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Gold(I) phosphine mediated selective inhibition of lymphoid tyrosine phosphatase

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Abstract

Selective protein tyrosine phosphatase (PTP) inhibition is often difficult to achieve owing to the high degree of similarity of the catalytic domains of this family of enzymes. Selective inhibitors of the lymphoid specific tyrosine phosphatase, LYP, are of great interest due to the involvement of LYP in several autoimmune disorders. This manuscript describes a study into the mechanistic details of selective LYP inhibition by a Au(I)-phosphine complex. The complex, [Au($(CH_2CH_2CN)_2$PPh)Cl], selectively inhibits LYP activity both *in vitro* and in cells, but does not inhibit other T-cell derived PTPs including the highly homologous PTP-PEST. The mode of inhibition was probed by investigating inhibition of LYP, the LYP mutant C129/231S, and PTP-PEST. Inhibition of LYP and PTP-PEST was competitive, while the LYP double mutant appeared mixed. Wild-type LYP was inhibited more potently than LYP C129/231S, indicating an important role for at least one of these residues in Au(I) binding. Coordination of Au(I) by both the active site cysteine residue as well as either Cys129 or 231 is suggested as a potential mechanism for LYP selective inhibition.

Keywords

Gold; Rheumatoid arthritis; Protein tyrosine phosphatase; Autoimmunity

1. Introduction

The medicinal history of gold dates back as far as 2500 BC to ancient Chinese civilizations [1]. Since then, various forms of gold have been used for many purposes from amulets to ward off evil spirits, to the beginning of systemic gold pharmacology inspired by the discovery that gold cyanide inhibits the growth of *Mycobacterium tuberculosis* at the turn of the 20th century [2,3]. Although gold has been promoted as a treatment for diseases as diverse as Crohn's disease, ulcerative colitis, bronchial asthma, and systemic lupus erythematosus with mixed results [4], the only recognized treatment in the United States originated in a 1935 report that

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[2]The equations used in calculating these values were $K_i = V'_{max}[I]/V_{max} - V'_{max}$ for LYP C129/231S and $K_i = K_m + K_m[I]/K'_m$ for wild-type LYP and PTP-PEST where K_m and V_{max} were values from samples containing no inhibitor and K'_m and V'_{max} were obtained from those samples containing inhibitor.

gold salts could relieve the symptoms of rheumatoid arthritis [5]. Since then, the only major advancement in gold based therapeutics came in the 1970s with the introduction of the orally available triethylphosphine(2,3,4,6-tetra-O-acetyl-β-D-thiopyranosato-S)gold(I) (auranofin) [6]. The primary reason for this lack of progress stems from the promiscuity of Au(I) as well as a poor understanding of the mechanism of action of Au(I)-based drugs. Gold(I) interacts with many biological macromolecules and shows a preference for thiolates with reduced pK_a values [1]. Recently, the protein tyrosine phosphatase (PTP) family of cysteine-dependent enzymes was shown to be inhibited by available Au(I) drugs as well as several analogs [7–9].

The PTPs dephosphorylate phosphotyrosine residues, play a prominent role in many cellular signaling events [10–12] and have been implicated in several human diseases and conditions including autoimmunity [13]. One recently discovered autoimmune-associated PTP is the lymphoid specific tyrosine phosphatase (LYP, PTPN22), where a single nucleotide polymorphism (SNP) in the *PTPN22* gene leading to a gain-of-function variant has been connected to several autoimmune disorders including type 1 diabetes [14], systemic lupus erythematosus [15], and rheumatoid arthritis [16], among others [17]. Another SNP in *PTPN22* resulting in a loss-of-function variant is reported to be protective against systemic lupus erythematosus [18], supporting the hypothesis that LYP is an important target in the treatment of autoimmune disorders [13]. To this end, we screened a library of Au(I)-phosphine complexes and identified several potent, selective LYP inhibitors [9].

Achieving selectivity in PTP inhibitor development has been challenging due to the high degree of homology in the active sites of these enzymes. However, selectivity is crucial for therapeutic applications, because nonspecific inhibition of PTP activity is detrimental. For example, PTP-PEST, a PTP with a C-terminal PEST motif, is 70% identical to LYP in the catalytic domain and is required for embryonic development. A PTP-PEST knockout in mice results in embryonic lethality [19], while a knockout of the mouse LYP homolog (termed PEP) lacks a noticeable phenotype [13], but demonstrates enhanced memory T cell responses. PTP inhibitors with selectivity for LYP over PTP-PEST had not been identified prior to our work with the Au(I)-phosphine library. By understanding the details of the LYP-selectivity of the Au(I) complexes, we hoped to shed some light on the key differences between LYP and PTP-PEST and further the development of selective inhibitors with therapeutic potential for the treatment of human autoimmunity.

