**Picomolar Concentrations of Free Zinc(II) Ions Regulate Receptor Protein-tyrosine Phosphatase β Activity**

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**Background:** Protein-tyrosine phosphatases (PTPs) are critical regulators of phosphorylation signaling.

**Results:** Zinc(II) ions are reversible inhibitors of receptor PTPβ with a remarkably low $K_i$ of $21 \pm 7$ pm.

**Conclusion:** The inhibition implicates cellular zinc(II) ions as physiological regulators of PTPs.

**Significance:** The results suggest that zinc modulates signal transduction in endothelial cells affecting angiogenesis and wound healing.

As key enzymes in the regulation of biological phosphorylations, protein-tyrosine phosphatases are central to the control of cellular signaling and metabolism. Zinc(II) ions are known to inhibit these enzymes, but the physiological significance of this inhibition has remained elusive. Employing metal buffering for strict metal control and performing a kinetic analysis, we now demonstrate that zinc(II) ions are reversible inhibitors of the cytoplasmic catalytic domain of the receptor protein-tyrosine phosphatase β (also known as vascular endothelial protein-tyrosine phosphatase). The $K_i(20)$ value is $21 \pm 7$ pm, 6 orders of magnitude lower than zinc inhibition reported previously for this enzyme. It exceeds the affinity of the most potent synthetic small molecule inhibitors targeting these enzymes. Inhibition is in the range of cellular zinc(II) ion concentrations, suggesting that zinc regulates this enzyme, which is involved in vascular physiology and angiogenesis. Thus, for some enzymes that are not recognized as zinc metalloenzymes, zinc binding inhibits rather than activates as in classical zinc enzymes. Activation then requires removal of the inhibitory zinc.

Phosphatases are major control enzymes in phosphorylation signaling, and they do not simply oppose the action of kinases (1). Protein-tyrosine phosphatases (PTPs) are one subfamily of phosphatases. The human genome contains 107 PTPs that belong to families of soluble (nonreceptor-type) and membrane-resident (receptor-type) proteins (2). PTPs have pivotal functions in cellular processes and are extensively regulated. At the protein level, this regulation includes redox modulation, phosphorylation, sumoylation, dimerization, and proteolysis (3). With the exception of class IV PTPs, the catalytic group in their active sites is a cysteine with a remarkably low thiol $pK_a$ (about 4.4–5.5 in PTP-1B). The cysteine forms a phosphoenzyme intermediate in the reaction with substrate. Redox modulation of PTP-1B involves oxidation of the catalytic cysteine to a sulfenic acid, which reacts with a backbone nitrogen of an adjacent serine to form a cyclic sulfenyl amide (4, 5). Other PTPs are redox-modulated differently. They form a disulfide between the two “backdoor” cysteines or between the catalytic cysteine and one of the backdoor cysteines (6, 7).

Vanadium compounds and zinc(II) ions are classical inhibitors of PTPs. Meta- and orthovanadate mimic the phosphate group in the transition states of the enzyme and therefore have been valuable in delineating the reaction mechanism (8). In contrast to vanadium, zinc is nutritionally essential with total cellular concentrations in the range of a few hundred micromolar. Because zinc(II) ions at $10 \mu M$ concentrations completely inhibit PTP, it was suggested that they are a physiologically important inhibitor (9). However, cellular zinc(II) ions are not available at these concentrations. Free zinc(II) ions are orders of magnitudes lower than total cellular zinc concentrations. They are estimated to be in the picomolar range and to fluctuate in this range (10–14). Because the observed zinc inhibition of PTPs is much higher than the available cellular zinc(II) ion concentrations, zinc inhibition was discussed as a consequence of toxic exposure to zinc or at elevated intracellular zinc(II) ion concentrations in oxidative neuronal death (15, 16). More recent investigations, however, demonstrated that zinc binds significantly tighter: 200 nm ($IC_{50}$) for human T-cell PTP (17) versus 85% inhibition at 100 nm ($IC_{50}$) for human T-cell PTP (18); 17 nm ($K_i(app)$) for human PTP-1B (19, 20); and 93 nm for PTP1C (SHP-1) (19). Such tight inhibition suggests that zinc modulates PTP activity under physiological conditions in a variety of signaling pathways (21).

