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Hsp90 chaperones have an energetic hot-spot for binding inhibitors

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Abstract

Although Hsp90-family chaperones have been extensively targeted with ATP-competitive inhibitors, it is unknown whether high affinity is achieved from a few highly stabilizing contacts or from many weaker contacts within the ATP-binding pocket. A large-scale analysis of Hsp90α:inhibitor structures shows that inhibitor hydrogen-bonding to a conserved aspartate (D93 in Hsp90α) stands out as most universal among Hsp90 inhibitors. Here we show that the D93 region makes a dominant energetic contribution to inhibitor binding for both cytosolic and organelle-specific Hsp90 paralogs. For inhibitors in the resorcinol family, the D93:inhibitor hydrogen-bond is pH-dependent because the associated inhibitor hydroxyl group is titratable, rationalizing a linked-protonation event previously observed by the Matulis group. The inhibitor hydroxyl group pK_a associated with the D93 hydrogen-bond is therefore critical for optimizing the affinity of resorcinol derivatives, and we demonstrate that spectrophotometric measurements can determine this pK_a value. Quantifying the energetic contribution of the D93 hotspot is best achieved with the mitochondrial Hsp90 paralog, yielding 3–6 kcal/mol of stabilization (35–60% of the total binding energy) for a diverse set of inhibitors. The Hsp90 Asp93→Asn substitution has long been known to abolish nucleotide binding, yet puzzlingly, native sequences of structurally similar ATPases, such as Topoisomerasese II, have an asparagine at this same crucial site. While aspartate and asparagine sidechains can both act as hydrogen bond acceptors, we show that a steric clash prevents the Hsp90 Asp93→Asn sidechain from adopting the necessary rotamer, whereas this steric restriction is absent in Topoisomerasese II.

KEYWORDS

ATPase, chaperone, energetics, heat shock protein 90 (Hsp90), inhibitor

1 | INTRODUCTION

The Hsp90 family of ATP-dependent chaperones aid in the folding and stabilization of "client proteins". Human Hsp90 paralogs include Hsp90α and Hsp90β in the cytosol, Grp94 in the endoplasmic reticulum (ER), and Trap1 in mitochondria.[1] Many oncogenic proteins depend on Hsp90 for their stability, and consequently, Hsp90 has been targeted with ATP-competitive inhibitors.[2] Longstanding development efforts have yielded high affinity inhibitors from diverse structural classes, such as those based on geldanamycin, resorcinol, purine, pyrimidines, or azoles.[2]

Fragment-based drug discovery (FBDD) has produced compounds with high affinity for Hsp90.[3] A premise of FBDD is that energetic hotspots can be identified by analyzing the binding properties of diverse small molecules, and that key stabilizing contacts can then be integrated into a single compound that achieves high affinity. While the general success of FBDD suggests that energetic hotspots are a common feature of drug targets,[4] their exact energetic contributions are challenging to quantify. Structure-based calculations are being developed to predict energetic hot-spots.[5–7] Improvements in these computational methods will require benchmarks of experimentally validated hot-spots and their associated energetic contributions.

The Hsp90 binding pocket has been described as having several distinct regions. For example, Taldone et al. identify Pocket A, which ATP and the majority of inhibitors contact, Pocket B, a hydrophobic pocket that is opened upon the binding of some inhibitors, and Pocket C, which is at the opening leading into these deeper pockets.[8] Each of these regions contains potentially important contacts for inhibitor binding. In Hsp90α, these are N51, D93, and T184 in Pocket A; L107 and F138 in Pocket B; and G97 and K58 in Pocket C. Areas in the vicinity of Pocket B have been subdivided into Sites 1, 2, and 3 which are distinguished by paralog-specific conformations.[9] For example, PU-H54, a Grp94 selective inhibitor, interacts with Site 1 in Hsp90α and Site 2 in Grp94. In Grp94 the movement of F199 allows access to a deeper hydrophobic area.[10] While paralog-specific differences in residues and conformations are being utilized in attempts to create more selective inhibitors, it is not known how much each of these regions contributes to Hsp90 inhibitor binding affinity. Indeed, it is not known whether inhibitor affinity arises from a few energetically dominant contacts or from many widely distributed contacts.

