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Helical β-Peptide Inhibitors of the p53-hDM2 Interaction

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β-Peptides differ from α-peptides by one additional backbone carbon atom and by resistance to metabolism and proteolysis.[1a−c] β-Peptides fold into helices, sheets, and turns and have well-recognized potential as peptidomimetics.[2] Two β-peptides that interact with discrete macromolecular targets are known: a β-tetrapeptide hairpin that binds the somatostatin receptor with nanomolar affinity,[1d,e] and a highly cationic β^3-decapeptide that binds TAR RNA.[1f] Here, we report a set of β^3-peptides that possess significant 14-helix structure in water; one recognizes a cleft on the surface of the human oncogene product double minute 2 (hDM2) with nanomolar affinity.

hDM2 is recognized in vivo by a short α-helix within the activation domain of p53 (p53AD, Figure 1A), the transcription factor that controls cell fate in response to stress. hDM2 negatively regulates p53 function, and disruption of the p53·hDM2 interaction[3a] is an important cancer therapy goal.[3b] Three residues on one face of p53AD (F19, W23, and L26) comprise the functional epitope that contributes heavily to the binding energy.[3c−e] Modification of a p53AD-based α-peptide with nonnatural α-amino acids that improve helix stability and surface complementarity results in a potent inhibitor that activates apoptosis in vivo.[3f,g] The first small molecule inhibitors with submicromolar potency were reported in 2004.[3h]

Our design began with a β^3-decapeptide with significant 14-helix stability in aqueous solution due to electrostatic macrodipole stabilization and side chain−side chain salt bridges on one helical face.[4] Although the dimensions of a 14-helix differ from those of an α-helix,[2] we hypothesized that the p53AD functional epitope would be recapitulated if the side chains of F19, W23, and L26 were presented at successive positions three residues apart on a stabilized 14-helix (Figure 1B). Because the 14-helix has almost exactly three residues per turn, these side chains should align upon folding. Four β^3-peptides were designed in which these side chains are appended in both possible orientations on each of the two available 14-helix faces (***β*53-1**−**4**, Figure 2).

We compared the circular dichroism (CD) spectra of ***β*53-1**−**4** in aqueous buffer to estimate their 14-helix content (Figure 3A).[1g−j,2] The 14-helix signature is clearly evident, and the relative minima at 214 nm suggest helical contents between 30% and 50% for ***β*53-1**, **3**, and **4**.[5] Two-dimensional NMR spectroscopy[6] in CD_3OH confirmed the presence of 14-helix structure in ***β*53-1**: ROESY spectra showed four of seven possible $C_\alpha H(i) \rightarrow C_\beta H(i+3)$ ROEs and two of six possible $C_N(i) \rightarrow C_\beta(i+3)$ ROEs. Additional ROEs may be present but were obscured by resonance overlap; no ROEs inconsistent with 14-helical structure were observed. Analytical ultracentrifugation[5,6] revealed that ***β*53-1**, **3**, and **4** were monomeric at concentrations between 80 and 400 μM, confirming that these 14-helices are stabilized by intramolecular interactions.

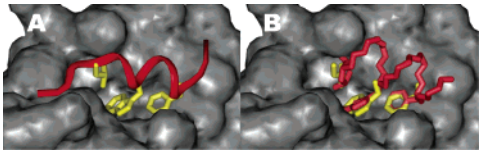

Figure 1. (A) Crystal structure of $p53AD_{15-29}$ (red) bound to $hDM2_{17-124}$ (gray surface); the side chains of F19, W23, and L26 are in yellow.[3a] (B) Model of an idealized β-peptide 14-helix (red) bound to $hDM2_{17-124}$ in the p53AD-binding cleft. The side chains of F19, W23, and L26 from p53AD (yellow) are retained for comparison.

