Inhibition of glycogen synthase kinase-3β by bivalent zinc ions: insight into the insulin-mimetic action of zinc

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Abstract

Zinc is an important trace element found in most body tissues as bivalent cations and has essential roles in human health. The insulin-like effect of zinc cations raises the possibility that they inhibit glycogen synthase kinase-3β (GSK-3β), a serine/threonine protein kinase linked with insulin resistance and type 2 diabetes. Here we show that physiological concentrations of zinc ions directly inhibit GSK-3β in vitro in an uncompetitive manner. Treatment of HEK-293 cells with zinc enhanced glycogen synthase activity and increased the intracellular levels of β-catenin, providing evidence for inhibition of endogenous GSK-3β by zinc. Moreover, zinc ions enhanced glucose uptake 3-fold in isolated mouse adipocytes, an increase similar to activation with saturated concentrations of insulin. We propose that the in vivo insulin-mimetic actions of zinc are mediated via direct inhibition of endogenous GSK-3β.

Keywords: Glycogen synthase kinase-3; Zinc ions; Insulin signaling

Zinc is a transition metal found in most body tissues as bivalent cations. Zinc ions are essential supplement in human diet and their involvement in the regulation of numerous cellular processes is well documented (reviewed in [2,14,19]). Zinc deficiency may lead to impaired immune functions, developmental abnormalities, and alterations in carbohydrate metabolism [2]. Human plasma normally contain about 15 μM Zn²⁺ [3], most of it bound to proteins, but some tissues such as muscle and liver contain much higher concentrations [2,14,19]. Still, the molecular mechanism of zinc-induced cellular responses is not fully understood.

One of the remarkable in vivo features of zinc is its insulin-like function and its potential link with insulin resistance and type 2 diabetes. Zinc was shown to stimulate lipogenesis and glucose transport in adipocytes [7,12,20] and its supplementation in diet attenuated hyperglycemia in db/db mice [23]. In zinc-deficient rats, glucose tolerance was abnormal and could be reversed by repletion of the metal ions. Finally, lower serum zinc concentrations were detected in type 2 diabetic patients compared to healthy subjects [1,4], suggesting that zinc deficiency may be linked with insulin resistance.

Glycogen synthase kinase-3 is a serine/threonine kinase that was originally discovered by its ability to phosphorylate and inhibit the enzyme glycogen synthase [6,27]. It was first cloned in 1990 and shown to exist as two isoforms, α and β, sharing 98% homology in their catalytic domain and similar biochemical (but not identical) properties [29]. In recent years, it was realized that GSK-3 is a key component of intracellular signaling, and that it differs from other known intracellular protein kinases by being constitutively active in resting cells, and inhibited upon cell activation by extracellular signals (reviewed in [13,15]). It was also shown that GSK-3 activity is negatively regulated by insulin [8,28]. Thus, GSK-3 inhibition might be essential for normal function of the insulin-activated signaling pathway. Further support came from studies showing that GSK-3β limits insulin receptor-mediated signaling via the phosphorylation of its substrate IRS-1 [10]. The involvement of abnormal GSK-3β activity in type 2 diabetes was demonstrated in fat tissue of obese diabetic...
mice [11] and in skeletal muscle of type 2 diabetes patients, in which GSK-3 α and β activities were significantly higher than in healthy subjects [21]. This could fit well with the notion that elevated activity of GSK-3 presumably contributed to impairment of insulin action.

Here we report that GSK-3β is inhibited by physiological concentrations of Zn2+ and that their addition to cells produces intracellular effects similar to those achieved by GSK-3β inhibition. We propose that at least some of the in vivo actions of zinc, in particular, those related to its insulin-mimetic action, are mediated via inhibition of endogenous GSK-3β.

