New Insulinomimetic Zinc(II) Complexes of Nicotinamide and its Derivatives: X-ray Structure and Biochemical Activity

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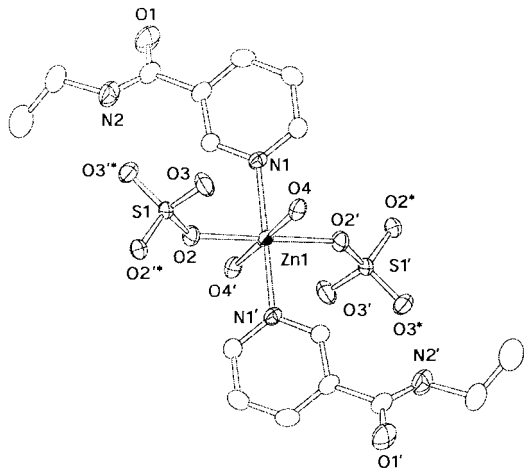
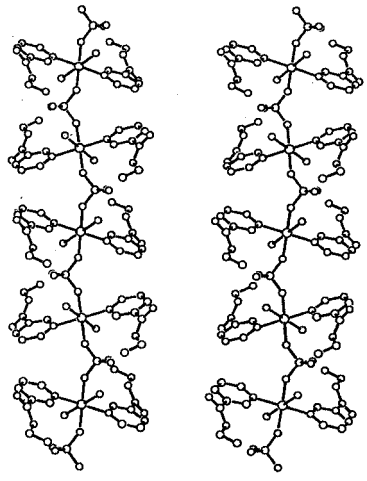
Three Zn(II) complexes with nicotinamide(na), nicotinemethylamide(nma), and nicotineethylamide(nea) were found to have in vitro insulinomimetic activities. X-ray structure analysis of $[Zn(nea)_2(H_2O)_2(SO_4)]_n$ showed that each nitrogen atom of two nea molecules locates trans, and the two water molecules and each oxygen atom of the two sulfates coordinate to a Zn(II) ion, forming an octahedral geometry of $Zn(N_2O_4)$ unit in an inorganic polymer crystal. The Zn(II) complexes with na, nma, and nea exhibited higher insulinomimetic activities, respective IC_{50} values (50% inhibition concentration of the complex in the free fatty acids release from rat adipocytes) being 1.34, 1.12, and 0.96 mM, than that of $ZnSO_4$ (IC_{50} = 1.52 mM).

We have reported that Zn(II) complexes have high insulinomimetic activities in in vitro and in vivo systems.[1–4] However, all the complexes have been molecular complexes. Moreover, it has been believed that it is essentially important for molecular complexes to show antineoplasmic activity in cisplatin and its related complexes.[5] Recently, many researchers have proposed metal-containing therapeutic agents of cationic complexes such as BBR3464 of cisplatin derivatives[6] and ^{99m}Tc-tetrofosmin complex of diagnostic nuclear medicine.[7] On the other hand, nicotinamide has been known to protect type 1 diabetes mellitus,[8–10] and researchers have proposed the action mechanism of the compound.[11,12] For example, Bedoya et al. described that the type 1 diabetes mellitus (DM) induced by streptozotocin (STZ) is counteracted by scavenging reactive oxygen species with nicotinamide.[11] Also, Kolb and Burkart reported that the treatment of nicotinamide prevents or delays insulin-deficient type 1 DM model animals and protects islet cells against cytotoxic actions in vivo.[12] In addition, it was found that Zn(II) induces metallothionein and partially prevents the development of DM by STZ.[13] On the basis of the observations, we have planned to synthesize new cationic Zn(II) complexes with nicotinamide and its derivatives and to estimate the insulinomimetic activities in in vitro experiments. In this study, we examined the relationship between structures and insulinomimetic activities of Zn(II) complexes with nicotinamide and its derivatives.

