Circular dichroism measurement of peptide helix unfolding

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Introduction

α-Helix formation in a monomolecular reaction in aqueous solution is now well demonstrated for a few short peptides: the C-peptide (residues 1–13) [1, 2] and the S-peptide (residues 1–20) [3–5] of RNase A (bovine pancreatic ribonuclease A), both of which contain residues 3–13 that form a helix in RNase A, as well as chemically modified derivatives [6, 7] and chemically synthesized analogs [8] of these peptides. Some short alanine-based peptides of de novo design that contain pairs of (Glu, Lys) residues in simple, repetitive sequences also form an α-helix in aqueous solution (Marqusee and Baldwin, in preparation).

Those results focus interest on the quantitative determination of helix content and the unfolding transitions of these peptides. Circular dichroism (CD) is particularly wellsuited to the measurements of overall helix content for several reasons:

(i) It can be measured at low peptide concentration (our measurements are typically made at 0–40 μM) which is important for avoiding aggregation of helical molecules. Sedimentation equilibrium measurements [1], lack of concentration dependence for CD signal [2], and gel filtration experiments [9] all indicate that helix formation by C-peptide and its analogs is monomolecular.

(ii) The $n \rightarrow \pi^*$ band centered at 222 nm is a reliable indicator of the α-helix [11], provided that the spectrum shows a genuine minimum at 222 nm.*

(iii) The change in ellipticity for $0 \rightarrow 100\%$ helix is nearly the same for different residues and to a first approximation depends only on helix length [14]. Moreover, the relation between the CD spectrum and peptide conformation is reasonably well understood theoretically [15].

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* CD spectra resembling that of α-helical polypeptides have been observed for small cyclic peptides possessing type I β-bends [12, 13].

We consider here the problem of obtaining a reliable CD baseline for 0% helix when guanidinium chloride (GuHCl) and/or temperature is used for unfolding. This problem has been discussed previously in the context of C-peptide and S-peptide by Brown and Klee [1] and by Filippi et al. [16, 17], respectively. Recently, helix-enhancing and -destabilizing replacements have been found for C-peptide [8, 9] and we use them here to study the unfolding behavior of strong, moderate and weak helix-formers. Data are also given for a shorter peptide (P-peptide, residues 1–8 of RNase A) that shows no observable helix formation and provides a control for the effects of GuHCl on the CD spectrum of a random-coil peptide.

The problem of how to obtain 100% helix is not discussed here, but complete helix formation by residues 3–13 can be obtained by adding S-peptide [16, 18] or analogs of S-peptide 1–15 [10] to folded S-protein, and the change in ellipticity for 0 → 100% helix for residues 3–13 has been derived from these data, subject to certain assumptions. The approach of adding trifluoroethanol (TFE) to induce complete helix formation has to be treated with caution in the C-peptide system, both because the TFE transitions are broad (almost noncooperative) and large amounts of TFE must be added [16, 17, 19], and because the C-peptide helix is stabilized by specific side-chain interactions [2, 6–9, 20] that are likely to be affected by TFE.

Results and Discussion

(a) *P-peptide as a model for a random-coil peptide*

Fig. 1A shows CD spectra from 260 to ~206 nm for P-peptide om 0.1 M NaCl at temperatures from 3.0°C to 58.9°C. A minor positive band, centered at ~215 nm, is present at low temperatures; its rotational strength decreases with increasing temperature. This band has been seen in earlier studies of random-coil model compounds [21–23], as well as C-peptide [1], S-peptide [24], and S-peptide derivatives [16, 17]. It is primarily responsible for the temperature dependence of the mean residue ellipticity at 222 nm ($[\theta]_{222}$), the wavelength used for measurement of α-helix content. The decrease in intensity of this band with temperature is also unaffected by GuHCl, as shown in Fig. 1B, which compares the plots of $[\theta]_{222}$ versus temperature in 0 M and 3 M GuHCl. Fig. 1C shows that there is little change in $[\theta]_{222}$ with GuHCl concentration at 3°C. The value in 8 M GuHCl (~+3,000 deg cm^2 dmol^{-1} at 3°C) is close to the value (+3600) reported by Filippi et al. [16] for [Pro^6, Orn^{10}]-S-peptide analog in 8 M GuHCl at 1°C. Neglecting sequence-specific effects, we conclude that P-peptide provides a good model for a random-coil peptide in 0.1 M NaCl and that the CD spectrum of P-peptide is nearly unaffected by GuHCl.

