BioMetals (2005) 18:319–323
DOI 10.1007/s10534-005-3688-8

The pharmacology of the insulinomimetic effect of zinc complexes

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Key words: Zn(II) complexes, allixin, insulin mimes, diabetes

Abstract

In developing new insulinomimetic zinc(II) complexes with different coordination structures and with a blood glucose-lowering effect to treat type 2 diabetic animals, we found a potent bis(maltolato)zinc(ll) complex, $Zn(mal)_2$. Using the complex as the leading compound, we examined the *in vitro* and *in vivo* structure-activity relationships of $Zn(mal)_2$ and its related complexes in respect to the inhibition of free fatty acids (FFA) release and the enhancement of glucose uptake in isolated rat adipocytes treated with epinephrine (adrenaline), and hypoglycemic activity. Among the compounds tested, a new Zn(II) complex with allixin that was isolated from garlic, bis(allixinato)Zn(II), $Zn(alx)_2$, was found to exhibit the highest insulin-mimetic and hypoglycemic activities in type 2 KK-A^y diabetic mice. On the basis of the results, $Zn(alx)_2$, complex was proposed to be a potent candidate for the treatment of type 2 diabetes.

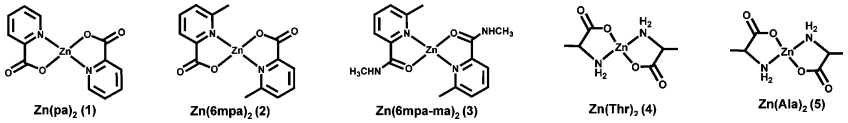
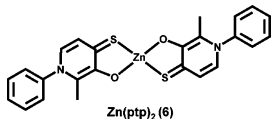
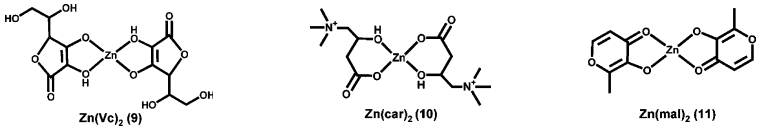
Introduction

A finding in 1980 that zinc(II) chloride ($ZnCl_2$) stimulated lipogenesis in rat adipocytes similarly to the action of insulin (Coulston & Dandona 1980) prompted many researchers to demonstrate the compound as an insulin mime. After many trials, $ZnCl_2$ was found to improve the hyperglycemia of type 1 diabetic mice in 1992 (Shisheva *et al*. 1992) and type 2 diabetic ob/ob mice in 1998 (Chen *et al*. 1998). In such studies, however, the hypoglycemia was achieved only by high doses and for long administration term of $ZnCl_2$.

Previously, several therapeutic agents involving gold, platinum, zinc, and aluminum have been developed for clinical use to treat a number of diseases or physiological disorders, including gold-containing auranofin as an antirheumatic, platinum-containing cisplatin and carboplatin as antineoplastics, and zinc-containing polaprezinc and aluminum-containing sucralfate as antiulcer-atives. These compounds are all metal complexes, in which the metal ion is expected to be incorporated in organs or tissues by complex formation higher than that of each metal ion itself. These examples encouraged us to develop new types of insulin mimes. We have already proposed several vanadyl(IV) (+4 oxidation state of vanadium) complexes, emphasizing the usefulness of vanadyl-picolinate complexes to treat types 1 and 2 diabetic animals (Sakurai 2002). Following our results with vanadyl complexes, we extended our work to the development of other types of metal complexes. For this purpose, we used zinc(II) complexes, which enabled us to lower the administration doses to diabetic animals by enhancing Zn incorporation into the organs of the animals (Sakurai 2002).

The mechanism of the insulin-mimetic action of Zn(II) has been examined since 1982, with respect to glucose oxidation and lipolysis stimulation (James & Charles 1982), glucose transport and glycogen synthesis (Ezaki 1989), and inhibition of endogenous glycogen synthase kinase-3β (Ronit *et al*. 2002). The results of these studies demonstrated that both the incorporation of Zn(II) into cells through the cell membrane and the subsequent interactions of Zn(II) with several biological systems in cells were essential for developing the insulin-mimetic activity of Zn(II).

