Crystal Structure of the Yersinia Protein-tyrosine Phosphatase YopH Complexed with a Specific Small Molecule Inhibitor*

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The pathogenic bacteria Yersinia are causative agents in human diseases ranging from gastrointestinal syndromes to bubonic plague. There is increasing risk of misuse of infectious agents, such as Yersinia pestis, as weapons of terror as well as instruments of warfare for mass destruction. Because the phosphatase activity of the Yersinia protein tyrosine phosphatase, YopH, is essential for virulence in the Yersinia pathogen, potent and selective YopH inhibitors are expected to serve as novel anti-terror agents. We have identified a specific YopH small molecule inhibitor, p-nitroacetate sulfate (pNCS), which exhibits a Ki value of 25 μM for YopH and displays a 13–60-fold selectivity in favor of YopH against a panel of mammalian PTPs. To facilitate the understanding of the underlying molecular basis for tight binding and specificity, we have determined the crystal structure of YopH in complex with pNCS at a 2.0-Å resolution. The structural data are corroborated by results from kinetic analyses of the interactions of YopH and its site-directed mutants with pNCS. The results show that while the interactions of the sulfonyl moiety and the phenyl ring with the YopH active site contribute to pNCS binding affinity, additional interactions of the hydroxyl and nitro groups in pNCS with Asp-356, Gln-357, Arg-404, and Gln-446 are responsible for the increased potency and selectivity. In particular, we note that residues Arg-404, Gln-290, Asp-356, and a bound water (WAT185) participate in a unique H-bonding network with the hydroxyl group ortho to the sulfonyl moiety, which may be exploited to design more potent and specific YopH inhibitors.

Protein tyrosine phosphatases (PTPs) are involved in the regulation of numerous cell functions including growth, differentiation, motility, cell-cell interactions, metabolism, gene transcription, and the immune response (1, 2). In vivo, tyrosine phosphorylation is a reversible and dynamic process. The phosphorylation states of proteins are governed by the opposing actions of protein tyrosine kinases, which catalyze protein tyrosine phosphorylation, and PTPs, which are responsible for dephosphorylation. Hundreds of protein kinases and protein phosphatases and their substrates are integrated within an elaborate signal-transducing network. The defective or inappropriate operation of this network is at the root of such widespread diseases including cancers and diabetes.

The importance of PTP regulation in cellular function was highlighted by the identification of a PTP in the pathogenic bacteria Yersinia (3). The genus Yersinia comprises three species of bacteria that are causative agents in human diseases ranging from gastrointestinal syndromes to bubonic plague (4). Yersinia pestis is the pathogen responsible for the bubonic plague, also known as the Black Death, because it reduced the population of Europe by ~25 million in the 15th century (4). Credible estimates of the number of people killed by this bacterium during the course of human history approach 200 million (5). Although plague has long been considered a “vanquished” disease, the recent outbreak of the pneumonic plague caused by Y. pestis in Surat, India (6) proves that its biological potential can be expressed under appropriate environmental conditions. Despite efforts to eradicate the disease, it has recently been recognized by the World Health Organization as a re-emerging public health concern. In addition, there is increasing risk of misuse of infectious agents, such as Y. pestis, as weapons of terror as well as instruments of warfare for mass destruction (7). Thus, there is an urgent need to devise effective protective strategies that could be implemented soon after a bioterrorist attack.

Pathogenic Yersinia contain a plasmid that encodes several of the bacterial virulence factors known as Yops (Yersinia outer membrane proteins) (8). The expression of Yops is correlated with the capacity of the bacterium to avoid host defense mechanisms (9). Surprisingly, one of the virulence factors, YopH, is homologous to eukaryotic PTPs with potent tyrosine phosphatase activity (3). In fact, YopH is the most active PTP characterized to date (10). The yopH gene is obligatory for pathogenesis as plasmids that have a nonfunctional yopH gene are avirulent (11–13). Moreover, conversion of the essential cysteine residue to alanine in YopH abolishes the PTP activity in Yersinia pseudotuberculosis and eliminates the virulence of the bacterium in a murine infection model (14, 15). These observations suggest that the Yersinia PTP activity is essential for the bacterial pathogenicity.