In order to more thoroughly examine the LYP-selectivity of the gold(I) phosphine complexes, we set out to probe the mechanistic details of LYP inhibition. LYP is a cysteine-dependent enzyme containing a cysteine residue with lowered pK_a in its active site. In addition to this highly conserved cysteine residue, LYP has two additional cysteine residues within disulfide bonding distance to the catalytic cysteine [20]. Based on previous work from our laboratory and others [7,8,21,22], it is reasonable to assume that the gold(I) phosphines interact with the catalytic cysteine residue. We hypothesized that the LYP-selectivity of the gold(I) complexes identified in our library screen could be due to interactions with the other active site cysteine residues in addition to direct coordination to the catalytic cysteine. The complex chosen for this study was bis(cyanoethyl)phenylphosphine–Au(I)–Cl (**1**)(Fig. 1), which is selective for LYP over several other PTPs (Table 1) [9]. Kinetic analyses of complex **1** with LYP, PTP-PEST and several LYP mutants support the suggestion that this selective inhibitor has a higher affinity for LYP at least in part because of synergistic binding by a non-catalytic cysteine in the active site.

2. Materials and methods

2.1. General considerations

All reagents were purchased from commercial suppliers and used without further purification. Complex **1** was synthesized as previously reported [9]. Catalytic domains of LYP and PTP-PEST were expressed and purified as previously described [7]. The catalytic domains of the LYP mutants, C129S, C231S, and C129/231S were generous gifts from Xiaojiang Chen at the University of Southern California (Department of Molecular and Computational Biology). Fluorescence data were collected on a Molecular Devices Spectramax M5 multimode plate reader with excitation and emission at 360 nm and 455 nm, respectively.

2.2. Enzyme inhibition assays

Inhibition assays were performed at room temperature in buffer containing 50 mM Tris, pH 6.5, 100 mM NaCl, 1 mM EDTA, 0.1 mM dithiothreitol (DTT) and 0.01% Brij 35. The final concentrations of enzymes used were 5 nM for wild-type LYP, PTP-PEST, LYP C129S, and LYP C231S, and 20 nM for LYP C129/231S. Solutions of substrate and inhibitor were made in DMSO and added such that the final concentration of DMSO was 5% of the total reaction volume. Prior to the reaction, enzymes were activated by incubation with 0.1 mM DTT for 30 min (1 h for LYP C129/231S). Enzyme activity was measured in 96-well plates using 1.5 μM 6,8-difluoro-4-methylumbelliferryl phosphate (DiFMUP) as the substrate. Assays were conducted in triplicate measuring the fluorescence at 455 nm every 60 s for 30 min and the results averaged and converted from relative fluorescence units to concentration of 6,8-difluoro-4-methylumbelliferrone (DiFMU) using a standard curve.

2.3. Reversibility assays

The reversibility of Au(I)-mediated LYP inhibition was shown in experiments in which 5 nM LYP was incubated with 0.1 mM DTT for 30 min, followed by incubation with the Au(I)-phosphine inhibitor for 30 min. These mixtures were then incubated for an additional 30 min with varying concentrations of L-cysteine (0–50 mM). Finally, 1.5 μM DiFMUP was added to all and the activity of LYP was measured in triplicate at each concentration of L-cysteine.

2.4. Mode of inhibition assays

The activity of LYP, PTP-PEST, and LYP C129/231S was measured in the absence and presence of fixed concentrations of Au(I)-phosphine inhibitor at a series of substrate concentrations. Assays were conducted in triplicate for each concentration of DiFMUP substrate. Inhibitor concentrations used were chosen such that they were higher than, lower than, and at approximately the IC_{50} value of the complex investigated. The reciprocal of the reaction rate was plotted as a function of the reciprocal of the substrate concentration for each concentration of inhibitor (Lineweaver–Burk plot). These data were also used to plot the reciprocal reaction rate as a function of substrate concentration (Dixon plot).

2.5. Antibodies

The polyclonal pTyr319-Zap-70, anti-ZAP-70, anti-pTyr505-Lck and anti-pTyr416-Src (cross-reactive with LckpTyr394) antibodies were obtained from Cell Signaling Technology, Inc., while the anti-ERK2 and monoclonal anti-Lck antibodies were from Santa Cruz Biotechnology. The Anti-ACTIVE MAPK (anti-pERK) polyclonal antibody was obtained from Promega. The ECL-Plus Chemiluminescence kit was obtained from GE-Amersham Biosciences.

2.6. Cells and Cell treatments

Jurkat T leukemia cells expressing SV-40 large T Antigen (JTAg) [23] were kept at logarithmic growth in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES pH 7.3, 2.5 mg/mL D-glucose, and 100 units/mL of penicillin and 100 μg/mL streptomycin.

