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Rational design of allosteric-inhibition sites in classical protein tyrosine phosphatases

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ABSTRACT

Protein tyrosine phosphatases (PTPs), which catalyze the dephosphorylation of phosphotyrosine in protein substrates, are critical regulators of metazoan cell signaling and have emerged as potential drug targets for a range of human diseases. Strategies for chemically targeting the function of individual PTPs selectively could serve to elucidate the signaling roles of these enzymes and would potentially expedite validation of the therapeutic promise of PTP inhibitors. Here we report a novel strategy for the design of non-natural allosteric-inhibition sites in PTPs; these sites, which can be introduced into target PTPs through protein engineering, serve to sensitize target PTPs to potent and selective inhibition by a biarsenical small molecule. Building on the recent discovery of a naturally occurring cryptic allosteric site in wild-type Src-homology-2 domain containing PTP (Shp2) that can be targeted by biarsenical compounds, we hypothesized that Shp2's unusual sensitivity to biarsenicals could be strengthened through rational design and that the Shp2-specific site could serve as a blueprint for the introduction of non-natural inhibitor sensitivity in other PTPs. Indeed, we show here that the strategic introduction of a cysteine residue at a position removed from the Shp2 active site can serve to increase the potency and selectivity of the interaction between Shp2's allosteric site and the biarsenical inhibitor. Moreover, we find that 'Shp2-like' allosteric sites can be installed de novo in PTP enzymes that do not possess naturally occurring sensitivity to biarsenical compounds. Using primary-sequence alignments to guide our enzyme engineering, we have successfully introduced allosteric-inhibition sites in four classical PTPs—PTP1B, PTPH-1, FAP-1, and HePTP—from four different PTP subfamilies, suggesting that our sensitization approach can likely be applied widely across the classical PTP family to generate biarsenical-responsive PTPs.

1. Introduction

Protein tyrosine phosphatases (PTPs) catalyze the dephosphorylation of phosphotyrosine residues in protein substrates.[1] Regulation of PTP activity is therefore essential to cellular signaling pathways, which hinge on tight control of myriad phosphate-mediated biochemical events. Indeed, a large—and growing—body of work implicates aberrant PTP activity, arising from mutations in PTP genes, in the pathogenesis of human diseases; these diseases range from metabolic disorders to neurodegeneration to cancer.[2,3] The emerging therapeutic relevance of PTPs has elicited intensive efforts on PTP-inhibitor-discovery from the pharmaceutical community.[4] Despite these and other efforts towards developing tools for deconvoluting PTP signaling, however, the precise biological roles of many individual PTPs have yet to be determined,[5] and the potential of PTPs as therapeutic targets has not, to date, been validated in the clinic. Furthermore, chemical approaches for the interrogation of PTP function are hindered by a shortage of selective, cell-permeable PTP inhibitors, as many of the challenges that generally beset inhibitor discovery are exacerbated in PTPs, owing to the high degree of structural similarity between classical-PTP active sites and the low cellular permeability of many active-site-directed PTP inhibitors.[4]

Small-molecule sensitization through protein engineering presents one putative remedy for the problem of chemically targeting members of a large and homologous protein family.[6,7] By introducing a molecular change into a target through protein engineering, novel and specific protein/small-molecule pairs can potentially be generated more efficiently than by the methods of conventional inhibitor design. For PTP inhibitors specifically, a unique inhibitor-sensitizing feature could, in principle, be introduced artificially into the catalytic domain of a PTP. The sensitized mutant would thus represent a distinctive pharmacological target that can be inhibited

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specifically in the presence of many wild-type PTPs inside a cell. Ideally, the mutant would retain wild-type-like activity in the absence of its small-molecule partner; thus, when expressed in cells in lieu of the wild-type PTP, the mutant would serve as a functional surrogate that can be chemically interrogated in a specific manner. Although the need to engineer a protein target of interest presents a significant limitation for chemical-biology studies (the sensitized mutant must be expressed in place of the wild-type enzyme in an engineered cellular system), inhibitor sensitization through protein engineering potentially presents two key advantages over standard medicinal chemistry. First, high target specificity can be achieved with relatively little small-molecule synthesis and/or screening. Second, if a conserved feature of a large enzyme family (such as the PTPs) is altered in the sensitization strategy, it is likely that the sensitizing mutation(s) can be applied widely across the protein family. In this scenario, the specific sensitizing mutations for different members of the protein family of interest may be easily determined from primary-sequence alignments, and a single inhibitor (or a small number of related compounds) could be used to selectively target many members of the protein family (in separate experiments, in which only a single target in a specifically engineered cell harbors the sensitizing mutation). Successful approaches for the introduction of non-natural inhibitor sensitivity have been described on a number of protein families;[6,8,9] particularly noteworthy examples include the 'bump-hole' sensitization of the protein kinases[10,11] and, more recently, BET bromodomains.[12]

Our lab has previously generated inhibitor-sensitive mutants of classical PTPs.[13–17] Specifically, we have shown that introduction of cysteine-rich motifs into a conserved and catalytically important loop of the PTP domain (the WPD loop) is sufficient to sensitize both non-transmembrane (NT) and receptor-like (R) PTPs to inhibition by the cell-permeable biarsenical compound FlAsH-EDT_2 (henceforth 'FlAsH' for brevity, Fig. 1A).[15–18] To sensitize a target PTP to FlAsH, two to four cysteine residues are placed in the WPD loop (either as point mutations or insertions); binding of FlAsH to these cysteines induces inhibition of phosphatase activity, presumably by altering the WPD loop's conformational switching between 'open' and 'closed' states.[19,20] Targeting engineered cysteine-enriched WPD loops with biarsenicals has proven to be a general approach for PTP inhibition.[16,21,22] However, the WPD loop constitutes part of the PTP active site (in its closed state) and plays a key role in the catalytic mechanism of PTPs, presenting the possibility that mutations on the loop itself could alter the inherent kinetic activity or substrate specificity of the enzyme. Moreover, the presence of catalytically essential residues on the WPD loop significantly constrains the options for its engineering in a manner, that is, functionally silent (e.g., the 'D' of WPD loop is a conserved aspartate residue that functions as a general acid–base in the PTP catalytic mechanism[23]).

