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Structure–Activity Relationship of Insulinomimetic Activity of Zinc(II) Complexes with Pyridine-2-sulfonic Acid Derivatives

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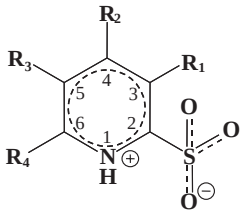
We prepared zinc(II) complexes with a series of monomethyl-substituted pyridine-2-sulfonic acid derivatives to clarify the relationship between the structure and insulinomimetic activity. Towards in vitro insulinomimetic activity, bis(3-methylpyridine-2-sulfonato)zinc(II) gave the best result among the zinc(II) complexes with methylpyridine-2-sulfonates.

Zinc(II) ion, of which the human body contains approximately 2 grams, plays key roles in more than 300 enzymes and proteins, and it is especially important as an essential trace element.[1,2] For example, zinc(II) (1) accelerates metabolism, (2) maintains degustation, olfaction, brain function, and immune systems, (3) suppresses cell senescence or canceration, and (4) detoxifies poisonous metals, such as lead. Since Coulston and Dandona first reported the insulinomimetic activity of zinc(II) ion in 1980,[3] a few researchers have investigated the insulinomimetic activity or the blood glucose normalizing effect of zinc(II) ion.[4,5] We first revealed that bis(maltolato)-zinc(II) complex with $Zn(O_4)$ coordination mode has higher insulinomimetic activity than zinc(II) ion in 2000.[6] We have found that molecular zinc(II) complexes of amino acid, picolinic acid, thione, and the derivatives with $Zn(N_2O_2)$, $Zn(S_2O_2)$, or $Zn(O_4)$ coordination modes have insulinomimetic activity in an in vitro experiment and blood glucose normalizing effects in an in vivo experiment.[6,7] We have also reported the in vitro insulinomimetic activity of ionic zinc(II) complexes with hydrophilic vitamins, oligopeptides, amino acid amide derivatives, picolinamide derivatives, and amine derivatives, which have some significant physiological functions in human body.[8,9] Additionally, we have reported that the lipophilicity of zinc(II) complex is an important factor in developing insulinomimetic complexes because zinc(II) complexes act on several action points within the cells,[10,11] as named "ensemble mechanism."[12] However, the systematic study of the structural effect on the insulinomimetic activity, particularly the substituent-positional dependence of the activity, has so far not been reported. We have used pyridine-2-sulfonic acid ($pySO_3$) as a pilot compound and have prepared ligands that contain a methyl group at the C3 to C6 position of $pySO_3$ (Fig. 1). The structures and physicochemical properties of the ligands and the zinc(II) complexes were assessed by using NMR, IR, and mass spectroscopy and elemental analyses. Some of the spectra have been published elsewhere.[13] In this study, we synthesized the zinc(II) complexes by using a new method to improve the yield.



	R_1	R_2	R_3	R_4
$pySO_3$	H	H	H	H
3m-$pySO_3$	CH_3	H	H	H
4m-$pySO_3$	H	CH_3	H	H
5m-$pySO_3$	H	H	CH_3	H
6m-$pySO_3$	H	H	H	CH_3

Fig. 1. Structures of pyridine-2-sulfonic acid and its derivatives.

Table 1. Estimated IC_{50} Values for the Free Fatty Acid (FFA) Release from Isolated Rat Adipocytes and Partition Coefficient of the Complexes

Complex	IC_{50} value/mM	Partition coefficient
$[Zn(pySO_3)_2(H_2O)_2]$	1.00	0.013 ± 0.003
$[Zn(3m\text{-}pySO_3)_2(H_2O)_2]$	0.75 ± 0.07[a)]	0.022 ± 0.004[a)]
$[Zn(4m\text{-}pySO_3)_2(H_2O)_2]$	1.09 ± 0.05	0.007 ± 0.003
$[Zn(5m\text{-}pySO_3)_2(H_2O)_2]$	1.09 ± 0.12	0.002 ± 0.002[a)]
$[Zn(6m\text{-}pySO_3)_2(H_2O)]$	1.12 ± 0.03	0.011 ± 0.003

a) $p < 0.05$ vs $[Zn(pySO_3)_2(H_2O)_2]$.

