BioMetals (2005) 18:333–338
DOI 10.1007/s10534-005-3707-9

Protein tyrosine phosphatases as targets of the combined insulinomimetic effects of zinc and oxidants

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Key words: diabetes, insulin signaling, PTP 1B, redox, zinc

Abstract

Zinc ions have an insulin-like (insulinomimetic) effect. A particularly sensitive target of zinc ions is protein tyrosine phosphatase 1B (PTP 1B), a key regulator of the phosphorylation state of the insulin receptor. Modulation of insulin signaling by zinc chelating agents and the recognition of temporal and spatial fluctuations of zinc suggest a physiological role of zinc in insulin signal transduction. Tyrosine phosphatases seem to be regulated jointly by insulin-induced redox (hydrogen peroxide) signaling, which results in their oxidative inactivation, and by their zinc inhibition after oxidative zinc release from other proteins. In diabetes, the significant oxidative stress and associated changes in zinc metabolism modify the cell's response and sensitivity to insulin. Zinc deficiency activates stress pathways and may result in a loss of tyrosine phosphatase control, thereby causing insulin resistance.

Introduction

In 1966, Quarterman and colleagues concluded that "zinc-deficient animals are much less sensitive to insulin" and "that there is an, as yet, undefined function of zinc involved" (Quarterman *et al.* 1966). Subsequent work attempted to define this function. Because most insulin preparations contain zinc and because insulin is stored as a zinc complex in the pancreatic β-cells, it was tested whether or not zinc potentiates the effect of the hormone.

Indeed, zinc has a potent stimulatory effect on lipogenesis (Coulston & Dandona 1980). This insulin-like (insulinomimetic) effect was thought to be mediated by extracellular hydrogen peroxide formation and by zinc inhibition of intracellular glutathione reductase (May & Contoreggi 1982). Zinc affects lipogenesis more noticeably in chemically-induced diabetes than in normal tissue (Shisheva *et al.* 1992), and it alleviates the hyperglycemia of ob/ob (leptin-deficient) (Chen *et al.* 1998) and db/db (leptin receptor-deficient) (Simon & Taylor 2001) genetically obese mice. Zinc increases the phosphorylation state of the insulin receptor and hence protein phosphorylation downstream in its signaling pathways (Tang & Shay 2001). In the following report, protein tyrosine phosphatases (PTPs) are discussed as one molecular target of the insulinomimetic effect of zinc. It is suggested that zinc has a regulatory role in phosphorylation signaling of insulin, and that perturbation of cellular zinc homeostasis is at least one cause for insulin resistance.

Receptor phosphorylation as the central event in insulin signal transduction

Tyrosine phosphorylation of the insulin receptor is the central switch in the activation of insulin-dependent signals. Binding of insulin to the

extracellular α-chains of the receptor induces receptor autophosphorylation on its cytosolic tyrosines 1158, 1162, and 1163 of the β-chains. Phosphorylation creates a binding interface for adaptor molecules such as the IRS 1–4 (insulin receptor substrate) proteins, which become phosphorylated themselves through the kinase activity of the insulin receptor and recruit proteins with SH2 (Src homology 2) domains. The assembled multiprotein complex then relays the signal to different pathways such as a pathway for translocation of a transporter for glucose uptake and the protein kinase Akt and mitogen-activated protein (MAP) kinase pathways for metabolic and growth effects (Figure 1).

