Solution Structure of a β-Peptide Ligand for hDM2

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Recently, we described a β-peptide foldamer, β53–1 (Figure 1A), that assembles into a 14-helix in aqueous solution, binds the oncoprotein hDM2 with submicromolar affinity, and inhibits the interaction of hDM2 with a peptide derived from the activation domain of p53 (p53AD).† The intact recognition epitope of β53–1, including a high degree of helical structure, is required for selective inhibition of the p53AD-hDM2 interaction. Here, we present the solution structure of β53–1 in methanol. The structure reveals details of a helix-stabilizing salt bridge on one helical face, novel “wedge into cleft” packing along another, and distortions in the β53–1 14-helix that may maximize presentation of the p53AD recognition epitope. These details deepen our understanding of how β-peptides fold and how they can be designed to form higher order structures,‡ and bind macromolecules.‡§

Two-dimensional NMR spectroscopy was performed using 5 mM β53–1 in CD3OH at 10 °C. Previous circular dichroism and analytical ultracentrifugation experiments§ and the NMR line widths observed herein are consistent with a monomeric, 14-helical structure for β53–1 under these conditions. The proton resonances of β53–1 were assigned unambiguously using TOCSY and natural abundance 1H–13C HSQC spectra. § ROESY experiments were then performed using mixing times of 200, 350, and 500 ms. ‡ The observed series of NH–CaH ROEs confirmed the sequential assignment by providing a backbone “ROE walk”. Three classes of medium-range ROEs characterize a 14-helical conformation: those between Hα(i) and Hα(i+2), Hα(i) and Hα(i+3), and Hα(i) and Hα(i+3). § All 20 potential medium-range interactions of this type were observed in the ROESY spectra of β53–1; in addition, 27 additional medium-range ROEs between side chains three positions apart were also observed. § The large number of medium-range ROEs observed by NMR provides clear evidence for a high level of 14-helix structure in β53–1; 449 ROEs quantified using a 350 ms mixing time were subsequently assigned and integrated using SPARKY. ‡ Peak volumes were converted to 151 upper-limit distance constraints ‡ and used to perform simulated annealing torsional dynamics on 100 random starting configurations of β53–1 using DYANA. ‡ No constraint violations were reported among the resulting 20 lowest-energy structures, which are shown in Figure 1B.

The ensemble of calculated structures of β53–1 (Figure 1B) shows a 14-helix with an average backbone atom RMSD from the mean structure of 0.17 ± 0.07 Å. The backbone torsions of individual structures deviate little from the mean, even at the termini (Figure 1C), illustrating the robustness of the β53–1 14-helix in methanol. The helix is characterized by approximately 1.61 Å rise per residue and 3.0 residues per turn for residues 1–6, with a slight unwinding to approximately 1.49 Å rise per residue and 3.3 residues per turn for residues 7–10. This unwinding appears to be unique to β53–1, as it was not observed in NMR structures of unrelated β-peptides with and without side chain ion pairing. § Side chains are also well-defined among the lowest-energy structures, with an overall average heavy atom RMSD from the mean of 0.60 ± 0.10 Å.

β53–1 contains four charged side chains arranged to favor formation of helix-stabilizing salt bridges on one 14-helix face. ‡ In all 20 low-energy structures, the terminal nitrogen of βE10 and the nearest terminal oxygen of βE10 are characterized by a consistent separation of 5.5 ± 0.6 Å. The relative positions of the remaining two ion pairs fall into two subpopulations (Figure 2D). In 17 structures, the terminal nitrogen of βO1 and the nearest terminal oxygen of βE10 are closer (5.4 ± 0.9 Å) than the equivalent atoms of βE4 and βO7 (6.8 ± 0.9 Å). By contrast, in the remaining three structures, the terminal nitrogen of βO7 and the nearest terminal oxygen of βE4 are closer (3.6 ± 0.4 Å) than the equivalent atoms of βO1 and βE4 (7.7 ± 1.3 Å). This interplay

Figure 1. (A) Chemical structure of β53–1, shown with N-terminus at left. (B) Solution structure of β53–1 in CD3OH at 10 °C, shown as a bundle of 20 lowest-energy structures, with C-terminus at left. (C) Ribbon representation of the backbones of 20 lowest-energy structures. (D) Two subpopulations of ion pairing configurations. Superposed at left are 17 structures in which ββO1 and ββE4 are proximal; superposed at right are three structures in which ββE4 and ββO7 are proximal. (E) Conformations of ββ-homovaline residues illustrating the “wedge into cleft” packing found in all 20 lowest-energy structures.

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Figure 2. Overlay of the methanol solution structure of β53–1 (red ribbon and side chains) with the crystal structure of a p53AD-derived peptide (gold ribbon and side chains) bound to hDM2 (gray surface).

Importantly, this subtle distortion allows the side chains comprising the β53–1 recognition face to better mimic those on the p53AD α-helix. Overlays between β53–1 in an idealized 14-helical conformation and p53AD bound to hDM2 revealed an imperfect alignment between the two ligands; while the β53L3, β53W6, and β53F9 side chains of β53–1 could superimpose with their counterparts on p53AD, the 14-helix backbone could not completely fit within hDM2’s binding groove. The comparable overlay with the solution structure of β53–1 (Figure 2) shows no such conflict. In its solution conformation, β53–1 can access all three of hDM2’s hydrophobic pockets while occupying the same binding groove as p53AD with no steric clashes. This fits demands subtle unwinding near the β53–1 C-terminus that staggers the side chains, producing a β53-peptide that is uniquely suited for α-helix mimicry. The solution structure of β53–1 suggests that the extended, highly variable surface presented by a 14-helical β53-peptide oligomer could be used as a platform to design small, metabolically stable inhibitors of protein interfaces containing one or more α-helices.

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Supporting Information Available: Assignment tables, ROE-derived upper-distance limits. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(6) Please see Supporting Information for details.
(7) ROE intensities were linear in this range.
(25) Retinoverse peptides are another promising class of metabolically stable mimics for α-helical peptides. For a recent related example, see: Sakurai, K.; Chung, H. S.; Kuhne, D. J. Am. Chem. Soc. 2004, 126, 16288.