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Potent Small-Molecule Binding to a Dynamic Hot Spot on IL-2

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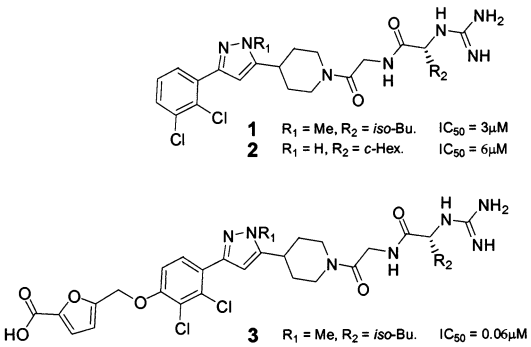
The disruption of protein−protein interactions represents one of the most challenging target classes for small-molecule drug discovery.[1] The difficulty is due in part to the nature of protein interfaces, which are often flat and quite large (1200−3000 Å^2).[2] However, despite the vast size of these interfaces, the binding energy of the interaction is not evenly dispersed throughout each surface. Rather, hot spots exist, which are much smaller subsets of the interface, and represent energetic focal points of the interaction.

Small peptides isolated from naïve phage libraries often bind at hot spots,[3] suggesting that small molecules could be found to inhibit this target class. However, like phage display, large libraries must be surveyed to search the chemical diversity needed to discover novel leads. Small-molecule fragment assembly methods such as tethering[4] offer a possibility because tens of millions of potential fragment combinations can be screened.

Tethering was used to enhance the affinity of low micromolar lead compounds **1** and **2** for IL-2. A furanoic acid fragment was merged with **1** to make **3**, which has an $IC_{50} = 60$ nM for IL-2, corresponding to a 50-fold improvement in affinity.[5] To determine the structural basis for the enhanced affinity**,** we crystallized IL-2 in the presence of each molecule. Diffraction-quality crystals were isolated with IL-2 in the presence of either **2** or **3**, and the structures were refined to a resolution of 2.6 and 2.8 Å, respectively (Supporting Information Table 1 and ($2F_o - F_c$) electron density map).



These compounds bind to a portion of IL-2 corresponding to the hot spot for IL-2Rα.[6] The structures solved here were compared with an apo form of IL-2 (Figure 1A) and a complex of IL-2 with a tethering "hit" ($IC_{50} > 1$ mM, Figure 1B). Each of the compounds is anchored to IL-2 via a buried polar interaction between a guanido group and the carboxylate side chain of Glu 62 (Figure 1B−D). Interestingly, this residue is a critical hot spot position for IL-2 binding to IL-2Rα.[6] Thus these inhibitors appear to mimic a functional determinant used by the receptor for specificity toward IL-2.

Hot spots have the characteristics of being accessible, hydrophobic, and adaptive.[3] While proteins are dynamic molecules, little is known about their adaptivity because crystal structures often only provide a snapshot of a particular protein conformation. We observed a high level of plasticity in the hot spot of IL-2 and captured the protein in multiple forms.

Along with Glu 62, Phe 42 is a hot spot position previously shown to be critical for IL-2Rα binding.[6] We noticed two different conformations of this residue in our structures. In the apo form and in the tethered structure, Phe 42 is in an "up" position (Figure 1A−B). However, in the complexes of IL-2 with both **2** and **3**, Phe 42 is in a "down" conformation and Leu 72 is shifted out. This conformational change opens up a greasy binding site for the dichlorophenyl moiety that is not observed in either the apo form or the tether bound form. Because the binding modes of either compound are incompatible with the IL-2 apo form, Phe 42 and Leu 72 appear to be gatekeeper residues, with a conformational shift necessary for binding to occur.

In addition to these movements, a striking conformational change was observed in the hot spot of IL-2. Tyr 31 is found in two distinct conformations, 14 Å apart. The first form is a solvent-exposed position with the tyrosyl side chain pointing out from the protein (Figure 1C). Tyr 31 is in a buried position in the second form, with the tyrosyl side chain pointing into the core of the protein (Figure 1D). In the structure of **2** with IL-2, each form is seen in the asymmetric unit. However, the binding site for the added furanoic acid in **3** is only available when Tyr 31 is in the buried conformation. In this form, helix A′ is elongated from one turn to two full turns (residues 34−42). The added helical turn organizes the binding pocket for the furanoic acid, which is positioned in a greasy yet charged pocket between Pro 34 and two positively charged residues, Lys 35 and Arg 38. Thus, while the binding of **2** is compatible with either conformation of Tyr 31, the binding of **3** is strictly limited to IL-2 with Tyr 31 in the buried conformation.

It is tempting to speculate that the binding mode of IL-2 observed in complex with **3** is the preferred conformation of IL-2 for its natural ligand, IL-2Rα, which has only 6-fold greater affinity for IL-2, despite the vast disparity in size. This binding mode creates an elongated and unobstructed groove from Pro 34 to Glu 62, which elaborates the hot spot of IL-2 by increasing the accessibility and solvent-exposed hydrophobicity of the site. Conformations of IL-2 similar to those seen here have been reported elsewhere;[7] however, the elongated groove is only visible when IL-2 is complexed with **3**. Furthermore, the binding of **3** is incompatible with the binding mode of IL-2 seen in complex with **2** (Figure 1C), suggesting that the form of IL-2 observed when bound to **3** is better suited for high-affinity ligand binding.