Bacteria do not contain tyrosine-phosphorylated proteins (16). The fact that YopH possesses PTP activity and is essential for pathogenicity suggests that it mediates a novel mechanism of bacterial pathogenesis. YopH may act on target pTyr-containing proteins within infected eukaryotic cells to disrupt the normal signaling pathways and to disarm the immune responses. Indeed, production of YopH during Yersinia infection
is associated with dephosphorylation of proteins in human epithelial cells and murine macrophages (14, 15, 17, 18). Two of the major cellular targets of the Yersinia PTP have been identified as the focal adhesion kinase and the focal adhesion protein p130Cas (19, 20). Because YopH is known to cause inhibition of phagocytic uptake of Yersinia and oxidative burst by cultured macrophages (18, 21, 22), the YopH-mediated dephosphorylation of focal adhesion kinase and p130Cas or related proteins is probably of importance for the ability of the Yersinia to escape the host immune response.

Because the PTP activity of YopH is essential for the virulence of Yersinia, specific inhibitors targeted to the Yersinia PTP are expected to render the bacteria avirulent. Thus, potent and specific inhibitors for the Yersinia PTP could serve as effective agents to block the spread and proliferation of Y. pestis infection. In this paper, we describe the identification of a specific small molecule YopH inhibitor, p-nitroacetohal sulfate (pNCS). We present the crystal structure of YopH in complex with pNCS solved to a 2.0-Å resolution. Finally, we report the binding interactions between YopH and pNCS probed with a combination of site-directed mutagenesis and kinetic studies. Together, our results reveal the structural basis of pNCS selectivity for YopH and lay the groundwork for the design of more potent and specific YopH inhibitors that may serve as powerful anti-plague agents.

**EXPERIMENTAL PROCEDURES**

**Materials**—p-Nitroacetohal sulfate (2-hydroxy-5-nitrophenyl sulfate) was purchased from Sigma. p-Nitrophenyl phosphate (pNPP) was purchased from Fluke Co. All of the other chemicals and reagents were of the highest grade available commercially.

**Protein Purification**—The catalytic domain (residues 163–468) of YopH was expressed and purified as described previously (10). The protein was further purified using hydrophobic chromatography. The catalytic domain of CD45 and LAR (containing both D1 and D2) was expressed and purified as a recombinant glutathione S-transferase fusion protein. Other recombinant PTPs, PTP1B, VHR, T cell PTP, SHP1, and the N-terminal His6-tagged HePTP were expressed in Escherichia coli and purified as described previously (23, 24). The expression and purification of catalytic domain of Cdc42 will be published elsewhere.

All of the mutants of YopH were expressed and purified using a procedure similar to the wild type enzyme.

**Site-directed Mutagenesis**—All of the mutant forms of YopH were generated using the QuikChange kit from Stratagene. The oligonucleotide primers used for mutagenesis were from Sigma. Because the PTP activity of YopH is essential for the virulence of Yersinia, specific inhibitors targeted to the Yersinia PTP are expected to render the bacteria avirulent. Thus, potent and specific inhibitors for the Yersinia PTP could serve as effective agents to block the spread and proliferation of Y. pestis infection. In this paper, we describe the identification of a specific small molecule YopH inhibitor, p-nitroacetohal sulfate (pNCS). We present the crystal structure of YopH in complex with pNCS solved to a 2.0-Å resolution. Finally, we report the binding interactions between YopH and pNCS probed with a combination of site-directed mutagenesis and kinetic studies. Together, our results reveal the structural basis of pNCS selectivity for YopH and lay the groundwork for the design of more potent and specific YopH inhibitors that may serve as powerful anti-plague agents.

