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Action mechanism of metallo-allixin complexes as antidiabetic agents*

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Abstract: The metabolic syndrome is a group of factors associated with an increased risk of atherosclerosis and diabetes. Diabetes mellitus (DM) is classified into 2 major types—type 1 DM and type 2 DM—characterized by chronic hyperglycemia resulting from defects in insulin secretion and insulin action, respectively. Several synthetic pharmaceuticals have been developed and clinically used for treating DM; however, these pharmaceuticals continue to cause side effects. Recently, we proposed that oxovanadium(IV) (vanadyl) and zinc(II) (zinc) complexes are potent antidiabetic agents for both type 1 and type 2 DM therapy. This article reviews the vanadyl- and zinc-allixin and their related complexes that are being currently developed as novel types of antidiabetic metal complexes, focusing on their action mechanism in terms of regulation of the insulin signaling pathway and inhibition of lipolysis signaling in adipocyte cells.

Keywords: metal complexes; diabetes; action mechanisms; vanadium; zinc.

INTRODUCTION

Cross-link between diabetes and trace elements

The worldwide prevalence of diabetes was estimated to rise approximately 4.4 % of the population or 366 million people in 2030 [1]. Diabetes mellitus (DM) is a syndrome of impaired glucose, lipid, and protein metabolisms caused by insulin dysfunction [2]. Insulin is an important hormone that regulates both glucose and lipid metabolism [2,3]. In peripheral tissues such as the liver, muscle, and adipose tissues, insulin acts on insulin receptors; this leads to the activation of downstream signaling molecules including insulin receptor substrate (IRS), phosphatidylinositol 3-kinase (PI3K), and Akt/protein kinase B (Akt/PKB) [3]. Akt/PKB is a key signal transduction molecule that regulates many proteins involved in glucose and lipid metabolism and transcription factors [4]. In particular, Akt/PKB activates the translocation of glucose transporter 4 (GLUT4) to the plasma membrane, resulting in the stimulation of the uptake of extracellular glucose [3,5]. In addition, Akt/PKB activates phosphodiesterase 3B (PDE3B), leading to the suppression of the intracellular cAMP concentration and PKA activity [6–9]; this in turn mediates the inhibition of lipolysis in the cells.

Deficiencies of trace elements are associated to chronic diseases [10]. Among chronic diseases, DM has been proposed that the essential trace elements, such as zinc, chromium, or manganese, are deficient [11–13]. Chronic hyperglycemia may cause alterations in the status of trace elements in the body.

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Therefore, trace elements may play important functions for glucose and lipid metabolisms, particularly insulin function in DM.

Several trace elements, such as vanadium and zinc, exert insulin-mimetic effects in in vitro and in vivo systems [14–16]. The complexes of these metals and small organic compounds (ligands) improved glucose utilization in both diabetic model animals and human diabetic patients [14–16]. However, the detail of action mechanism of such metal complexes has yet been unsolved.

Action mechanisms of vanadyl and zinc complexes

Recently, we have proposed possible action mechanisms for some oxovanadium(IV) (vanadyl) and zinc(II) (zinc) complexes in isolated rat adipocytes [14,15,17,18]. Both vanadyl and zinc complexes enhanced glucose uptake into the adipocytes without the addition of any hormones [19,20]. Under the same experimental conditions, these complexes inhibited epinephrine-induced free fatty acid (FFA) release [17,18]. These results indicated that vanadyl and zinc complexes have common "insulin-mimetic" activities. In order to examine the action mechanism of these complexes, we used several inhibitors such as the selective insulin receptor β-subunit (IRβ) inhibitor [HNMPA-$(AM)_3$], PI3K inhibitor (wortmannin), glucose transporter inhibitor (cytochalasin-B), and PDE inhibitor (cilostamide) [17,18]. The inhibition of FFA release by vanadyl and zinc complexes was reversed by these inhibitors, indicating that the complexes simultaneously act on several target sites such as IRβ, PI3K, PDE3B, and GLUT4. Thus, we termed this mechanism as an "ensemble mechanism" (Fig. 1) [14]. However, the critical action mechanism of the vanadyl and zinc complexes with respect to the regulation of the insulin and lipolysis signaling pathways still remains unclear.

