New Insulinomimetic Zinc(II) Complexes of α-Amino Acids and Their Derivatives with $Zn(N_2O_2)$ Coordination Mode

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Zinc(II) complexes of α-amino acids and their derivatives with a $Zn(N_2O_2)$ coordination mode were found to have *in vitro* insulinomimetic activity as estimated with the inhibition of free fatty acid release in isolated rat adipocytes treated with epinephrine. It was revealed that the insulinomimetic activities of zinc(II) complexes with over-all stability constants ($\log\beta$) less than 10.5 are higher than those of $ZnSO_4$ and $VOSO_4$. The high blood glucose level of KK-A^y mice with type 2 diabetes mellitus was lowered by daily intraperitoneal injections of a zinc(II) complex, *cis*-[Zn(L-Thr)$_2$(H_2O)$_2$], for 14 d. The improvement of diabetes mellitus was confirmed with the oral glucose tolerance test.

Key words zinc(II) complex; amino acid; insulinomimetic activity; NIDDM; blood glucose normalizing effect; glucose tolerance test

Zinc(II) ion is known to be one of the important essential trace elements found in biological systems and also in many metalloproteins and metalloenzymes which exist in living organisms. Physico-chemical properties of a zinc(II) ion, which functions as a Lewis acid, is entirely unlike those of vanadium ions with a redox property and insulinomimetic activity. Among many pharmacological and nutritional roles of zinc(II) ion,[1] an interesting pharmacological role was reported in 1980, in which a zinc(II) ion acts as an insulinomimics.[2] The administration of $ZnCl_2$ to streptozotocin-induced diabetes rats (STZ rats) or ob/ob mice normalized their high blood glucose levels,[3,4] when a high dose[3] and a long-term (8 weeks) administration were given.[4] However, the insulinomimetic activities of zinc(II) complexes have not yet been studied. Thus, we have tried to develop insulinomimetic zinc(II) complexes with various coordination modes around a zinc(II) ion, because zinc(II) ion is generally less toxic than vanadium ions.[5] During our investigation, we found first that zinc(II) maltolate complex with a $Zn(O_4)$ coordination mode exibits high insulin-like activities in *in vitro*.[6] On the basis of the results, we examined the structure–activity relationship of Zn(II) complexes, and found that zinc(II) complexes with $Zn(N_2O_2)$ coordination mode exibit higher insulinomimetic activity than those of $ZnSO_4$ and $VOSO_4$. In this paper, we report the insulinomimic zinc(II) complexes with naturally occurring amino acids and their derivatives.

The Zn(II) complexes used in this study were readily prepared by adding $ZnSO_4 \cdot 7H_2O$ to an aqueous solution of lithium or barium salt of the appropriate ligand (generated *in situ* from ligands and lithium or barium hydroxide) at room temperature. The choice of lithium or barium as cation was made based upon the aqueous solubility of the desired complex. Lithium was preferred instead of barium, because the complex is prone to coprecipitate with barium sulfate in the reaction. All complexes were purified from hot water by recrystallization, identified by the elemental analyses and IR and ^{1}H-NMR spectra, and found to be molecular complexes without a counter ion.

The insulinomimetic activities of the complexes were evaluated by using the isolated rat adipocytes treated with epinephrine in terms of the inhibition of free fatty acid release.[7] The inhibitory effects of the complexes were compared with those of $ZnSO_4$ and $VOSO_4$ as positive control. It was revealed that zinc(II) complexes with lower over-all stability constants ($\log\beta$) than 10.5 exhibited higher insulinomimetic activities than those of $ZnSO_4$ and $VOSO_4$ or were comparable to them except Zn(GtG) (IC_{50}=3.18) (Table 1). On the other hand, zinc(II) complexes with His, GeG, and mGeGm ($\log\beta$=12.05, 11.22, and 11.83, respectively) with higher $\log\beta$ values than 11.0 showed essentially no insulinomimetic activity. As shown in Fig. 1, the structure of zinc(II) complex with *N*,*N*′-ethylene-bis-glycine (GeG) was revealed to be an octahedral geometry [Zn(GeG)(H_2O)$_2$] **1** with a *cis*-β configuration of only C_1 symmetry in an aqueous solution based on 2 separate signals for ν_{NH} of IR and for Gly-CH_2 and ethylenic CH_2 of ^{1}H-NMR.[14] It is interesting to note that the geometry around the zinc(II) ion in **1** without the insulinomimetic activity is similar to that of a VO complex [VO(GeG)(H_2O)][9,15] having high insulinomimetic activity except the differences of an O^{2-} and a H_2O molecule, and *cis*-α and *cis*-β (Table 1). Although the coordination geometry[21] of [Zn(L-Thr)$_2$(H_2O)$_2$] **2** ($\log\beta$=8.46) is similar to that of **1** with *cis* configuration of two H_2O molecules, the insulinomimic activities of **1** (IC_{50}=none) and **2** (IC_{50}=0.54) are completely different. Accordingly, the difference of the insulinomimetic activities of **1** ($\log\beta$=11.22) and **2** ($\log\beta$=8.46) appeared to depend on the difference of their stability constants.