PTPs and insulin receptor dephosphorylation

Protein tyrosine phosphatases (PTPs) regulate the phosphorylation state of tyrosines by dephosphorylation. The conclusion that PTP 1B is the key phosphatase that dephosphorylates the insulin receptor (Kenner *et al.* 1996; Byon *et al.* 1998) is supported by *in vivo* data from PTP 1B knock-out mice (Elchebly *et al.* 1999). To emphasize the significance of these studies, we cite from a recent review (Ross *et al.* 2004): 'Mice homozygous for the gene-knock-out were generally healthy with normal weight gain and food intake and had plasma glucose levels after feeding that were 13% lower than those of controls. They exhibited increased insulin sensitivity as verified by glucose and insulin tolerance tests. The knockout mice also demonstrated a decreased tendency toward obesity when placed on a high-fat diet.... Therefore, therapeutic intervention via small molecule inhibitors of PTP 1B would appear to be a promising treatment option.... Although numerous reports have appeared describing small molecule inhibitors of PTP 1B, none have yet to emerge from phase III clinical testing.' The significance of PTP 1B in controlling insulin sensitivity (Johnson *et al.* 2002) is further supported by the observation of insulin resistance in mice that overexpress PTP 1B (Zabalotny *et al.* 2004) and by increased insulin sensitivity when diabetic mice are treated with PTP 1B antisense oligonucleotides (Zinker *et al.* 2002). These findings have directed attention to PTP 1B as a major drug target for the therapy of diabetes.

Regulation of PTPs by phosphorylation and redox

Regulatory networks superimposed on PTPs, referred to as 'phosphatase cascades' (Alonso *et al.* 2004), afford additional control of insulin signaling. Phosphatases are not dephosphorylating their substrates continuously. Dephosphorylation is tightly controlled by mechanisms that are at least as complex as the ones that control phosphorylation (Fischer 1999). At least three molecular mechanisms regulate PTP activity post-translationally and shape the insulin signal. First, phosphatases can be regulated by their phosphorylation. Upon interaction with the insulin receptor, tyrosine phosphorylation of PTP 1B (Bandyopadhyay *et al.* 1997) increases its catalytic activity (Dadke *et al.* 2001). Phosphorylation of PTP 1B by several other kinases such as PKC on Ser-378 (Flint *et al.* 1993) or by kinases of the CLK family on Ser-50 (Moeslein *et al.* 1999) establishes crosstalk between insulin signaling and other signaling pathways. Second, *in vitro*, hydrogen peroxide oxidizes the sulfhydryl group of the catalytic cysteine of PTP 1B reversibly to a sulfenic acid or irreversibly to either a sulfinic or a sulfonic acid. Which oxidant is responsible for this conversion *in vivo* is unknown at present. A case has been made for reversible regulation by *S*-nitrosothiols in intact cells (Li & Whorton 2003). The reversible oxidation to a sulfenic acid seems to be the predominant reaction, because a covalent cyclic sulfenyl amide intermediate between the sulfur atom of Cys-215 and the mainchain amide of the neighboring Ser-216 protects the cysteine from further oxidation (Salmeen *et al.* 2003; van Montfort *et al.* 2003). Insulin attenuates its own signal by modulating PTP 1B activity (Cheng *et al.* 2002). One mechanism is the insulin-stimulated production of cellular hydrogen peroxide (Mahadev *et al.* 2001), which enhances the insulin signal and establishes a feedback loop by inactivating PTP 1B. The pathway of generating reactive oxygen species includes the NADPH oxidase Nox4 and the G protein $G_{\alpha i2}$ (Krieger-Brauer *et al.* 1997; Mahadev *et al.* 2004) (Figure 1).

Zinc inhibition of PTPs

Third, the enzymatic activity of PTP 1B is inhibited reversibly by zinc ions (Haase & Maret 2003). It has

