Effect of a C-Terminal Cationic Group on the Competition between α-Helical Turn and β-Turn in a Model Depsipeptide

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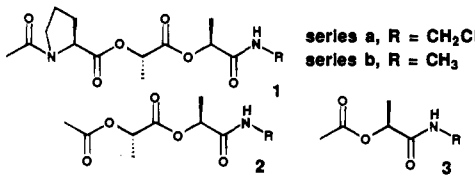
The α-helical conformation is only marginally stable, at best, relative to disordered states for peptides of moderate length (≤15 residues).[1] This situation has been rationalized in terms of a competition between the substantial entropic cost of initiating an α-helix and the small energetic gain of adding a new residue to the end of an existing helix.[2] It has been proposed that α-helices in proteins are often stabilized by special sets of interactions at helix termini, including interactions between the net dipole of the helix and an ionic group.[3] Model studies in water with peptides that can form α-helices several turns in length provide evidence for a stabilizing interaction between the conformation-dependent dipole and an appropriate terminal charge.[3c] Here, we describe results with an even simpler system which shows that a terminal charge promotes formation of an α-helical turn relative not only to "unfolded" states but also to alternative folded states.

We recently introduced model systems for formation of a single α-helical turn, examplified by **1b**, a depsipeptide analogue of an end-capped prolyl-alanyl-alanine tripeptide.[4] Use of esters

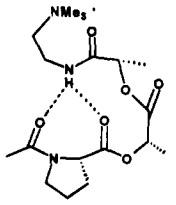


in place of amides for the two internal linkages promotes formation of the 13-membered ring H-bond (α-helical turn), because two potential hydrogen bond donor groups (NH) have been eliminated and the alternative acceptor sites for the lone amide NH are esters, which are weaker H-bonding partners than is the N-terminal amide. Despite these inducements, only about 30% of **1b** adopts the α-helical turn in a dilute CH_2Cl_2 solution at room temperature. Another 43% of **1b** has a β-turn conformation (10-membered ring H-bond), and the remainder is not H-bonded.[4] We have now found that addition of a tetralkylammonium group to the C-terminus of this depsipeptide selectively enhances the α-helical turn over the β-turn in CH_2Cl_2.

Preliminary studies indicated that SbF_6^- does not interact strongly with amide protons in dilute CH_2Cl_2 solution, a prerequisite for our experiments. The IR spectrum of 1 mM **3a** (not shown) has only a single N−H stretch band, at 3419 cm^{-1}. Neutral analogue **3b** shows a single band at 3457 cm^{-1},[4] and precedent indicates that this band arises from a non-H-bonded methyl amide N−H group.[4,5] The 38 cm^{-1} $\Delta\tilde{\nu}_{N-H}$ between **3a** and **3b** may be attributed in part to the steric difference between the nitrogen substitutents (*N,N,N*-trimethylammonioethyl vs methyl), since *N*-propylacetamide and *N*-methylacetamide show a non-H-bonded $\Delta\tilde{\nu}_{N-H}$ of 14 cm^{-1} in CH_2Cl_2. The remainder of the $\Delta\tilde{\nu}_{N-H}$ between **3a** and **3b** may stem from a weak interaction between the amide proton and SbF_6^- [6] and/or from a slight inductive weakening of the N−H bond by the nearby electron-withdrawing ammonium group in **3a**.

Figures 1a,b show N−H stretch region IR data for **2a,b**, 1 mM each in CH_2Cl_2. The bands at 3458 and 3416 cm^{-1} for **2b** result from non-H-bonded and β-turn (10-membered ring) H-bonded N−H, respectively.[4] For **2a**, curve fitting analysis suggests the presence of two bands, at 3419 and 3375 cm^{-1}. The higher energy band is assigned to N−H free of internal H-bonding, based on the data for **3a**, and the lower energy band is assigned to N−H involved in a β-turn H-bond. There appears to be a consistent shift of ca. 40 cm^{-1} between each of the analogous N−H stretch bands of **2a** and **2b**. The cationic group seems to enhance modestly the tendency for β-turn folding.

Figures 1c,d show N−H stretch IR data for **1a,b**.[7] The bands at 3454, 3409, and 3365 cm^{-1} for **1b** result from non-H-bonded, β-turn H-bonded, and α-helical turn H-bonded N−H, respectively.[4] For **1a**, curve fitting implies the presence of a major band at 3320 cm^{-1} and a minor band at 3290 cm^{-1}. The broad major band is assigned to the 13-membered ring H-bond (α-helical turn). According to this assignment, the α-helical H-bonded band is shifted ca. 40 cm^{-1} to lower energy in the cationic system, which is similar to the behavior of the β-turn and non-H-bonded bands. The minor band for **1a** is tentatively assigned to a folding pattern in which the N−H is H-bonded to both N-terminal carbonyls, as indicated below (this type of local folding pattern, involving simultaneous 10- and 13-membered ring H-bonds, has been observed at the C-termini of α-helices in crystalline proteins[8]). The most important implication of these data is that **1a** is largely folded into the α-helical turn, while nonionic analogue **1b** equilibrates among three different folding patterns.