Previous findings have yielded perplexing results about the impact of specific Hsp90 residues on the binding of both inhibitors and nucleotides. For example, the D93N substitution dramatically weakens nucleotide binding,[11] which has been rationalized to arise from a loss of a hydrogen-bond in which the nucleotide N6 amine group acts the proton donor and Asp93 acts as the hydrogen-bond acceptor.[12] This explanation is at best incomplete because asparagine and aspartate sidechains can both act as hydrogen-bond acceptors. Asparagine and aspartate sidechains have identical hydrogen-bonding energy as measured by helix-capping stabilization.[13]

Hsp90 is part of the GHKL superfamily, whose members (DNA gyrase, Hsp90, histidine Kinase, mutL) have similar ATP binding pockets.[14] Shortly after D93N in Hsp90α was shown to disrupt ATP binding, the homologous D73N variant of DNA gyrase B was found to abolish the binding of ATP and two classes of inhibitors: coumarins and cyclothialidines.[11,15] However, in stark contrast, other GHKL family members, such as Topoisomerase II, have an asparagine (N99 in yeast numbering) at the equivalent position of D93. The seemingly disparate observations about influence of the D93 position for Hsp90 and the entire GHKL superfamily have contributed to difficultly in understanding which inhibitor contacts to Hsp90 provide the most energetic stabilization and whether the energetic influence of these contacts is conserved across the GHKL superfamily.

2 | RESULTS

We questioned whether a large-scale structural analysis could yield insights into whether certain regions of Hsp90 are critical to the binding of most inhibitors. The Hsp90 NTD readily crystalizes and as a result, over 200 NTD:inhibitor crystal structures are currently deposited in the PDB. We analyzed these crystal structures to quantify which types of inhibitor contacts are observed most frequently (Table S1). The D93 residue in Hsp90α stands out in this analysis. Figure 1a,b catalogs the frequency of direct and water-mediated hydrogen bonds made between Hsp90 inhibitors and residues on the Hsp90α NTD. The D93 sidechain forms these two types of hydrogen-bonds in 94.5% of the NTD:inhibitor crystal structures examined. All available NTD:inhibitor structures for Grp94, Trap1 and Hsp90β have an inhibitor hydrogen-bond at the equivalent of D93 (Table S1).

Of the 200 Hsp90α structures examined only 11 inhibitors do not make a direct or water-mediated hydrogen bond with D93. Of these, 8 are less than 300 Da in size and therefore likely are fragments from screening efforts. One of the remaining three inhibitors (Ligand ID: 592) binds near D93 but in an orientation not compatible with a D93 hydrogen bond.[16] Only two structures show inhibitors larger than 300 Da that do not contact D93 (Ligand IDs: YJX and IK9). These two inhibitors bind at Pocket B; a large hydrophobic pocket which is formed upon the binding of some inhibitors.[10] A roughly equal proportion of these structures show inhibitor hydrogen bonding with D93 through nitrogen- or oxygen-associated hydrogens, 45% and 50%, respectively (Figure S1A). Further examination reveals that the nitrogen-associated hydrogen-bond donors are substituents of highly variable inhibitor scaffolds (Figure S1B). In contrast, the oxygen-associated hydrogen-bond donors are predominantly aromatic OH groups of resorcinol derivatives (Figure S1C).

Figure 1c shows examples of inhibitor hydrogen-bonding to D93. The geometry of nitrogen-associated

hydrogen-bond donors (ADP, EC144) is distinct from the resorcinols (radicicol, AUY922). However, in all cases the same group of residues on Hsp90α (D93, T184, G97) participates in the hydrogen-bonding network. Indeed, Figure 1b shows that T184 and G97 make water-mediated hydrogen-bonds in approximately 90% of all Hsp90α:inhibitor structures. The consistency of the D93-centered hydrogen bonding network despite the high structural and chemical diversity of Hsp90 inhibitors suggests that this region is critically important

2.1 | Residue D93 is critical for the binding of highly diverse Hsp90 inhibitors

Inhibitor-induced melting temperature (T_m) shifts of the Hsp90 NTD are frequently used as an indicator of inhibitor affinity.[17] Thermal shift assay (TSA) measurements were performed using a fluorescent dye that monitors the exposure of hydrophobic surface associated with unfolding of the Hsp90α NTD (Methods). Both inhibitors and ADP show concentration-dependent increases in T_m (Figure S2). To compare many different inhibitors we evaluated T_m shifts (ΔT_m) at a single concentration of 50 μM. Figure 2 shows that the D93N variant has severely impaired inhibitor binding properties, as indicated by the smaller ΔT_m for all inhibitors.

We next sought a method to determine the specific energetic contribution of the D93 position in a wild-type, full-length Hsp90 dimer under the biologically relevant conditions in which the inhibitor is competing against ATP under turnover conditions. As described next, a discovery from the Matulis group provides this opportunity.

The Matulis group previously discovered that radicicol binding to Hsp90α NTD is associated with a linked protonation event and speculated that protonation of the D93 carboxyl group is responsible.[18] We can reproduce the pH-dependent stabilization of the Hsp90α NTD by radicicol as measured by TSA (Figure 3) and observe similar pH-dependent stabilization for Grp94 and Trap1. Importantly, we observe that this pH-dependence is abolished for the D93N variant (Figure 3a) indicating that this residue is indeed the responsible group. A second resorcinol-based inhibitor, AUY922 also shows an analogous, albeit weaker, pH dependence. We later show this difference in pH dependence is related to the pK_a of the hydroxyl group which contacts D93. In contrast,