For example, PTP-1B inhibition causes the IGF-1/insulin receptor to remain in a phosphorylated state. Thus, zinc inhibition of PTP-1B and the ensuing sustained insulin signaling have been suggested to account for the insulinomimetic effects of zinc(II) ions (22, 23). Also, zinc inhibition of a MAPK tyrosine phosphatase in Erk signaling is involved in hippocampus-dependent memory (24).

In this study, we investigated the effects of zinc on PTPs in more detail. Specifically, we optimized the assay conditions, employed a sensitive fluorometric assay, and performed a kinetic analysis of the inhibition. We demonstrate that zinc(II) ions reversibly inhibit the cytoplasmic catalytic domain of RPTPβ (R3 subtype) with unparalleled potency. Zinc inhibition is 6 orders of magnitude tighter than reported previously for RPTPβ (25). In the context of cellular zinc(II) ion concentrations and their fluctuations, the data suggest that cellular zinc...
inhibits RPTPβ tonically and that the enzyme must be activated by zinc chelation.

**EXPERIMENTAL PROCEDURES**

**Materials**—HEPES, molecular biology grade, was purchased from Calbiochem; EDTA was from BDH (VWR International); tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was from Thermo Fisher; bovine serum albumin, nitrilotriacetic acid (NTA), and zinc sulfate (ZnSO₄·7H₂O) were from Sigma-Aldrich; and 3,6-fluorescein diphosphate (FDP) was from AnaSpec (San Jose, CA). Human recombinant PTP-RB (RPTPβ), residues 1675–1996, was from Cell Sciences (Canton, MA), supplied in 25 mM Tris-HCl, 75 mM NaCl, 0.05% Tween 20, 50% glycerol, 2 mM EDTA, 1 mM DTT, 10 mM glutathione, pH 8.0.

**PTP Assay**—RPTPβ was assayed fluorometrically for enzymatic activity at 23 °C in a freshly prepared buffer containing final concentrations of 50 mM HEPES/Na⁺, 100 μM TCEP, and 1 mM NTA, pH 7.4. The enzyme was added to this buffer to yield final concentrations of 0.5–5 nM. The reaction was initiated by adding FDP, a fluorogenic phosphate substrate (26), to a final concentration of 0.8 μM. Assays were performed in triplicate in a total volume of 100 μl in 96-well black optical bottom plates (p-clear plate black, reference number 655090, Greiner Bio-One Ltd., Stonehouse, UK). The increase in fluorescence due to the hydrolysis of FDP to fluorescein was measured at 485 ± 20 nm excitation and 530 ± 20 nm emission for up to 1 h, using a fluorescence plate reader (Synergy HT, BioTek, Winooski, VT). Initial velocities were determined from the linear portion of the progress curves. Kinetic parameters were obtained from nonlinear regression analyses of hyperbolic velocity versus [S] plots.

**Zinc Inhibition of RPTPβ**—RPTPβ was assayed for enzymatic activity in the presence of free zinc(II) ions. Zinc was buffered in the pico- and nanomolar range (0.3 pm–1.7 μM) using 1 mM NTA containing 0.1 μM to 1 mM ZnSO₄. Zinc was added to the buffer prior to enzyme and substrate. Free zinc(II) ion concentrations were calculated using the software programs Maxchelator (27) and MINEQL version 4.6 (Environmental Research Software, Hallowell, ME). PTP activity at each free zinc(II) ion concentration was compared with the maximum activity determined in the presence of 1 mM NTA. Data were plotted semilogarithmically and fitted to Hill’s equation (20) using SigmaPlot version 12 (SYSTAT Software Inc.). Zinc inhibition at 0.8 μM FDP was measured at three different enzyme concentrations (0.5, 2, and 5 nM). The data for the Dixon plots were obtained by single readings at each substrate and inhibitor concentration.

**Determination of Protein and Metal Concentrations**—Protein was quantitated spectrophotometrically (Synergy HT plate reader) using the Bio-Rad protein assay reagent (Bio-Rad) with bovine serum albumin as a standard, but using a modified Bradford assay with a 10-fold increased sensitivity (28). Inductively coupled plasma mass spectrometry (Perkin-Elmer Life Sciences, model Elan 6100 DRC plus) analysis was performed on all components of the assay to determine whether zinc contamination of reagents affects the free zinc(II) ion concentrations in the buffer system. Samples were diluted in a minimum volume of 1 ml of HNO₃ (1 or 5% v/v) in 15-ml polypropylene tubes. The final concentrations were the same as those used in the assay.