Table 1 insulinomimetic Zn(II) complexes with different coordination modes

Coordination mode	Complex
N_2O_2	
S_2O_2	
N_4	
(8) O4	

1),2) Yoshikawa Y et al. 2002 *J Biol Inorg Chem* **7**. 68. 3) Yoshikwa Y et al. 2002 *Chem Pharm Bull* **50**, 337. 4),5) Yoshikawa Y et al. 2001 *Chem Pharm Bull* **49**, 652. 6) Koath A et al. 2002 *Lett*,114. 7),8) Kondo M et al. 2001 *Trace Nutr Res* **18**, 73. 9) Kojima Y et al. 2003 *Vitamin* **77**, 571–576. 10) Yoshikawa Y et al. 2003 *Chem Pharm Bull* **51**, 230. 11) Yoshikawa Y et al. 2001 *Biochem Biophys Res Commun* **281**, 1190.

The development of new compounds for treating diabetes is currently important to reduce the need for insulin injection in type 1 diabetic patients and to replace the clinically used synthetic therapeutics, which have several severe side effects for type 2 diabetic patients.

On the bases of these findings and observations, we conclude that Zn(II) complexes have promise as new insulin mimes. For this reason, we have been developing several types of Zn(II) complexes with different coordinating structures.

Anti-diabetic Zn(II) complexes

We evaluated the pharmacology of insulinomimetic Zn(II) complexes with respect to the *in vitro* interaction of Zn(II) and isolated Wistar rat adipocytes for the first step, and then made an *in vivo* appraisal by using type 2 diabetes model animal, KK-A^y mice, who received *i.p.* injection or oral administration of Zn(II) complexes for the second step. In *in vitro* evaluation, we used simple and convenient test systems composed of examining suppression of free fatty acids (FFA) release from the adipocytes (Nakai *et al.* 1995) and the enhancement of glucose uptake in the cells treated with epinephrine (adrenaline) (Adachi & Sakurai 2004). In the *in vivo* appraisal, male KK-A^y mice received the prepared complexes daily at a dose of approximately 3 mg Zn kg^{-1} body weight for two consecutive weeks. When the blood glucose levels were below 200–250 mg dl^{-1} after two weeks, we considered the hyperglycemia of the mice to be improved as a result of the complex administration. We also looked for improvement of the oral glucose tolerance test in the treated animals who were given glucose solution at a dose of 1 g kg^{-1} body weight.

Since 2000 (Yoshikawa *et al.* 2000), we have prepared many Zn(II) complexes and subjected them to both *in vitro* and *in vivo* experiments. After many trials, we have proposed several types of potent Zn(II) complexes, as shown in Table 1 (Sakurai 2002), as potential insulin mimes.

A colorless single crystal of $Zn(mal)_2$ (bis(maltolato)Zn(II)) (11) was determined to be *trans*-$[Zn(mal)_2(H_2O_2)][Zn(mal)_2(H_2O)](H_2O)_2$ by X-ray structure analysis (Yoshikawa *et al.* 2000), revealing the occurrence of two different geometries around the Zn atoms with octahedral and

square pyramidal conformation. In a colorless single crystal of $[Zn(6mpa)_2(H_2O)]H_2O$ (bis(6-methylpicolinato)Zn(II)) (Yoshikawa *et al.* 2002), the coordination geometry was a distorted trigonal hipyramidal structure. These two complexes, $Zn(mal)_2$ and $Zn(6mpa)_2$, have been found to be the most useful complexes to treat type 2 diabetes model animals.

Possible action mechanism of Zn(II) complexes

As part of developing new Zn(II) complexes as mimes of insulin, we examined the action mechanism of the complexes. Previously, researchers found that the Zn(II) effect on both glucose oxidation and lipolysis stimulation was due to the inhibition of catalase (May & Contoreggi 1982), and in a later study it was found that Zn(II) stimulated both lipogenesis and glucose transport in the adipocytes (Ezaki 1989). In addition, *in vivo* insulinomimetic activity of Zn(II) was found to mediate through a direct inhibition of endogenous glycogen synthase kinase-3β (Ronit *et al.* 2002). We examined the action mechanism of Zn(II) complexes in respect of the FFA release from adipocytes by using several inhibitions, such as hydroxy-2-naphthalenylmethylphosphonic acid tri-acetoxy methyl ester ($HNMPA\text{-}(AM)_3$) and wortmannin, which are involved in glucose uptake; cytochalasin which is involved in the glucose transporter 4 (GLUT4); and cilostamide, which affects the activation of phosphodiesterase, for both fatty acids and glucose metabolisms in the adipocytes. By comparing the obtained results on $ZnSO_4$, $Zn(pic)_2$, $Zn(mal)_2$, and $Zn(thr)_2$ (bis(threoninato)Zn(II)), we found that such Zn(II) complexes seemed to promote the glucose uptake into the adipocytes by affecting simultaneously at least three sites in the adipocytes (Yoshikawa *et al.* 2004). This multifunctional action mechanism of Zn(II) complexes has resulted in reference to the system as an ensemble mechanism (Adachi *et al.* 2004).

