Enantiomeric Binding Elements Interacting at the Same Site of an SH3 Protein Receptor

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Sensitivity to chirality in protein−ligand interactions is of particular interest in molecular recognition and enzyme catalysis. That enzymes catalyze reactions involving only one enantiomer of a chiral substrate is commonly explained by the inherent chirality of proteins.[1−3] However, an apparent lack of stereospecificity has also been observed in biochemical studies of protein−ligand binding.[4,5] For example, calmodulin is able to bind two enantiomeric peptides with comparable affinity.[5] The structural details of how a protein receptor can recognize ligands having mirror image relationship are scarce.

Two ligands relevant to this issue that bind SH3 protein domains were recently discovered using split-pool synthesis and an affinity selection assay.[6] Although they contain key SH3-binding elements with a mirror image relationship that bind to the same pocket in the protein, these ligands bind the Src SH3 domain with similar affinity. Multidimensional NMR has now been used to uncover the structural basis for this result. A comparison of the structures of two synthetic ligands, NL2 and NL2R (Figure 1), complexed to enantiomerically pure Src SH3 reveals that a subsite on the binding surface maintains key intermolecular contacts with the mirror image elements. The results illustrate a mechanism through which a protein receptor interacts with binding elements having opposite chirality. Similar topographical surfaces of the chiral ligands coupled with a hydrophobic binding site lacking directional hydrogen bonds or charge−charge interactions appear to be important features of a receptor−ligand system having minimal sensitivity to chirality.

The NMR structure of the Src SH3−NL2 (Ac-Mn18-Mn1-PLPPLP-NH_2; P = Pro, L = Leu) complex has been reported.[7] Since Mn18 is a chiral monomer, its enantiomer Mn20, which was not used in the original library synthesis, was synthesized[8,9] in order to investigate the effect of inverting the stereochemistry on binding. The corresponding diastereomeric compound Ac-Mn20-Mn1-PLPPLP-NH_2 (NL2R) was found to bind the Src SH3 domain with affinity comparable to that of NL2 (NL2, K_d = 11 μM; NL2R, K_d = 5.4 μM; Figure 1).[7] Both Mn18 and Mn20 are crucial for high affinity binding to the Src SH3 domain as evidenced by the weak affinities of the two truncation ligands. The ligand Ac-Mn1-PLPPLP-NH_2 has a K_d of 220 μM to Src SH3, and the K_d for Ac-PLPPLP-NH_2 is larger than 1 mM (the binding was too weak to be measured accurately using fluorescence perturbation[6]). The structure of the SH3−NL2R complex[10] was determined using multidimensional NMR and compared to that of the SH3−NL2 complex.

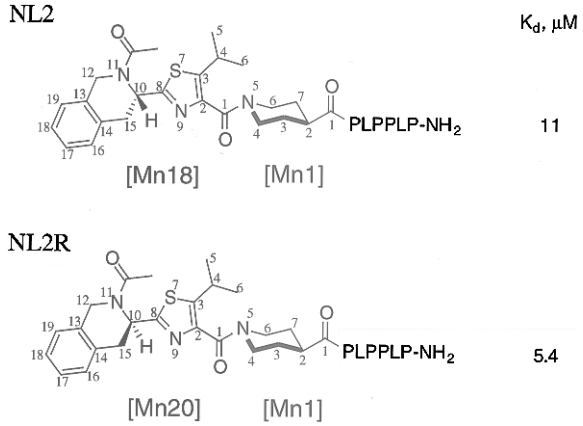

Figure 1. Ligands NL2 and NL2R. Mn1, Mn18, and Mn20 are synthetic monomers. The numbering of the heavy atoms and the corresponding protons in Mn1, Mn18, and Mn20 is used in the text to describe the structures. The dissociation constants (K_d) to Src SH3[6,7] were measured by a fluorescence perturbation assay at pH 7.4.[17]

Although the unbound forms of NL2 and NL2R have highly similar 1D NMR spectra (Figure 2A), the two SH3-bound ligands have very different 2D ^{13}C-filtered TOCSY spectra for the respective aromatic protons on Mn18 and Mn20 (the TOCSY spectra were acquired using samples consisting of a 1:1 ratio of the uniformly ^{13}C-labeled SH3 protein and unlabeled ligand) (Figure 2B). The binding site serves as a chiral shift reagent, dispersing otherwise degenerate resonances. The spectra indicate that racemization did not occur during synthesis.