**RESULTS**

**PTP Assays**—A major issue when assaying PTPs, and a confounding factor in published data, is the control of the concentrations of metal ions and the redox state of the enzymes. The catalytic cysteine of PTPs is prone to oxidation and rapid inactivation. PTPs are prepared and supplied with rather high (mm) concentrations of the chelating agent EDTA and a reducing agent, typically DTT, which is also a relatively strong zinc-chelating agent (29). Removal of the agents results in rapid loss of activity. It is virtually impossible to dilute enzyme solutions in such a way that the agents do not interfere with assays of metal binding. Zinc is a ubiquitous contaminant of laboratory chemicals and biochemicals. Metal contamination in chemicals is usually given as ppm (parts per million). However, 1 ppb (parts per billion) corresponds to 15 nM zinc, a concentration that inhibits PTP-1B 50% and is clearly too high for investigating zinc inhibition of PTPs. Measuring zinc in the reagents used in the assay demonstrated that all the chemicals contained zinc at concentrations greater than 1 ppb. Notably, reagents, such as the substrate FDP, are usually not thought to be a source of zinc contamination and thought to be free of inhibitors (26). However, FDP has zinc concentrations far exceeding the concentrations that would cause 50% inhibition of PTP-1B (27.5 ppb at 0.42 μM FDP). The RPTPβ solution contains at least stoichiometric amounts of zinc. It was not feasible to remove zinc from all the solutions to control its concentrations at the sub-ppb level in the assay, although substantial efforts were made to avoid and control metal contamination. A solution to this problem is the use of metal buffering techniques, measurement of zinc concentrations in all solutions, and subsequently inclusion of measured zinc concentrations from the reagents in the calculations of free zinc(II) ion concentrations in the zinc-buffered solutions. PTPs have pH optima at slightly acidic pH (30). Because protons compete with zinc at zinc-binding sites, enzymatic activity should not be measured at the pH optimum when investigating zinc inhibition. Also critical in the experiments is the choice of the reducing agent to protect PTP from oxidation and to maintain its enzymatic activity. Standard reducing agents, such as DTT, glutathione, or 2-mercaptoethanol, are thiols and are not suitable because they are also chelating or zinc-binding agents. Therefore, we used TCEP as a reducing agent with low zinc binding capacity (31). We chose HEPES as a pH buffer because it has a low complexing capacity for zinc at pH 7.4 (32). As a metal buffering system, we used NTA, which has a logKᵣ of 8.3, at a sufficiently high zinc buffering capacity of 1 mM (33). NTA forms 1:1 and 2:1 complexes with zinc (34). Including the 2:1 complex in the calculations does not change the free zinc(II) ion concentrations significantly. Both TCEP and NTA are also a source of zinc with 16 and 5 ppb zinc at 244 and 76 nM, respectively. These concentrations were considered in the calculations of free zinc(II) ion concentrations.

**PTP Inhibition**—3,6-Fluorescein diphosphate was used as a PTP substrate because it allows assayng the enzyme at sub-
nanomolar concentrations. Using this substrate and the optimized assay conditions, we found that zinc inhibits RPTPβ/H9252 with a Ki(app) value of 98 ± 9 pM (Fig. 1A). The inhibition is 5 orders of magnitude stronger than previously reported (50% inhibition between 1 and 25 μM zinc) (25). The zinc inhibition is reversible as adding EDTA to the assay restores RPTPβ activity (Fig. 1B).