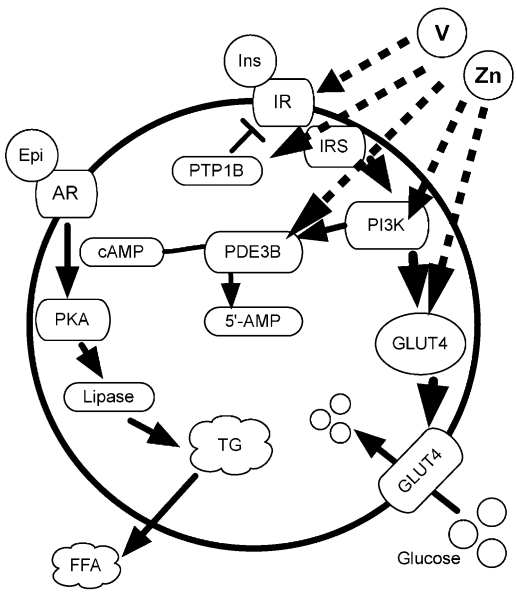

Fig. 1 Ensemble mechanism of vanadyl and zinc complexes in adipocytes. Vandyl and zinc complexes act on IRβ, PTP1B, PI3K, GLUT4, and PDE3B (dashed arrows). When the complexes activate these proteins, GLUT4 translocates to the plasma membrane; this in turn stimulates the extracellular uptake of glucose (thick arrows). In addition, when the complexes activate PDE3B, the intracellular cAMP level is decreased; this in turn inhibits the activities of PKA and lipase and subsequently FFA release (thin arrows). Abbreviations: ins, insulin; IR, insulin receptor; PTP1B, protein tyrosine phosphatase 1B; IRS, insulin receptor substrate; PI3K, phosphatidylinostitol 3-kinase; GLUT4, glucose transporter; PDE3B, phosphodiesterase 3B; Epi, epinephrine (adrenaline); AR, adrenogenic receptor; PKA, cAMP-dependent kinase; TG, triglyceride; FFA, free fatty acid.

Bis(allixinato)oxovanadium(IV) [$VO(alx)_2$] (Fig. 2B) has been found to be a potent antidiabetic agent from the study on the structure–activity relationship of bis(3-hydroxypyronato)oxovanadium(IV) [$VO(3hp)_2$] (Fig. 2A) and its related complexes, involving bis(maltolato)oxovanadium(IV) [$VO(ma)_2$] [16,21,22]. To examine the action site of $VO(alx)_2$, we measured the levels of phosphoproteins in the insulin signaling pathway in the 3T3-L1 adipocytes. $VO(alx)_2$ enhanced not only the tyrosine phosphorylation of IRβ and IRS but also of Akt and mitogen-activated protein kinase (MAPK) (Fig. 3) [23]. In addition, $VO(alx)_2$ regulated the downstream effects of Akt, such as the stimulation of GLUT4 translocation to the plasma membrane and the activation of forkhead transcription factor class O 1 (FoxO1) (Fig. 3) [23].

