New Insulin-Mimetic Zinc (II) Complexes; Bis-maltolato Zinc(II) and Bis-2-hydroxypyridine-*N*-oxido Zinc(II) with $Zn(O_4)$ Coordination Mode

Yutaka Yoshikawa, Eriko Ueda, Kenji Kawabe, Hiroyuki Miyake, Hiromu Sakurai,† and Yoshitane Kojima*
Department of Chemistry, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585
†Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414

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Zinc(II) complexes with a $Zn(O_4)$ coordination mode were found to have insulinomimetic activity. A bis-maltolato zinc(II) ($Zn(Mal)_2$) complex was revealed to be in an octahedral and a square pyramidal geometries in a unit cell. Both $Zn(Mal)_2$ (IC_{50} = 0.59 mM) and bis-2-hydroxypyridine-*N*-oxide zinc(II) (IC_{50} = 0.41 mM) complexes exhibited higher insulinomimetic activity than $VOSO_4$(IC_{50} = 1.00 mM) and $ZnSO_4$(IC_{50} = 0.81 mM) in terms of IC_{50} values, which show 50% inhibition concentration of the complex in the free fatty acids release from rat adipocytes.

A recent research shows that the number of people suffering from diabetes mellitus (DM), one of the life-style related disease, has risen up to nearly 14 million, including the figure of potential patients in Japan.[1] DM is a disease associated with absolute or relative insulin deficiency, and the development and research of many drugs for the treatment of DM have been performed all over the world. Based on the reported *in vitro* experiment in 1980 that zinc(II) ion acts as an insulinomimics,[2] the administration of $ZnCl_2$ to streptozotocin-induced diabetes rats (STZ rats) or ob/ob mice was found to normalize their high blood glucose levels.[3,4] However, they used a high dose[3] or a long-term (8 weeks) in zinc(II) ion administration.[4] On the other hand, we have reported that the insulinomimetic activity of many oxovanadium(IV) (VO) complexes are higher than that of free VO ion.[5,6] Also, it was reported that bis(maltolato = Mal) VO complex is more effective than free VO ion.[7] Since zinc(II) is generally less toxic than VO,[8] we tried to develop insulinomimetic zinc(II) complexes with different coordination modes around zinc(II). During our efforts, we found that zinc(II) complexes with a $Zn(O_4)$ coordination mode exhibit high insulinomimetic activity. In this paper, we will report the first results on the insulinomimetic $Zn(Mal)_2$ **1**[9] and Zn (2-hydroxypyridine-*N*-oxide = HPNO)$_2$ **2**[10] complexes.

The ligand of **1** is well-known as a food additive.[11] The complexes **1** and **2** were prepared according to a simple procedure. A colorless single crystal of $Zn(Mal)_2$ suitable for X-ray structure analysis was proposed as *t*-$[Zn(Mal)_2(H_2O)_2]$-$[Zn(Mal)_2(H_2O)]\cdot(H_2O)_2$ **1**.[12] Two different geometries around the zinc(II)s in the complex **1** were revealed to be in a unit cell, where two Mal and two H_2O coordinate to a zinc(II) by *trans* forms in an octahedral conformation, and two Mal and an apical H_2O coordinate to a zinc(II) in a square pyramidal conformation (Figure 1).[12]

The insulinomimetic activities of the zinc(II) complexes have been evaluated by *in vitro* experiments.[13] The inhibitory effects of **1** and **2** were compared with those of $VOSO_4$ as a positive control and of $ZnSO_4$ as a free zinc(II) ion (Figure 2).