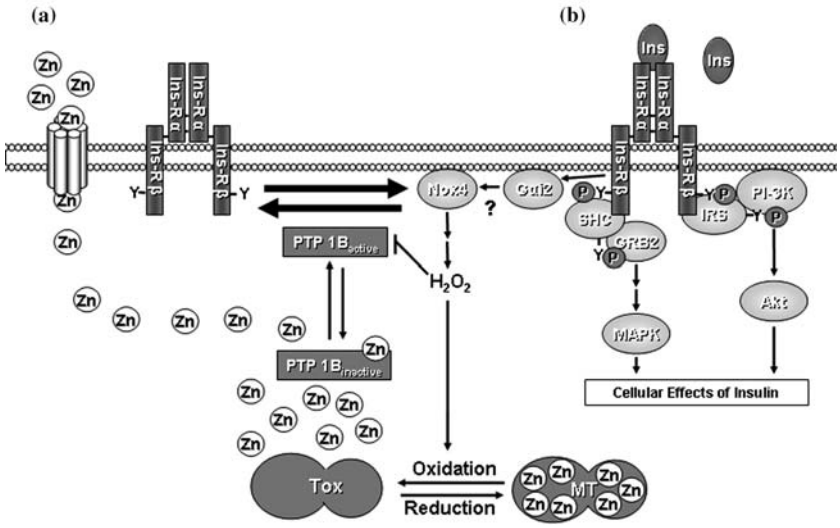

Figure 1. Zinc and redox modulation of insulin phosphorylation signaling. (a) Extracellular zinc enters the cell and inhibits PTP 1B, thus increasing net phosphorylation of the insulin receptor and enhancing activation of downstream pathways such as MAP, PI-3 and Akt kinases. (b) Insulin binding to its receptor induces autophosphorylation, assembly of multiprotein complexes with adaptor proteins such as IRS and SHC/GRB2, and activation of insulin signaling cascades. Through a pathway involving a G-protein and the oxidase Nox4, insulin induces the production of hydrogen peroxide. The hydrogen peroxide can oxidize PTP 1B or release protein-bound zinc from proteins such as metallothionein (MT). In this process oxidized thionein (Tox) is formed. Both pathways inactivate PTPs and attenuate the insulin signal.

long been known that zinc inhibits PTPs, but the reported inhibition was in the micromolar range (10 μM) (Brautigan *et al*. 1981). Such zinc concentrations have toxicological relevance as demonstrated for zinc inhibition of PTP 1B in airway epithelial cells (Samet *et al*. 1999). Possible physiological relevance for the zinc interaction was established only recently when the concentration range of available zinc and the much tighter zinc inhibition of PTP 1B were defined. The concentration of available cellular zinc is in the nanomolar or even picomolar range (Beyersmann & Haase 2001) and much lower than total cellular zinc, which is in the range of a few hundred micromolar (Arslan *et al*. 1985; Palmiter & Findley 1995). Zinc inhibition of PTPs occurs in this low range of available zinc. Thus, T-cell PTP is inhibited 50% at 200 nM zinc (Maret *et al*. 1999), while PTP 1B has an even lower IC_{50} value of 17 nM (Haase & Maret 2003). Because nanomolar concentrations of zinc inhibit a truncated form of SHP-2 that contains only the catalytic domain (Haase & Maret 2003), this highly conserved domain (Alonso *et al*. 2004) must contain the inhibitory zinc binding site. Experiments in cell cultures provide further evidence for a physiological role of zinc in modulating PTP activity. Incubation of cells with zinc increases tyrosine phosphorylation of the insulin receptor and this effect is mediated by inhibition of its dephosphorylation rather than enhanced phosphorylation (Haase & Maret 2003). In order to determine whether there is enough cellular zinc available to inhibit PTPs, a zinc-specific fluorophore, FluoZin-3, was employed. Owing to its zinc binding constant of 15 nM, this chelating fluorophore detects nanomolar concentrations of zinc in C6 rat glioma cells, suggesting that zinc is indeed available for PTP inhibition *in vivo* (Haase & Maret 2003). When the concentration of intracellular, available zinc is lowered with a membrane-permeable zinc-specific chelating agent, phosphorylation of the insulin receptor decreases. These experiments confirm that under normal conditions of cell culture zinc inhibits PTP 1B (and some other PTPs) partially and affects the phosphorylation state of the insulin receptor and insulin signal transduction.