**Inhibition Studies**—Inhibition constants were calculated using the Lineweaver-Burk double-reciprocal plot by fitting program KINETASYST to the appropriate equations. The following buffered solution was used for activity measurements: 0.1 M Hepes, pH 7, containing 0.1 mM EDTA, and the ionic strength of 0.15 M adjusted by the addition of sodium chloride. The reaction (300 μl) was quenched after 2–3 min by the addition of the phosphatase followed by the production of inorganic phosphate. The reaction (300 μl) was quenched after 2–3 min by the addition of the phosphatase followed by the production of inorganic phosphate. The reaction (300 μl) was quenched after 2–3 min by the addition of the phosphatase followed by the production of inorganic phosphate. The reaction (300 μl) was quenched after 2–3 min by the addition of the phosphatase followed by the production of inorganic phosphate. The reaction (300 μl) was quenched after 2–3 min by the addition of the phosphatase followed by the production of inorganic phosphate.
YopH and PTP1B-catalyzed hydrolysis of
esters (Table II). The ability of these compounds to inhibit the
screened several aryl phosphonate, sulfonate, and sulfate esters of
the mammalian PTPs with similar potency. As an initial effort to
order water molecules.

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The final model comprises 279 YopH residues, 1 NCS molecule, and 219 ordered water molecules.

RESULTS AND DISCUSSION

Identification of p-Nitrocatechol Sulfate as a Specific Inhibitor for YopH—Structural and kinetic studies suggest that pTyr plays a major role in substrate binding by the PTPs, including YopH (25, 30–33). Consequently, major efforts have been made to develop nonhydrolyzable pTyr surrogates that contain both a phosphate mimic that substitutes the phospho-
yl group and an aromatic scaffold that can occupy the active site pocket in a manner reminiscent of the benzene ring in
pTyr. A variety of nonhydrolyzable pTyr surrogates have been reported for the PTPs (34, 35). Because of the conserved nature of the pTyr-binding pocket, these nonhydrolyzable pTyr surro-
gates in general do not display any selectivity against different PTPs. However, inhibitor potency and selectivity can be achieved by incorporation of an appropriate structural element to the nonhydrolyzable pTyr mimetic to target both the active site and a unique adjacent site (2).

Because the YopH catalytic domain shares only 20–30% sequence identity with the mammalian PTPs (36), it may seem quite feasible to acquire YopH specific inhibitors. Previous reported YopH inhibitors include suramin (37) and \( \alpha \)-ketocarboxylic acids (38). Unfortunately, these compounds also inhibit mammalian PTPs with similar potency. As an initial effort to search for selective small molecule inhibitors for YopH, we screened several aryl phosphonate, sulfonate, and sulfate esters (Table II). The ability of these compounds to inhibit the YopH and PTP1B-catalyzed hydrolysis of pNPP was assessed at pH 7 and 25 °C (for details see “Experimental Procedures”). As shown previously, sulfate monoesters were not hydrolyzed by the PTPs but instead inhibited the PTP reaction reversibly and competitively with respect to the substrate (Fig. 1). Simi-
larly, the aryl phosphonate and sulfonate also inhibited the
PTPs reversibly and competitively. Given the structural simi-
larity of aryl sulfate/sulfonate/phosphonate moieties to pTyr, the competitive inhibition pattern observed for these com-
ounds was expected. As shown in Table II, the \( K_i \) values for benzyl phosphonate, \( p \)-nitrophenyl sulfate, 2,4,6-nitrophenyl sulfate, and sulfosalicylic acid are in the millimolar range, similar to the \( K_m \) values for pNPP and pTyr (10, 31). Moreover, they failed to distinguish between YopH and PTP1B. This is perhaps not surprising because the majority of the invariant residues conserved in all of the PTPs from bacteria to mammals (36) is located in and around the enzyme active site (27, 32). The lower affinity for 2,4,6-nitrophenyl sulfate may simply be caused by steric hindrance at the ortho positions. Remarkably, \( p \)NCS competitively inhibited YopH with a \( K_i \) of 25 \( \mu \)M, which ranks \( p \)NCS among the most potent pTyr mimetics identified to date. Furthermore, the \( K_i \) values of \( p \)NCS toward YopH and PTP1B differ dramatically (25 \( \mu \)M for YopH and 1.2 mM for PTP1B).