The structures reveal how the same receptor binds two mirror image elements. Upon complexation, the common peptidic PLPPLP fragments of the two ligands adopt essentially the same polyproline type II (PPII) helix conformation as expected from studies of other Src SH3 ligands (Figure 3A).[7,11−13] Mn1 serves as a bridging element linking the PPII helix to the "monomer" residing in the pocket between the n-Src and RT loops. NL2 and NL2R differ from each other by the chirality of the C_{10} stereocenters of Mn18 and Mn20 (Figure 1). In the two structures, opposite faces of the tetrahydroisoquinoline group pack against Thr96 and Thr98 in the RT loop as a result of the opposite stereochemistry, but the bound conformations of the two enantiomeric moieties are remarkably similar (Figure 3B). NL2 and NL2R can be interconverted mentally by disconnecting the $N_{11}-C_{10}$ and $C_{15}-C_{10}$ bonds in one monomer, flipping the disubstituted phenyl ring by 180°, and rejoining the bonds to form the enantiomeric monomer. Flipping of the tetrahydroisoquinoline rings is accompanied by a 180° rotation of the thiazolyl groups along the C_8-C_{10} bond, thereby preserving the $N_9-C_8-C_{10}-N_{11}$ dihedral angle in the two complexes. For

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(10) The structure of the SH3−NL2R complex in an aqueous buffer was determined using isotope-edited and isotope-filtered 2D and 3D NMR spectroscopies as described previously.[7] The c-Src SH3 contains residues 85−140, and the numbering system used in the text is that of full-length c-Src from chicken. The NMR samples contain 2−3 mM of a 1:1 complex of the uniformly ^{13}C/^{15}N-labeled SH3 domain and unlabeled NL2R so that the resonances belonging to the protein and the ligand can be distinguished from each other. The average root-mean-square deviations (rmsds) of the 23 calculated structures of the SH3−NL2R complex versus their mean coordinate are 0.75 Å for the backbone and 1.26 Å for the heavy atoms, respectively. There are a total of 126 intermolecular NOEs. The heavy atom rmsd for the nonpeptide Mn20−Mn1 portion is 0.21 Å and is 0.66 Å for the entire ligand.
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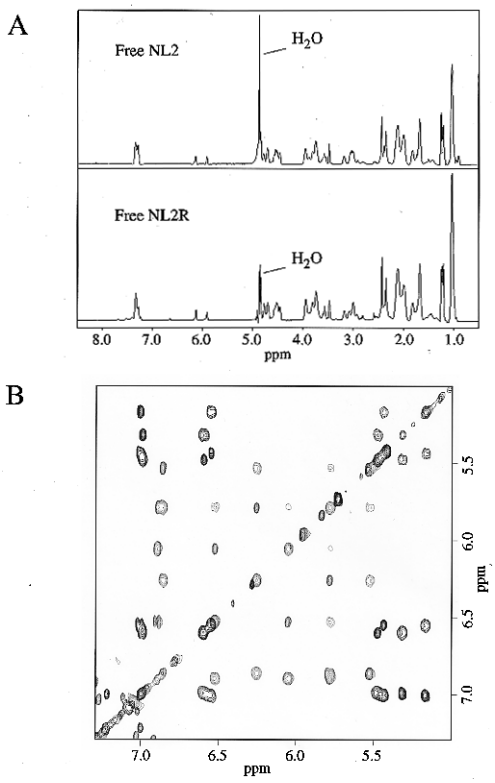

Figure 2. (A) Comparison of the 1D NMR spectra of free NL2 and NL2R in a D_2O buffer. (B) Overlay of the 2D ^{13}C-filtered TOCSY spectra of NL2-SH3 (red) and NL2R-SH3 (black) complexes. All cross peak signals shown in the 2D NMR spectra are from the SH3-bound ligands; the signals from the ^{13}C-labeled SH3 have been purged through isotope filtering.[18,19]

Mn18, the isopropyl substituent on the thiazole has weak NOE contacts with an H_β of Trp118 in the Src SH3 protein. In contrast, the isopropyl group of the thiazole in Mn20 points toward solvent and has no NOEs to the receptor. Instead, the Mn20 isopropyl has NOEs to the nearby hydrogens attached to C_6 on the following monomer Mn1.