The enzyme follows Michaelis-Menten kinetics with a K_m value of 0.21 ± 0.02 μM for FDP and a V_max value of 27.5 ± 0.8 nM/min (Fig. 2A). If substrate competes with zinc binding, the kinetically determined inhibition constant at a fixed substrate concentration (Fig. 1A) reflects an apparent K_i only. Therefore, a kinetic analysis of zinc inhibition was performed for RPTPβ by varying the substrate concentration below and above the K_m value for FDP. Zinc indeed competes with substrate. Hence the zinc inhibition constant in the absence of substrate is even lower than the estimated value of 98 pm determined at about 4K_m (FDP). Analysis of the data in the form of a Dixon plot (Fig. 2B) gives a K_i value of 21 ± 7 pm. The Dixon plot does not discriminate between purely competitive and mixed inhibition. When we analyzed the data with the SigmaPlot kinetics program, both competitive and mixed (intersecting, linear non-competitive) inhibition passed the statistical tests. In both models, the slope effect, representative of the substrate competing with the inhibitor for the enzyme, is virtually the same (K_i values of 21 and 28 pm). The small intercept effect reflecting inhibitor binding to the enzyme-substrate complex with a 14-fold weaker affinity needs confirmation with other substrates. In summary, using enzymatic activity has been the only feasible approach to investigating zinc inhibition of PTPs, but it has the inherent limitation that the substrate affects the measured zinc inhibition. Only a full kinetic analysis provides the correct inhibition constant for zinc(II) ions.

DISCUSSION

Zinc inhibits PTPs much more tightly than generally appreciated. Methodological issues have precluded observing the tight zinc inhibition of these important enzymes. To maintain activity, they are prepared and supplied with high concentrations of chelating agents, such as EDTA, reducing agents, such as DTT and/or glutathione, and pH buffers, such as Tris, all of which have metal-chelating capacity. These conditions are not
conducive for investigating metal binding. Critical for studies of metal inhibition is the use of buffers and a reducing agent with low zinc-complexing capacity and control of metal ion concentrations by metal buffering. When controlling these parameters, using a sensitive fluorometric assay, and performing a kinetic analysis of the inhibition, a remarkably strong zinc inhibition of RPTPβ is revealed.

The strong zinc inhibition indicates physiological rather than pathophysiological or toxicological significance with regard to recent studies on cellular free zinc(II) ion concentrations and their regulation (10, 13). The distinction between free and total zinc concentrations is not widely appreciated, and many investigations claim physiological significance at micromolar zinc(II) ion concentrations because total cellular zinc concentrations are a few hundred micromolar. However, this concentration of total cellular zinc is strongly buffered by cytosolic proteins binding zinc with high affinity, and thus, the free zinc(II) ion concentration and not the total zinc concentration is the important parameter to be considered for physiological significance. Investigators have employed different experimental conditions, zinc-chelating fluorophores, and methods, such as quantitation with a fluorescence microscope or a spectrofluorometer, and demonstrated that free zinc(II) ion concentrations in various cultured cells are in the range of tens to hundreds of picomolar (10–12, 14). Critical for the physiological significance of zinc regulation of PTPs is the recent interest in a role of cellular zinc(II) ions in information transfer and regulation. Free zinc(II) ions fluctuate in the picomolar range of concentrations, and these fluctuations are influenced by various physiological stimuli (13, 35–37).

RPTPβ is a receptor-type PTP, also known as also known as vascular endothelial PTP (VEPTP), that controls the phosphorylation state of the angiopoietin receptor Tie-2. Its expression is restricted to endothelial cells (38, 39). Mice lacking the RPTPβ gene die on embryonic day 10 because of effects on angiogenesis in the yolk sac and in the embryo, but not on vasculogenesis (39, 40). It is well known that zinc is important for wound healing, but the molecular mechanism is not known (41). The results indicate a molecular basis for zinc in controlling growth arrest and maintenance of quiescent cells in angiogenesis, which occurs in the proliferative phase of wound healing. The only other instance where such a strong zinc inhibition has been observed is erythrocyte Ca$^{2+}$-ATPase, which zinc inhibits with an IC$_{50}$ of 80 ps (42). Because the free zinc(II) ion concentration in erythrocytes is 24 pm and indeed in the range of this inhibition (43), the data also suggest physiological zinc inhibition of an enzyme that is not known to be a zinc enzyme.

Zinc inhibition of PTPs within the range of resting zinc(II) ion concentrations in the cell raises the possibility that zinc is a tonic inhibitor of some of these enzymes. Such inhibitory control has been postulated as one of the main functions of zinc in biology (44). A way of activating zinc-inhibited enzymes is the reaction with thionein, the apo-form of metallothionein (17). The reox state of the cell is one of the main regulators of zinc loading onto metallothionein. Given that cellular zinc(II) ions are not freely available, the induction, extensive regulation, and changing zinc load make metallothionein/thionein a candidate for controlling zinc activation and zinc inhibition of enzymes (45).