We evaluated insulinomimetic activity of zinc(II) complexes by using isolated rat adipocytes as described in Refs. 14 and 15. The results are shown in Table 1 as IC_{50} values.[15] The zinc(II) complex with 3-methylpyridinesulfonic acid (3m-$pySO_3$) introduced methyl group at the C3 position of $pySO_3$ showed higher insulinomimetic activity than that of the pilot zinc(II) complex with $pySO_3$ (Table 1).

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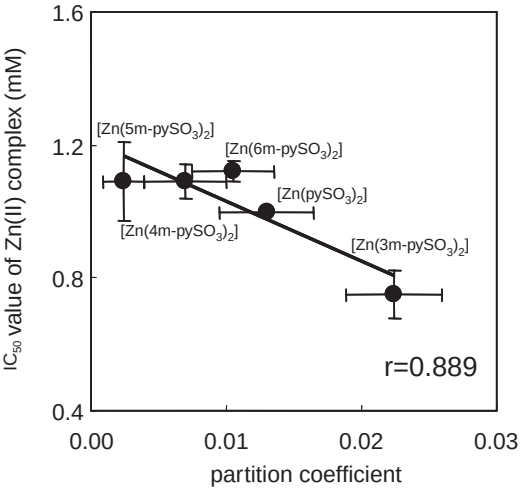

Fig. 2. Correlation between the IC_{50} value and partition coefficient[16] of the zinc(II) complexes with pyridine-2-sulfonic acid derivatives.

On the other hand, the zinc(II) complexes of $pySO_3$ containing methyl group at the C4, C5, or C6 position did not show an increase in the insulinomimetic activity compared to [Zn-$(pySO_3)_2(H_2O)_2$].

The difference in the insulinomimetic activity may be related to many factors. The observed small but clear difference may be due to the affinity of the complex toward the cell membrane. The partition coefficients of the five zinc(II) complexes were measured by using UV spectrophotometry. The values of partition coefficients altered with a change in the position of methyl substituent on the pyridine ring (Table 1 and Fig. 1). Many researchers have previously reported that simple diffusion process is an important factor of the transport mechanism through the membrane.[17,18] Thus, the high lipophylicity is the most important factor for transport through the membrane. When a methyl group was substituted in place of hydrogen at the C3 position of $pySO_3$, we found that the lipophylicity of the $[Zn(3m\text{-}pySO_3)_2(H_2O)_2]$ was significantly higher (Table 1 and Fig. 2). Furthermore, the crystal structures of [Zn(nm-$pySO_3)_2$] ($n = 0, 3$, and 5) have been analysed,[13] and they have a common molecular structure of the formula [Zn(nm-$pySO_3)_2(H_2O)_2$] ($n = 0, 3$, and 5) with arrangements of *cis* for H_2Os, *cis* for SO_3s, and *trans* for pyridines. In this geometry, the 3-methyl group effectively covers the adjacent polar sulfonate group (distance between the methyl carbon atom and the closest sulfonate oxygen atom is 3.119(3) Å). This shielding effect may prevent water solvation around sulfonate group, thus making $[Zn(3m\text{-}pySO_3)_2(H_2O)_2]$ more hydrophobic compared to the other derivatives.

In summary, we studied the insulinomimetic activity of zinc(II) complexes by changing the position of methyl substituent on the pyridine ring and found that $[Zn(3m\text{-}pySO_3)_2(H_2O)_2]$ showed the highest activity. We are continuing to investigate why introducing methyl group at the C3 position shows high insulinomimetinc activity in in vitro experiments.

Experimental

General Procedures. FT-IR spectra were recorded with KBr pellets on a Jasco FT/IR-420 spectrophotometer (Tokyo, Japan). ^1H NMR spectra were recorded on a JEOL Lambda300 spectrometer, and chemical shifts were referenced to tetramethylsilane. Electrospray ionisation (ESI) mass spectrometry measurements were performed on an Applied Biosystem Mariner spectrometer using HPLC grade solvents. Elemental analyses were performed by the Analytical Research Service Centre at Osaka City University on Perkin-Elmer 240C or FISONS Instrument EA108 elemental analyzers.

Synthesis of Ligands. The ligands, pyridine-2-sulfonic acid, 1*H*-3-methylpyridine-2-thione, 1*H*-4-methylpyridine-2-thione, 1*H*-5-methylpyridine-2-thione, and 1*H*-6-methylpyridine-2-thione, were prepared by the method in Ref. 13.