The structures reveal that the overall "L-shape" geometry of Mn18 is maintained in Mn20 upon binding, allowing the binding interactions occurring in one complex to be preserved in the other. Comparing resonances for the Mn18 and Mn20 aromatic protons shows that H_{16} in Mn18 and H_{19} in Mn20 have similar chemical shifts, as do H_{19} in Mn18 and H_{16} in Mn20 (Figure 3B). The ^1H-^{13}C HMQC spectra of the ^{13}C-labeled SH3 in the two complexes are almost identical, suggesting that the two ligands cause the same resonance perturbations at the binding site. These chemical shift data faithfully represent the relative chemical environment in which the corresponding nuclei reside in the two complexes and are in accord with the structures defined by intermolecular NOEs.

The conservation of the key interactions in binding explains the similar K_d data for the two complexes. The accessible surface areas on the SH3 domain buried by NL2 and NL2R are 482 and 431 Å^2, respectively (calculated with GRASP[14] using a probe of radius 1.4 Å). The similar affinities of the two ligands are analogous to other examples in which L-amino acid peptide ligands selected through natural evolution can be mimicked in terms of protein binding by artificial D-peptides.[4,5,15,16]

The comparative structural studies involving NL2 and NL2R illustrate a mechanism through which a protein can recognize

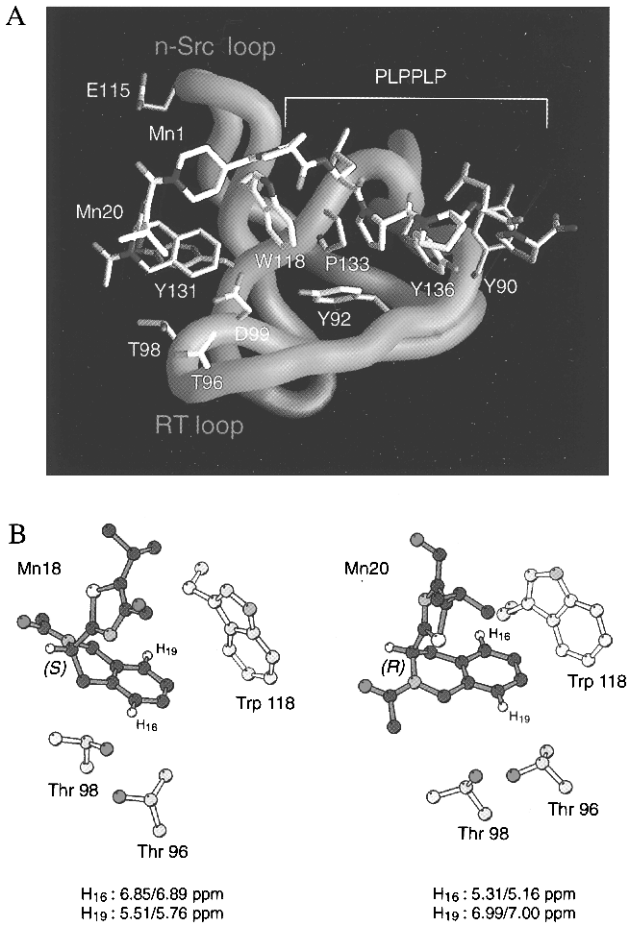

Figure 3. (A) Structure of NL2R at the binding site (average minimized structure). The C_α trace of the SH3 domain is shown as a red worm. The SH3 residues are labeled yellow, and the ligand residues are white. (B) Schematic representation of the bound conformations of Mn18 in the ligand NL2 and Mn20 in NL2R. The two sets of resonances correspond to the *trans*/*cis* rotamers of the Ac-amide. The chemical shifts of H_{16} and H_{19} in Mn18 resemble those of H_{19} and H_{16} in Mn20, respectively, as they reside in similar chemical environment on the receptor surface. Also shown are binding site residues Thr96 and Thr98 in the RT loop as well as Trp118. Carbon is black/gray, hydrogen is white, oxygen is red, nitrogen is blue, and sulfur is yellow.

mirror image binding elements. As a result of their unique cyclic features, monomers Mn18 and Mn20 can adopt conformations that present overall similar molecular surfaces contacting the SH3 domain. The binding is largely mediated by van der Waals and hydrophobic contacts involving primarily side chain packing and lacks the geometrically precise registers such as charge−charge and hydrogen-bonding interactions. These latter forces often play a prominent role in the highly stereospecific enzyme−substrate recognition observed in enzyme active sites, where an intricate catalytic machinery requires accurate alignment with the substrate in order to achieve catalysis.

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Supporting Information Available: Text describing materials and methods (2 pages). See any current masthead page for ordering and Internet access instructions.

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