The structural basis of zinc inhibition of PTPs is not known. It could involve the sulfhydryl group of the catalytic cysteine (residue 1094 in RPTPβ) as a ligand for zinc, the general acid/base aspartate (residue 1870), and/or an adjacent histidine (residue 1871). The latter two residues are on the conformationally flexible WPD loop (46).

Zinc ions are much better inhibitors than the classic vanadium complexes or any other drug. Experimental drugs bind with micromolar affinity, but exploitation of another substrate-binding site has resulted in nanomolar inhibition (47). Proper design of the ligand environment of zinc complexes or modulation of cellular zinc availability could provide additional avenues in the intensive ongoing efforts to develop therapeutic drugs for these important enzymes. The remarkable zinc inhibition also raises questions of how perturbed cellular zinc homeostasis affects major cellular signaling pathways.

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REFERENCES

1. Fischer, E. H. (1999) Cell signaling by protein tyrosine phosphorylation. Adv. Enzyme Regul. 39, 359–369
2. Alonso, A., Sasin, J., Bottini, N., Friedberg, I., Friedberg, I., Osterman, A., Godzik, A., Hunter, T., Dixon, J., and Mustelin, T. (2004) Protein-tyrosine phosphatases in the human genome. Cell 117, 699–711
3. Yip, S. C., Saha, S., and Cherrnoff, J. (2010) PTP1B: a double agent in metabolism and oncogenesis. Trends Biochem. Sci. 35, 442–449
4. Salmeen, A., Andersen, J. N., Myers, M. P., Meng, T. C., Hinks, J. A., Tonks, N. K., and Barford, D. (2003) Redox regulation of protein-tyrosine phosphatase 1B involves a sulfenyl amide intermediate. Nature 423, 769–773
5. van Montfort, R. L., Congreve, M., Tisi, D., Carr, R., and Ihoti, H. (2003) Oxidation state of the active-site cysteine in protein-tyrosine phosphatase 1B. Nature 423, 773–777
6. Chen, C. Y., Willard, D., and Rudolph, J. (2009) Redox regulation of SH2 domain-containing protein-tyrosine phosphatases by two backdoor cysteines. Biochemistry 48, 1399–1409
7. Tsai, S. J., Sen, U., Zhao, L., Greenleaf, W. B., Dasgupta, J., Fiorillo, E., Orrü, V., Bottini, N., and Chen, X. S. (2009) Crystal structure of the human lymphoid tyrosine phosphatase catalytic domain: insights into redox regulation. Biochemistry 48, 4838–4845
8. Brandão, T. A., Hengge, A. C., and Johnson, S. J. (2010) Insights into the reaction of protein-tyrosine phosphatase 1B: crystal structures for transition state analogs of both catalytic steps. J. Biol. Chem. 285, 15874–15883
9. Brautigan, D. L., Bornstein, P., and Gallis, B. (1981) Phosphotyrosyl-protein phosphatase: specific inhibition by Zn. J. Biol. Chem. 256, 6519–6522
10. Krezel, A., and Maret, W. (2006) Zinc-buffering capacity of a eukaryotic cell at physiological pZn. J. Biol. Inorg. Chem. 11, 1049–1062
11. Bozym, R. A., Thompson, R. B., Stoddard, A. K., and Fierke, C. A. (2006) Measuring picomolar intracellular exchangeable zinc in PC-12 cells using a ratiometric fluorescence biosensor. ACS Chem. Biol. 1, 103–111
12. Vinkenborg, J. L., Nicolson, T. J., Bellomo, E. A., Koay, M. S., Rutter, G. A., and Merkx, M. (2009) Genetically encoded FRET sensors to monitor intracellular Zn$^{2+}$ homeostasis. Nat. Methods 6, 737–740
13. Li, Y., and Maret, W. (2009) Transient fluctuations of intracellular zinc ions in cell proliferation. Exp. Cell Res. 315, 2463–2470
