like β-strand. Valine tripeptides adopt only the latter type of structure. Upon heating, the CD minimum of the alanine peptide undergoes a red shift with a clear isodichroic point as in model coils (e.g., poly-(Glu)) studied by Tiffany and Krimm.[1] The strength of the minimum is reduced in D_2O solution, indicating a role for hydrogen bonding.

Here we use two types of model peptide to show that complete PPII CD is seen in compounds containing only one peptide bond and that the contributions of the peptide bonds on either side of the chiral alpha-carbon are distinct. Brand and Erlanger in 1950[6] showed by optical rotatory dispersion that Ala-Gly and Gly-Ala dipeptides exhibited opposite and unequal optical rotations, and CD spectra corresponding to this study are shown in Figure 1. The contribution from the peptide bond N-terminal to the alanine (Gly-Ala) corresponds to the 195 nm negative maximum of the PPII spectrum, while the C-terminal peptide bond (Ala-Gly) shows a broad, weak positive signal. The Ala-Ala spectrum is identical to the sum of these two.

Ac-Ala and Ac-AlaNH_2 both show 195 nm negative maxima. AlaNH_2 shows a much weaker positive signal, comparable to Ala-Gly. Thus the negative maxima of Ac-Ala and Ac-AlaNH_2 are due to the effects of the chiral alpha carbon on their N-terminal peptide bonds, and the wavelength indicates that a $\pi-\pi^*$ transition is responsible. The side chain has an effect since other *N*-acetyl-L-amino acids have very different spectra, especially when it contains a strong chromophore (Ac-Phe) (Table 1).

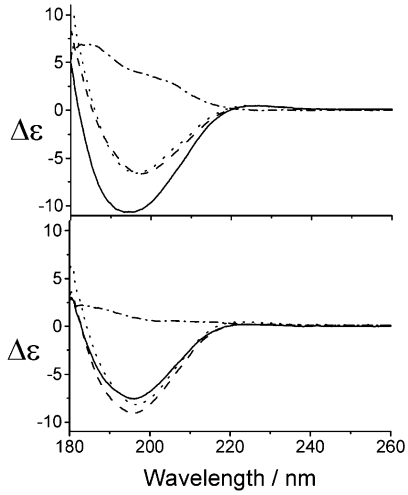

Figure 1. UV−CD of model peptides. Upper frame: Ala-Gly (dot−dash), Ala-Ala (dash), Gly-Ala (solid), Ala-Gly + Gly-Ala (dot). Lower frame: Ala-amide (dot−dash), Ac-Ala-NH_2 (dash), Ac-Ala (solid), Ala-NH_2 + Ac-Ala (dot).

Table 1. Main Maxima of CD Spectra for Selected Model Compounds[a]

model compound	λ (nm); intensity (Δε) negative maximum	positive maximum
Ac-Val	200; −6.02	n/a
Ac-Ala	195; −7.56	224; 0.18
Ac-Asp	200; −5.68	219; 1.47
Fm-Asp	200; −12.11	226; 0.77
Ac-Glu	200; −3.86	219; 0.56
Ac-Lys	198; −4.23	231; 0.14
Ac-Met	196; −2.03	215; 0.94
Ac-Asn	195; −6.00	224; 0.27
Ac-Pro	210; −7.36	n/a
Ac-Phe	184; −31.2	196; 23.1
Ac-Ala pH 2	188; −9.7	211;1.94
Ac-Asp pH 2	187; −3.82	207; 0.54
Ac-Glu pH2	188; −4.83	209; 2.81
Gly-Ala	195; −10.64	n/a
Ala-Gly	n/a	182; 6.86
Ala-Ala	198; −6.63	226;0.46

[a] Concentrations 1 mg/ml, pH 7.0 50 mM sodium phosphate or pH 2 buffered with KCl/HCL 100mM. Path length 0.1 mm. Jasco 810 at 25°C. Ac, *N*-acetyl; Fm, N-formyl. All stereoisomers were L. Compounds were supplied by Sigma or Bachem and used without further purification.

Fm-Asp (Fm = N-formyl) and Ac-Asp both have negative maxima at 200 nm, but the former has a much stronger signal. At pH 2, Ac-Ala, Ac-Glu, and Ac-Asp have negative maxima shifted to shorter wavelengths.

