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Inhibition of the Lymphoid Tyrosine Phosphatase: The Effect of Zinc(II) ions and Chelating Ligand Fragments on Enzymatic Activity

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Abstract

A 96-member chelator fragment library (CFL-1.1) was screened to identify inhibitors of the lymphoid tyrosine phosphatase in the absence and presence of zinc acetate. Fragments that inhibit LYP activity more potently in the presence of zinc, fragments that rescue LYP activity in the presence of inhibitory concentrations of zinc, and fragments that inhibit LYP activity independent of zinc concentration were identified. Of these, 1,2-dihydroxynaphthalene was the most potent inhibitor with an IC_{50} value of $2.52 \pm 0.06 \mu M$ after 2 h of incubation. LYP inhibition by 1,2-dihydroxynaphthalene was very similar to inhibition by 1,2-naphthoquinone ($IC_{50} = 1.10 \pm 0.03$), indicating that the oxidized quinone species is likely the active inhibitor. The inhibition was time-dependent, consistent with covalent modification of the enzyme.

The protein tyrosine phosphatases (PTPs) are a family of signaling enzymes that play critical roles in human health. For example, aberrant PTP activity has been implicated in diseases as diverse as cancer, metabolic disorders and autoimmunity.^{1–3} Reliant on a nucleophilic cysteine residue for activity, the PTPs are also susceptible to inhibition by heavy metals⁴ and oxidation.^{5, 6} Because the PTPs play such instrumental roles in human biology, there is great interest in developing inhibitors that could serve as chemical probes for dissecting the biological roles of the PTPs as well as potential lead compounds for therapeutic development.^{2, 7, 8}

One PTP of particular therapeutic interest is the lymphoid tyrosine phosphatase (LYP).⁹ LYP serves as a negative regulator of early T cell receptor signaling and has been implicated in the development of autoimmunity.^{10–12} Based on the known susceptibility of LYP to metal ions,⁴ oxidizing agents^{5, 6} and phosphotyrosine mimetic compounds such as salicylic acids,^{13–16} we decided to undertake a small-scale, fragment-based screen to identify metal

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binding fragments that inhibit LYP activity either alone or in complex with metal ions. The chelator fragment library used in this work, CFL-1.1 (Figure 1), incorporates a variety of metal-binding motifs in a total of 96 fragments.¹⁷ Included in this library are phosphotyrosine mimetic moieties such as salicylic acids and picolinic acids and redox active fragments including catechols.

Initial investigations into the effect of zinc(II) on LYP activity under our standard assay conditions demonstrated that zinc is an effective inhibitor of LYP, with complete inhibition achieved in the presence of 100 μ M zinc(II) acetate. This is not surprising, as thiophilic metal ions have been shown to act as competitive, pseudo-irreversible inhibitors of PTP activity, interacting with the catalytic cysteine residue.^{4, 18–20} As shown in Figure 2, in the presence of 40 μ M of zinc acetate, the LYP activity was reduced to 20% of the control, facilitating the identification of chelators that may rescue zinc-mediated enzyme inhibition by binding to and removing the zinc from the enzyme active site. At 5 μ M zinc acetate, the activity of LYP was reduced to 80% of the control, providing a useful starting point from which to identify chelators that may act synergistically with zinc to inhibit LYP activity. Using the information from the initial dose-response data with zinc acetate, three separate screens of the fragment library CFL-1.1 were carried out: (1) in the presence of 40 μ M zinc acetate to identify chelators capable of removing zinc from the active site of LYP and rescuing the enzyme from zinc-mediated inhibition, (2) in the presence of 5 μ M zinc acetate in order to identify compounds that display enhanced inhibition in the presence of zinc and (3) in the absence of zinc in order to identify fragments capable of inhibiting LYP activity on their own.

As indicated in Figure 3, di-(2-picolyl)-amine (**3g**), 5-chloro-8-quinolol (**12b**) and 2,6-pyridine dicarboxylic acid (**8a**) had little effect on enzymatic activity alone, but were each capable of rescuing the enzyme from zinc-mediated inhibition. These compounds are all known zinc chelators, and their ability to restore enzyme activity in the presence of zinc is consistent with hypothesis that they may sequester zinc, removing it from the enzyme active site. The observation that the chelators are able to activate LYP slightly in the absence of added zinc is consistent with the sensitivity of the enzyme to inhibition by adventitious metal. Indeed, tyrosine phosphatase assays are usually carried out in a buffer containing EDTA to avoid this problem.²¹ It appears that, under the conditions of this assay, approximately two equivalents of each chelator (relative to zinc) are required to restore full activity.