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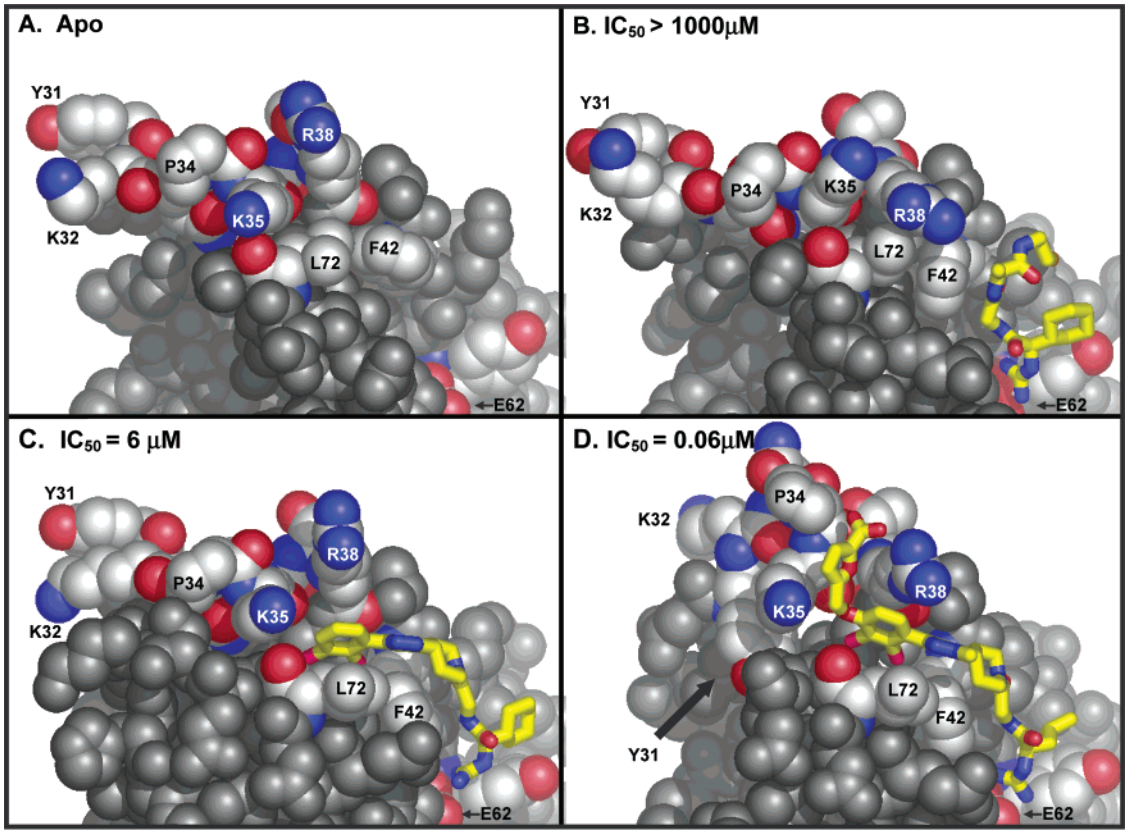

Figure 1. Comparison of four IL-2 crystal structures. (A) An unliganded form of IL-2. F42 is in the "up" position, and no binding site for the small molecule is observed. (B) A complex with a guanido-containing tethering hit. F42 is also in the "up" position. (C) Structure of **2** with IL-2 F42 is in the "down" position, and L72 is shifted out of the way to accommodate the dichlorophenyl moiety in **2**. Y31 is in the solvent-exposed form. (D) Structure of **3** with IL-2. F42 is in the down position, L72 is shifted, and Y31 is buried into the core of the protein. A binding pocket is created for the furanoic acid between P34, K35, and R38. The binding of **3** creates an elongated groove which stretches from P34 to E62. A $\pi-$cation interaction between the dicholoroaryl moiety of **3** and R38 could facilitate binding of the compound. Figures were made with Pymol (http://pymol.sourceforge.net). The PDB accession numbers are 1PW6 for **2**/IL-2 and 1PY2 for **3**/IL-2 and are available at www.pdb.org.

IL-2 is a dynamic molecule which binds this class of inhibitors like a zipper, with the conformation of the protein changing as necessary to bind each novel functionality added to the compound (Figure 1A−D, and two movies published as Supporting Information). Binding-site rigidity is often thought to play a role in developing high-affinity compounds. However, in this case, specific contacts between small molecule and protein are made, despite the inherent plasticity of the binding site.

Structure-guided, rational drug design relies on crystal structures to provide a path for optimizing lead compounds. This approach is suitable in the case of most enzymes, which have rigid, well-defined pockets and often have added structural data from substrate complexes. Given the adaptivity observed in IL-2, it is unlikely that **3** could have been found by rational design. Therefore, when targeting hot spots, fragment-assembly methods offer the stochastic advantage of finding fragments in highly adaptable protein regions where structural changes are unpredictable.

Supporting Information Available: X-ray crystallography statistics (PDF) and two movies (AVI). This material is available free of charge via the Internet at http://pubs.acs.org.

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