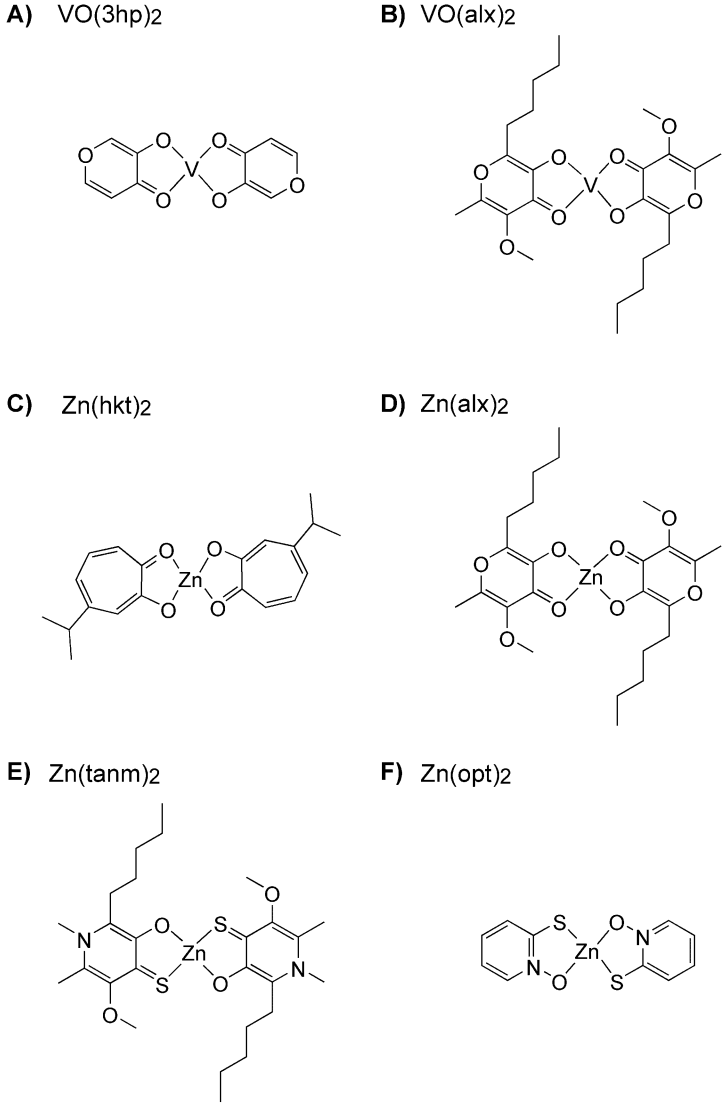

Fig. 2 Chemical structures of novel types of vanadyl and zinc complexes. (A) bis(3-hydroxypyronato)oxovanadium(IV) [$VO(3hp)_2$], (B) bis(allixinato)oxovanadium(IV) [$VO(alx)_2$], (C) bis(hinokitiol)zinc(II) [$Zn(hkt)_2$], (D) bis(allixinato)zinc(II) [$Zn(alx)_2$], (E) bis(thioallixin-*N*-methyl)zinc(II) [$Zn(tanm)_2$], and (F) bis(1-oxy-2-pyridine-thiolato)zinc(II) [$Zn(opt)_2$].