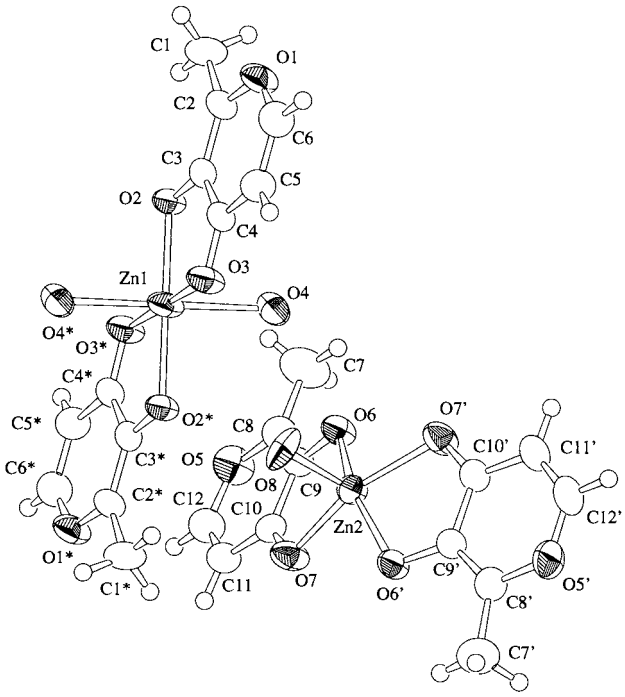

Figure 1. ORTEP drawing of *t*-$[Zn(Mal)_2(H_2O)_2]$ $[Zn(Mal)_2(H_2O)]\cdot(H_2O)_2$ **1**: Selective bond distances (Å) are as follows: Zn1-O2, 2.027(2); Zn1-O3, 2.072(3); Zn1-O4, 2.282(3); Zn2-O6, 2.014 (3); Zn1-O7, 2.078 (3); Zn2-O8, 2.015(4). Two crystal water molecules are omitted.

Both zinc(II) complexes at 5×10^{-4} M inhibited the free fatty acids (FFA) released from the epinephrine-stimulated rat adipocytes more than $VOSO_4$ and $ZnSO_4$. The effects were dose-dependent in the concentration range of 10^{-4}–10^{-3} M. From these results, the apparent IC_{50} values, a 50% inhibitory concentration of the FFA release in each complex, was estimated to be 0.59 and 0.41 mM for **1** and **2**, respectively. Zinc(II) complexes **1** and **2** were more active than $VOSO_4$ (IC_{50} = 1 mM) and $ZnSO_4$ (IC_{50} = 0.81 mM). The conclusion suggests that a $Zn(O_4)$ coordination mode around the zinc(II) is advantageous to free zinc(II) ions and metal–oxo ion, like V=O, in giving insulinomimetic activity. We proposed here that **1** and **2** are potent insulinomimetic complexes, and further study to know the action mechanism of **1** and **2** in under way.

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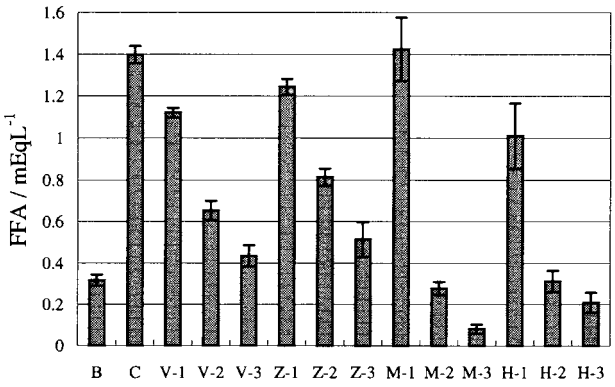

Figure 2. Inhibitory effects of $VOSO_4$, $ZnSO_4$, **1**(M) and **2**(H) on free fatty acid (FFA) release from rat adipocytes treated with epinephrine (EP). Rat adipocytes were prepared as reported.[14] Each column is expressed as the mean ±SD for 3 experiments. B: blank, cells only; C: control, cells plus 1×10^{-5} M epinephrine. V-1-3, Z-1-3, M-1-3, and H-1-3 are $VOSO_4$, $ZnSO_4$, **1** and **2**, respectively: In each system, adipocytes (1.0×10^6 cells / ml) were treated with 10^{-4}, 5×10^{-4}, 10^{-3} M of the compound in each numerical order, respectively, for 30 min and then incubated with 10^{-5} M EP for 3 h at 37 °C.

University for elemental analyses and ESI MASS measurements.

