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Use of a Retroinverso p53 Peptide as an Inhibitor of MDM2

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MDM2 regulates the transcription factor p53 by binding to its transactivation domain and promoting its ubiquitin-dependent degradation.[1] Because p53 plays a central role as a tumor suppressor by inducing cell cycle arrest or apoptosis in response to DNA damage,[2] its overly rapid degradation can result in tumorigenesis. While overexpression of MDM2 has been observed in many tumors,[3] it has also been shown that p53 function can be restored by disrupting its interaction with MDM2.[4,6e] Therefore the p53−MDM2 interface has emerged as an important target for chemotherapeutic agents.[5,6]

The X-ray crystal structure of a 15-residue peptide fragment of p53 (**1**, Figure 1) complexed with human MDM2 reveals that the p53 peptide binds in an α-helical conformation in a deep hydrophobic groove of MDM2, making three critical contacts with p53 residues Phe19, Trp23, and Leu26, all of which lie on one face of the helix (Figure 2a).[7] While side chains of these three residues interact with MDM2 with high steric complementarity, the backbone of the p53 peptide makes no contacts except for one hydrogen bond. In the absence of MDM2, the 15-residue peptide has no distinct secondary structure.[8] If the peptide backbone simply provides a scaffold[9] for the side-chain groups required for binding,[6e−g] then D-peptides may also bind if they can adopt conformations in which the spacing and orientation of key side chains are maintained.[10] To test this hypothesis, we synthesized peptide isomers of the p53 peptide (**2**−**5**, Figure 1) and evaluated their ability to interact with MDM2. Our results show that the retroinverso isomer **5** of the natural p53 peptide **1** inhibits MDM2 with potency comparable to that of **1**. Although D-peptides preferentially adopt left-handed helices, this result suggests that the all-D peptide **5** is capable of adopting a helical conformation with at least two successive right-handed turns.

Peptides **2**−**5** contain the same side-chain groups and compositions as **1** but have acyl and amide caps at the ends. Peptide **2**, the capped isomer of **1**, serves as a control. Peptide **3** is the mirror image isomer of **2** composed of all-D amino acid residues.[10] Because of the enantiomeric relationship, the side-chain orientations of **2** and **3** are not superimposable (Figure 2a,b). Peptide **4** is the retro isomer of **2**, meaning that the sequence of amino acids from the N- to the C-terminus is identical to the sequence of **2** reading from the C- to N-terminus. Peptide **5** is the retroinverso isomer of **2**, meaning that both the direction of the sequence and the chirality of the amino acids are opposite to those of **2**.[11,12] Thus, peptide **5** is the D-peptide enantiomer of **4**. The side chains of a peptide and its retro isomer are oriented in opposite directions, making it impossible for a retro peptide to establish the same contacts as the parent peptide even if the order of amino acids is maintained (compare a and c of Figure 2). The side-chain orientations of a peptide and its retroinverso isomer are similar in extended

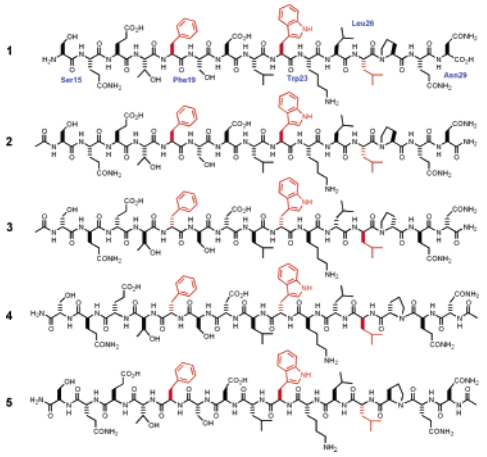

Figure 1. **1**: The natural p53 15-mer peptide (Ser15-Asn29); **2**: the end-capped natural p53 peptide; **3**: the mirror image isomer, all-D peptide; **4**: the retro isomer; **5**: the retroinverso isomer, all-D peptide. The key hydrophobic residues are highlighted in red.

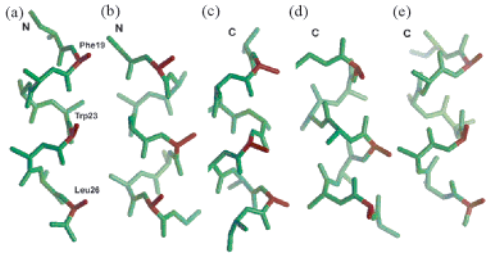

Figure 2. (a) MDM2 bound conformation of the natural p53 peptide (Thr18-Pro27).[7] The side-chain orientations of the key hydrophobic residues are represented by the $C^{\alpha}-C^{\beta}$ bond in red. The side-chain orientation of Phe, Trp, and Leu (from the top) for (b) the mirror image isomer in an idealized right-handed helical conformation; (c) the retro isomer in a right-handed helical conformation; (d) the retroinverso isomer in a left-handed helical conformation; (e) the retroinverso isomer in a right-handed helical conformation. Labels N and C show the N- and C-terminus, respectively. α-Helix models were created with Insight II (Biosym) and were rendered using Viewer Lite 3.2 (Accelrys Inc.).