To examine the specificity of \( p \)NCS for YopH, its inhibitory activity toward a panel of PTPs including cytosolic PTPs, PTP1B, \( T \) cell PTP, HePTP, and SHP1, the receptor-like PTPs, CD45 and LAR, and the dual specificity phosphatases, Cdc14 and VHR, was determined. As shown in Table III, with the exception of Cdc14, \( p \)NCS acts as a competitive inhibitor for every PTP examined. In addition, \( p \)NCS is highly selective for YopH, exhibiting a 13–80-fold preference for YopH relative to all of the phosphatases examined. We recently showed that suramin, a molecule containing multiple aryl sulfonate moieties, is a competitive and reversible PTP inhibitor with \( K_i \) values in the low micromolar range (37). Unfortunately, sura-

![Graph](image)

**Table II**

| Compounds | \( K_i \) (\( \mu \)M) |
|-----------|------------------|
| YopH | PTP1B |
| benzyl phosphonate | 5,000 ± 300 | 3,000 ± 200 |
| \( p \)-nitrophenyl sulfate | 2,500 ± 150 | 10,000 ± 800 |
| 2,4,6-nitrophenyl sulfate | 25 ± 0.7 | 1,260 ± 120 |
| sulfosalicylic acid | 14,000 ± 1,500 | 24,000 ± 2,000 |
| sulfite monoester | 6,500 ± 400 | 21,000 ± 1,200 |

All measurements were performed at pH 7 and 25 °C using pNPP as a substrate.

**Table III**

| PTPs | \( K_i \) (\( \mu \)M) | Mode of inhibition |
|------|------------------|------------------|
| YopH | 25 ± 0.7 | Competitive |
| SHP1 | 750 ± 56 | Competitive |
| HePTP | 1,500 ± 54 | Competitive |
| PTP1B | 1,200 ± 120 | Competitive |
| TCPTP | 330 ± 42 | Competitive |
| CD45 | 500 ± 68 | Competitive |
| LAR | 830 ± 270 | Competitive |
| VHR | 350 ± 49 | Competitive |
| Cdc14 | 1,400 ± 60 | Uncompetitive |

All measurements were performed at pH 7 and 25 °C using pNPP as a substrate.
min binds YopH and PTP1B with equal affinity. Thus, \( pNCS \) is the most specific YopH inhibitor reported to date. Considering its relatively modest structural framework, the inhibitory efficacy and specificity of \( pNCS \) for YopH is unprecedented. To determine the molecular basis for the selectivity of \( pNCS \) for YopH, we have crystallized YopH with \( pNCS \) and solved the three-dimensional structure of the complex.

Structure of YopH\( pNCS \) Complex—Using conditions similar to those described previously (10, 27), we produced high quality crystals of the YopH\( pNCS \) complex that diffracted to a 2.0-Å resolution. The structure was solved by molecular replacement and was refined to an \( R \) factor of 22.6% (\( R_{\text{free}} = 24.3\% \)) for data from 30 to 2.0 Å (Table I). The final model for the YopH\( pNCS \) complex included YopH residues 186–468 and all of the atoms in \( pNCS \). \( pNCS \) was unambiguously fitted to well defined electron density (Fig. 2A). Consistent with the competitive inhibition pattern, \( pNCS \) occupies the YopH active site (Fig. 2B). The overall structure of YopH in the complex is quite similar to that observed in the complex between YopH and \( \text{WO}_4^{2-} \) (27). As observed in the YopH\( \text{WO}_4^{2-} \) complex, the WPD loop (residues 351–359) adopts a closed conformation in the YopH\( pNCS \) structure. The sulfate group in \( pNCS \) assumes the same position in the active site of YopH taken by tungstate in the YopH\( \text{WO}_4^{2-} \) complex. Consequently, the phosphate-binding loop (P-loop, residues 403–410) in YopH\( pNCS \) is superimposable to that in the YopH\( \text{WO}_4^{2-} \) complex (Fig. 3). In the YopH\( pNCS \) structure, the terminal nonbridge sulfate oxygens of \( pNCS \) form an extensive array of hydrogen bonds with the main-chain nitrogens of the P-loop and the guanidinium side chain of Arg-409 similar to that observed in the YopH\( \text{WO}_4^{2-} \) complex (Fig. 4A).