14. Qin, Y., Dittmer, P. J., Park, J. G., Jansen, K. B., and Palmer, A. E. (2011) Measuring steady-state and dynamic endoplasmic reticulum and Golgi
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Zn2+ with genetically encoded sensors. Proc. Natl. Acad. Sci. U.S.A. 108, 7351–7356
15. Ho, Y., Samarasinge, R., Knoch, M. E., Lewis, M., Aizenman, E., and DeFranco, D. B. (2008) Selective inhibition of mitogen-activated protein kinase phosphatases by zinc accounts for extracellular signal-regulated kinase 1/2-dependent oxidative neuronal cell death. Mol. Pharmacol. 74, 1141–1151
16. Samet, J. M., and Tal, T. L. (2010) Toxicological disruption of signaling homeostasis: tyrosine phosphatases as targets. Annu. Rev. Pharmacol. Toxicol. 50, 215–235
17. Maret, W., Jacob, C., Vallee, B. L., and Fischer, E. H. (1999) Inhibitory sites of the combined insulinomimetic effects of zinc and oxidants. Proc. Natl. Acad. Sci. U.S.A. 96, 1936–1940
18. Zander, N. F., Lorenzen, J. A., Cool, D. E., Tonks, N. K., Daum, G., Krebs, E. G., and Fischer, E. H. (1991) Purification and characterization of a human recombinant T-cell protein-tyrosine phosphatase from a baculovirus expression system. Biochemistry 30, 6964–6970
19. Haase, H., and Maret, W. (2003) Intracellular zinc fluctuations modulate protein-tyrosine phosphatase activity in insulin/insulin-like growth factor-1 signaling. Exp. Cell Res. 291, 289–298
20. Krezel, A., and Maret, W. (2008) Thionein/metallothionein control Zn(II) availability and the activity of enzymes. J. Biol. Inorg. Chem. 13, 401–409
21. Hogstrand, C., Kille, P., Nicholson, R. L., and Taylor, K. M. (2009) Zinc transporters and cancer: a potential role for ZIP7 as a hub for tyrosine kinase activation. Trends Mol. Med. 15, 101–111
22. Coulston, L., and Danda, P. (1980) Insulin-like effect of zinc on adipocytes. Diabetes 29, 665–667
23. Haase, H., and Maret, W. (2005) Protein-tyrosine phosphatases as targets of the combined insulinomimetic effects of zinc and oxidants. BioMetals 18, 333–338
24. Sindicu, C., Palmiter, R. D., and Storm, D. R. (2011) Zinc transporter ZnT-3 regulates presynaptic Erk1/2 signaling and hippocampus-dependent memory. Proc. Natl. Acad. Sci. U.S.A. 108, 3366–3370
25. Harder, K. W., Owen, P., Wong, L. K., Aebersold, R., Clark-Lewis, I., and Jirik, F. R. (1994) Characterization and kinetic analysis of the intracellular domain of human protein-tyrosine phosphatase β (HPTPβ) using synthetic phosphopeptides. Biochem. J. 298, 395–401
26. Huang, Z., Wang, Q., Ly, H. D., Gorvindarajan, A., Scheigetz, J., Zamboni, R., Desmarais, S., and Ramachandran, C. (1999) 3,6-Fluorescein diphosphosphate: a sensitive fluorogenic and chromogenic substrate for protein-tyrosine phosphatases. J. Biomol. Screen. 4, 327–334
27. Bers, D. M., Patton, C. W., and Nuccitelli, R. (2010) A practical guide to the preparation of Ca2+ buffers. Methods Cell Biol. 99, 1–26
28. Zot, T., and Selinger, Z. (1996) Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. Anal. Biochem. 236, 302–308
29. Krezel, A., Lesniak, W., Jezowska-Bojczuk, M., Mlynarz, P., Brusañ, J., Kozlowski, H., and Bal, W. (2001) Coordination of heavy metals by dithiothreitol, a commonly used thiol group protectant. J. Inorg. Biochem. 84, 77–88
30. Zhao, Z., Bouchard, P., Diltz, C. D., Shen, S. H., and Fischer, E. H. (1993) Purification and characterization of a protein-tyrosine phosphatase containing SH2 domains. J. Biol. Chem. 268, 2816–2820