An ideal inhibitor-sensitized enzyme is one in which the sensitized enzyme functions exactly like the wild-type in the absence of ligand; it follows that an ideal PTP-sensitization strategy may be one in which PTP activity is controlled *allosterically*, at a binding site well removed from the catalytic site. Engineering of allosteric sites, however, represents an exceedingly difficult protein-design challenge, and most inhibitor-sensitization strategies described to date utilize one or more space-creating mutations ('holes') in the protein target's active-site. It is difficult to imagine how allosteric-inhibition sites could be designed in PTP domains from first principles.

Recently, however, we serendipitously discovered a rare, naturally occurring PTP allosteric-inhibition site that potentially provides a blueprint for engineering allosteric-inhibitor sensitivity into PTP domains.[24] Specifically, we found that the oncogenic PTP Src-homology-2 domain containing PTP (Shp2) is unusual among classical PTPs in that its wild-type catalytic domain is sensitive to potent inhibition by FlAsH (and other biarsenicals), even in the absence of engineering.[24] Shp2's unusual biarsenical sensitivity derives from an allosteric site comprising two cysteine residues—C367, which is situated on PTP Motif 7 and is conserved across the classical PTP subfamily, and C333, which is situated on PTP Motif 4 and lies at a position occupied by proline in almost all other classical PTPs (Fig. 1B and C).[1] The Shp2-specific cysteine (C333) plays a determinative role in conferring Shp2's unusual sensitivity to biarsenicals, and when the residue is mutated to proline (C333P), Shp2's biarsenical sensitivity is abolished.[24]

The discovery of Shp2's allosteric site has significant ramifications from both pharmaceutical and inhibitor-sensitization perspectives. Because of the potential importance of Shp2 inhibitors in pharmaceutical development, the enzyme's unique allosteric site may present a novel target for Shp2-directed drug discovery. More germane to the work presented herein, the 'naturally sensitized' Shp2 catalytic domain raises important questions regarding the inhibitor sensitization of PTP domains through protein engineering. First, can the Shp2 allosteric site be engineered to optimize the potency and efficiency of its inhibition? Second, does the existence of Shp2's allosteric site suggest a strategy for engineering allosteric-inhibitor sensitivity into the catalytic domain of other PTPs? Here we report that the biarsenical sensitivity of the Shp2-specific allosteric site can be optimized through rational design. Moreover, we show that the optimized Shp2 allosteric site presents a template for designing allosteric-inhibition sites in other classical PTPs that are not sensitive to biarsenicals, including PTPs for which no allosteric inhibitors are known. The PTP-engineering strategy we report here could thus

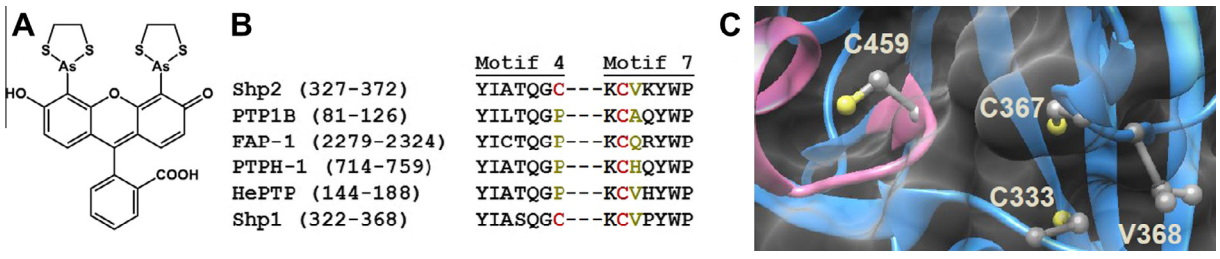

Figure 1. Design of classical PTP mutants that possess unnatural sensitivity to biarsenical compounds. (A) Chemical structure of the biarsenical compound FlAsH. (B) Partial amino-acid sequence alignment of the human PTPs discussed in this study, showing only structural motifs 4 and 7 of the PTP domain (as assigned by Andersen et al.[1]). Highlighted in red are Shp2's C333 and C367 and their cysteine counterparts in other PTPs. Highlighted in dark yellow are the amino acid residues substituted with cysteine in the present study. (The PTP-domain primary-sequence numbering varies widely due to the diversity in protein size and structure of the PTP family outside of the conserved PTP domain.[42]) (C) Three-dimensional structure of Shp2's catalytic domain (PDB ID: 3B7O).[30] Shp2 is shown as a blue ribbon, with the conserved active-site motif highlighted in pink and featuring the catalytic cysteine C459. The side chains of C333, C367, V368, and C459 are colored by atom type. The enzyme's surface is rendered transparently so that the buried residues C333 and C367 can be visualized.

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