conformations and in helical conformations having the same handedness (compare a and e of Figure 2) but differ in helical conformations having opposite helical twists (compare a and d of Figure 2).[12] Helical handedness is correlated with amino acid chirality, and all-D peptides such as **3** and **5** would be expected to adopt a left-handed conformation similar to that in Figure 2b,d.[13] The helical propensity of peptides **1**−**5** was measured by CD with increasing amounts (0−60 v/v %) of 2,2,2-trifluoroethanol (TFE), an α-helix-stabilizing solvent. None of the peptides showed distinct secondary structures in PBS.[8] Addition of up to 60% TFE did not dramatically affect the conformation of **1**−**3** (Figure 3a). However, the CD spectra for **4** and **5** changed significantly, showing distinct minima and maxima respectively at 208 and 222 nm, consistent

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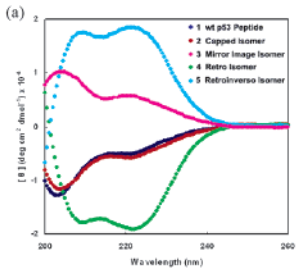

(b)

peptide	IC_{50} (μM)
1	4.12
2	17.4
3	>1000*
4	>1000*
5	15.4

Figure 3. (a) CD spectra of the p53 peptide isomers (100 μM) in 10 mM PBS, pH 7.2/60% (v/v) TFE at 5 °C. (b) Observed inhibitory activity of the p53 peptides against p53−MDM2. *Inhibition did not reach 50% up to 1 mM.

Table 1. Alanine Substitutions of the Hydrophobic Residues of Peptides **2** and **5** and the Inhibitory Activity (IC_{50}; μM) of the Mutants Against p53−MDM2

peptide	F19A	W23A	L26A
2	750	>1000	600
5	600	>1000	500

[a] The positions of the alanine substitution are designated according to the residues for the p53 peptide.

with induction of an α-helical conformation. Reflecting the enantiomeric relationship, the CD spectra for **2**−**3** and **4**−**5** are symmetric: **3** and **5** preferentially form a right-handed α-helix, and **2** and **4** form a left-handed α-helix.

We next evaluated the ability of the five peptides to inhibit the p53−MDM2 interaction by inhibition ELISA using a biotinylated wild-type p53 peptide immobilized on a streptavidin-coated microtiter plate and GST-hMDM2 (1-118). As shown in Figure 3b, **1** and **2** have similar IC_{50} values. The 4-fold difference in activity between **1** and **2** suggests that at least one of the end charges in **1** may play a modest role in binding. In contrast, the binding of peptides **3** and **4** to MDM2 is not detectable (IC_{50} > 1 mM), consistent with their inability to present the side chains in the appropriate orientation for binding (Figure 2b,c). Retroinverso peptide **5**, however, has the same inhibitory potency as **2**. Because **4** and **5** possess identical properties except for their interaction with chiral molecules, these results suggest that **5** interacts specifically with MDM2.

To determine whether **5** makes similar contacts to MDM2 as peptides **1** and **2**, we individually mutated the Phe, Trp, and Leu residues to Ala and evaluated the inhibitory potencies of each of the three mutant peptides by inhibition ELISA. Mutations of Phe, Trp, or Leu to Ala in **2** as well as in **5** resulted in the severe loss of inhibitory activity (Table 1). Therefore, all three hydrophobic residues in **5** play a critical role in MDM2 binding, as they do for peptides **1** and **2**.

From the perspective of a chiral receptor, peptides **2** and **5** differ in at least three fundamental ways: (i) the positions of the nitrogen and oxygen atoms in the amide bonds are reversed, (ii) the interresidue hydrogen bonds point in opposite directions, and (iii) the chirality of corresponding amino acid residues is inverted. Our results show that none of these fundamental changes in backbone structure significantly affects peptide binding, supporting the hypothesis put forth by others that the backbone of the p53 peptide functions primarily as a scaffold to display side chains.[6e−g] Nevertheless, the ability of the retroinverso peptide **5** to mimic the parent peptide **2** in MDM2 binding is remarkable.[12] For **5** to make similar contacts to MDM2 as **1** and **2**, it must adopt a helical conformation having two successive right-handed turns (Figure 2e). D-Peptides preferentially adopt left-handed conformations (Figure 3a), and in such a conformation the side chains of **5** would not be oriented in a similar manner as in **1** or **2**. However, right-handed turns are not prohibited in D-peptides,[13] and AM1 calculations indicate that **5** can adopt roughly two right-handed helical turns without incurring a severe energetic penalty (Supporting Information). Thus, **5** may represent a novel example of a D-peptide binding to a protein in a right-handed, loosely helical conformation, which would position the key side chains appropriately. If this hypothesis is borne out, retroinverso D-peptides may have unanticipated utility as metabolically stable mimics of natural α-helical recognition elements.[9,14,15]

