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Catch the Kinase Conformer

Protein kinases exist in inactive and active states, but little attention has been paid to which state is or should be the target in drug discovery efforts. In this issue of *Chemistry & Biology*, Okram et al. [1] tackle this issue and show that inhibitors can be designed specifically to bind to inactive Abl.

Over 500 protein kinases comprising approximately 2% of the human genome have been identified; however, a limited number are well characterized functionally and structurally [2]. Kinases' abilities to activate or inactivate other proteins (including sometimes themselves) by phosphorylation make them key controllers in various cellular processes such as cell growth, differentiation, metabolism, and apoptosis. Deregulation of kinase activities is well associated with a multitude of disease states, particularly cancer in which excessive kinase activity is associated with cellular transformation. The kinase family thus provides a treasure trove of therapeutic targets, second only to G protein-coupled receptors [3]. The basis for therapeutic intervention is through chemical inhibition of kinase activity.

A major issue with respect to protein kinases as therapeutic targets has been the lack of selectivity of ATP-competitive inhibitors toward their targets. Since the ATP binding pocket is homologous in the majority of protein kinases, it is highly probable that an ATP-directed small molecule will bind to a number of targets albeit with varying degrees of potency. The observation that ATP [4] and small molecule ATP antagonists [5] can bind with different affinities to active and inactive kinases raises the possibility of designing inhibitors specific for inactive confirmations resulting in higher ligand selectivity. Conventional "type I" inhibitors bind to the ATP cleft in an open (DFG in) active conformation (Figure 1) and this conformation is generally similar in most kinases [6]. In contrast, the ATP binding site of the inactive conformation (DFG out) is more unique, and ligand design based on this scaffold results in "type II" molecules that are not readily accommodated in binding sites of active kinases, illustrated for Abl in Figure 1.

The fact that kinases exist in active and/or inactive states in cells has been known for more than 20 years [7]. The activation of protein kinases occurs mainly through conformational adjustments induced by post-translational modification (principally phosphorylation) and, in some cases, by binding of a regulatory subunit. Only recently have differences between active and inactive kinases been considered in drug discovery and thought given to how this could be exploited in the design of specific inhibitors of ATP binding [1, 8, 9]. An example of this is the binding of cyclins to CDKs, an event triggering a large shift in the position of the activation loop and subsequent alignment of the residues comprising the catalytic domain. Conformational mobility of the loop regions can be exploited in structure-based design, and recently has resulted in serendipitous discoveries of ligands that either sequestered unique conformations that are incompatible with ATP binding [10], or utilized a novel allosteric binding site [11].

Despite these new developments in targeting inactive kinase conformations, the majority of efforts in drug discovery do not consider the kinase activation state. In some instances, studies present activity data for potent inhibitors of a kinase, obviously determined in an assay using the active enzyme, but, surprisingly, designed or explained using X-ray crystal structures of the inactive form. This is particularly common for CDK2 inhibitor discovery where the significant structural changes occurring upon activation of the kinase can result in dramatic differences in inhibitor binding [12, 13].

The first attempt to quantify the differences in ligand binding between active and inactive kinases by Davis et al. [14] raised some concerns. Although the authors suggested that differences do exist with implications for drug design, the observation was made that the inhibitor studied bound with an overall similar mode to the active and inactive forms. More substantial proof that activation changes result in significant differences in ligand binding with direct implications for drug design emerged recently [9]. A series of CDK2 inhibitors studied in monomeric (inactive) and cyclin bound (active) forms demonstrated that small changes in the ATP binding site could have a profound effect on ligand binding.

