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On the Stability of a Single-Turn α-Helix: The Single versus Multiconformation Problem

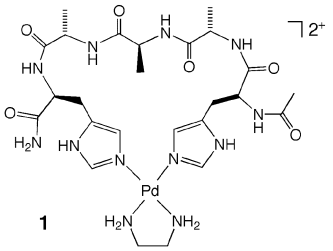
James P. Snyder,*,† Ami S. Lakdawala,† and Michael J. Kelso‡

Department of Chemistry, Emory University, Atlanta, Georgia 30322, and The Centre for Drug Design and Development, Institute for Molecular Bioscience, University of Queensland, St. Lucia, Brisbane, 4072, Australia

Received July 23, 2002; E-mail: snyder@euch4e.chem.emory.edu

Insights into the design-based folding of peptides and proteins carry the potential to contribute to the long-standing protein folding problem, the development of new materials, and the conception of novel drug entities. Efforts to conscript β-peptides for roles previously served by α-peptides[1] and to refold natural proteins into unnatural shapes,[2] for example, represent two fruitful and recently traversed discovery pathways. In the same spirit, mere fragments of well-known secondary structures are being coaxed to adopt three-dimensional shapes normally reserved for larger molecules. Peptides rich with side chains bearing a propensity for α-helix formation rarely do so when the molecule contains less than 15 residues. Nonetheless, strategies for stabilizing α-helical peptides have taken advantage of the intrinsic helix dipole, capping motifs, organic templates, hydrophobic interactions, salt bridges, metal ion chelation, unnatural amino acids, and covalent side chain tethers.[3,4]

A particularly striking example of the latter is the recent report of the first stable single α-helical turn in solution. The work employed a palladium-centered clip to bridge histidines at the ends of the pentapeptide Ac-HAAAH-NH_2 to give $[Pd(en)Ac\text{-}HAAAH\text{-}NH_2]^{2+}$ (**1**).[4]



Although the 22-membered ring includes four amide bonds, two imidazole rings, and the inherent capacity for three $i, i+4$ α-helical hydrogen bonds, it still retains 14 easily rotated single bonds. Nonetheless, interpretation of the compound's geometry-discerning NMR properties (44 ROE distances and three $^3J_{HH}$'s), assisted by H-bond constrained simulated annealing with XPLOR, led to the proposal of a well-defined α-helix with two orientations of the C-terminal amide.[4] Our examination of the conformational profile for **1**, on the contrary, suggests a much more complex ensemble with little or no contribution from the ideal helical turn structure.

While the previous authors performed their modeling studies with the Ac-HAAAH-NH_2 pentapeptide alone, we chose to include the metal clip in the context of the AMBER* force field and the GBSA/H_2O continuum solvent model.[5] Parametrization of the Pd center[6] in AMBER* involved a density functional optimization of the dicationic complex **2** (DFT: Becke3LYP/LANL2DZ,[7] Figure 1). Examination of the torsional potential around the Pd−N bond for

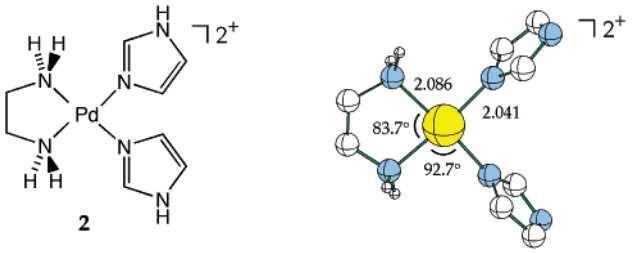


Figure 1. The $[Pd(NH_2(CH_2)_2NH_2)Im_2]^{2+}$ complex optimized by DFT and reproduced by the AMBER*/GBSA/H_2O force field; AMBER* selected distances (Å) and angles (deg).

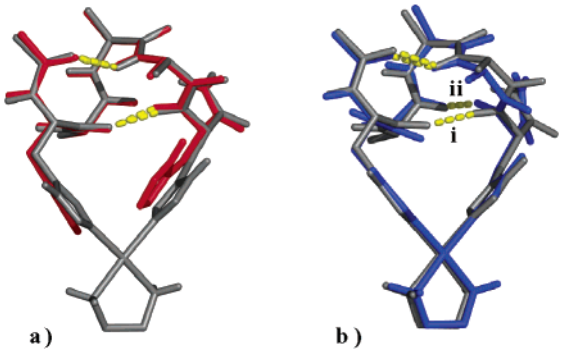


Figure 2. (a) Superposition of idealized α-helical **1-h** (grey) and the Kelso et al. α-helical Ac-HAAAH-NH_2 (red) derived by simulated annealing.[4] (b) Overlap of **1-h** (grey) and unconstrained optimized **1-h′** (blue); hydrogen bonds in yellow. H-bond **i** is $i, i+4$; **ii** $i, i+3$.

a single imidazole with the same method provided a rotation barrier of 3.0 or 3.7 kcal/mol depending on the in-plane orientation of the rotating heterocyclic ligand. Both geometric and energetic features were incorporated into AMBER* as illustrated by its reproduction of the global minimum DFT structure (Figure 1).