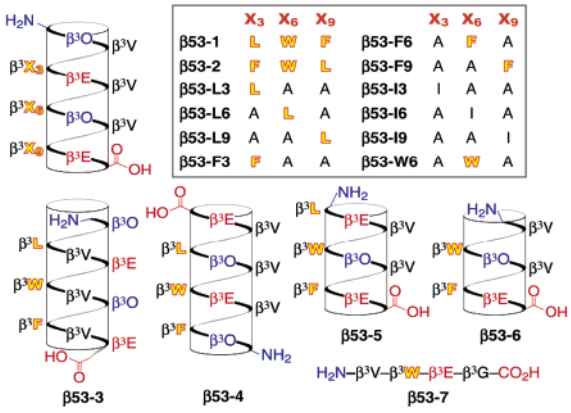

Figure 2. Helical net diagrams of β^3-peptides studied herein. β^3X denotes a β^3-homoamino acid where X is the one-letter code for the corresponding α-amino acid. Red and blue accentuate electrostatic features; residues that comprise the p53AD epitope are in yellow.

We designed a competition fluorescence polarization (FP) assay[7] using $hDM2_{1-188}$ (hDM2) and a fluorescein-labeled $p53AD_{15-31}$ peptide[6] ($p53AD^{Flu}$) to monitor inhibition of p53AD·hDM2 complexation by ***β*53-1**−**4**. The K_d of $p53AD^{Flu}$·hDM2 measured by direct FP analysis was 233 ± 32 nM (Figure 3C), consistent with previous work.[3a−d,7] $p53AD_{15-31}$ inhibited the $p53AD^{Flu}$·hDM2 interaction with an IC_{50} of 2.47 ± 0.11 μM, as expected.[7] Two β^3-peptides, ***β*53-1** and ***β*53-3**, inhibited $p53AD^{Flu}$·hDM2 complexation with IC_{50} values of 94.5 ± 4.4 and 1589 ± 104 μM, respectively (Figure 3B), but only ***β*53-1** failed to inhibit formation of the unrelated CREB KID·CBP KIX complex;[8] ***β*53-3** was not studied further.[6] To ensure that the inhibition was due to direct binding, two fluorescein conjugates of ***β*53-1** were prepared. ***β*53-1**^{Flu}, labeled on the C-terminus, bound hDM2 with a K_d of 583 ± 88 nM, while ^{Flu}***β*53-1**, labeled on the N-terminus, bound hDM2 with a K_d of 368 ± 76 nM (Figure 3C). The similarity of the two K_d values, as well as the inability of other fluorescein-β-peptide conjugates to bind hDM2,[9] implies that the dye contributes

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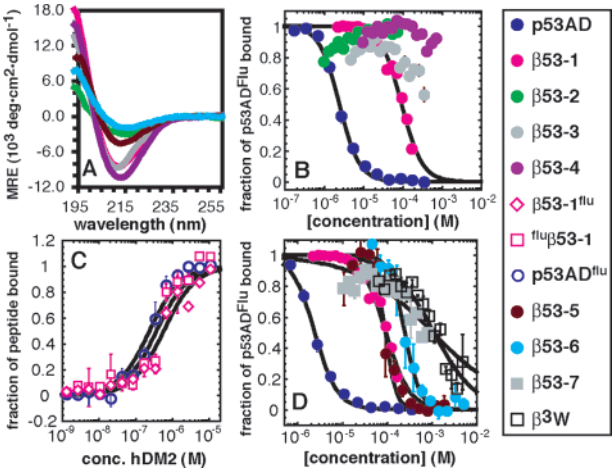

Figure 3. (A) CD spectra of ***β*53-1**−**6** in PBC buffer (pH 7.0).[6] Peptide concentrations were 160 μM, except ***β*53-2**, which was 22 μM. (B) Inhibition of p53AD^{Flu}·hDM2 complexation by p53AD and ***β*53-1**−**4**. (C) Direct binding of p53AD^{Flu}, ^{Flu}***β*53-1**, and ***β*53-1^{Flu}** to hDM2. (D) Inhibition of p53AD^{Flu}·hDM2 complexation by p53AD, ***β*53-1**, ***β*53-5**−**7**, and β^3-homotryptophan.

little to the binding energy. The affinity of ***β*53-1** for hDM2 is only 1.6 to 2.5-fold lower than that of p53AD.