2.7. Inhibition studies with JTAg cells

Fixed concentrations of inhibitor (50 μM) or DMSO (control) was added to 20×10^6 cells suspended in 800 μL RPMI 1640, and incubated for 1 h at room temperature. The volume of DMSO added was held constant at less than 2% of the total volume. JTAg cells pre-incubated with DMSO or inhibitor were divided into 400 μL aliquots containing 10×10^6 cells and stimulated with supernatants of C305 hybridomas [24] for 2 min or left untreated. Cells were lysed in 20 mM Tris–HCl pH 7.5, 150 mM NaCl, 5 mM EDTA pH 8.0 containing 1% NP-40, 10 μg/mL aprotinin and leupeptin, 10 μg/mL soybean trypsin inhibitor, 1 mM Na_3VO_4 and 1 mM phenylmethylsulfonyl fluoride after which lysates were clarified by centrifugation at 13,200 rpm for 20 min. The total protein concentration in each cell lysate was determined by the Bradford protein assay (Bio-Rad) in order to normalize the amount of protein used in SDS–PAGE.

2.8. SDS–PAGE and immunoblots

Aliquots of lysates were suspended in SDS sample buffer, heated at 95 °C for 5 min and the boiled samples run on 10% SDS–polyacrylamide gels. Proteins resolved by gel electrophoresis were transferred onto nitrocellulose membranes (Hybond ECL, GE Healthcare) and treated with appropriate dilutions per manufacturers instructions of unconjugated primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies (purchased from GE Healthcare). Blots were developed with the enhanced chemiluminescence detection system, ECL-Plus, following manufacturers directions.

3. Results and discussion

Initially, the reversibility of complex **1** mediated inhibition was examined. Because Au(I) forms a labile bond with cysteine residues in the enzyme, particularly the active site cysteine, exogenously added cysteine should compete with the active site cysteine for Au(I) binding and restore enzyme activity. Addition of a large excess of thiolate (>1000-fold) was required to achieve reversibility, demonstrating the high affinity of Au(I) for the active site of LYP (Fig. 2). The full recovery of LYP activity to 100% was not seen because the addition of extremely high concentrations (>20 mM) of L-cysteine begins to inhibit the enzyme. However, these results are consistent with previously published data on the reversible inhibition of PTPs by gold compounds [7,8].

Recently, two cysteine residues (Cys129 and Cys231) in close proximity to the active site cysteine of LYP (Cys227, PDB: 2P6X, 3H2X) were found to be important in the regulation of LYP activity [20]. Because of the thiophilicity of Au(I), we postulated that complex **1** may bind both to the active site cysteine and also to a second cysteine residue in the active site, either C129 or C231. In order to examine the effect these residues have on the inhibitory properties of complex **1**, inhibition studies were carried out on a series of LYP mutants (C129S, C231S, and C129/231S) and the values compared with those of wild-type LYP and PTP-PEST (Table 1). Inhibition of the single LYP mutants C129S and C231S was not significantly different from that of wild-type LYP. This suggests that these residues individually are not crucial to the inhibitory properties of **1**. However, with the double mutant LYP C129/231S, the IC_{50} is roughly 10-fold higher than that of wild-type LYP and single mutants. This indicates that either of these residues can assist in the binding of **1** and that at least one of them is required

for maximal affinity. Upon coordination of Au(I) to the active site cysteine, one of the adjacent thiol(ate)s could either substitute for the phosphine ligand of the inhibitor creating an Cys–S–Au–S–Cys complex or bind in addition to the phosphine ligand creating a species with the general formula $[Au(PR_3)(S–Cys)_2]$. This type of interaction has been seen in the active site of human glutathione reductase co-crystallized with a gold complex [25]. Scheme 1 depicts this potential ligand substitution reaction with adjacent cysteine residues 129 and 231.

In order to further probe the LYP selectivity of **1**, we pursued determining the mode of inhibition by incubating LYP as well as PTP-PEST and the LYP double mutant with varying substrate and inhibitor concentrations. Double reciprocal plots of inverse rate as a function of inverse substrate concentration in the presence of several concentrations of **1** were constructed for each enzyme (Fig. 3A–C). In the LYP and PTP-PEST plots (Fig. 3A andB), the lines cross near the origin, indicating competitive inhibition. In the LYP C129/231S plot (Fig. 3C), the lines cross near the negative *x*-axis, indicating mixed-type inhibition. Calculated K_i values[2] for LYP, LYP C129/231S, and PTP-PEST were 2.38 ± 1.1 μM, 58.6 ± 5.3 μM, and 53.3 ± 16.3 μM respectively. These results agree with the observed IC_{50} values and further demonstrate that complex **1** has a higher affinity for LYP than either the LYP C129/231S mutant or PTP-PEST.