Although several x-ray crystal structures are available for the unliganded and oxyanion-bounded forms of YopH (27, 39, 40), our structure represents the first in which YopH is bound with a pTyr mimic. Aside from the similarities between the structures of YopH\( pNCS \) and YopH\( \text{WO}_4^{2-} \), there are also noticeable differences. For example, although the WPD loop in YopH\( pNCS \) is in the closed conformation, the positions of Asp-356 and Gln-357 are shifted away from the active site Cys-403 by 0.5 Å and move toward Glu-290 in comparison with the YopH\( \text{WO}_4^{2-} \) complex (Fig. 3). In addition, compared with YopH\( \text{WO}_4^{2-} \), the \( \gamma_4 \) of Arg-404 in the P-loop rotates 79° toward helix \( \alpha 3 \) (residues 292–294) due to the presence of the phenyl ring in \( pNCS \) (Fig. 3). Finally, the phenolic oxygen of the inhibitor is rotated 16° toward the WPD loop and the side chain of Arg-404 compared with the apical oxygen of the \( \text{WO}_4^{2-} \) (Fig. 3). These structural changes are necessary for YopH to engage in several specific interactions with the inhibitor as detailed below.

The remarkable potency and selectivity of \( pNCS \) for YopH are the results of numerous specific interactions. Thus, in addition to the polar interactions between the terminal sulfate oxygen atoms and the backbone amides of the P-loop and the side chain of Arg-409, there are several specific H-bonds between YopH and \( pNCS \) (Fig. 4A). For example, Asp-356 forms two hydrogen bonds to the hydroxyl oxygen in \( pNCS \). The hydroxyl oxygen also makes a polar interaction with the Ne of Arg-404. The Ne of Arg-404 side chain also participates in a water (WAT185)-mediated H-bond network with the carboxyl...
groups of Asp-356 and Glu-290. This water-mediated H-bond network may be important in stabilizing the interactions between Asp-356 and the hydroxyl group in pNCS. Furthermore, the phenolic oxygen is hydrogen-bonded to the carboxyl group of Asp-356, the Ne of Gln-357, and a bound water molecule (WAT74), which is coordinated by three H-bonds from the Oe of Gln-446, Ne of Gln-450, and one of the terminal oxygens from the sulfate (Fig. 4A). The distance between Asp-356 and the phenolic oxygen is consistent with its role as a general acid in PTP catalysis (36). WAT74 is conserved and has been observed in a number of PTP inhibitor/substrate complexes (27, 32, 41). Finally, due to the presence of the phenyl ring in pNCS, the side chain of Gln-446 is unable to form a H-bond with the phenolic oxygen as observed with the equivalent apical tungstate oxygen in the YopH-WO$_4^{2-}$ structure. Instead, the Ne of Gln-446 is engaged in a H-bond with one of the oxygens in the nitro group of pNCS. The observed interactions of YopH with the OH and NO$_2$ groups in pNCS are consistent with the fact that pNCS is a better inhibitor than benzyl phosphonate and p-nitrophenyl sulfate.