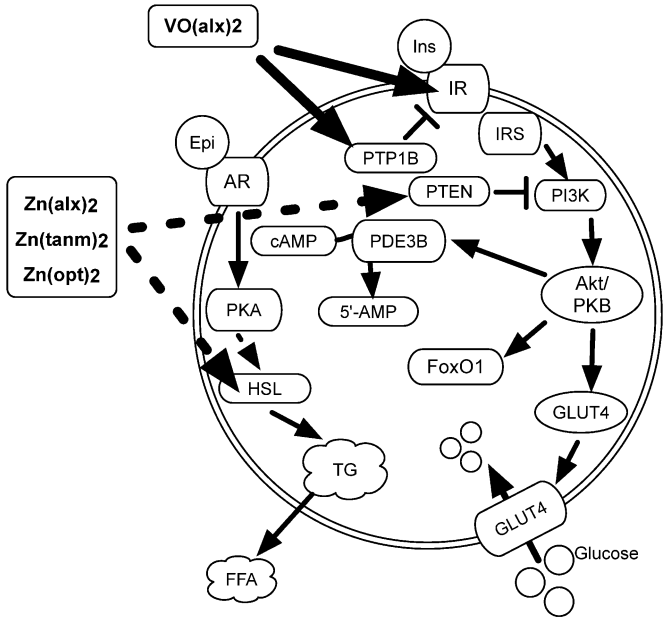

Fig. 3 Possible action sites of vanadyl and zinc complexes in adipocytes. $VO(alx)_2$ acts on IRβ and/or PTP1B, leading to the activation of downstream insulin receptor proteins (thick arrows). The activation of AKT/PKB by $VO(alx)_2$ is essential for the translocation of GLUT4 to the plasma membrane and the regulation of the transcription factor FoxO1. $Zn(alx)_2$, $Zn(tanm)_2$, and $Zn(opt)_2$ suppress the activity of PTEN (dashed arrow) and subsequently activate the downstream proteins of PI3K. Moreover, Zn complexes also activate Akt/PKB; this in turn stimulates the translocation of GLUT4 to the plasma membrane. In addition, the activation of Akt by Zn $(alx)_2$ and $Zn(tanm)_2$ induces the enzymatic activity of PDE3B; this in turn suppresses the activities of PKA and HSL and consequently FFA release is suppressed (dashed arrow). Abbreviations: IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase; PTP1B, protein tyrosine phosphatase-1B; PDK1, phosphoinositide-dependent protein kinase 1; PTEN, phosphatase and tensin homolog deleted on chromosome 10; Akt/PKB, Akt/protein kinase B; GLUT4, glucose transporter 4; FoxO1, forkhead transcription factor class O1; PDE3B, phosphodiestarase 3B; AC, adenylate cyclase; PKA, cAMP-dependent kinase; HSL, hormone-sensitive lipase; FFA, free fatty acid.

We also investigated the physiological function of $VO(alx)_2$ in two murine models of diabetes: streptozotocin (STZ)-induced type 1-like and obesity-linked KKA^y type 2 mice [22,24]. $VO(alx)_2$ improved hyperglycemia in not only STZ mice but also KKA^y mice. Interestingly, $VO(alx)_2$ improved almost all parameters relevant to the metabolic disease, such as the level of insulin and hypertension in KKA^y mice; however, it could not rescue the adiponectin level in the serum [24]. Adiponectin is an adipokine, which is synthesized and released by adipocytes [25]. Our result suggests that $VO(alx)_2$ affects the organs such as muscle and liver rather than the adipose tissues in both STZ and KKA^y mice. However, why $VO(alx)_2$ is unable to rescue the level of adiponectin in diabetic animals remains unresolved.

Antimetabolic syndrome activity of zinc complexes

Since $VO(alx)_2$ was not able to improve the adiponectin level in the KKA^y mice, we evaluated the ability of zinc complexes in treating type 2 diabetes and metabolic disease in the animals. During the study, we noticed that the bis(hinokitiol)zinc [$Zn(hkt)_2$] complex (Fig. 2C) slightly improved the adiponectin level in the KKA^y mice [26]. Following these studies, we found that bis(allixinato)zinc(II) [$Zn(alx)_2$] (Fig. 2D) and its related complex bis(thioallixin-*N*-methyl)zinc(II) [$Zn(tanm)_2$] (Fig. 2E) are effective

in treating the diabetic state of KKA^y mice [18,27]. Surprisingly, the chronic administration of both $Zn(alx)_2$ and $Zn(tanm)_2$ not only improved the hyperglycemia and the levels of insulin and leptin but also increased the adiponectin level, which is reduced in the KKA^y mice [28]. These findings indicated that $Zn(alx)_2$ and $Zn(tanm)_2$ affect the adipose tissues rather than the liver and muscle tissues. Our results suggest for the first time that both $Zn(alx)_2$ and $Zn(tanm)_2$ are alternative candidates to clinically useful synthetic compounds in treating metabolic syndrome [28–30].

$Zn(alx)_2$ and $Zn(tanm)_2$ induced Akt/PKB activation, leading to the translocation of GLUT4 to the plasma membrane and glucose uptake. In addition, zinc was incorporated into the adipocytes in a dose- and time-dependent manner when both complexes were added to the cells; thus, we concluded that the activation of Akt/PKB-GLUT4 signaling was dependent on the intracellular zinc concentration [31]. An imbalance in the intracellular zinc concentration has been reported in diabetes and other disorders [32,33]. Therefore, our findings suggest that $Zn(alx)_2$ and $Zn(tanm)_2$ improve the imbalance in the intracellular zinc concentration and consequently exhibit insulin-mimetic activity.