Dixon plots were also constructed using the reciprocal of the rate as a function of inhibitor concentration (Fig. 4A–C). The Dixon plots of the data for LYP and PTP-PEST yield lines that all appear to cross just above the negative *x*-axis consistent with either mixed or competitive inhibition. The same appears to be true for LYP C129/231S with the exception of the line from the lowest concentration of substrate, which would be likely to contain the most error. By obtaining estimated K_i values from these plots (K_i= −intersection point) it appears that LYP, PTP-PEST and the LYP double mutant have K_i values of approximately 1.2, 80 and 70 μM respectively. These values are consistent with the previous calculated values, demonstrating the ability of **1** to bind with higher affinity to wild-type LYP than LYP C129/231S or PTP-PEST.

It is interesting that the K_i value of complex **1** with the LYP double mutant is closer to that of PTP-PEST than wild-type LYP. It is evident from this study that one of these two non-catalytic cysteine residues assists in the binding of complex **1**, supporting the recently reported role of these cysteine residues as important modulators of LYP activity [20]. However, PTP-PEST also contains two cysteine residues analogous to LYP Cys 129 and Cys 231, as seen in the sequence alignment in Fig. 5. The configuration of these corresponding residues in PTP-PEST is unknown and it is possible that these residues are not structurally and functionally analogous to the cysteines in the active site of LYP. Detailed structural information about the Au(I)-enzyme interaction will be required to fully explain the increased affinity of complex **1** for LYP over PTP-PEST.

To further explore the inhibitory properties of complex **1**, cellular inhibition studies were conducted to evaluate this inhibitor's potential to inhibit LYP in cells. Early stage T cell receptor (TCR) signaling involves the negative regulation of Lck (a lymphocyte specific tyrosine kinase) by dephosphorylation at position Y394 by LYP, as well as activation of Lck by dephosphorylation at Y505 by CD45 [26,27]. Therefore, the inhibition of cellular LYP and CD45 activity can be monitored by following the phosphorylation levels at these two sites on Lck as previously described [7]. Should inhibition of these enzymes occur, an increase in phosphorylation will be observed at the respective position on Lck. Fig. 6A shows the western blot results of cells incubated in the presence and absence of inhibitor, with and without TCR stimulation. It is clear from these blots that, in the TCR stimulated cells (lanes 2 and 4), an increase in phosphorylation at Y394 is seen in the presence of complex **1** (lane 4), demonstrating its ability to inhibit LYP activity in cells. There is no change in phosphorylation

levels at position Y505 of Lck in the presence of **1**, supporting the *in vitro* data, which indicates that this inhibitor does not significantly inhibit CD45 activity.

Events further down the T cell signaling pathway include activation of ZAP-70 and ERK1/2 by their phosphorylation. Inhibitors of LYP that affect early T cell signaling would be expected to yield an increase in the phosphorylation of these enzymes as well. The phosphorylation of ZAP-70 and ERK1/2 was examined after cellular incubation with complex **1**. As seen in Fig. 6B, the lysates of TCR stimulated cells incubated with inhibitor (lane 4) show increased phosphorylation levels of both ZAP-70 and ERK1/2 (first and third rows, respectively) over the lysates of untreated cells (lane 2). These blots establish the ability of complex **1** to inhibit LYP activity in early TCR signaling selectively over CD45, as well as the effect of LYP inhibition on events further down the signaling pathway, demonstrating the ability of **1** to restore T cell signaling.

4. Conclusions

Taken together, these data provide insight into the selectivity of Au(I)-phosphine inhibitors for LYP over the highly homologous PTP-PEST. The selective Au(I)-phosphine complexes appear to have a higher affinity for wild-type LYP than PTP-PEST and the LYP double mutant. The differences in IC_{50} and K_i values of the LYP double mutant underscore the importance of these cysteine residues in inhibitor binding either directly or indirectly. Cellular inhibition studies demonstrate the ability of complex **1** to inhibit LYP activity in more complex biological environments and corroborate the observed *in vitro* selectivity. These studies have shed some mechanistic light on the selective inhibition of LYP by Au(I)-phosphines and pave the way for the future development of potent, selective LYP inhibitors for therapeutic use.

References

[1]. Fricker SP. Gold Bull 1996;29:53–60.

[2]. Brown DH, Smith WE. Chem. Soc. Rev 1980;9:217–240.

[3]. Shaw CF III. Chem. Rev 1999;99:2589–2600. [PubMed: 11749494]

[4]. Eisler R. Inflamm. Res 2003;52:487–501. [PubMed: 14991077]

[5]. Forestier J. J. Lab. Clin. Med 1935;20:827–840.