Structure **2** was augmented with Ac-HAAAH-NH_2 (**3**) to give the linkage isomer of $[Pd(en)(peptide)]^{2+}$ that is purported to form an α-helix in water (**1**),[4] and then subjected to a 75 000 step Monte Carlo conformational search[7] with AMBER*/GBSA/H_2O and a 10 kcal/mol energy cutoff. The resulting 8850 optimized conformers were supplemented with two helical conformations. The first was generated by constrained torsional optimization of **1** to give the idealized α-helical form with two $i, i+4$ hydrogen bonds, **1-h**. The metal complex is superimposed on the α-helical peptide in Figure 2a. The second related conformer was obtained by unconstrained optimization of **1-h** providing **1-h′**, 6.0 kcal/mol lower in energy. It retains aspects of the helical features, but a somewhat different hydrogen-bonding pattern (one each $i, i+3$ and $i, i+4$ H-bonds, Figure 2b, blue). The combined 8852 conformers and the NMR parameters measured by Kelso et al.[4] (44 ROE distances and five $^3J_{HH}$'s) were subjected to a NAMFIS[8] conformational deconvolution, resulting in an eight conformer "best fit" of the data

† Emory University.
‡ University of Queensland.

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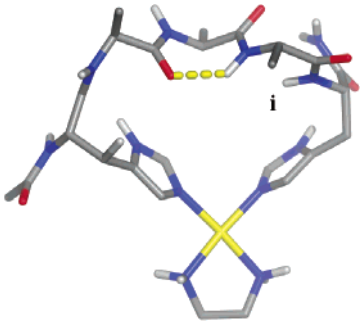

Figure 3. Inverse [γ]-turn conformer **a** is predicted to have a 55% population in solution. The hydrogen bond, indicated by a dashed yellow line, and the sequestered His5 NH (**i**) satisfy reported VT-NMR data.[4]

(SSD[9] = 102) with populations ranging from 2 to 55%. The most populated conformer (**a**, 55%, Figure 3) possesses one i, $i + 2$ hydrogen bond, signifying the presence of an inverse γ-turn.[7,10−13] The intramolecular hydrogen bond at Ala4 is consistent with the reported variable temperature NH shifts,[4] as is the sequestered His5 NH directed into the molecular cavity (Figure 3). The next three most-populated conformers also sustain a γ-turn (**b**, **c**, **d**; 16, 7, and 4%, respectively). The four γ-turn conformers differ qualitatively by at least one torsional angle.[7] The fifth conformer (**e**, 3%) is a β-turn in which His5 is the NH H-bond donor to the Ala2 C=O. While **1-h** is not among the NAMFIS conformers, **1-h′** appears as the sixth most populated form at 3%. Submission of the **1-h** and **1-h′** pair alone to a NAMFIS matching of the same NMR data yields only **1-h′** (100%) with an SSD = 171.

To evaluate the situation in the context of constraint-guided simulated annealing, we presented the linear structure of peptide **3**, the NMR constraints, and the assumption of two i, $i + 4$ hydrogen bonds to CNSsolve/XPLOR.[14] The 15 resulting structures and the optimized average are α-helical similar to Figure 2a (red). The simulated annealing exercise was repeated with the same NMR variables, but with H-bonding assumptions corresponding to **1-h′**. The desired structure was obtained directly. Within the context of CNSsolve, **1-h′** satisfies the data and exhibits constraint violations similar to those of the ideal α-helical structure. The constraint violation comparison is noteworthy considering that **1-h′** is a local AMBER* minimum and satisfies only a subset of the NMR data. Conformer **a** is also located by the CNSsolve treatment.[15] In view of these observations, the absence of the α-helical form from the NAMFIS conformers, and the fact that **1-h** is a 6 kcal/mol destabilized virtual conformation (AMBER*), a single-turn α-helix appears to be an unrealistic solution to the NMR-derived metrics. It is clear that relaxation of the requirement that **1** adopts a single α-helical conformation, as necessitated by the CNSsolve/XPLOR treatment (see Supporting Information),[4,14] permits the NMR spectra to be interpreted by NAMFIS in terms of a rapidly equilibrating mixture of eight conformers with a variety of hydrogen-bonding patterns.

This example illustrates a general problem facing workers applying 2-D NMR to the structure determination of small molecules in solution.[16] Intuitive analysis of structure leading to the conclusion that only a single form exists in solution is a self-fulfilling exercise. Simulated annealing and related techniques which combine the totality of the NMR constraints and any user-conceived assumptions in a search for a lone structure will certainly deliver a family of such structures.[17] However, unlike soluble proteins where a single overall conformation is the norm, small molecules with one or more single bonds in general experience conformational averaging. While it may be attractive to computationally constrain a compound to a single conformation in solution, we believe it advisable to clearly demonstrate the fact outside the limits of assumption. NAMFIS is but one of a number of methods available[18] to test whether an alternative multiconformational interpretation fits the data equally well or better.

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Supporting Information Available: Computational results (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) MacroModel 6.5; cf. http://www.schrodinger.com.

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(7) See the Supporting Information for details.

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(9) The sum of square differences gives the goodness of fit; cf. refs 8 and 17.

(10) γ-Turns are about one-third as frequent as helices in proteins[11] and participate in [γ][γ]-turns,[12] while cyclic peptides commonly sustain γ-turns.[13]

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