Effect of Hexafluoroisopropanol on the Thermodynamics of Peptide Secondary Structure Formation

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Cold denaturation studies have had a pivotal role in elucidating the thermodynamics of protein folding,[1] but this phenomenon has rarely been encountered for peptides in aqueous solution.[2] Cold denaturation is expected when the solution of the unfolded state has a substantially larger heat capacity than that of the folded state. Circular dichroism (CD) studies have now shown that many peptide helices unfold upon cooling when limited amounts of hexafluoroisopropanol (HFIP) are added to an aqueous solution.[3] This phenomenon, and a major portion of the peptide structuring effect of HFIP, has been attributed to an HFIP-induced enhancement of the hydrophobic effect.[2a] Cold-induced unfolding of helices has not been confirmed by other spectroscopies such as NMR or IR. Peptide β hairpin formation also has been reported to be hydrophobically driven and enhanced by fluoroalcohol addition;[4,5] however, fluoroalcohol enhancment of cold denaturation has not been reported. In this account, we provide these confirmations and demonstrate that isotope-edited FT-IR is particularly well-suited for studies of peptide structuring transitions. The use of this strategy provides residue-specific structural information, and T-jump kinetics should afford the timescales for secondary structure formation in aqueous HFIP.

AC-2738, an analogue of the helical domain of pancreatic amylin,[6] provided a confirmation of the phenomenon by ^{1}H NMR. Figure 1 is a direct comparison, for AC-2738, of CD measures of fractional helicity and another accepted measure of helicity, the α methine chemical shift deviations from random coil reference values.[7,8] The temperature-induced changes in the upfield shifts of α methines throughout the helix are in direct proportion to the changes in fractional helicity evident by CD, suggesting substantial cooperativity in this helix/coil transition and that the dramatic helix unfolding upon cooling is uniform throughout the sequence.

FT-IR and ^{13}C NMR were employed for two members of Baldwin's YGG-3X series of helical peptides (YGG-3X = AcYGGK$\underline{A}$XAAK$\underline{A}$X$\underline{A}$AKAX$\underline{A}$AKNH$_2$, X=V or A)[9] with sequence-specific ^{13}C′-Ala labels located centrally ($\underline{A}$) or terminally ($\underline{A}$) in the helix. In 8−9% HFIP,[3] YGG-3A displays a monotonic decrease in $-[\theta]_{222}$, while YGG-3V goes from a fractional helicity of 0.33 at 3 °C to 0.60 at 31 °C based on CD; NMR and CD studies confirmed that cold denaturation also occurs in HFIP/D_2O mixtures at the ca. millimolar concentrations used for FT-IR studies (see Supporting Information). Thermal difference FT-IR spectra that show warming-induced helix formation for YGG-3V and the steady monotonic loss of helicity for YGG-3A appear in Figure 2.[10] For the centrally labeled species, a temperature increase from 3 to 29 °C produces a 34% *increase* in the intensity of the solvated helix amide-I′ IR band (1589 cm^{-1} for the ^{13}C-labeled amide site[11]) of YGG-3V and a 12% *decrease* for the same band of YGG-3A.[12]

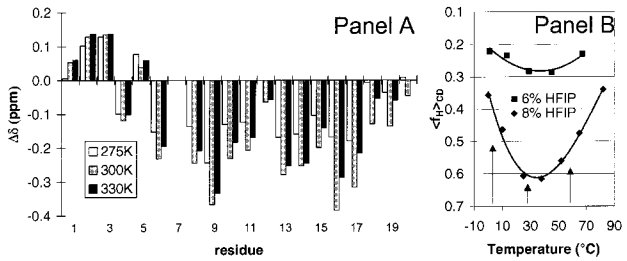

Figure 1. Parallel study of the helicity of peptide AC-2738 by NMR and CD. The CD measure of fractional helicity is $\langle f_H \rangle_{CD} = -[\theta]_{221}/29000$. Previously published[8] coil values for H_a were employed to calculate $\Delta\delta$; δ_{ref} was assumed to be temperature independent. The arrows on the CD plot correspond to the temperatures employed in the NMR study.