[6]. Sutton BM, McGusty E, Walz DT, DiMartino MJ. J. Med. Chem 1972;15:1095–1098. [PubMed: 4654656]

[7]. Krishnamurthy D, Karver MR, Fiorillo E, Orru V, Stanford SM, Bottini N, Barrios AM. J. Med. Chem 2008;51:4790–4795. [PubMed: 18605719]

[8]. Wang Q, Janzen N, Ramachandran C, Jirik F. Biochem. Pharmacol 1997;54:703–711. [PubMed: 9310347]

[9]. Karver MR, Krishnamurthy D, Kulkarni RA, Bottini N, Barrios AM. J. Med. Chem 2009;52:6912–6918. [PubMed: 19888762]

[10]. Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T. Cell 2004;117:699–711. [PubMed: 15186772]

[11]. Stoker AW. J. Endocrinol 2005;185:19–33. [PubMed: 15817824]

[12]. Zhang Z-Y. Annu. Rev. Pharmacol. Toxicol 2002;42:209–234. [PubMed: 11807171]

[13]. Vang T, Miletic AV, Arimura Y, Tautz L, Rickert RC, Mustelin T. Annu. Rev. Immunol 2008;26:29–55. [PubMed: 18303998]

[14]. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T. Nat. Genet 2004;36:337–338. [PubMed: 15004560]

[15]. Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VEH, Chang M, Ramos P, Baechler EC, Batliwalla FM, Novitzke J, Williams AH, Gillett C, Rodine P, Graham RR, Ardlie

KG, Gaffney PM, Moser KL, Petri M, Begovich AB, Gregersen PK, Behrens TW. Am. J. Hum. Genet 2004;75:504–507. [PubMed: 15273934]

[16]. Begovich AB, Carlton VEH, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT, Chang M, Chang S-YP, Saiki RK, Catanese JJ, Leong DU, Garcia VE, McAllister LB, Jeffrey DA, Lee AT, Batliwalla F, Remmers E, Criswell LA, Seldin MF, Kastner DL, Amos CI, Sninsky JJ, Gregersen PK. Am. J. Hum. Genet 2004;75:330–337. [PubMed: 15208781]

[17]. Bottini N, Vang T, Cucca F, Mustelin T. Sem. Immunol 2006;18:207–213.

[18]. Orru V, Tsai SJ, Rueda B, Fiorillo E, Stanford SM, Dasgupta J, Hartiala J, Zhao L, Ortego-Centeno N, D'Alfanso S, Arnett FC, Wu H, Gonzalez-Gay MA, Tsao BP, Pons-Estel B, Alarcon-Riquelme ME, He Y, Zhang Z-Y, Allayee H, Chen XJ, Martin J, Bottini N. Hum. Mol. Genet 2009;18:569–579. [PubMed: 18981062]

[19]. Sirois J, Cote J-F, Charest A, Uetani N, Bourdeau A, Duncan SA, Daniels E, Tremblay ML. Mech. Dev 2006;123:869–880. [PubMed: 17070019]

[20]. Tsai SJ, Sen U, Zhao L, Greenleaf WB, Dasgupta J, Fiorillo E, Orru V, Bottini N, Chen XJ. Biochemistry 2009;48:4838–4845. [PubMed: 19371084]

[21]. Chircorian A, Barrios AM. Bioorg. Med. Chem. Lett 2004;14:5113–5116. [PubMed: 15380210]

[22]. Gunatilleke SS, Barrios AM. J. Med. Chem 2006;49:3933–3937. [PubMed: 16789749]

[23]. Clipstone NA, Crabtree GR. Nature 1992;357:695–697. [PubMed: 1377362]

[24]. Weiss A, Stobo JD. J. Exp. Med 1984;160:1284–1299. [PubMed: 6208306]

[25]. Urig S, Fritz-Wolf K, Reau R, Herold-Mende C, Toth K, Davioud-Charvet E, Becker K. Angew. Chem. Int. Ed 2006;45:1881–1886.

[26]. Mustelin T, Alonso A, Bottini N, Huynh H, Rahmouni S, Nika K, Louis-ditSully C, Tautz L, Togo SH, Bruckner S, Mena-Duran AV, al-Khouri AM. Mol. Immunol 2004;41:687–700. [PubMed: 15220004]

[27]. Wu J, Katrekar A, Honigberg LA, Smith AM, Conn MT, Tang J, Jeffery D, Mortara K, Sampang J, Williams SR, Buggy J, Clark JM. J. Biol. Chem 2006;281:11002–11010. [PubMed: 16461343]