Zinc as a potential physiological regulator of insulin signal transduction

The concentration of cellular, available zinc is not constant, but fluctuates under various conditions. Such 'zinc transients' in space and time provide a possible mechanism of regulating phosphotyrosine

signals. Zinc fluctuations are controlled by zinc buffering systems, and one system comprises metallothionein (MT) and its apoprotein thionein (T), which are both present in most cells (Yang *et al*. 2001). MT-null mice with targeted disruptions of the MT-1 and MT-2 genes become mildly obese and develop hyperinsulinemia (Beattie *et al*. 1998). The cysteines that bind zinc in MT are redox-sensitive and confer redox control on the availability of zinc (Maret & Vallee 1998). Under oxidative conditions, MT releases its zinc and forms the oxidized apoprotein (Haase & Maret 2004). Reductants restore the zinc-binding capacity of T (Chen & Maret 2001), and T has the capacity to remove the inhibitory zinc and to re-activate zinc-inhibited PTP *in vitro* (Maret *et al*. 1999). Thus, zinc inhibition of PTP can be modulated by redox reactions that affect other proteins such as MT and T (Figure 1). Remarkably, there seems to be crosstalk between insulin and MT via both redox reactions and induction of MT by insulin (Imbra & Karin 1987). First, hydrogen peroxide itself is insulinomimetic (Czech *et al*. 1974) and releases zinc from MT *in vitro* (Quesada *et al*. 1996). The insulin-stimulated hydrogen peroxide could increase the availability of cellular zinc *in vivo* and inhibit PTPs. Second, on a longer time scale, insulin induces T, which re-activates PTPs by removing the inhibitory zinc. In this way, the zinc homeostatic protein MT might establish feedback regulation of the insulin signal. Future experiments need to address the relative significance of redox versus zinc modulation of PTPs. Zinc binding at the active site of PTP may protect the enzyme against reversible or irreversible oxidation and re-direct the redox signal to other targets. Alternatively, if zinc binds at another site in the catalytic domain, PTPs might be regulated by both zinc and redox. Considering the tight binding of zinc and ligand selectivity of zinc coordination sites, the 'zinc signal' would seem to provide higher specificity than hydrogen peroxide, which causes wide-spread sulfenic acid formation (Saurin *et al*. 2004).

Hypothesis: Development of insulin resistance from local zinc deficiencies

Since both massive zincuria (Chausmer 1998) and oxidative stress (Robertson 2004) are a hallmark of diabetes, it occurred to us that oxidative stress-induced zinc release could result in a cellular zinc deficiency in insulin-responsive tissue. The ensuing constitutive activation of PTPs could be one mechanism of how insulin resistance develops. Constitutive activation of tyrosine phosphatases in a zinc-deficient diabetic state would explain the observed shift from tyrosine to serine phosphorylation of IRS-1 in diabetes, which has been suggested as a general pathway of how insulin resistance develops (Le Marchand-Brustel *et al*. 2003). Zinc deficiency also elicits an oxidative stress that activates stress pathways (Oteiza *et al*. 2000). Both activation of stress pathways and modulation of enzymes involved in cellular redox control result in insulin resistance (McClung *et al*. 2004; Nakatani *et al*. 2004; Xie *et al*. 2004). In any event, zinc and redox stress are interdependent in such a way that nutritional or conditional zinc deficiency and oxidative stress are potential etiological factors for diabetes. Reducible selenium compounds, which also have insulinomimetic effects (McNeill *et al*. 1991) and release zinc from MT (Jacob *et al*. 1998), may exert their effects via zinc modulation of PTPs. They could be an alternative or complement to chromium and vanadium compounds that effect insulin signaling (Hulley & Davison 2003; Anderson 2003), and yet other inorganic micronutrients. In addition to the significant potential for prevention, approaching zinc dysregulation through specific nutritional intervention is a cost-saving alternative to multibillion dollar investments to find drugs that modulate PTP 1B activity.

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