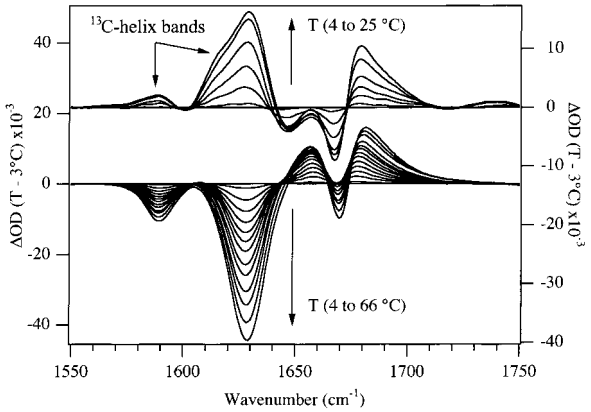

Figure 2. Thermal FT-IR difference signals for YGG-3V (upper traces; T = 4.4, 8.4, 12.4, 16.5, 20.7, 25 °C) and YGG-3A (lower traces; T = 4.4→66.2 °C, at 4−7 °C intervals, minus T = 3 °C) bearing two central ^{13}C-Ala residues. The spectra were recorded in 9% (YGG-3A) and 10% (YGG-3V) HFIP/D_2O.[10] The difference feature at 1670/1680 cm^{-1} is due to TFA, which displays a temperature-dependent ν_{max}.

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(11) Two solvated helix bands are observed for YGG-3A and YGG-3V at 1590 (^{13}C═O) and 1629 cm^{-1} (^{12}C═O). There are two additional helix bands present in the YGG-3V spectra, at 1615 (shoulder; ^{13}C═O) and 1655 cm^{-1} (^{12}C═O). We attribute these two additional bands to solvent-protected helical sites; the reduced solvation is probably due to the branched valine side chains. Parallel ^{13}C NMR studies also reveal distinct behavior for the two centrally labeled sites of YGG-3V. All four helix bands of YGG-3V increase with temperature over the range from 3 to 25 °C, indicating a cooperative cold denaturation transition that includes both the solvated and solvent-protected helical sites. At temperatures higher than 25 °C, YGG-3V becomes less helical upon further warming.

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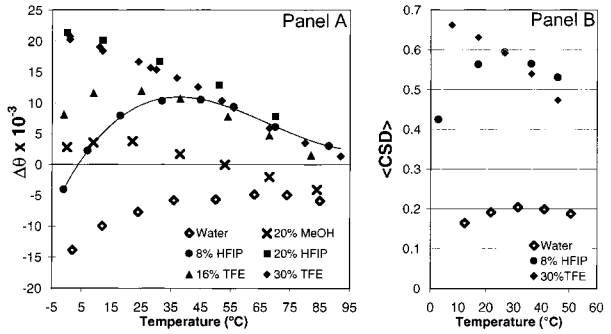

Figure 3. Melting behavior of peptide βH-1 as monitored by CD (A) and NMR (B). In the CD study, $[\theta]_{196} - [\theta]_{216}$, the β signature amplitude, is plotted versus temperature; the points for the 8% HFIP medium are connected by a polynomial best-fit line to guide the eye. Panel B shows the average downfield shift of the α methines of residues Lys[2,11], Thr[4,13], and Ser[6,15].

Thus CD, IR, ^{1}H, and ^{13}C NMR (Supporting Information) all indicate cold-induced unfolding of helical peptide states in this medium. The ΔC_p values derived using a standard two-state model can be as large as +2 kJ K^{-1} mol^{-1}.[2a] In water, however, none of the monomeric helical peptides examined to date display sufficient curvature in their CD melting curves to allow the extraction of ΔC_p values with any certainty. Alternate methods[8] suggest, but do not prove, that ΔC_p for helix unfolding in water could be positive, perhaps as large as 20−40 J K^{-1} residue^{-1} for helices with a few hydrophobic residues—still far short of the values seen in aqueous HFIP. Measurements in HFIP-containg media could provide a previously unavailable measure of the extent to which secondary structure contributes to the net stabilization of protein folds if the HFIP effect on ΔC_p could be quantified. A β hairpin (βH-1, AcKKYTVSINGKKITVSI), reported to cold-denature in aqueous buffer but not in 50% methanol,[5] has provided a means for determining the "solvophobic" factor for a wide variety of water−alcohol mixtures. Chemical shift and NOE comparisons[13] reveal that βH-1 in 30% TFE or 20% HFIP populates a β hairpin state very similar to that observed[5] in 50% methanol, and CD reveals a "β sheet" signature (maximum at 196, minimum at 216 nm; see Supporting Information). Key data from our studies appear in Figure 3. Panel A shows the "β CD amplitude" versus temperature for six media. Some degree of curvature is observed for water, which decreases upon methanol addition but increases with addition of limited quantities of fluoroalcohol, particularly with HFIP. To emphasize β strand association (rather than turn formation), we selected the six inwardly directed Hα resonances (Lys[2,11], Thr[4,13], Ser[6,15]) of the β strands which appear far downfield in the folded state as our probe of hairpin formation. Panel B of Figure 3 shows a plot of this chemical shift probe versus temperature in water, 8% HFIP, and 30% TFE. Once again, cold denaturation is dramatic in 8% HFIP.