(b) *GuHCl-induced unfolding of strong, moderate and weak helix-formers*

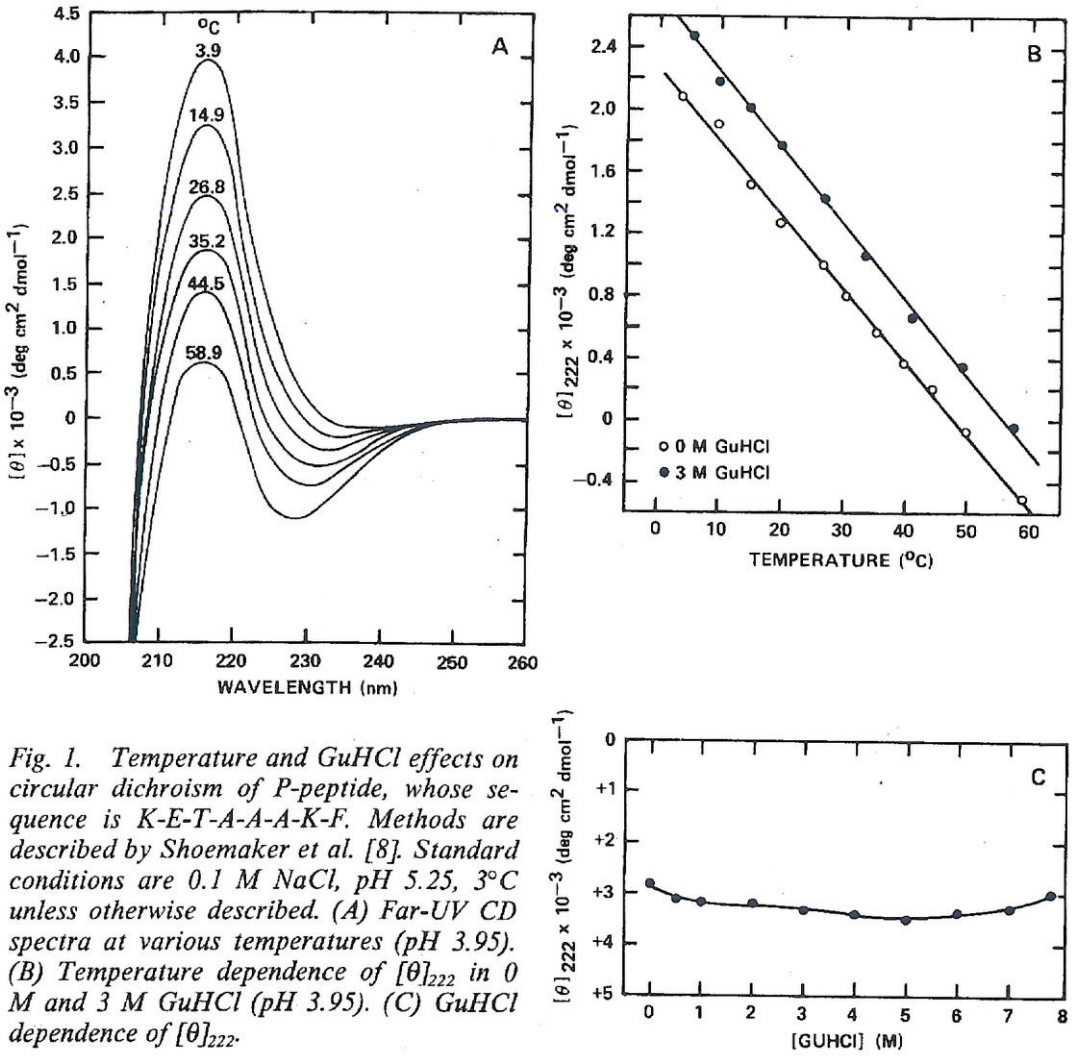

Fig. 1. Temperature and GuHCl effects on circular dichroism of P-peptide, whose sequence is K-E-T-A-A-A-K-F. Methods are described by Shoemaker et al. [8]. Standard conditions are 0.1 M NaCl, pH 5.25, 3°C unless otherwise described. (A) Far-UV CD spectra at various temperatures (pH 3.95). (B) Temperature dependence of $[\theta]_{222}$ in 0 M and 3 M GuHCl (pH 3.95). (C) GuHCl dependence of $[\theta]_{222}$.

Three analogs of C-peptide were chosen as representatives of strong (RN 44), moderate (RN 54), and weak (RN 56) helix-formers. Their sequences are given in the legend to Fig. 2. All three peptides show broad helix-coil transitions induced by GuHCl. At 3°C, complete unfolding is approached in 8 M GuHCl by all three peptides, as judged by the P-peptide control (Fig. 2A). At 70°C, the helix contents at 0 M GuHCl are considerably reduced compared to 3°C, but again the unfolding transitions are broad and complete unfolding is barely reached at 8 M GuHCl (Fig. 2B).