In addition, the zinc(II) complexes with L- and D-amino acids, Asn, Pro, Thr, and Val, exhibited similar insulinomimetic activities to each other as shown in Table 1. Accordingly, the difference of the insulinomimetic activities of zinc(II) complexes was not seen on the basis of the absolute configurations of the α-amino acids.

Because complex **2** exhibited the highest *in vitro* insulinomimetic activity, we evaluated the *in vivo* blood glucose lowering effect of complex **2** in KK-A^y mice (8 weeks old: CREA Japan Inc., Tokyo) with type 2 diabetes mellitus. They received daily intraperitoneal (i.p.) injections of **2** at about 10

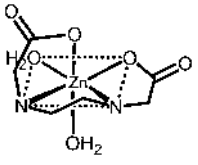

Fig. 1. Proposed Structure of *cis*-β-[Zn(GeG)(H_2O)$_2$] **1** in an Aqueous Solution

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Table 1. Estimated IC_{50} (mM) Values for the Free Fatty Acids (FFA) Release from Isolated Rat Adipocytes in the Presence of Glucose and the Over-all Stability Constants (log β[a]) of Zinc(II) Complexes

Complex	IC_{50}/mM (±S.D.)[b]	log β[c]	Complex	IC_{50}/mM (±S.D.)[b]	log β[c]
Zn(L-Asn)$_2$	0.65 (0.03)*	8.55[d]	Zn(D-Asn)$_2$	0.65 (0.09)*	8.55[e]
Zn(L-Pro)$_2$	0.89 (0.07)	9.75	Zn(D-Pro)$_2$	0.89 (0.07)	9.75[e]
Zn(L-Thr)$_2$	0.54 (0.03)**	8.46	Zn(D-Thr)$_2$	0.48 (0.03)**	8.46[e]
Zn(L-Val)$_2$	0.77 (0.08)	8.24	Zn(D-Val)$_2$	0.87 (0.04)	8.24[e]
Zn(Gly)$_2$	0.63 (0.05)*	9.19	Zn(L-Asp)$_2$	1.25 (0.08)	10.15
Zn(L-Ala)$_2$	0.55 (0.05)**	8.61	Zn(L-Gln)$_2$	0.84 (0.07)	9.17
Zn(L-His)$_2$	None	12.05			
Zn(GeG[f])	None	11.22	Zn(mGeGm[g])	None	11.83[d]
Zn(MeM[h])	None	—[i]	Zn(βAeAβ[j])	0.82 (0.05)	7.6
Zn(GtG[k])	3.18 (0.04)	10.27[d]	Zn(VtV[l])	0.92 (0.04)	8.63[d]
$VOSO_4$	1.00		$ZnSO_4$	0.81 (0.1)	

a) Refer to ref. 16. *b*) Each datum is expressed as the mean±S.D. for 3 experiments. *c*) Refer to ref. 17. *d*) Unpublished data, refer to ref. 18. *e*) The over-all stability constant is applied the identical data of L-amino acid. *f*) GeG; *N*,*N*′-ethylene-bis-glycine=EDDA. *g*) mGeGm; *N*,*N*′-ethylene-bis-sarcosine. *h*) MeM; *N*,*N*′-ethylene-bis-L-methionine. *i*) The value could not be obtained because of the precipitations occurred during the course of titration. *j*) βAeAβ; *N*,*N*′-ethylene-bis-β-alanine. *k*) GtG; *N*,*N*′-trimethylene-bis-glycine. *l*) VtV; *N*,*N*′-trimethylene-bis-L-valine. **Significance at $p<0.01$ *vs.* $ZnSO_4$. *Significance at $p<0.05$ *vs.* $ZnSO_4$.