31. Krezel, A., Latajka, R., Bujacz, G. D., and Bal, W. (2003) Coordination properties of tris(2-carboxyethyl)phosphine, a newly introduced thiol reductant, and its oxide. Inorg. Chem. 42, 1994–2003
32. Anwar, Z. M. (2005) Complexation equilibria of Zn(II), Pb(II), and Cd(II) with reduced glutathione (GSH) and biologically important zwitterionic buffers. J. Chin. Chem. Soc. 52, 863–871
33. Krezel, A., and Maret, W. (2007) Dual nanomolar and picomolar Zn(II) binding properties of metallothionein. J. Am. Chem. Soc. 129, 10911–10921
34. Rabenstein, D. L., and Kula, R. J. (1969) Ligand-exchange kinetics and solution equilibria of cadmium, zinc, and lead nitrilotriacetate. J. Am. Chem. Soc. 91, 2492–2503
35. Yamasaki, S., Sakata-Sogawa, K., Hasegawa, A., Suzuki, T., Kabu, K., Sato, E., Kurotski, T., Yamashita, S., Tokunaga, M., Nishida, K., and Hirano, T. (2007) Zinc is a novel intracellular second messenger. J. Cell Biol. 177, 637–645
36. Taylor, K. M., Vichova, P., Jordan, N., Hiscox, S., Hendley, R., and Nicholson, R. I. (2008) ZIP7-mediated intracellular zinc transport contributes to aberrant growth factor signaling in anthromone-resistant breast cancer cells. Endocrinology 149, 4912–4920
37. Haase, H., Ober-Blöbaum, J. L., Engelhardt, G., Hebcl, S., Heit, A., Heine, H., and Rink, L. (2008) Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. J. Immunol. 181, 6491–6502
38. Fachinger, G., Deutsch, U., and Risau, W. (1999) Functional interaction of vascular endothelial protein-tyrosine phosphatase with the angiopoietin receptor Tie-2. Oncogene 18, 5948–5953
39. Bäumer, S., Keller, L., Holtmann, A., Funke, R., August, B., Gamp, A., Wolburg, H., Wolburg-Buchholz, K., Deutsch, U., and Vestweber, D. (2006) Vascular endothelial cell-specific phosphotyrosine phosphatase (VE-PTP) activity is required for blood vessel development. Blood 107, 4754–4762
40. Dominguez, M. G., Hughes, V. C., Pan, L., Simmons, M., Daly, C., Anderson, K., Nogueru-Troise, I., Murphy, A. J., Valenzuela, D. M., Davis, S., Thurston, G., Yancopoulos, G. D., and Gale, N. W. (2007) Vascular endothelial tyrosine phosphatase (VE-PTP) null mice undergo vasculogenesis but die embryonically because of defects in angiogenesis. Proc. Natl. Acad. Sci. U.S.A. 104, 3243–3248
41. Jansen, J., and Rink, L. (2011) in Zinc in Human Health (Rink, L., ed) pp. 514–529, IOS Press, Amsterdam, The Netherlands
42. Hogstrand, C., Verbost, P. M., and Wendelaar Bonga, S. E. (1999) Inhibition of human erythrocyte Ca2+-ATPase by Zn2+. Toxicology 133, 139–145
43. Simons, T. J. (1991) Intracellular free zinc and zinc buffering in human red blood cells. J. Membr. Biol. 123, 63–71
44. Williams, R. J. (1984) Zinc: what is its role in biology? Endeavour 8, 65–70
45. Maret, W. (2011) Redox biochemistry of mammalian metallothioneins. J. Biol. Inorg. Chem. 16, 1079–1086
46. Evdokimov, A. G., Pokross, M., Walter, R., Mekel, M., Cox, B., Li, C., Bechard, R., Genbaufe, F., Andrews, R., Diven, C., Howard, B., Rastogi, V., Gray, J., Maier, M., and Peters, K. G. (2006) Engineering the catalytic domain of human protein-tyrosine phosphatase β for structure-based drug discovery. Acta Crystallogr. D Biol. Crystallogr. 62, 1435–1445
47. Barr, A. J. (2010) Protein-tyrosine phosphatases as drug targets: strategies and challenges of inhibitor development. Future Med. Chem. 2, 1563–1576