A new insulin mime bis(allixinato)Zn(II) complex

Because Zn $(mal)_2$ complex has been found to exhibit significant insulinomimetic effect in type 2 diabetic model animals, we used this $Zn(mal)_2$ as the leading compound to examine the *in vitro* and *in vivo* structure-activity relationships of $Zn(mal)_2$ and its related complexes, such as $Zn(mal)_2$, $Zn(3hp)_2$ (bis(3-hydroxypyranato)Zn(II)), $Zn(ema)_2$ (bis(2-ethylmaltolato)Zn(II)), $Zn(ka)_2$ (bis(kojato)Zn(II)), and $Zn(alx)_2$ (Adachi *et al.* 2004).

A new Zn(II) complex with allixin (3-hydroxy-5-methoxy-6-methyl-2-pentyl-4-pyrone, Halx) isolated from garlic, $Zn(alx)_2$, exhibited the highest insulinomimetic activity among the complexed as evaluated in relation to the inhibition of FFA release (Figure 1a) and the enhancement of glucose uptake in isolated rat adipocytes treated with epinephrine (adrenaline) (Figure 1b). Interestingly, the insulinomimetic activity of the Zn(II)

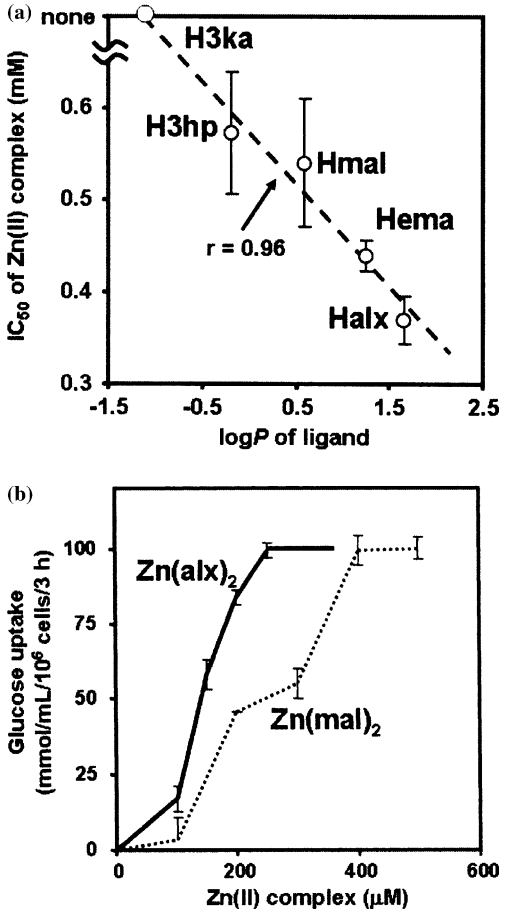


Figure 1. (a) Correlation between IC_{50} value and partition coefficient (log *P*) of *maltol* and its related complexes (IC_{50}) value: the 50% inhibitory concentration of the Zn(II) complex in the FFA-release from rat adipocytes treated with epinephrine). (b) Concentration-dependent glucose-uptake enhancement by $Zn(mal)_2$ and $Zn(alx)_2$ in isolated rat adipocytes treated with epinephrine in the presence of 2% DMSO. Data are expressed as the means ± SDs for three experiments