A handful of compounds showed the potential for chelator-enhanced inhibition in the presence of 5 μ M zinc acetate in our initial screen. Of these, only compound **5g** (2-hydroxy-1,4-naphthoquinone) showed consistent concentration-dependent inhibition of LYP in the presence of zinc (Figure 4) in follow-up studies. The interpretation of these results is ambiguous because 2-hydroxy-1,4-naphthoquinone is a Michael acceptor.

Similar compounds have been shown to form pseudo-irreversible adducts with the catalytic cysteine residue.²² Indeed, in the absence of zinc, compound **5g** showed time- dependent inhibition, with essentially no inhibition at the initial time point but an IC₅₀ value of $5.6 \pm$

0.3 μM after 2 h of incubation. In the presence of zinc, there appears to be an additive effect in the inhibition of LYP by compound **5g** (Figure S2).

The most potent hit from CFL-1.1 was compound **1h**, 1,2-dihydroxynaphthalene. Compound **1h** was also a time-dependent inhibitor of LYP activity, with an IC_{50} of $2.52 \pm 0.06 \mu\text{M}$ after 2 h of incubation (Figure 5). Compounds related to 1,2-dihydroxynaphthalene have been reported as PTP inhibitors previously, although it is likely the oxidized version of this compound that is active.²³ In the presence of oxygen, **1h** is readily oxidized to 1,2-naphthoquinone, an oxidant that has been reported to inhibit PTP activity.²⁴ Indeed, 1,2-naphthoquinone shows very similar activity against LYP (Figure 5), with an IC_{50} value of $1.10 \pm 0.03 \mu\text{M}$ after 2 h of incubation. As mentioned previously, similar compounds have been shown to form pseudo-irreversible adducts with the catalytic cysteine residue of the PTPs.²² However, 1,4-naphthoquinone derivatives have also been reported as selective allosteric inhibitors of PTP activity, acting through a non-redox-mediated mechanism.²⁵

Interestingly, compound **1h** has modest selectivity for LYP over PTP1B, CD45, and YopH (Figure 6). This selectivity is not dramatic, but could serve as the starting point for the building a more potent and selective LYP inhibitor.

From a library of 96 metal-binding fragments, we have identified a series of inhibitors of the lymphoid tyrosine phosphatase, LYP. The most potent inhibitors were the naphthoquinones **5g** and **1h**, which displayed time-dependent LYP inhibition consistent with covalent adduct. The 1,2-dihydroxynaphthalene scaffold has modest inherent selectivity for LYP over a handful of other PTPs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PTP	protein tyrosine phosphatase
LYP	lymphoid tyrosine phosphatase
LYPcat	catalytic subunit of LYP
DiFMUP	6,8-difluoro-4-methylumbelliferyl phosphate
DiFMU	6,8-difluoro-4-methylumbelliferone

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Figure 1.
Chelator fragment library.

Figure 2.

Inhibition of LYP activity by zinc acetate. Enzyme activity (defined as 100% in the absence of Zn) decreases in a dose dependent manner as Zn(OAc)₂ is added, with complete inhibition achieved at 100 μM added Zn(II). Inset shows the response to low concentrations of Zn(II).

Figure 3.

Compounds **3g**, **12b**, and **8a** have little effect on LYP activity on their own (open circles) but are all capable of restoring LYP activity in the presence of 40 μM zinc acetate (solid circles). Two equiv of chelator (80 μM) is sufficient to restore activity in the case of **3g** and **12b**, with slightly more than two equiv required for **8a**.

Figure 4.

Left panel: Compound **5g** inhibits LYP activity more potently in the presence of zinc (solid circles) than in the absence of zinc (open circles). Right panel: Compound **5g** is a time-dependent inhibitor of LYP activity, showing increased inhibition as incubation time increases from 0 min (open circles) to 30 min (solid circles) to 60 min (triangles) and finally 120 min (squares).

Figure 5.

Compound **1h** (left panel) and 1,2-naphthoquinone (right panel) inhibit LYP activity in a time-dependent manner, showing increased inhibition as incubation time increases from 0 min (open circles) to 30 min (solid circles) to 60 min (triangles) and finally 120 min (squares).

Figure 6.
Inhibition of LYP (circles) CD45 (squares), PTP1B (diamonds) and YopH (triangles) by compound **1h**, 1,2-dihydroxynaphthalene.