Fig. 4. Interactions between pNCS and YopH. A, H-bonding and polar interactions with a cutoff distance of 3.2 Å for hydrogen bonds and 4.0 Å for electrostatic or polar interactions. B, hydrophobic interactions with a cutoff distance of 4.5 Å.
In addition to the polar interactions, there are also van der Waals contacts between YopH and the inhibitor. The phenyl ring of the pNCS is engaged in extensive hydrophobic interactions with the active site cavity flanked by Arg-404, Phe-229, and Ile-232 at one side and Asp-356, Gln-357, Ala-405, Val-407, and Gln-446 at the other (Figs. 2B and 4B). Phe-229 assumes the same position as Tyr-46 in PTP1B, forming stacking interactions with the phenyl ring of the inhibitor. A hydrogen bond is observed between the OH group of Tyr-46 and the side chain of Arg-404, Phe-229, and Gln-446 at the other (Figs. 2B and 4B). This enables the aliphatic portion of the side chain to make several van der Waals contacts with the active site cavity flanked by Arg-404, Phe-229, and Gln-446 (Fig. 5). Ser-216 is invariant among almost all of the mammalian PTPs. Interestingly, in YopH, this residue is Arg-404 (Fig. 5). As mentioned above, in the structure of YopH-pNCS, Arg-404 is found in a conformation that is distinct from those observed in the apo- or oxoanion-bound YopH structures (27, 39, 40). This enables the aliphatic portion of the side chain of Arg-404 to make several van der Waals contacts with the oxoanion binding site residues (Val and Ile are interchangeable at position 407). In conclusion, most of the interactions between the side chain of Arg-404 and the phenyl ring of pNCS are less extensive than those between Phe-229 and pTyr (Fig. 4B). However, as shown in Fig. 4A, the side chain of Gln-357 does have the capacity to form an H-bond with the phenolic oxygen in pNCS. Residues Asp-356, Ala-405, Val-407, and Gln-446 make similar van der Waals contacts with the phenyl ring as those observed for the corresponding residues (Asp-181, Ala-217, Ile-219, and Gln-262) in PTP1B.

**Mutational Analysis of the Interactions between YopH and pNCS**—To further investigate the structural basis for the potency of pNCS and specificity for YopH, we selectively altered several amino acids in YopH that are shown to be involved in pNCS binding and evaluated the effect on YopH phosphatase activity and inhibitor binding affinity. The wild type and mutant YopHs were expressed in E. coli and purified to homogeneity as described previously (10). All of the kinetic measurements were performed at 25 °C in pH 7.0, 50 mM 3,3-dimethylglutarate buffer containing 1 mM EDTA and an ionic strength of 0.15 M. Table IV summarizes the kinetic parameters (k_{cat} and K_{m}) for pNPP hydrolysis and the K_{i} values for arsenate observed for the wild type and YopH mutants. With only a few exceptions, the K_{i} values for the YopH mutants are not significantly different from that of the wild type enzyme, suggesting no structural perturbations in the active site by the mutations. We have shown previously that the rate-limiting step for the YopH-catalyzed reaction is largely determined by the hydrolysis of the phosphoenzyme intermediate (42, 43). Thus, the moderate decreases in k_{cat} for Q357H, Q357F, Q357A, and Q446A are consistent with the removal of a hydrogen bond with the attacking water nucleophile in a dissociative transition state (44). Large changes in kinetic parameters are expected for D356N and D356A, because Asp-356 performs general acid/base functions in PTP catalysis (44). Because Phe-229 is involved in hydrophobic stacking with the phenyl ring, drastic alteration of the side chain may affect the precise positioning of active site residues important for catalysis (45). Interestingly, mutations at Asp-356 and Phe-229 do not affect the binding affinity for arsenate, suggesting that these two residues may not play an important role in oxoanion binding. When Arg-404 was replaced with the corresponding Ser residue in mammalian PTPs (Fig. 5), a 7-fold decrease in k_{cat} and a 6-fold increase in K_{i} for arsenate were observed. In addition, the affinity of R404S for phosphate was also decreased by 3-fold. Because Arg-404 constitutes part of the P-loop, it is possible that substitution at position 404 may perturb the interactions between the phosphoryl moiety and the P-loop.

An Ile is often found at the position taken by Val-407 in many mammalian PTPs. Replacement of Val-407 by an Ile causes little change in the kinetic parameters for pNPP hydrolysis and K_{i} values for arsenate and pNCS. This finding suggests that Val and Ile are interchangeable at position 407. In contrast, deletion of the side chain from Gln-446 reduces the affinity of YopH for pNCS by 4-fold, consistent with an H-bond between Gln-446 and the nitro group in the inhibitor. In accordance with the π–π interaction between the side chain of Phe-229 and the phenyl ring of pNCS, removal of the side chain from Phe-229 decreases the affinity of YopH for pNCS by 14-fold. Collectively, these results are in agreement with the structural data, which indicate direct interactions of Phe-229 and Gln-446 with pNCS. The lack of mutational effects on arsenate binding further supports that the observed effects on pNCS binding are unlikely due to nonspecific structural alterations.