Materials and methods

In vitro assays. Purified recombinant rabbit GSK-3β [9] was incubated with peptide substrates PGS-1 (YRRAVPSPSLRHISS PSQS(p)EDEEE) together with ZnCl2 at the indicated concentrations. The reaction mixture included 50 mM Tris-HCl, pH 7.3, 10 mM MgAc, [γ-32P]ATP (100 μM), and 0.01% β-mercaptoethanol and was incubated for 10 min at 30 °C. Reactions were spotted on phosphocellulose paper (p81), washed with 100 mM phosphoric acid, and counted for radioactivity as described [9]. For the initial velocity experiments, the enzyme was incubated with various concentrations of the p9CREB peptide substrate [26] with different concentrations of GSK-3 was determined the p9CREB peptide substrate [26] with different concentrations of GSK-3 was determined (Fig. 2). Zn2+ concentrations without substrate. 3P intensity was measured by exposure of polycrylamide gel to PhosphoImager SI (Molecular Dynamics). The effect of Zn2+ on cdc2 kinase (New England Bio-labs, MA, USA) was tested. Cdc2 (1 U) was incubated with indicated concentrations of Zn2+. Histone H1 (Calbiochem, CA, USA) substrate (5 μg), and reaction mixture containing [γ-32P]ATP (Amersham, NJ, USA). The reactions were boiled with SDS sample buffer, separated on gel electrophoresis, and autoradiographed.

β-catenin analysis. HEK 293 cells grown in 10 cm plates were treated with the indicated concentrations of ZnCl2 for 3 h. The cells were collected and extracted with buffer G (20 mM Tris, pH 7.3, 10 mM β-glycerophosphate, 10% glycerol, 1 mM EGTA, 1 mM EDTA, 50 mM NaF, 5 mM NaPPI, 25 μg/mL leupeptin, 25 μg/mL aprotinin, 500 mM micromolar, and 1% Triton X-100). Cell extracts were subjected to gel electrophoresis, followed by immunoblot analysis with monoclonal antibodies directed against β-catenin or GSK-3β (Transduction Laboratories, KY, USA).

Glycogen synthase assays. Glycogen synthase activity was assayed according to the method of Thomas et al. [25] and based on the incorporation of uridine 5′-diphosphate[14C]glucose (UDPG) into glycogen. Cell lysates were prepared as previously described and aliquots of GSK-3 were incubated with indicated concentrations of Zn2+ for 1h, followed by addition of 2-deoxy-α-[3H]glucose (Amersham, NJ, USA, 0.5 μCi/vial) for 10 min. The assays were terminated by centrifugation of cells through dinitrophenol (ICN, CA, USA) and [3H] was quantitated by liquid scintillation analyzer (Packard).

Results

We tested the ability of Zn2+ ions to directly inhibit GSK-3β in vitro. The purified enzyme was incubated with various concentrations of ZnCl2 and its ability to phosphorylate PGS-1 peptide substrate was determined. As shown in Fig. 1A, Zn2+ inhibited purified GSK-3β at low concentrations with IC50 = 15 μM, similar to mean human serum physiological concentrations [3]. We next examined whether Zn2+ ions inhibit the autocatalytic activity of GSK-3β. Autophosphorylation of GSK-3β was determined in the presence of varying Zn2+ concentrations (Fig. 1B). The results demonstrated that Zn2+ ions interact directly with the enzyme and inhibit its catalytic activity. We also tested the inhibitory effect of two divalent transition metal ions Co2+ and Mn2+ that have ionic radii similar to that of zinc on GSK-3β activity. Neither Co2+ nor Mn2+ inhibited GSK-3β at concentrations of 10, 20, 50, and 100 μM (data not shown). Thus, the inhibition of GSK-3β by Zn2+ represents distinct interaction between the protein kinase and zinc cations. In addition, we examined whether Zn2+ inhibits cyclin-dependent protein kinase-cdc2, which is closely related to GSK-3β. The enzyme was incubated with various concentrations of ZnCl2 and the phosphorylation of Histone H1 substrate was determined (Fig. 2). Zn2+ did not inhibit cdc2, indicating that the inhibition of GSK-3β by this metal ion is unique.