Next, we prepared a series of β^3-decapeptides to assess whether the affinity of ***β*53-1** for hDM2 required all or part of the functional epitope composed of p53AD side chains F19, W23, and L26. β^3-Peptides ***β*53-W6**, ***β*53-F9**, and ***β*53-L3**, in which two of these three side chains were changed to β^3-homoalanine, inhibited p53AD^{Flu}-hDM2 complexation with IC_{50} values of 198.1 ± 10.0, 1701 ± 163, and >7000 μM, respectively.[6] ***β*53-W6**, which retained β^3-homotyptophan (β^3W), was the most potent inhibitor, with an IC_{50} value 2-fold higher than that of ***β*53-1**, whereas ***β*53-F9**, which retained β^3-homophenylalanine (β^3F), was moderately potent. Importantly, the relative arrangement of β^3W and β^3F was critical: β^3-peptides containing different arrangements of these residues, ***β*53-2** and **4**, showed no inhibition at 70 and 700 μM, respectively, and others with a single β^3F residue (***β*53-F3**, ***β*53-F6**) showed no inhibition at 1 mM. In addition, β^3-peptides with a single β^3L residue (***β*53-L3**, ***β*53-L6**, ***β*53-L9**) showed no inhibition at concentrations as high as 20 mM, and those with a single β^3I residue (***β*53-I3**, ***β*53-I6**, ***β*53-I9**) showed no inhibition at concentrations as high as 1 mM. A sequence-unrelated β^3-tetrapeptide containing β^3W (***β*53-7**) and β^3W itself were poor inhibitors (IC_{50} > 1 mM, Figure 3D). These data indicate that ***β*53-1** interacts with hDM2 with specific contributions from two of three residues comprising the p53AD functional epitope, β^3W and β^3F. The relative importance of β^3W, β^3F, and β^3L in the context of ***β*53-1** is consistent with data for p53AD-based α-peptides.[3d]

We next determined if ***β*53-1** could be minimized while retaining high affinity and selectivity for hDM2. ***β*53-5** and ***β*53-6** lack residues 1-2 or 1-4 (including the β^3L at position 3) of ***β*53-1**, respectively, and are less structured in aqueous solution (Figure 3A). ***β*53-5** inhibited the p53AD^{Flu}·hDM2 interaction slightly better (IC_{50} = 80.8 ± 3.2 μM, Figure 3D) than did ***β*53-1** whereas β^3-hexapeptide ***β*53-6** inhibited poorly (IC_{50} = 250 ± 12 μM). Importantly, ***β*53-5** and ***β*53-6** inhibited CREB^{KID}·CBP^{KIX} complexation with potencies similar to those for p53AD·hDM2 inhibition (60.1 ± 5.3 μM and 150.6 ± 15.0 μM, respectively), whereas ***β*53-1** did not.[6] As a whole, these data imply that the 14-helix structure of ***β*53-1** is a prerequisite for selective hDM2 recognition.

In summary, we describe a strategy for the design of protein surface ligands in which a functional epitope is presented by a compact, 14-helical β-peptide scaffold. This strategy may have advantages over one in which individual or multiple β-amino acids are introduced into a functional α-peptide,[1f,1k,7a] since it is based on homology of secondary structure, not primary sequence. Currently we are exploring the generality of this strategy and the potencies of ***β*53-1** and ***β*53-5** *in vivo*.

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Supporting Information Available: NMR analysis of ***β*53-1**, AU analysis of ***β*53-1**−**4**, and FP analysis of CBP KIX·CREB KID^{Flu} inhibition. This material is available free of charge via the Internet at http://pubs.acs.org.

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