Further evidence for the selective enhancement of the α-helical turn over the β-turn by the C-terminal ammonium group is found in the ester C=O/amide I IR data for **1**−**3**. Figure 2a shows that the ammonium group does not significantly affect the positions of the ester C=O stretch band (1741 cm^{-1}, with a higher energy shoulder[9]) or the amide I band (1683 cm^{-1}) of

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(2) For leading references, see: Creighton, T. E. *Proteins: Structures and Molecular Principles*, 2nd ed.; Freeman: New York, 1993.

(3) (a) First proposal of a favorable interaction between the α-helical dipole and terminal charges: Blagdon, D. E.; Goodman, M. *Biopolymers* **1975**, *14*, 241. (b) Reviews on the helix dipole: Wada, A. *Adv. Biophys.* **1976**, *9*, 1. Hol, W. G. J. *Prog. Biophys. Mol. Biol.* **1985**, *45*, 149. (c) Model studies with synthetic peptides: Fairman, R.; Shoemaker, K. R.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Proteins* **1989**, *5*, 1. Lockhart, D. J.; Kim, P. S. *Science* **1992**, *257*, 947.

(4) Gallo, E. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9774.

(5) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164.

(6) Anions other than SbF_6^- interacted more strongly with amide protons in CH_2Cl_2. The analogue of **3a** with BPh_4^- as counterion showed bands of similar intensity at 3428 and 3361 cm^{-1}, and use of I^- as counterion resulted in a minor band at 3432 cm^{-1} and a major band at ca. 3250 cm^{-1}. In both cases, the lower energy band is assigned to an anion−H−N interaction.

(7) For both **1a** and **1b** in CD_2Cl_2, ^1H NMR indicated that ca. 7% of the molecules exist with the N-terminal prolyl amide group in the *s-cis* conformation. The α-helical turn folding pattern requires that this amide group be in the *s-trans* conformation.

(8) Baker, E. N.; Hubbard, R. E. *Prog. Biophys. Molec. Biol.* **1984**, *44*, 97.

(9) This doubling of the ester C=O stretch band of **3b** has previously been noted: (a) Ingwall, R. T.; Gilon, C.; Goodman, M. *Macromolecules* **1976**, *9*, 802. These workers attributed the doubling to conformational isomerism about the C−C(=O) bond. (b) Boussard, G.; Marraud, M. *Biopolymers* **1981**, *20*, 169. These workers argued that the doubling arises from partial formation of a weak hydrogen bond between the amide proton and the ester π-electron system.

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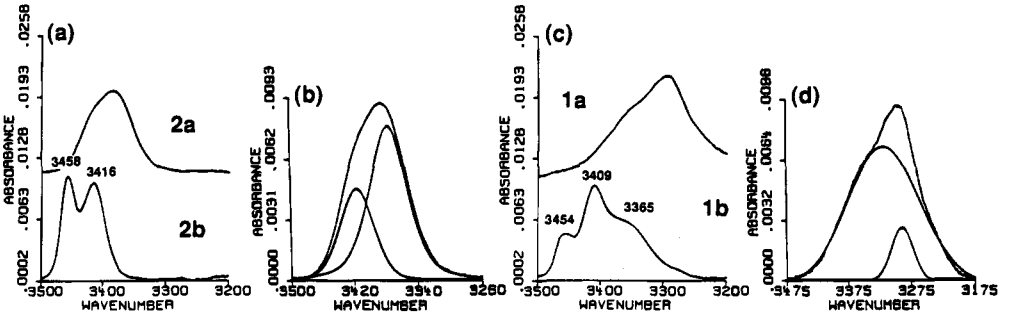

Figure 1. N−H stretch FT−IR data for 1 mM depsipeptide samples in CH_2Cl_2 at room temperature after subtraction of the spectrum of pure CH_2Cl_2. (a) Upper, **2a**; lower, **2b**. (b) Curve fitting result for **2a**. The computer-generated component bands (maxima at 3419 and 3375 cm^{-1}) are juxtaposed with the experimental spectrum. (c) Upper, **1a**; lower, **1b**. (d) Curve fitting result for **1a**. The computer-generated component bands (maxima at 3320 and 3290 cm^{-1}) are juxtaposed with the experimental spectrum.