Although both Phe-229 and Gln-446 contribute to the binding affinity of pNCS for YopH, they are not likely responsible for the selectivity of pNCS toward YopH because they are highly conserved among the PTPs. Since Gln-357 and Arg-404 are not conserved among PTPs (Fig. 5), we evaluated the possibility that these two residues could serve as determinants for the specificity of YopH for pNCS. Removal of the side chain at position 357 (YopH/Q357A) increases the K_{i} for pNCS by 4.8-fold. This finding is consistent with the observed interactions between Gln-357 and pNCS (Fig. 4). Interestingly, the replacement of Gln-357 by a His or Phe, residues commonly found at

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**TABLE IV**

| Kinetic parameters of pNPP hydrolysis and K_{i} values for arsenate and pNCS for the wild-type and mutant YopHs. |

| YopH | k_{cat} | K_{m} | K_{i} for arsenate | K_{i} for pNCS |
|------|--------|------|------------------|---------------|
| Wild type | 39 ± 1.3 | 1.7 ± 0.1 | 0.94 ± 0.09 | 25 ± 0.7 |
| R404S | 5.2 ± 0.42 | 11 ± 1.6 | 5.9 ± 1.0 | 680 ± 120 |
| Q357H | 22 ± 2.1 | 1.9 ± 0.21 | 2.6 ± 0.26 | 68 ± 12 |
| Q357F | 18 ± 2.5 | 0.91 ± 0.09 | 3.4 ± 0.35 | 5.2 ± 0.47 |
| Q357A | 21 ± 1.2 | 1.8 ± 0.16 | 1.3 ± 0.08 | 120 ± 6.4 |
| P229A | 18 ± 1.5 | 10 ± 1.6 | 1.6 ± 0.08 | 350 ± 38 |
| V407I | 29 ± 2.3 | 1.6 ± 0.14 | 1.5 ± 0.11 | 37 ± 4.7 |
| Q446A | 27 ± 1.8 | 1.3 ± 0.15 | 0.74 ± 0.06 | 96 ± 11 |
| D356N | 1.5 ± 0.02 | 2.5 ± 0.2 | 1.4 ± 0.2 | 39 ± 4.0 |
| D356A | 0.12 ± 0.07 | 0.17 ± 0.03 | 0.49 ± 0.055 | 86 ± 30 |
position 357 in mammalian PTPs, results in a 2.7-fold decrease and a 4.8-fold increase in pNCS binding affinity, respectively (Table IV). These results may be explained by the increased hydrophobic interactions between pNCS and the aromatic side chain of Phe and, perhaps to a smaller extent, the side chain of His. The lower affinity of YopH/Q357H suggests that a His at position 357 may not be sufficient to offset the lost interactions between the inhibitor and the side chain of Gln-357. This result suggests that structural difference at position 357 may be at least partially responsible for the selectivity of pNCS against PTPs, bearing a His at this position. However, Gln-357 is not likely responsible for selectivity of pNCS against PTPs, bearing a Phe (e.g. PTP1B and T cell PTP) at this position since a nearly 5-fold increase in pNCS binding affinity was observed for YopH/Q357P.