Fig. 3 compares spectra taken at 3°C in 0 and 8 M GuHCl for the strong helix-former RN44 (Fig. 3A) and for the weak helix-former RN56 (Fig. 3B). The spectra taken in 8 M GuHCl are similar and resemble that of P-peptide (Fig. 1), although the spectra in 0 M GuHCl are quite different.

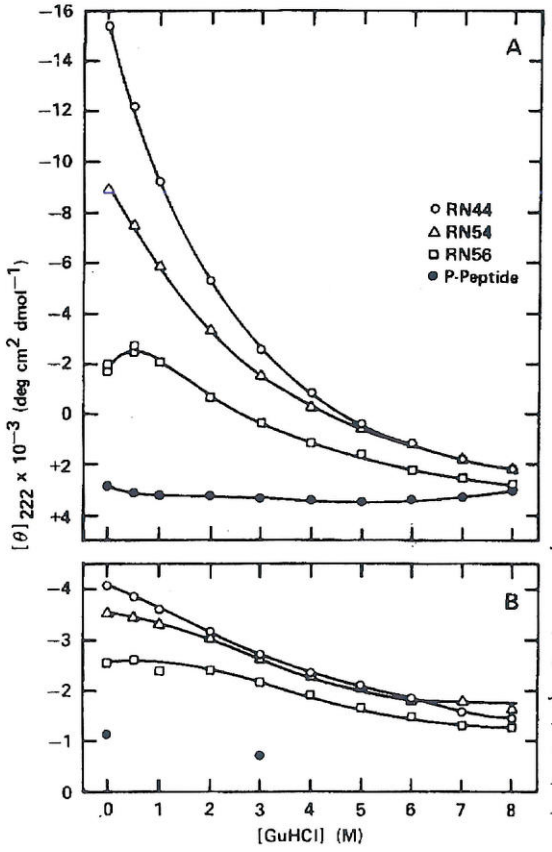

Fig. 2. Comparison of GuHCl-induced unfolding for strong (RN44), moderate (RN54), and weak (RN56) helix-formers. $[\theta]_{222}$ is measured at (A) 3°C and (B) 70°C in 0.1 M NaCl, pH 5.25. P-peptide (Fig. 1C) is used as reference in (A). In (B), the values of $[\theta]_{222}$ for complete unfolding, extrapolated from the P-peptide results (Fig. 1B), are also shown. Sequences: RN44: Succinyl-A-A-T-A-A-A-K-F-L-A-A-H-A-CONH$_2$; RN 54: RN 44 (Suc-Ala → acetyl-Ala); RN 56: RN 44 (Suc-Ala → Lys).