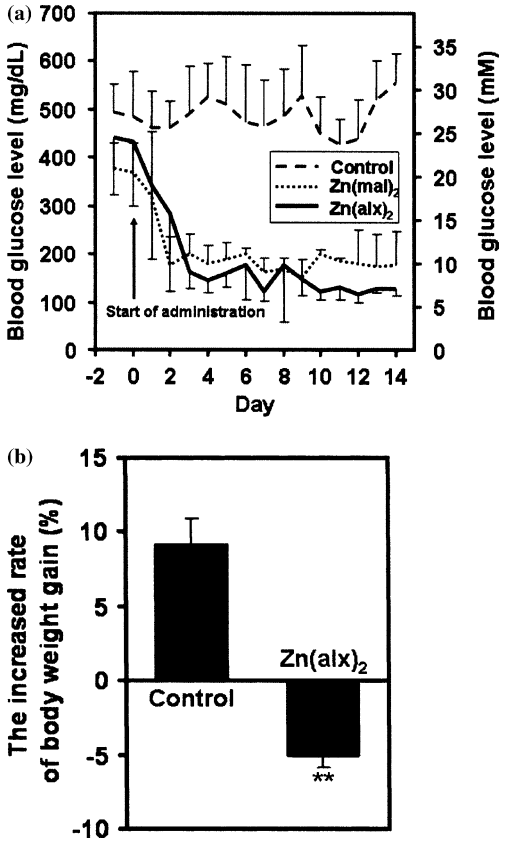


Figure 2. Changes of blood glucose level (a) and increased rate of body weight gain (b) in control KK-A^y mice and KK-A^y mice treated with $Zn(mal)_2$ or $Zn(alx)_2$ by daily *i.p.* injections for 14 days. (Doses were 4.5 mg Zn/kg for the first 2 days, and the doses were adjusted at 2.0–4.5 mg Zn/kg according to the daily changes of blood glucose levels after 3 days. Data are expressed as the means ± SDs for five mice. **Significance at $P < 0.01$ *versus control*

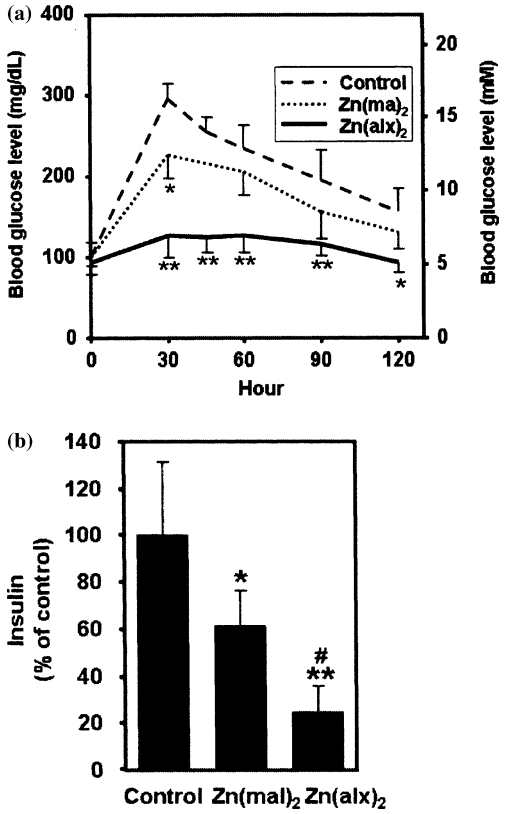


Figure 3. Oral glucose tolerance tests (OGTT) (a) and insulin level (b) for the control KK-A^y mice and the KK-A^y mice after daily *i.p.* administration of $Zn(mal)_2$ or $Zn(alx)_2$. OGTT tests were performed on mice fasted for 12 h, and then they were given oral glucose solution at a dose of 1 g/kg body weight. Data are expressed as the means ± SDs for 5 mice. *Significance at $P < 0.05$ *versus* control, **Significance at $P < 0.01$ *versus* control. #Significance at $P < 0.01$ *versus* $Zn(mal)_2$

complexes were strongly correlated (correlation coefficient = 0.96) with the partition coefficient (log P) of the ligand (Figure 1a), indicating that the activity of $Zn(mal)_2$-related complexes depended on the lipophilicity of the ligand. Using these results, we compared the blood glucose-lowering effects of $Zn(alx)_2$ and $Zn(mal)_2$, and found that both complexes normalized hyperglycemia in KK-A^y mice after a 14-day course of daily intraperitoneal injections (Figure 2a), which was supported also by the weight loss of obese KK-A^y mice (Figure 2b). However, $Zn(alx)_2$ improved glucose tolerance in KK-A^y mice much more than did $Zn(mal)_2$ (Figure 3a), demonstrating that $Zn(alx)_2$ possessed greater *in vivo* antidiabetic activity than that of $Zn(mal)_2$. In addition, $Zn(alx)_2$ improved the insulin level (Figure 3b) as well as leptin resistance (data not shown), thus suppressing the progress of obesity in type 2 diabetic KK-A^y mice. From these results, we concluded that the $Zn(alx)_2$ complex is a novel potent candidate for the treatment of type 2 diabetes mellitus.

Acknowledgements

We thank Dr. Yukihiro Kodera, Prof. Akira Katoh, Prof. Yoshitane Kojima, and Dr. Yutaka Yoshikawa for their excellent collaborations. This research was supported by a grant from the Ministry of Education, Science, Sports, and Culture of the Japanese government (Specially Promoted Research).

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