The present study from Okram et al. [1] provides the first description of the inhibitor determinants required for binding and stabilizing inactive kinase conformations (type II inhibitors), and demonstrates how these can be used in the design of a new generation of ligands. The principle strategy reported for the design of type II kinase ligands is to append appropriate pharmacophoric elements to type I ligands, thereby transforming them into compounds that preferentially interact with inactive kinases, targeting Abl as a proof of principle. In this work, several type I progenitors were modified through attachment of urea and benzamide groups and screened against a kinase panel [1]. Selective targeting of inactive kinase structures by these compounds was confirmed through assay results demonstrating that unphosphorylated Abl (inactive) was preferentially inhibited by type II inhibitors in contrast to the effects of type I inhibitors and in addition, a crystal structure of the complex between a type II molecule and c-Abl showed that a similar conformation to Gleevec (known to bind to inactive conformation) was obtained. A major observation is that type II inhibitors themselves can induce structural changes and result in an inactive conformation [5]. Activity data demonstrated that the type II compounds also can bind to the phosphorylated active Abl, thus implying that they can drag the protein from an active to an inactive conformation. This conclusion, however, requires further investigation since no direct comparison has been made of the same ligand/kinase complex in both the phosphorylated and unphosphorylated forms. For this case, it could be possible for the ligand either to induce the protein conformation [5] or to bind to a transitional activation state [15].

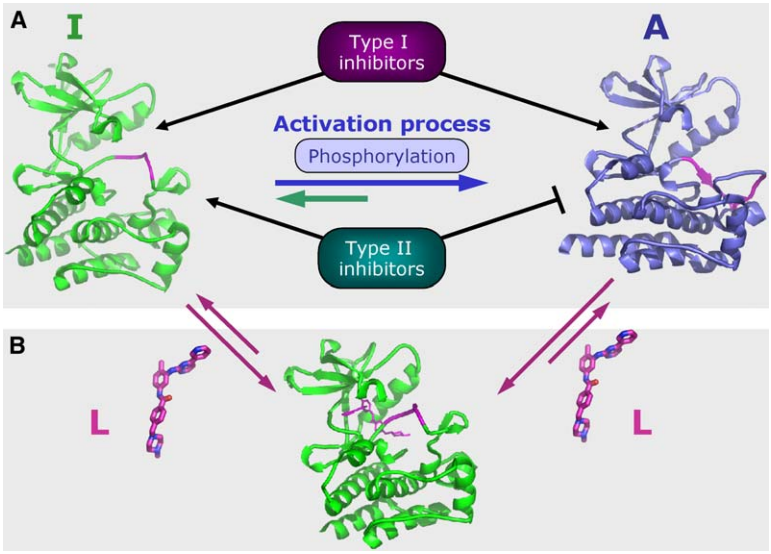

Figure 1. Inhibition of Active and Inactive Conformations of Abl

(A) Inactive Abl (**I**, green) transforms to the active (**A**, blue) form after an activation event (phosphorylation). The conformational alterations occurring upon activation adjust the activation loop (magenta), converting the ATP binding pocket to an open conformation where it can accommodate ATP and substrate. As the ATP binding pockets of active kinases (**A**) in general are homologous, it is highly probable that any small molecule design guided by an active kinase will bind to a number of other active kinases resulting in poor selectivity (type I). The ATP binding site of (**I**) has distinct differences from that of (**A**) and, in general, from other kinases and thus can be exploited to design type II inhibitors (**L**) that do not bind to (**A**), and are more selective for (**I**).

(B) A selective inhibitor (**L**), which cannot be accommodated in the binding site of (**A**), may drive the equilibrium to the predominantly (**I**) conformation, thereby resulting in less active protein available for biological processes.

PDB codes 1IEP and 1M52[5] were used for the above figure.

In this current study, convincing evidence is presented for the design of specific and selective inactive-conformation inhibitors of Abl and other protein kinases. The surprising results were that the type II compounds showed broader selectivity profiles compared to the type I inhibitors in addition to gaining potency. Based on these results, it is conceivable that the development of the next generation of specific and selective kinase drugs will be accelerated. This approach, however, will be dependent on the availability of more structural information for many of the kinases that remain uncharacterized. The authors correctly state that much more needs to be learned, but these results will provide a foundation on which to generate further knowledge of the determinants of type II inhibitors and thus will enable other kinases or other proteins families sharing similar features (i.e., activation, phosphorylation) to be inhibited in this way. We are coming to a stage where we can go beyond specific inhibition of a particular protein to inhibiting a specific conformation of that protein. This advance will lead to new tools to study the in vivo effects of conformer-specific inhibition and enable the impact of this on the dynamics of proteins in solution to be assessed.

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Selected Reading

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