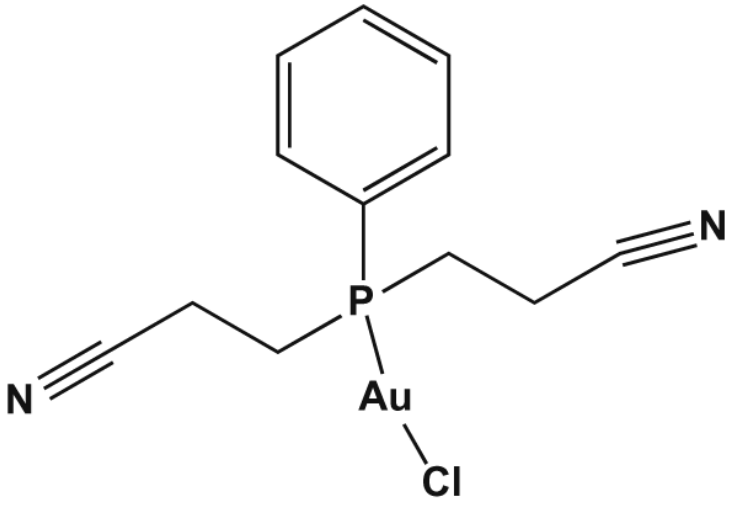


Fig. 1.
Chemical structure of bis(cyanoethyl)phenylphosphine–Au(I)–Cl (Complex **1**).

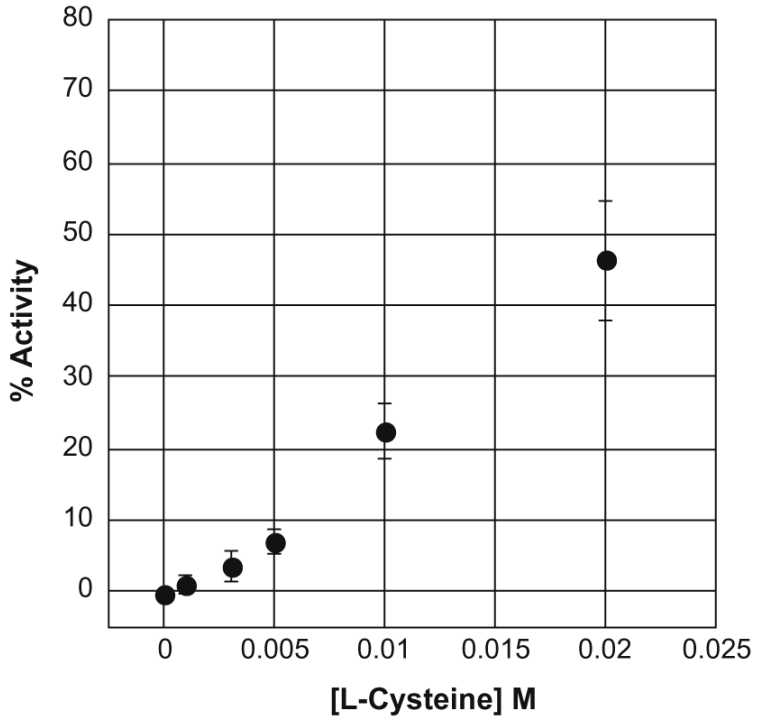


Fig. 2.
Reversibility of complex **1** mediated LYP inhibition in the presence of excess L-cysteine.

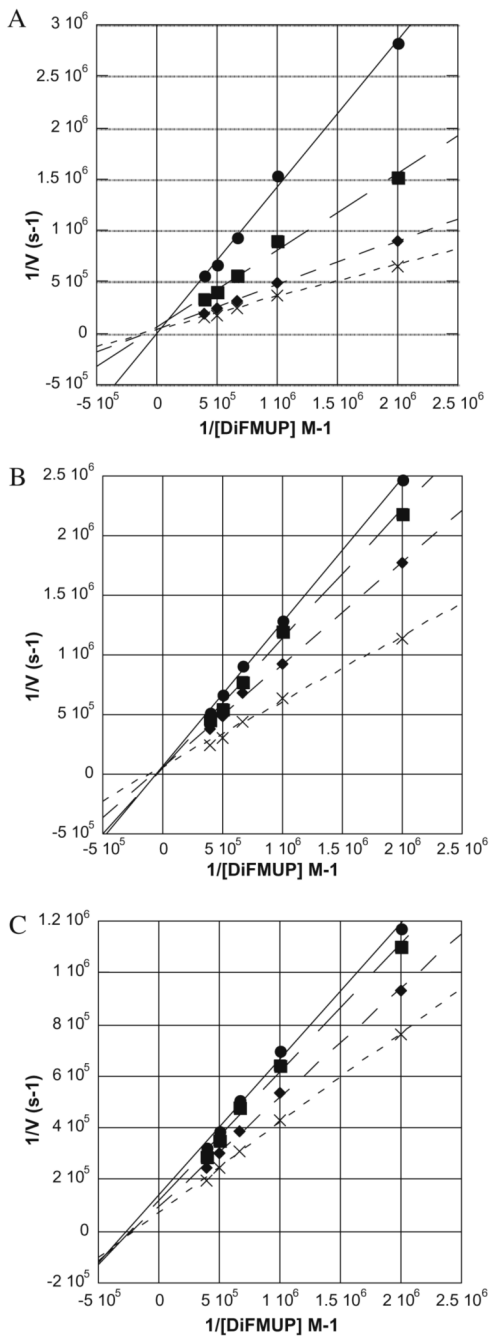


Fig. 3.