Three Zn(II) complexes **1**, **2**, and **3** with nicotinamide (na), nicotinemethylamide (nma), and nicotineethylamide (nea), respectively, were prepared.[14,15] A colorless single crystal of **3** suitable for X-ray structure analysis was obtained from a water/ethanol solution as $[Zn(nea)_2(H_2O)_2(SO_4)]_n$ (Figure 1).[16] Figure 1 (a) shows the ORTEP view of **3**. The Zn(II) ion of **3** has an octahedral geometry as a $Zn(N_2O_4)$ unit coordinated by each nitrogen atom of two nea molecules located in a trans



(a)



(b)

Figure 1. (a) The ORTEP drawing of $[Zn(nea)_2(H_2O)_2(SO_4)]$ **3**. Selective bond distance (Å) and angles (°) are as follows: Zn(1)–N(1) 2.129(2), Zn(1)–O(2) 2.102(2), Zn(1)–O(4) 2.126(2), O(2)–Zn(1)–O(2)' 180.0, O(2)–Zn(1)–O(4) 89.68(8), O(2)–Zn(1)–O(4)' 90.32(8), O(2)–Zn(1)–N(1) 89.32(8), O(2)–Zn(1)–N(1)' 90.68(8), O(2)'–Zn(1)–O(4) 90.32(8), O(4)–Zn(1)–N(1) 85.38(8), O(4)–Zn(1)–N(1)' 94.62(8). (b) The stereoview of **3**.

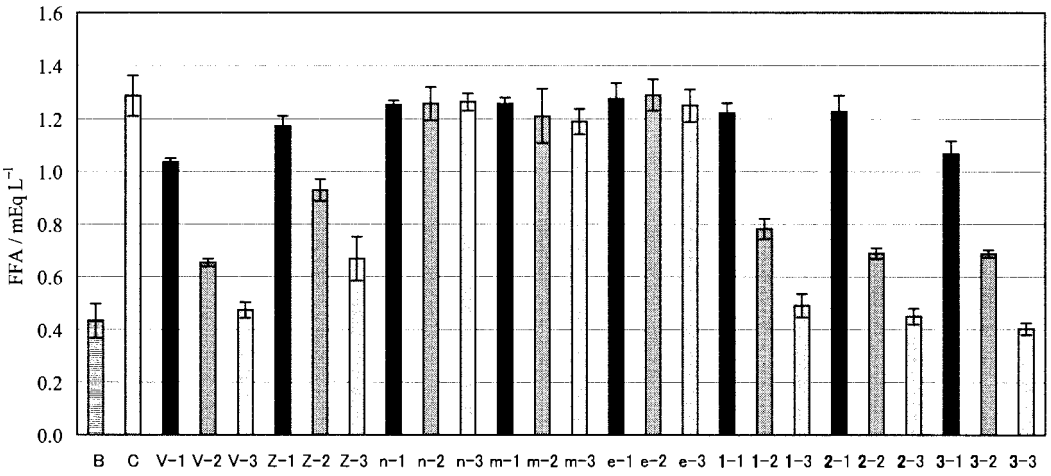


Figure 2. Inhibitory effects of $VOSO_4$, $ZnSO_4$, na, nma, nea, **1**, **2**, and **3** on free fatty acid (FFA) release from isolated rat adipocytes treated with epinephrine (EP). Rat adipocytes were prepared as reported [16]. Each column is expressed as the mean ± SDs for 3 experiments. B: blank, cells only; C: control, cells plus 10^{-5} EP. V-1–3, Z-1–3, n-1–3, m-1–3, e-1–3, **1**-1–3, **2**-1–3, **3**-1–3 are $VOSO_4$, $ZnSO_4$, na, nma, mea, **1**, **2**, and **3**, respectively. In each system, rat adipocytes (10^6 cells/mL) were treated with 10^{-4}, 5×10^{-4}, and 10^{-3} M of the compound in numerical order, respectively, for 30 min and then incubated with 10^{-5} M EP for 3 h at 37 °C.

position, each oxygen atom of two different sulfate ions and two water molecules. In the crystal, the **3**s form an one-dimensional coordination polymer bridged by sulfate ions (Figure 1 (b)).