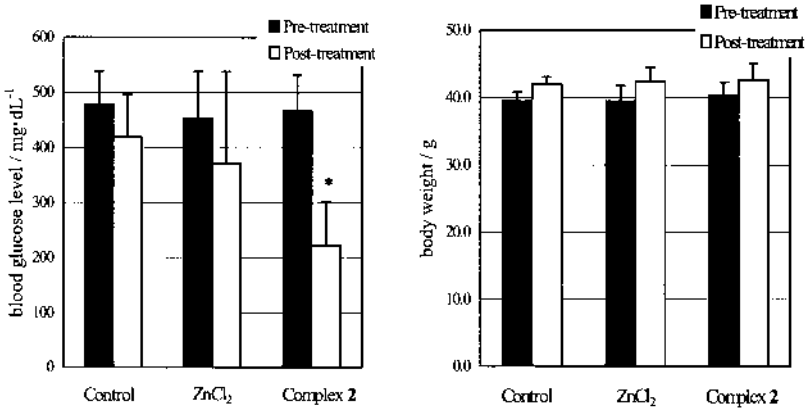

Fig. 2. Changes of Blood Glucose Levels (Left) and Body Weights (Right) before and after Supplementation of i.p. Injection

Hyperglycemic KK-A^y mice received daily i.p. injections of 5% acacia (n=9), $ZnCl_2$ at a dose of 3 mg Zn/kg body weights (n=5) or complex **2** at a dose of 3 mg Zn/kg body weights for 14 d (n=11). Values are means±S.D. for 5 to 11 mice. *Significance at $p<0.0001$ *vs.* post-treatment of the untreated KK-A^y mice.

a.m. for 2 weeks.[22] Blood samples were obtained from the mouse-tail vein, and the glucose levels were measured with a Glucocard (Arkray, Kyoto). The body weights of KK-A^y mice who were allowed free access to solid food (CREA Japan Inc.) and tap water were measured daily during the administration of **2**. The intakes of solid food and drinking water for each mouse were checked daily throughout the experiments. When the mice were given complex **2** at a dose of 3 mg Zn/kg body weight daily, the glucose levels were lowered and maintained at approximately 220—230 mg/dl (12.2—12.8 mM) for 2 weeks (Fig. 2, left). While the glucose levels of control mice and $ZnCl_2$ (3 mg Zn/kg body weight) treated mice for 2 weeks were almost unchanged. During the treatment, the body weight of the KK-A^y mice in each group increased slightly from 40.1±2.2 to 42.3±2.3 g (Fig. 2, right). The serum parameters, which indicate the degrees of renal disturbance (BUN) and liver disturbance (GOT and GPT), were not altered compared with those of the untreated KK-A^y mice.[23]

In conclusion, the present results have revealed that (1) the occurrence of the interrelationship between the stability constants and the insulinomimetic activities of zinc(II) complexes in *in vitro* studies and (2) the complex with the lowest IC_{50} value in the *in vitro* evaluation exhibits excellent blood glucose lowering effect, which in turn improves the diabetic state of the animals as estimated by the glucose tolerance test (Fig. 3).

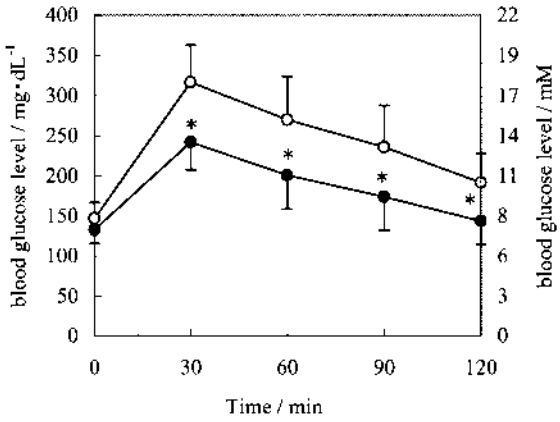

Fig. 3. Oral Glucose Tolerance Tests for the Untreated KK-A^y Mice (—○—) and KK-A^y Mice Treated with Complex **2** (—●—)

Oral glucose tolerance tests were performed on mice fasted for 12 h and then they were given glucose solution orally at a dose of 1 g/kg body weight. Values are means±S.D. for 9 or 11 mice. *Significance at $p<0.05$ *vs.* the untreated KK-A^y mice.

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23) The BUN, GOT, and GPT values of the untreated KK-A^y mice and KK-A^y mice administered to **2** were 32.9±2.4 and 24.5±2.6, 61±15 and 67±15, and 30±9 and 26±11, respectively.