Determinants for Specific Binding of pNCS to YopH—The residue that is most likely responsible for the observed potency and specificity of pNCS is Arg-404. YopH is the only PTP that contains an Arg at position 404 in the P-loop (Fig. 5). As shown in Fig. 4, the side chain of Arg-404 makes three van der Waals contacts with the carbon atom to which the hydroxyl group is attached and a long polar interaction with the hydroxyl group in pNCS. In addition, the guanidinium group of Arg-404 also participates in a unique H-bonding network involving residues Glu-290, Arg-409, Asp-356, and WAT185 (Fig. 4A). This network is essential for positioning the side chain of Asp-356 to engage in two H-bonds with the hydroxyl group and a third with the phenolic oxygen in pNCS. Understandably, the conversion of Arg-404 to a Ser, which is present in all of the mammalian PTPs, reduced the affinity of YopH for pNCS by 27-fold (Table IV), possibly because of the disruption of this H-bond network. To directly probe the role of Asp-356 in binding, we changed it to either an Asn or an Ala. YopH/D356N exhibits a binding affinity for pNCS similar to that of the wild type enzyme, indicating that an Asn can effectively substitute for Asp-356 and retain the H-bonding interactions with the inhibitor. However, removal of the side chain from Asp-356 (YopH/D356A) reduces the affinity of YopH for pNCS by only 3.4-fold (Table IV). This modest effect may be an underestimate of the contribution of Asp-356 to pNCS binding because mutation of the general acid Asp to an Ala is known to cause an increase in the binding affinity (5-fold) for pTyr-containing substrates and inhibitors including suramin (37, 46, 47). Consequently, the observed 3.4-fold decrease in pNCS binding affinity by YopH/D356A supports direct involvement of Asp-356 in pNCS binding.

Although Asp-356, Glu-290, and Arg-409 are invariant among all of the PTPs, Arg-404 and its interactions with these residues and pNCS are unique to YopH. This observation together with the large decrease in binding affinity when Arg-404 is replaced with a Ser strongly suggests that the major determinant for both potency and selectivity of pNCS for YopH is Arg-404. Although we only studied the first sphere residues that are in direct contact with the inhibitor, it is likely that second sphere residues may also exert significant influence on ligand binding potency and selectivity. This is highlighted by the finding that pNCS displays a 4-fold selectivity for T cell PTP against PTP1B, even though the catalytic domains of the two PTPs share greater than 75% sequence identity and have a virtually identical pTyr binding pocket (48).

Conclusions and Implications for YopH Inhibitor Design—The YopH phosphatase activity is essential for virulence in the Yersinia pathogen, which causes diseases such as the plague. Thus, potent and selective YopH inhibitors are expected to serve as novel anti-plague agents. Previously, it was suggested that the common active site structure for the PTPs would make it difficult to distinguish friend from foe when designing drugs against YopH (49). We have identified pNCS as a specific small molecule YopH inhibitor that exhibits a Ki value in the low micromolar range for YopH and displays a 13–60-fold selectivity in favor of YopH against a panel of mammalian PTPs. Given the fact that most nonhydrolysable pTyr surrogates exhibit low affinity and little selectivity toward PTPs, the observed potency and selectivity of pNCS toward YopH is striking. To reveal the binding interactions, we have determined the crystal structure of YopH in complex with pNCS. This structure together with results from kinetic analyses of the interactions of YopH and its site-directed mutants with the inhibitor provides a molecular explanation of the extraordinary potency and selectivity of pNCS.

The results described here show that while the interactions of the sulfuryl moiety and the phenyl ring with the YopH active site are important for binding affinity, additional interactions of the hydroxyl and nitro groups in pNCS with Asp-356, Gln-357, Arg-404, and Gln-446 are responsible for the increased potency and selectivity. The structure of YopH/pNCS should serve as an excellent starting point for future YopH inhibitor design. In particular, we note that residues Arg-404, Glu-290, and a bound WAT185 are in a unique H-bonding network with the hydroxyl group ortho to the sulfuryl moiety, which may be exploited to enhance potency and selectivity for YopH. Furthermore, we suggest based on our success in acquiring potent and selective PTP1B inhibitors (29, 41) that YopH inhibitors with even higher potency and selectivity can be devised by attacking pNCS or its close analogs to an appropriate structural scaffold to allow simultaneous engagement of both the active site and a unique adjacent peripheral site (2). The creation of novel reagents that can selectively target the essential virulence factor YopH will be an enormously important advance in our ability to prevent and control the spread of Y. pestis infection after a bioterrorist event.

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