References and Notes

1 K. Tsuda and Y. Seino, *J. Japan Diabetes Soc.*, **41**, 959 (1998).
2 L. Coulston and P. Dandona, *Diabetes*, **29**, 665 (1980).
3 A. Shisheva, D. Gefel, and Y. Schechter, *Diabetes*, **41**, 982 (1992).
4 M. D. Chen, S. J. Liou, P. Y. Lin, V. C. Yang, P. S. Alexander, and W. H. Lin, *Biol. Trace Elem. Res.*, **61**, 303 (1998).
5 K. Kawabe, M. Tadokoro, A. Ichimura, Y. Kojima, T. Takino, and H. Sakurai, *J. Am. Chem. Soc.*, **121**, 7937 (1999).
6 H. Sakurai, A. Tamura, T. Takino, K. Ozutsumi, K. Kawabe, and Y. Kojima, *Inorg. React. Mech.*, in press.
7 V. G. Yuen, P. Caravan, L. Gelmini, N. Glover, J. H. McNeill, I. A. Setyawati, Y. Zhou, and C. Orvig, *J. Inorg. Biochem.*, **68**, 109 (1997).
8 K. T. Smith and F. H. Nielsen in "Trace Minerals in Foods," ed. by K. T. Smith, Marcel Dakker, NewYork and Basel (1988) p. 209 and p. 257.
9 Zinc(II) complex **1**: To an aqueous solution of maltol (0.38 g , 3.0 mmol) and $LiOH \cdot H_2O$ (0.06 g , 3.0 mmol), an aqueous solution of $ZnSO_4 \cdot 7\,H_2O$ (0.43 g, 1.5 mmol) was added followed by stirring for 5 h at room temperature. The obtained white precipitate was washed with a small amount of water (0.36 g , yield ; 66%). Anal. Found: C, 39.66; N, 0.02; H, 4.14%. Calcd for $C_{12}H_{10}O_6Zn \cdot 2.5H_2O$; C, 39.97; N, 0.00; H, 4.19%. Mp 244–252 °C (dec.). IR (KBr disk); 1613 cm^{-1} for $\nu_{C=O}$. ESI MASS *m/z* = 315 $[M + H]^+$.
10 Zinc(II) complex **2**: To an aqueous solution of HPNO (0.33 g , 3.0 mmol) and $Ba(OH)_2 \cdot 8\,H_2O$ (0.48 g, 1.5 mmol), an aqueous solution of $ZnSO_4 \cdot 7\,H_2O$ (0.43 g, 1.5 mmol) was added followed by stirring for 5 h at room temperature. After the filtration of $BaSO_4$ and the removal of the solvent, the residue was dissolved with a small amount of hot water and reprecipitated with methanol. The obtained white precipitate was washed with a small amount of methanol (0.30 g , yield ; 71%). Anal. Found: C, 41.61; N, 9.76; H, 2.71%. Calcd for $C_{10}H_8N_2O_4Zn$: C, 42.06; N, 9.81; H, 2.81%. Mp 320–360 °C (dec.). IR (KBr disk); 1625 cm^{-1} for $\nu_{C=O}$. ESI MASS *m/z* = 285 $[M + H]^+$.
11 P. Caravan, L. Gelmini, N. Glover, F. G. Herring, H. Li, J. H. McNeill, S. J. Rettig, I. A. Setyawati, E. Shuter, Y. Sun, A. S. Tracey, V. G. Yuen, and C. Orvig, *J. Am. Chem. Soc.*, **117**, 12759 (1995).
12 Crystal data for *t*-$[Zn(Mal)_2(H_2O)_2][Zn(Mal)_2(H_2O)] \cdot (H_2O)_2$ **1**: Two independent half molecules exist in the asymmetric unit. $C_{12}H_{15}O_{8.5}Zn$, M = 360.63, orthorhombic, space group *P*bcn (#60), *a* = 16.1743 (6), *b* = 13.2102 (4), *c* = 13.3956 (4) Å, *V* = 2862.2 (2) $Å^3$, *F*(000) = 1480.00, *Z* = 8, *Dc* = 1.674 g/cm^3, μ(Mo Kα) = 17.57 cm^{-1}, 2 θ_{max} = 54.9°. Intensity data (15033 total (3238 independent) reflections) were collected on Rigaku RAXIS-RAPID Imaging Plate. The final cycle of full matrix least squares refinement was based on 1711 observed reflections ($I > 2.00\,\sigma(I)$) and 197 variable parameters, and converged to *R* = 0.036 and *Rw* = 0.048. Maximum peak in final differential map is 0.36 $eÅ^{-3}$. Programs used to solve structure: SHELX86 and DIRDIF. All calculations including data reduction: teXsan crystallographic software package (Molecular Structure Corporation).
13 Isolated male rat adipocytes (1.0×10^6 cells/mL) prepared as described in ref 14 were preincubated at 37 °C for 30 min with various concentrations (10^{-4}–10^{-3} M) of zinc(II) complexes in KRB buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$ and 5 mM glucose: pH 7.4) containing 2% BSA. A 10^{-4} M epinephrine was then added to the reaction mixtures and the resulting solutions were incubated at 37 °C for 180 min. The reactions were stopped by soaking in ice water and the mixtures were centrifuged at 3000 rpm for 10 min. For outer solution of the cells, FFA levels were determined with an FFA kit (Wako).
14 M. Nakai, H. Watanabe, C. Fujiwara, H. Kakegawa, T. Satoh, J. Takada, R. Matsushita, and H. Sakurai, *Biol. Pharm. Bull.*, **18**, 719 (1995).