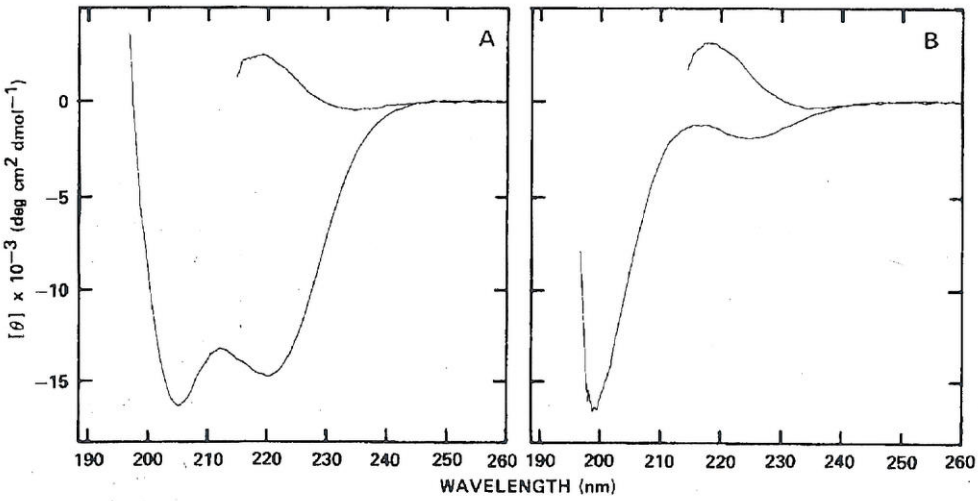

Fig. 3. Comparison of far-UV CD spectra for (A) RN 44 and (B) RN 56 in 0 M and 8 M GuHCl. Standard conditions are 0.1 M NaCl, pH 5.25, 3°C.

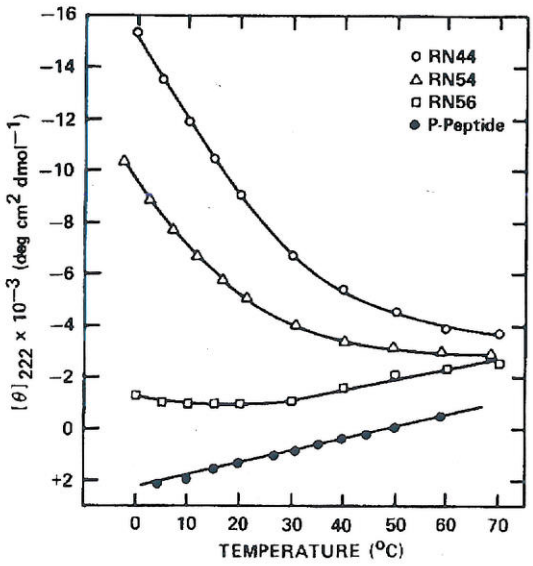

Fig. 4. Comparison of thermal unfolding for RN 44, RN 54, and RN 56, monitored by $[\theta]_{222}$. P-peptide is used as reference. Standard conditions are 0.1 M NaCl, pH 5.25, 3°C.

(c) *Thermal unfolding of strong, moderate, and weak helix-formers*

The thermal unfolding transitions of these three peptides in 0.1 M NaCl are broad (Fig. 4), although complete unfolding for all of them is approached by 70°C. It is not surprising that such short helices should show broad thermal transitions (compare the thermal unfolding curve of a 26-residue helix [25]), but we are not yet able to compare these experimental curves with theoretical ones, for two reasons. First, the enthalpy change of unfolding has not yet been measured for helix-forming peptides such as these. The transition curves from calorimetry are too broad to permit a reliable determination of ΔH (J.M. Sturtevant, personal communication). Second, these peptide helices are considerably more stable [8] than predicted using the Zimm-Bragg equation [26] with host-guest data [27], because specific intrahelical side-chain interactions help to stabilize the helix. Although the Zimm-Bragg theory has been generalized to take these side-chain interactions into account [28, 29], the extended theory has not yet been applied to the problem of predicting thermal unfolding curves.