6m-$pySO_3H$: MS (ESI in methanol) m/z: 174 $(M + H)^+$, IR spectra ν_{S-O} 1267, ν_{C-S} 1011 cm^{-1}, Anal. Calcd for $C_6H_7NO_3S$: C, 41.61; H, 4.07; N, 8.09%. Found: C, 41.72; H, 3.96; N, 7.86%; yield 50%.

Synthesis of Zinc(II) Complexes with Pyridine-2-sulfonic Acid Derivatives. Zinc(II) complexes with $pySO_3$, 4m-$pySO_3$, 5m-$pySO_3$, or 6m-$pySO_3$ were prepared in the manner similar to that in Ref. 19, in a simple term, an aqueous solution of the ligand and $ZnSO_4 \cdot 7H_2O$ were mixed in 2:1 mole ratio and $Ba(OH)_2 \cdot 8H_2O$ was added to the reaction solution. After removal of $BaSO_4$ by filtration and the solvent, the residue was washed with a small amount of methanol. For zinc(II) complexes with 3m-$pySO_3$, an aqueous solution of 3-methylpyridine-2-sulfonic acid and $ZnSO_4 \cdot 7H_2O$ were mixed in 2:1 mole ratio and $LiOH \cdot H_2O$ was added to the reaction solution. The obtained white precipitate was washed with a small amount of cold water.

$[Zn(pySO_3)_2(H_2O)_2]$: Anal. Calcd for $C_{10}H_{12}N_2O_8S_2Zn$: C, 28.75; H, 2.90; N, 6.71%. Found: C, 28.84; H, 2.82; N, 6.70%; yield 31%. $[Zn(3m\text{-}pySO_3)_2(H_2O)_2] \cdot 0.2H_2O$: Anal. Calcd for $C_{12}H_{16}N_2O_8S_2Zn \cdot 0.2H_2O$: C, 32.07; H, 3.68; N, 6.23%. Found: C, 32.07; H, 3.82; N, 6.18%; yield 58%. $[Zn(4m\text{-}pySO_3)_2(H_2O)_2] \cdot 0.5H_2O$: Anal. Calcd for $C_{12}H_{16}N_2O_8S_2Zn \cdot 0.5H_2O$: C, 31.69; H, 3.77; N, 6.16%. Found: C, 31.70; H, 3.54; N, 6.16%; yield 33%. $[Zn(5m\text{-}pySO_3)_2(H_2O)_2]$: Anal. Calcd for $C_{12}H_{16}N_2O_8S_2Zn$: C, 32.33; H, 3.62; N, 6.28%. Found: C, 32.32; H, 3.69; N, 6.22%; yield 42%. $[Zn(6m\text{-}pySO_3)_2(H_2O)]$: MS (ESI in H_2O) m/z: 409 $(M + H)^+$ for $C_{12}H_{12}N_2O_6S_2Zn$. IR spectra; ν_{S-O} 1279, ν_{C-S} 1022 cm^{-1}. Anal. Calcd for $C_{12}H_{14}N_2O_7S_2Zn$: C, 33.69; H, 3.30; N, 6.55%. Found: C, 33.49; H, 3.35; N, 6.46%; yield 49%.

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15 Isolated adipocytes prepared from male rats weighing 200–210 g were preincubated at 37 °C for 30 min with various concentrations (10^{-4}–10^{-3} M) of zinc(II) complexes in Krebs Ringer Bicarbonate buffer (pH 7.4) containing 2% bovine serum albumin. A 10^{-4} M epinephrine was then added to the reaction mixtures and the resulting solutions were incubated at 37 °C for 3 h. 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). From the curve for the zinc(II) complexes, the concentration-dependent inhibitory effect on FFA release from isolated rat adipocytes treated with epinephrine, the IC_{50} value, which shows the 50% inhibitory concentration of the complex, was determined.[14]

16 The partition coefficient values were obtained using the equation $P = C_{octanol}/C_{water}$, where $C_{octanol}$ and C_{water} are the equilibrium concentrations of the zinc(II) complexes in the *n*-octanol and HEPES buffer, respectively, after shaking for 60 min. The concentrations of zinc(II) complexes in each phase were determined using the characteristic wavelength at approximately 258–265 nm due to pyridine ring.

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