Hairpin/coil transitions are closer to a two-state equilibrium than is helix formation, allowing eq 1 to be used with greater confidence. Fits of both the CD and NMR data were performed with T^0 set to 298, 365, and 386 K. Details of the analyses of the

$$\Delta G(T) = \Delta H^0 - T\Delta S^0 + \Delta C_p[T - T^0 - T\ln(T/T^0)] \quad (1)$$

CD and NMR thermal stability curves appear in Table 1S (Supporting Information). For all NMR and CD data that display substantial curvature, the ΔC_p values are not sensitive to the T^0 selected for the fit nor highly dependent on the baseline assumptions. The two measures of folding are in reasonable accord, with the NMR data yielding larger estimates of ΔC_p. Due to uncertainties in the folded-state CD baseline and the observation of additional CD features that cannot be ascribed to β strand association, we view the thermodynamic parameters from the NMR data as the better estimates for the hairpin/coil transition. The medium dependencies of ΔC_p and ΔS^0_{298} that we have derived appear in Table 1. Italicized values are less secure since they represent extrapolations from the thermodynamic parameters derived from the CD melting curves.

Table 1

	medium					
	water	20% MeOH	8% HFIP	20% HFIP	16% TFE	30% TFE
ΔC_p[a]	675	560	1730	550	900	<520
ΔS^0_{298}[a]	−8 ± 3	*+27±3*	−39 ± 5	*+42*	0 ± 3	≥48

[a] Both ΔC_p and ΔS^0_{298} are given in units of J K^{-1} mol^{-1}.

In agreement with Maynard et al.,[5] we find that ΔC_p decreases upon methanol addition. In contrast, the addition of limited quantities of TFE, and particularly HFIP, increases ΔC_p. At room and physiological temperatures, peptide β hairpin formation is entropically driven in water and in 8% HFIP.

This report provides additional evidence for the importance of hydrophobic interactions in peptide secondary structure formation. For the hydrophobically driven β hairpin formation examined, the addition of HFIP to the 8% level increases hairpin formation *and* increases ΔC_p by nearly a factor of 3. Surprisingly, α helices bearing a few non-interacting hydrophobic residues can display even larger ΔC_p values. The initial phase of secondary structure induction during fluoroalcohol titrations of peptides appears to be largely the result of these effects rather than differential stabilization (or destabilization) of the folded versus coil conformation by alcohol/peptide binding interactions, as the latter would be reflected predominantly in the enthalpy term. The addition of limited quantities of fluoroalcohol may mimic the early hydrophobic collapse stage of protein folding.

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Supporting Information Available: Graphs of $[\theta]_{221}$ versus temperature for YGG-3V and YGG-3A in D_2O and H_2O with various levels of added HFIP and GdmCl, a figure showing the CD traces for peptide βH-1 in 8 and 20% HFIP at several temperatures, and Table 1S (a detailed comparison of the thermodynamic parameters derived from CD versus NMR data) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) The losses and gains in helicity over the same temperature jump in HFIP/H_2O media have been determined by ^{13}C NMR: YGG-3A (−14.4 ± 2%) and YGG-3V (+44 ± 10%), with $\Delta\delta$(0→100% helicity) = 3.040 + 0.00715T(°C) ppm for the ^{13}C′ resonance.

(13) The βH-1 peptide employed for these studies was prepared in our laboratory. Our NMR studies confirm the assignments and structural conclusions of Maynard et al.,[5] including the presence of cross-strand hydrophobic interactions.