Acknowledgements

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References

1. Brown, J.E. and Klee, W.A., Biochemistry, 10 (1971) 470.
2. Bierzynski, A., Kim, P.S. and Baldwin, R.L., Proc. Natl. Acad. Sci. U.S.A., 79 (1982) 2470.
3. Kim, P.S., Bierzynski, A. and Baldwin, R.L., J. Mol. Biol., 162 (1982) 187.
4. Rico, M., Nieto, J.L., Santoro, J., Bermejo, F.J., Herranz, J. and Gallego, E., FEBS Lett., 162 (1983) 314.
5. Kim, P.S. and Baldwin, R.L., Nature, 307 (1984) 329.
6. Rico, M., Gallego, E., Santoro, J., Bermejo, F.J., Nieto, J.L. and Herranz, J., Biochem. Biophys. Res. Comm., 123 (1984) 757.
7. Rico, M., Santoro, J., Bermejo, F.J., Herranz, J., Nieto, J.L., Gallego, E. and Jiménez, M.A., Biopolymers, 25 (1986) 1031.
8. Shoemaker, K.R., Kim, P.S., Brems, D.N., Marqusee, S., York, E.J., Chaiken, I.M., Stewart, J.M. and Baldwin, R.L., Proc. Natl. Acad. Sci. U.S.A., 82 (1985) 2349.
9. Shoemaker, K.R., Kim, P.S., York, E.J., Stewart, J.M. and Baldwin, R.L., Nature, 326 (1987) 563.
10. Mitchinson, C. and Baldwin, R.L., Proteins, 1(1986) 23.
11. Holzwarth, G. and Doty, P., J. Am. Chem. Soc., 87 (1965) 218.
12. Bandekar, J., Evans, D.J., Krimm, S., Leach, S.J., Lee, S., McQuie, J.R., Minasian, E., Nemethy, G., Pottle, M.S., Scheraga, H.A., Stimson, E.R. and Woody, R.W., Int. J. Pep. Prot. Res., 19 (1982) 187.
13. Gierasch, L.M., Deber, C.M., Madison, V., Niu, C.-H. and Blout, E.R., Biochemistry, 20 (1981) 4730.
14. Chen, Y.-H., Yang, J.T. and Chau, K.H., Biochemistry, 13 (1974) 3350.
15. Schellman, J.A. and Becktel, W.J., Biopolymers, 22 (1983) 171.
16. Filippi, B., Borin, G., Anselmi, U. and Marchiori, F., Biopolymers, 17 (1978) 2525.
17. Filippi, B., Borin, G., Moretto, V. and Marchiori, F., Biopolymers, 17 (1978) 2545.
18. Labhardt, A.M., Biopolymers, 20 (1981) 1459.
19. Nelson, J.W. and Kallenbach, N.R., Proteins, 1 (1986) 211.
20. Shoemaker, K.R., Fairman, R., Kim, P.S., York, E.J., Stewart, J.M. and Baldwin, R.L., Cold Spring Harbor Symp. Quant. Biol., 52 (1987) in press.
21. Greenfield, N. and Fasman, G.D., Biochemistry, 8 (1969) 4108.
22. Mattice, W.L., Lo, J.-T. and Mandelkern, L., Macromolecules, 5 (1972) 729.
23. Mattice, W.L., Biopolymers, 13 (1974) 169.
24. Klee, W.A., Biochemistry, 7 (1968) 2731.
25. Zimm, B.H., Doty, P. and Iso, K., Proc. Natl. Acad. Sci. U.S.A., 45 (1959) 1601.
26. Zimm, B.H. and Bragg, J.K., J. Chem. Phys., 31 (1959) 526.
27. Sueki, M., Lee, S., Powers, S.P., Denton, J.B., Konishi, Y. and Scheraga, H.A., Macromolecules, 17 (1984) 148.
28. Vasquez, M., Pincus, M.R. and Scheraga, H.A., Biopolymers, 26 (1987) 351.
29. Vasquez, M. and Scheraga, H.A., Biopolymers (1987) in press.