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10.1021/ja052632x CCC: $30.25

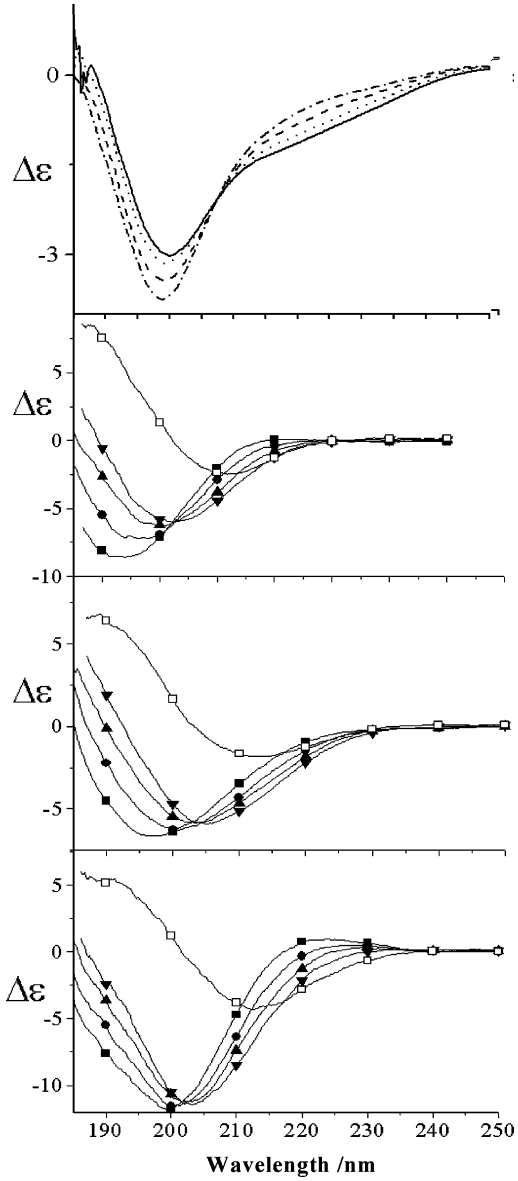

Figure 2. Temperature dependence of model peptides by far-UV−CD. Upper frame: colicin N translocation domain. Temperatures (°C) 15 (dot−dash), 35 (dash), 65 (dot), 90 (solid). Upper middle: Gly-Ala. Lower-middle: NAc-Val. Lower frame: NFor-Asp (■) 5, (●) 35, (▲) 65, (▼) 95, (□) 95 °C spectrum minus 5 °C spectrum. Pathlength 1 mm, concentration 0.1 mg/mL 50 mM Na phosphate pH 7 at 25 °C.

The positions of the negative maxima therefore depend on the side chain and the zwitterion-cation equilibrium. We replaced H_2O by D_2O in solutions of Ala-Gly and Gly-Ala. Both showed reduced intensities, and the Gly-Ala negative maximum ($\Delta\epsilon = -7.85$) occurred at a shorter wavelength of 192 nm. Thus the effect of hydrogen bonding on formation of the PPII structure in the alanine tripeptide [4] is also observed in this basic model.

The alanine tripeptide shows a two-state thermal unfolding.[4] As temperature is increased, the fraction of PPII structure decreases in favor of the β form, displaying a clear isodichroic point at 202 nm and a β-strand difference spectrum. Hence, as a final test of the validity of PPII structures formed by the single-peptide models, we collected a series of spectra from 185 to 260 nm at temperatures between 5 °C and 95 °C (Figure 2). In each case the peptides showed the expected two-state transition with an isodichroic point at 202 nm. The disordered 90-residue colicin N translocation domain peptide showed a similar shift, although the isodichroic point was 208 nm[3]. This may be due to its aromatic and proline residues. The change in intensity was smaller for Ac-Val, but all showed a similar form of difference spectrum. Ac-Pro showed little change, and Ala-Gly decreased in intensity with no clear evidence of a two-state transition (not shown).

The results show that the simplest peptides, e.g., *N*-acyl-amino acids, provide UV−CD spectra like those of unordered proteins and that they display two-state thermal transitions.

Why is the CD spectrum of $AlaNH_2$ so different from that of Ac−Ala? In $AlaNH_2$, the amide chromophore is in a nearly achiral environment because the CH_3 and NH_3^+ substituents on C_α are isoelectronic, differing only by the single additional nuclear charge on the N. By contrast, in Ac-Ala the three substituents on C_α are very different electronically. Moreover, nπ* and $\pi\pi$* transitions in the carboxyl group can couple with the nπ* and $\pi\pi$* transitions in the amide. The same argument applies to the nearly achiral Ala-Gly vs the distinctly chiral Gly-Ala.

The origin of the PPII spectrum can therefore be clarified. The characteristic negative maximum at <200 nm results from the coupling of a peptide bond N-terminal to the chiral α-carbon. Therefore the simplest peptide bonds have a preferred conformation that defines the spectrum of disordered proteins of any size. Furthermore, our results provide further evidence for the importance of the PPII conformation in unordered peptides because the CD spectra and wavelength shifts observed here, in the previous work by Eker,[4] and in so-called "random polypeptides", are indicative of a preference for the PPII conformation. In the absence of helix or sheet, polypeptides may appear unstructured and dynamic, but they are not random.

Acknowledgment. This work was supported by the BBSRC and the Wellcome Trust. G.A. was a FEBS Fellow., and J.H.L. was a BBSRC Fellow.

Supporting Information Available: CDSpectra (Doc) and numerical data (CSV) for all samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA052632X