The kinetic nature of Zn2+ inhibition was studied by measuring the initial velocity as the function of substrate concentration at several Zn2+ concentrations. Line-weaver–Burk plot analyses (Fig. 3) indicate that Zn2+ ions act as uncompetitive inhibitors of GSK-3β, suggesting that they interact with the enzyme at a site distinct from its substrate recognition site. This uncompetitive nature is similar to that previously reported for inhibition of GSK-3β by lithium cations [16]. We next examined if exposure of intact cells to Zn2+ can produce consequence similar to GSK-3β inhibition. We examined the impact of Zn2+ on two known physiological substrates of GSK-3β: glycogen synthase and β-catenin. Inhibition of GSK-3β should increase glycogen synthase activity and lead to up-regulation of cytoplasmic β-catenin [22,30]. As shown in Fig. 4A, treatment of HEK 293 cells with 20 μM Zn2+ for 2 h enhanced glycogen synthase activity 2-fold. In addition, treatment of HEK 293 cells with Zn2+ increased cytoplasmic β-catenin levels as determined by Western blot analysis (Fig. 4B). These results support the notion that Zn2+ ions inhibit endogenous GSK-3β.
GSK-3β attenuates insulin signaling; thus, we hypothesized that Zn$^{2+}$ should promote glucose uptake via its inhibitory effect on GSK-3β. It was previously shown that zinc activates glucose uptake in rat adipocytes, albeit, at relatively high non-physiological concentrations (1 mM) [12]. We examined the ability of Zn$^{2+}$ to promote glucose uptake in mice adipocytes. Mice adipocytes were incubated with various concentrations of ZnCl$_2$ and glucose uptake was determined by the incorporation of 2-deoxy-D-$^3$H-glucose. As shown in Fig. 5, 10 μM Zn$^{2+}$ increased hexose uptake 3-fold compared with non-treated cells. This activation was similar to that achieved by an optimal concentration of insulin (10 nM, not shown). These studies also indicated that low concentrations of ZnCl$_2$ (10 μM) could activate glucose uptake in adipocytes. When we examined the effect of Zn$^{2+}$ on glucose uptake in two cell lines, C6 rat glioma and GP8 rat brain endothelial cells, that express predominantly the glucose transporter-1 (GLUT1), we found that Zn$^{2+}$ did not affect glucose transport in these cells (data not shown). These results suggested that Zn$^{2+}$-induced activation of glucose uptake in adipocytes...
Discussion

Zinc is involved in diverse cellular processes, both as an integral part of the active site of numerous enzymes (carboxypeptidases, metalloproteases, etc.) and as a regulatory factor. The insulin-mimetic function of zinc was demonstrated previously, though, the molecular target(s) modulated by zinc were not identified. In these studies, we undertook to investigate the role of zinc ions in the regulation of the protein kinase GSK-3β, a negative modulator of insulin action [10]. Here we show that zinc inhibits GSK-3β and suggest that its in vivo insulin-like effects are mediated via direct inhibition of endogenous GSK-3β.

The in vitro studies revealed that GSK-3β inhibition by physiological concentrations (~15 μM) of Zn²⁺ is specific. In intact cells, zinc produced consequences similar to those achieved by inhibition of GSK-3β, namely, increased cytoplasmic β-catenin expression and activation of glycogen synthase, supporting the notion that it inhibits endogenous GSK-3β. The fact that zinc-induced glucose uptake gives further support for the insulin-mimetic action of Zn²⁺ and suggests that the inhibition of GSK-3β may be essential for insulin-induced glucose uptake. Our results correlate with studies done with lithium, another ion that is also an inhibitor of GSK-3β. Similar to Zn²⁺, lithium ions were shown to increase β-catenin expression level, glycogen synthase activity, and glucose uptake in intact cells [5,24], suggesting a possible common mechanism for the insulin-mimetic action of these cations.

It is noteworthy that zinc deficiency, determined from its fasting serum concentrations, was reported for type 2 diabetes and for major depression patients [1,4,18]. The latter observation may be of particular clinical significance, since both lithium ions and valproic acid are widely used in the maintenance treatment of bipolar depression and shown to inhibit GSK-3β at therapeutic concentrations [16,31]. According to our present observations, it is plausible that inhibition of GSK-3β by dietary zinc could possibly allow clinical benefits similar to those achieved with lithium or valproic acid.

Altogether, the insulin-mimetic action of Zn²⁺ and the intriguing correlation between zinc deficiency in type 2 diabetes and in bipolar depression may be explained by its inhibitory action towards GSK-3β and may suggest a potential therapeutic value for dietary zinc supplementation in these chronic disorders.
References