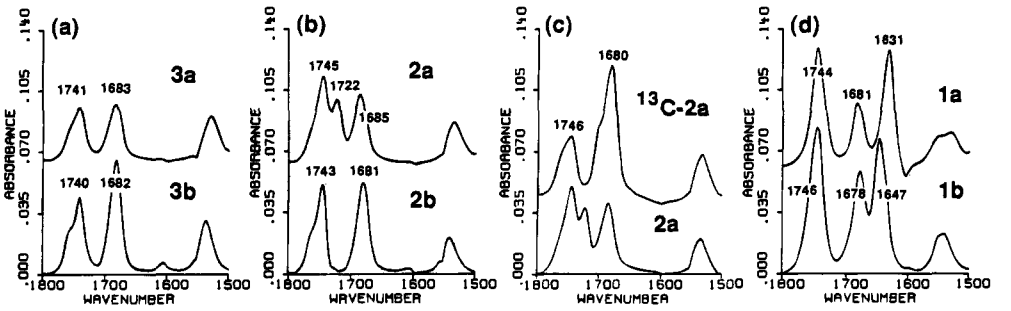

Figure 2. Ester C=O stretch/amide I FT−IR data for 1 mM depsipeptide samples in CH_2Cl_2 at room temperature after subtraction of the spectrum of pure CH_2Cl_2. (a) Upper, **3a**; lower, **3b**. (b) Upper, **2a**; lower, **2b**. (c) Upper, **2a** in which the C-terminal acetyl group has been ^{13}C-labeled at the carbonyl carbon; lower, **2a**. (d) Upper, **1a**; lower, **1b**.

3a relative to those of **3b**. In contrast, the ammonium appendage causes the appearence of a new ester C=O band at 1722 cm^{-1} for **2a** (Figure 2b). This new band is attributed to the carbonyl involved in the 10-membered ring H-bond, an assignment that is supported by the effect of labeling this carbonyl's carbon with ^{13}C (Figure 2c; a shift of ca. 40 cm^{-1} to lower energy is expected for ^{12}C to ^{13}C substitution at the carbonyl). Figure 2d shows that there is no ester C=O stretch band shifted to low energy for **1a** but that the amide I band of the N-terminal acyl group is 16 cm^{-1} lower than the analogous band for **1b**. (Analysis of the data for **1a,b** is aided by the fact that the amide I band of the N-terminal amide group [a tertiary amide] is well resolved from the amide I band of the C-terminal group [a secondary amide].) These carbonyl region data are consistent with the N−H stretch region data in indicating that **1a** experiences little or no β-turn folding and that the α-helical turn is predominant.

The existence of an equilibrium between intramolecularly H-bonded and non-H-bonded species in CH_2Cl_2 solutions of uncharged depsipeptides **1b** and **2b** cannot be detected from the ester C=O/amide I band data for these compounds. This situation presumably arises because the H-bonded and non-H-bonded states of **1b** and **2b** are populated to similar extents, and the extent of the H-bond-induced shifts in these bands is similar to the widths of the bands. The magnitude of H-bond-induced shifts in acceptor vibrational modes is thought to be proportional to H-bond strength;[10] therefore, it is noteworthy that the internal H-bonds in the cationic depsipeptides produce relatively large changes in ester C=O stretch and amide I bands. These large shifts might indicate a bidentate interaction of the N-terminal carbonyl group with both the C-terminal amide proton and one of the relatively electropositve protons adjacent to the ammonium nitrogen.[11] Alternatively, these shifts might result from enhanced strength of the H-bonds in the cationic molecules, stemming either from an inductive acidification of the N−H by the ammonium group or from a favorable interaction between the cation and the dipole of the C=O···H−N entity. Whatever the origin of this ester C=O/amide I effect, it is operative in both the β-turn and α-helical turn folding patterns and therefore does not appear to explain the selectivity for α-helical turn in **1a**.

The β-turn and α-helical turn conformations of **1b** have similar enthalpic stabilities in CH_2Cl_2,[4] which is striking since the α-helical turn involves an amide−amide H-bond, while the β-turn involves a substantially weaker amide−ester H-bond.[12] We have speculated that this behavior results from the fact that the α-helical turn (but not the β-turn) requires approximately parallel alignment of all four carbonyl groups, which should be unfavorable in a relatively nonpolar solvent.[4] In larger peptides, such alignment has been proposed to give rise to the net dipole associated with the α-helix.[3] The results reported here are consistent with previous hypotheses that interaction of the conformation-dependent dipole with an appropriately positioned charge stabilizes the α-helix relative to unfolded states.[3] Our data also demonstrate a new point: interaction between a conformation-dependent dipole and an ionic group can select among alternative folded states. These results are of interest with regard to the conformation-directing role of buried charges in folded proteins.[13] Our findings should also be useful for the design of peptides and other molecules with specified folding patterns.[14]

(10) (a) Pimentel, G. C.; McClellan, A. L. *The Hydrogen Bond*; Freeman: San Francisco, CA, 1960. (b) For a recent application of this phenomenon, see: Eberhardt, E. S.; Raines, R. T. *J. Am. Chem. Soc.* **1994**, *116*, 2149.

(11) For leading references on ammonium C−H groups as H-bond donors, see: Reetz, M. T.; Hütte, S.; Goddard, R. *J. Am. Chem. Soc.* **1993**, *115*, 9339.

(12) We have measured an enthalpic difference of ca. 1.6 kcal/mol between intramolecular C=O···H−N hydrogen bonds involving an amide vs an ester carbonyl as acceptor; see ref 4.

(13) (a) Rashin, A. A.; Honig, B. *J. Mol. Biol.* **1984**, *173*, 515. (b) Honig, B.; Hubbell, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 5412.

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