Critical action sites of zinc complexes

We examined the critical action sites of both the $Zn(alx)_2$ and $Zn(tanm)_2$ complexes. The PI3K inhibitor wortmannin inhibited the activation of Akt/PKB induced by both complexes. Nevertheless, both complexes did not induce the tyrosine phosphorylation of IRβ and IRS (unpublished data). In this regard, it has been reported that zinc induces the degradation of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [34]. Similarly, in our study we observed that zinc complexes inhibit the enzymatic activity of PTEN (W. Basuki, unpublished data). In addition, the overexpression of PTEN in mice impaired the insulin signaling pathway and resulted in insulin resistance [35]. Based on these observations, we propose that $Zn(alx)_2$ and $Zn(tanm)_2$ affect PTEN and activate PI3K-Akt/PKB signaling (Fig. 3). Similar results were also obtained with bis(1-oxy-2-pyridine-thiolato)Zn(II) [$Zn(opt)_2$], which is a different type of complex (Fig. 2F) [36].

Based on the above-mentioned results, we analyzed whether $Zn(alx)_2$ and $Zn(tanm)_2$ inhibit the secretion of FFA from adipocytes (the inhibition of lipolysis signaling). Adipocytes play a role in regulating the level of endogenous FFA in the body [37,38]. The degradation of triglycerides is controlled by hormone-sensitive lipase (HSL). This lipase is phosphorylated by cAMP-dependent kinase (PKA) that is activated by the increase in the intracellular cAMP concentration [37,39]. On the other hand, PDE3B that is activated by Akt/PKB catalyzes the conversion of cAMP to 5'-AMP [6,8,9], indicating that this enzyme is a negative regulator of PKA signaling. Adipocyte, treated with $Zn(alx)_2$ and $Zn(tanm)_2$, attenuated PKA-dependent HSL phosphorylation and consequently inhibited FFA secretion from the adipocytes [31]. Alternatively, the PI3K inhibitor reversed this effect. Therefore, the suppression of FFA release by $Zn(alx)_2$ and $Zn(tanm)_2$ indicates the activation of PI3K-Akt/PKB-PDE3B signaling (Fig. 3). In addition, the inhibitory effect of both complexes on FFA release suggests the direct inhibition of HSL or FFA release from the cells (Fig. 3).

DISCUSSION

We are currently developing new types of insulin-mimetic vanadyl and zinc complexes such as $VO(alx)_2$, $Zn(alx)_2$, and $Zn(tanm)_2$ [22–24,27,28]. These complexes are potent activators of the insulin signaling pathway. In addition, they perform other common or unique functions as examined in the 3T3-L1 adipocytes (Fig. 3). A common mechanism of action of $VO(alx)_2$ [23], $Zn(alx)_2$, and $Zn(tanm)_2$ is the induction of Akt/PKB activity that results in the translocation of GLUT4 to the plasma membrane. Although these complexes activate Akt/PKB, their critical action sites are slightly different from each other. $VO(alx)_2$ targets IRβ and/or PTPase [23], while $Zn(alx)_2$ and $Zn(tanm)_2$ target PTEN (Fig. 3). Such different action sites of the metal complexes may depend on the chemical characteristic of the

vanadyl and zinc metal ions. The characteristic of ligand is an important for the passing through the plasma membrane. Unfortunately, we do not yet determine the binding features of $VO(alx)_2$, $Zn(alx)_2$, and $Zn(tanm)_2$ after incorporating into the cells. The detail of metal complex structure in the cells needs to be verified in future experiments. On this point, previously we have determined the binding features of the complexes such as vanadyl-picolinates in several organs by using ESEEM (pulse electron paramagnetic resonance method), in which the ternary structures consisting of ligand-VO-proteins were suggested [40,41].

In addition, $VO(alx)_2$, $Zn(alx)_2$, and $Zn(tanm)_2$ also play unique roles in cells; $VO(alx)_2$ regulates the activation of the FoxO1 [23], while $Zn(alx)_2$ and $Zn(tanm)_2$ regulate the activation of HSL, resulting in the suppression of FFA release [31]. We believe that these results will be important for developing novel molecular targeting strategies for insulin-mimetic metal complexes.

In conclusion, the common mechanism of action of $VO(alx)_2$, $Zn(alx)_2$, and $Zn(tanm)_2$ is their effect on the insulin signaling pathway; this in turn regulates gene transcription and suppresses lipolysis signaling. The results of this study will provide researchers in this field valuable insights for creating and developing novel types of insulin mimetics and antidiabetic metal complexes.

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