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Supporting Information Available: Experimental procedures, peptide sequences, and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Freedman, D. A.; Wu, L.; Levine, A. J. *Cell Mol. Life Sci.* **1999**, *55*, 96.
(2) (a) Levine, A. J. *Cell* **1997**, *88*, 323. (b) Vogelstein, B.; Lane, D.; Levine, A. J. *Nature* **2000**, *408*, 307.
(3) (a) Jones, S. N.; Roe, A. E.; Donehower, L. A.; Bradley, A. *Nature* **1995**, *378*, 206. (b) Juven-Gershon, T.; Oren, M. *Mol. Med.* **1999**, *5*, 71.
(4) (a) Bötteger, A.; Bötteger, V.; Sparks, A.; Liu, W. L.; Howard, S. F.; Lane, D. P. *Curr. Biol.* **1997**, *7*, 860. (b) Wasylyk, C.; Salvi, R.; Argentini, M.; Sureuil, C.; Delumeau, I.; Abecassis, J.; Debusche, L.; Wasylyk, B. *Oncogene* **1999**, *18*, 1921. (c) Chène, P.; Fuchs, J.; Bohn, J.; García-Echeverría, C.; Furet, P.; Fabbro, D. *J. Mol. Biol.* **2000**, *299*, 245.
(5) (a) Chène, P. *Nat. Rev. Cancer* **2003**, *3*, 102. (b) Fischer, P. M.; Lane, D. P. *Trends Pharmacol. Sci.* **2004**, *25*, 343.
(6) (a) Reference 5 describes notable MDM2 inhibitors found earlier. (b) Knight, S. M. G.; Umezawa, N.; Lee, H.-S.; Gellman, S. H.; Kay, B. K. *Anal. Biochem.* **2002**, *300*, 230. (c) Schon, O.; Friedler, A.; Bycroft, M.; Freund, M. V.; Fersht, A. R. *J. Mol. Biol.* **2002**, *323*, 491. (d) Huber, V. J.; Arroll, T. W.; Lum C.; Goodman, B. A.; Nakanishi, H. *Tetrahedron Lett.* **2002**, *43*, 6729. (e) Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A. *Science* **2004**, *303*, 844. (f) Fasan, R.; Dias, R. L. A.; Moehle, K.; Zerbe, O.; Vrijbloed, J. W.; Obrecht, D.; Robinson, J. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 2109. (g) Kritzer, J. A.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. *J. Am. Chem. Soc.* **2004**, 126, 9468. (h) Galatin, P. S.; Abraham, D. J. *J. Med. Chem.* **2004**, *47*, 4163.
(7) Kussie, P. H.; Gorina, S.; Marechal, V.; Elenbaas, B.; Moreau, J.; Levine, A. J.; Pavletich, N. P. *Science* **1996**, *274*, 948.
(8) (a) Chang, J.; Kim, D. H.; Lee, S. W.; Choi, K. H.; Sung, Y. C. *J. Biol. Chem.* **1995**, *270*, 25014. (b) Botuyan, M. V. E.; Momand, J.; Chen, Y. *Folding Des.* **1997**, *2*, 331. (c) Uesugi, M.; Verdine, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14801.
(9) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479.
(10) Mirror image D-peptides have been shown to inhibit α-helical peptide−protein interactions. See: (a) Fisher, P. J.; Prendergast, F. G.; Ehrhardt, M. R.; Urbauer, J. L.; Wand, A. J.; Sedarous, S. S.; McCormick, D. J.; Buckley, P. J. *Nature* **1994**, *368*, 651. (b) Eckert, D. M.; Malashkevich, V. N.; Hong, L. H.; Carr, P. A.; Kim, P. S. *Cell* **1999**, *99*, 103.
(11) For pioneering works on the structure of retroinverso peptides, see: (a) Shemyakin, M.; Ovchinnikov, Y. A.; Ivanov, V. T. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 492. (b) Freidinger, R. M.; Verber, D. F. *J. Am. Chem. Soc.* **1979**, *101*, 6129.
(12) For reviews on the retroinverso peptidomimetics, see: (a) Goodman, M.; Chorev, M. *Trends Biotechnol.* **1995**, *13*, 438. (b) Fletcher, M. D.; Campbell, M. M. *Chem. Rev.* **1998**, *98*, 763. (c) van Regenmortel, M. H. V.; Muller, S. *Curr. Opin. Biotechnol.* **1999**, *9*, 337.
(13) Branden, C. I.; Tooze, J. *Introduction to Protein Structure*, 3rd ed.; Garland Publishing: New York, 1998; pp 8−10.
(14) A retroinverso peptide fused to a membrane permeable peptide sequence was shown to inhibit in vivo a protein−protein interaction involving α-helices; Pescarolo, M. P.; Bagnasco, L.; Malacarne, D.; Melchiori, A.; Valente, P.; Millo, E.; Bruno, S.; Basso, S.; Parodi, S. *FASEB J.* **2001**, *1*, 1.
(15) β-Peptides are a promising class of peptidomimetics for α-helical peptides with metabolic stability: (a) Chen, R. P.; Gellman, S. H.; DeGrado, W. F.; *Chem. Rev.* **2001**, *101*, 3219. (b) References 6b and 6g.

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