Insulinomimetic activities of three ligands (na, nma, and nea molecules) and their Zn(II) complexes have been estimated by in vitro experiments.[17] There were no inhibitory effects of the na, nma, and nea molecules (Figure 2). The inhibitory effects of **1**, **2**, and **3** were compared with those of $VOSO_4$ and $ZnSO_4$ as positive controls (Figure 2). All Zn(II) complexes at 5×10^{-4} M inhibited the release of free fatty acids (FFA) from epinephrine-stimulated rat adipocytes more than that of $ZnSO_4$. The effects were dose-dependent in the concentration range of 10^{-4}–10^{-3} M. From these results, the apparent IC_{50} value, 50% inhibitory concentration of the complex for the FFA release, was estimated to be 1.34 ± 0.11*, 1.12 ± 0.05*, and 0.96 ± 0.03** mM (*significance at $p < 0.01$ vs $ZnSO_4$ and **significance at $p < 0.005$ vs $ZnSO_4$) for **1**, **2**, and **3**, respectively, indicating that they are more active than $ZnSO_4$ (IC_{50} = 1.52 ± 0.05 mM). The insulinomimetic activity was enhanced by the addition of alkyl groups such as methyl and ethyl.

In conclusion, cationic Zn(II) complexes, in giving insulinomimetic activities, were found to be advantageous to free Zn(II) ions. Furthermore, these Zn(II) complexes of nicotinamide derivatives are expected to have not only the in vivo blood glucose normalizing activity but also the preventive activity against DM. In addition, we will examine action mechanism of molecular complexes in comparison with that of ionic complexes. The present results will be useful for developing new insulinomimetic Zn(II) complexes in future.

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14 Complex **1** was prepared in aqueous solution of na by adding aqueous solution of $ZnSO_4 \cdot 7H_2O$, followed by adding aqueous solution of $Ba(ClO_4)_2$, and filtering the precipitated $BaSO_4$ at room temperature. The complex was recrystallized from hot water. **1**: Anal. Found: C, 24.45; N, 9.55; H, 3.40%. Calcd for $C_{12}H_{12}N_4O_2Zn \cdot (ClO_4)_2 \cdot 4.5H_2O$: C, 24.45; N, 9.50; H, 3.59%. Mp 141–146 °C. IR (KBr)/cm^{-1}; 1697 for $\nu_{c=o}$ and 1170–1050(vs) and 939(w) for ClO_4^{2-}.
15 Complexes **2** and **3** were prepared in each aqueous solution of nma and nea, respectively, by adding aqueous solution of $ZnSO_4 \cdot 7H_2O$. The complexes were recrystallized from hot water. **2**: Anal. Found: C, 31.97; N, 10.61; H, 5.04%. Calcd for $C_{14}H_{16}N_4O_2Zn \cdot (SO_4) \cdot 5H_2O$: C, 32.10; N, 10.70; H, 5.00%. Mp 115–119 °C. IR (KBr)/cm^{-1}; 1645 for $\nu_{c=o}$ and 1119(vs) for SO_4^{2-}. **3**: Anal. Found: C, 37.49; N, 10.71; H, 5.01%. Calcd for $C_{16}H_{20}N_4O_2Zn \cdot (SO_4) \cdot 3H_2O$: C, 37.25; N, 10.86; H, 5.08%. Mp 130–133 °C. IR (KBr)/cm^{-1}; 1656 for $\nu_{c=o}$ and 1092(vs) for SO_4^{2-}.
16 Crystal data for **3**: One independent molecule exists in the asymmetric unit. $C_{16}H_{24}N_4O_8ZnS$ (fw = 497.83), monoclinic, space group $C2/c$ (#15), a = 17.697(4) Å, b = 9.108(4) Å, c = 12.674(3) Å, β = 93.65(2)°; V = 2038.7(9) Å^3. $F(000)$ = 1032.00, Z = 4, Dc = 1.622 g/cm^3, μ (Mo Kα) = 13.60 cm^{-1}, $2\theta_{max}$ = 55.0°. Intensity data (2640 total (2463 independent) reflections) were collected on a Rigaku AFC7R (rotating anode). The final cycle of full matrix least squares refinement was based on 2191 observed reflections ($I > 3.00\sigma(I)$) and 187 variable parameters, and converged R = 0.034 and R_w = 0.071. Maximum peak in final differential map is 0.60 eA^{-3}.
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