Lineweaver–Burk plots. (A) LYP with 5 μM (●), 3 μM (■), 1 μM (◆), and 0 μM (**X**) **1**. (B) PTP-PEST with 100 μM (●), 80 μM (■), 60 μM (◆), and 0 μM (**X**) **1**. (C) LYP C129/231S with 50 μM (●), 40 μM (■), 20 μM (◆), and 0 μM (**X**) **1**.

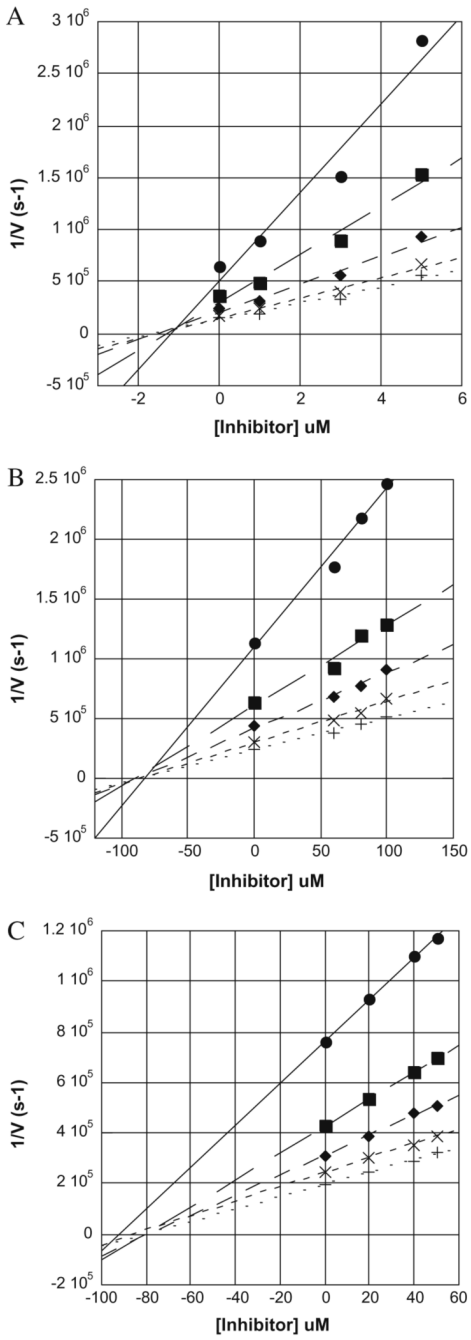


Fig. 4.

Dixon plots of (A) LYP, (B) PTP-PEST and (C) LYP C129/231S with 0.5 μM (●), 1 μM (■), 1.5 μM (◆), 2 μM (**X**), and 2.5 μM (+) DiFMUP substrate using complex **1** as the inhibitor. Error bars representing the standard deviation for each data point have been plotted, but are smaller than the data point symbols.

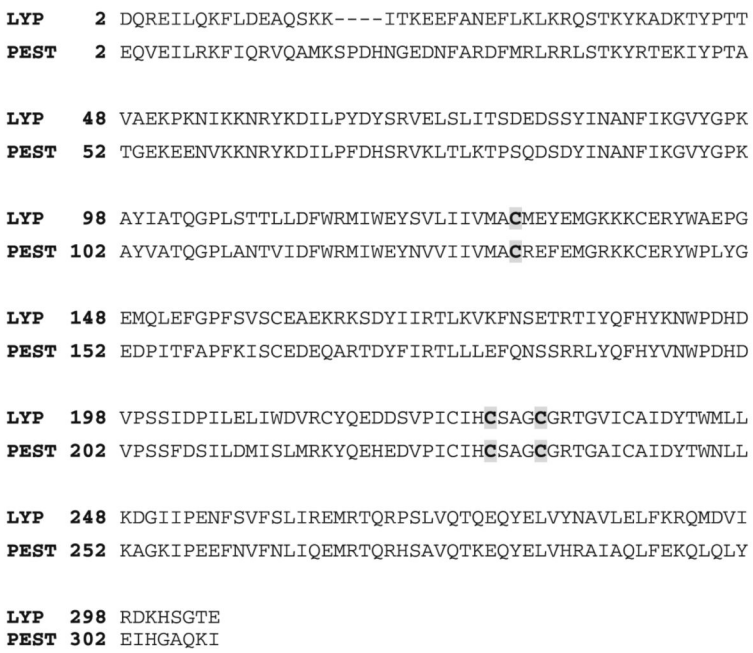


Fig. 5.

Amino acid sequence alignment of the catalytic domains of LYP (2-305) and PTP-PEST (2-309). Highlighted and in bold are three conserved cysteine residues; Cys129, Cys227 (catalytic), and Cys231 (LYP numbering).

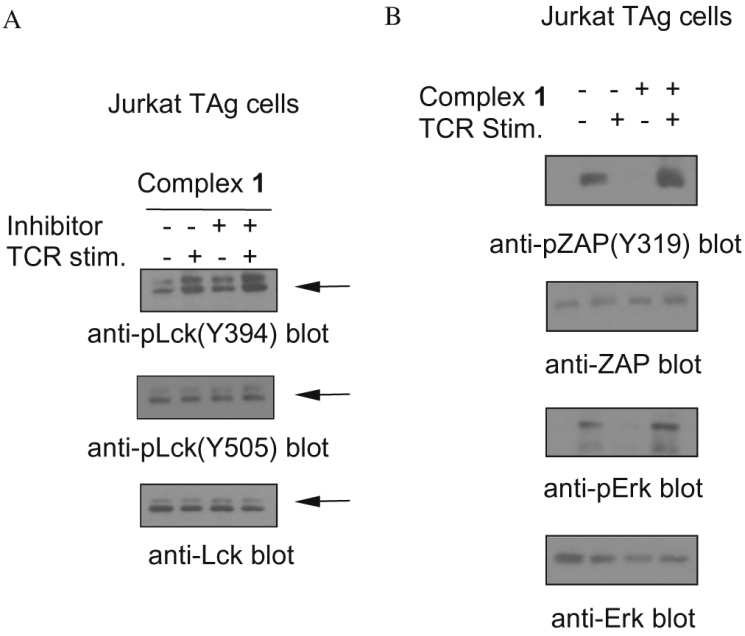
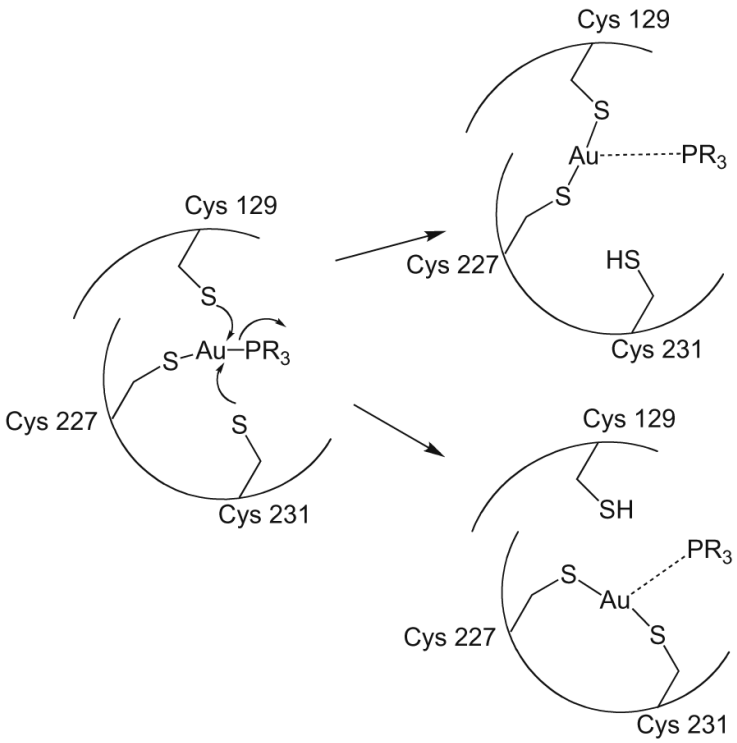


Fig. 6.

Intracellular inhibition of LYP by gold complexes. Immunoblots of lysates of Jurkat TAg cells treated with 50 μM of complex **1** (lanes 3 and 4 in each panel) or untreated (lanes 1 and 2 in each panel) and either left unstimulated (lanes 1 and 3 in each panel) or stimulated (lanes 2 and 4 in each panel) with C305 antibodies for 2 min. (A) Top panel: anti-pLck(Tyr394) Middle panel: anti-pLck(Tyr505) blot of the same sample. Bottom panel: anti-Lck blot of the same sample (loading control). Arrows indicate the position of Lck (56 KDa) in each panel. (B) Panels show blots of total lysates with the following antibodies: top panel, anti-pZAP70 (Y319); second from top, anti-ZAP70 loading control; second from bottom, anti-pERK; bottom panel, anti-ERK loading control.



Scheme 1.

Representation of the ligand exchange reactions that may occur upon coordination of **1** to the active site of LYP.

Table 1

IC_{50} values in μM of different PTPs with complex **1**. LYP, PTP-PEST, HePTP and CD45 values were reported previously [9]

Enzyme	IC_{50}
LYP	3.5 ± 0.1
PTP-PEST	>80
HePTP	>50
CD45	>70
LYP C129S	4.0 ± 0.2
LYP C231S	4.0 ± 0.8
LYP C129/231S	40 ± 4