Insulinomimetic Zn(II) Complexes as Evaluated by Both Glucose-Uptake Activity and Inhibition of Free Fatty Acids Release in Isolated Rat Adipocytes

Midori NISHIDE,[a] Yutaka YOSHIKAWA,[b] Eriko U. YOSHIKAWA,[a] Kinuyo MATSUMOTO,[a] Hiromu SAKURAI,[b] and Naemi M. KAJIWARA*,[a]

[a] *Laboratory of Nutrition Physiology, Graduate School of Life Science, Kobe Women's University; 2–1 Aoyama, Suma, Kobe 654–8585, Japan: and* [b] *Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University; 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607–8414, Japan.*

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We prepared 4 new Zn(II) complexes with $Zn(O_4)$, $Zn(N_2O_2)$, and $Zn(S_2O_2)$ coordination modes and evaluated their insulinomimetic activities in an *in vitro* study. The insulinomimetic activities of bis(pyrrole-2-carboxylato)zinc ($Zn(pc)_2$), bis(α-furonic acidato)zinc ($Zn(fa)_2$), bis(thiophene-2-carboxylato)zinc ($Zn(tc)_2$), and bis(thiophene-2-acetato)zinc ($Zn(ta)_2$) complexes were found to be higher than that of zinc sulfate ($ZnSO_4$). $Zn(ta)_2$ showed the highest insulinomimetic activity among the Zn(II) complexes because of its high lipophilicity.

Key words Zn(II) complex; insulinomimetic activity; inhibition of free fatty acid-release; glucose-uptake activity

Diabetes mellitus (DM) is emerging as one of the most significant diseases of the 21st century with the worldwide increase in the number of patients suffering from this disease.[1—3] According to the 2002 report of the Ministry of Health, Labor and Welfare of Japan, the number of people in Japan at "a high risk of developing diabetes" (glycosylated hemoglobin [HbA_{1c}] higher than 6.1% or receiving treatment for DM) has increased from 6900000 to 7400000 over 5 years.[4] In addition, the number of people with "the possibility of developing DM" (HbA_{1c} in the range of 5.6—6.1% and receiving no treatment for DM) has also increased from 13700000 in 1997 to 16200000.[4] On the other hand, no agents other than insulin have been developed for the treatment of either type 1 DM or severe type 2 DM. Therefore, there is an urgent need for developing new types of therapeutic agents for the treatment of DM. Several researchers have attempted to confirm the insulinomimetic activity of the Zn^{2+} ion.[5,6] We focused on the finding that Zn(II) complex bound to sulfur showed a strong insulinomimetic activity and a blood glucose lowering effect.[7,8] In this study, we synthesized the Zn(II) complexes with the $Zn(S_2O_2)$ coordination mode by using thiophene carboxylic acid or thiophene acetic acid, both of which contain thiophene as the basic skeleton, and Zn(II) complexes with furan or pyrrole wherein nitrogen of pyrrole or oxygen of furan was substituted for sulfur of thiophene carboxylic acid. We measured the insulinomimetic activity and investigated the correlation between their activities and lipophilicity.

Experimental

Materials All reagents and solvents used in this study were of the highest commercially available grade and were used as obtained. D-(+)-Glucose, $ZnSO_4$, thiophene-2-carboxylic acid, pyrrole-2-carboxylic acid, and α-furoic acid were purchased from Wako Pure Chemical Co. (Osaka, Japan); thiophene-2-acetic acid was obtained from Tokyo Kasei Inc. (Tokyo, Japan); and (±)-epinephrine hydrochloride, collagenase, and bovine serum albumin (BSA; fraction V) were purchased from Sigma Chemical Co., (St. Louis, MO, U.S.A.).

Synthesis of 4 Zn(II) Complexes The intended Zn(II) complexes were readily prepared by adding $ZnSO_4 \cdot 7H_2O$ and barium hydroxide to an aqueous solution of various ligands at room temperature.[9,10] The Zn(II) complexes were purified with hot water to obtain bis(thiophene-2-carboxylato)zinc ($Zn(tc)_2$), with 2-propanol to obtain bis(α-furoic acidato)zinc ($Zn(fa)_2$), and with methyl alcohol to obtain bis(thiophene-2-acetato)zinc ($Zn(ta)_2$) and bis(pyrrole-2-carboxylato)zinc ($Zn(pc)_2$).

Inhibitory Effects of the Zn(II) Complexes on Free Fatty Acid (FFA) Release and Glucose Uptake Enhancing Ability in Isolated Rat Adipocytes Treated with Epinephrine Male Wistar rats (age, 7—8-weeks; weight, 200—250 g) were obtained from CLEA Japan Inc., (Japan). The animals were maintained in a 12-h light/dark cycle in our central animal facility, and they were given free access to solid food (CE-2: CLEA Japan Inc.) and tap water. The animal experiments were approved by the Experimental Animal Research Committee of Kobe Women's University (KWU) and were performed according to the Guidelines for Animal Experimentation of KWU. In order to evaluate the *in vitro* insulinomimetic activity of the Zn(II) complexes, we first performed a simple and convenient *in vitro* assay based on the inhibition of FFA (Free Fatty Acids) release from isolated rat adipocytes treated with epinephrine (adrenaline).[11] This assay has been used by many researchers over 10 years.[12—14] The glucose-uptake enhancing ability of the Zn(II) compounds was evaluated based on the decrease in the glucose concentrations in the medium; the following equation was used: glucose-uptake level $= C_{control} - C_{compound}$ (μmol/ml), where $C_{control}$ is the glucose concentration in the medium containing cells without compounds such as the Zn(II) compounds after incubation time,[15] and $C_{compound}$ is the residual glucose concentration in the medium containing cells treated with these compounds after incubation time. The glucose-uptake ability of the compounds was evaluated by the apparent EC_{50} values, that is, the 50% enhancing concentration of the compound with respect to the maximal glucose-uptake concentration during the 3-h incubation. These complexes were confirmed to have dose-dependent concentrations of 5×10^{-4}, 4×10^{-4}, 3×10^{-4}, 2×10^{-4}, and 10^{-3} M. Furthermore, the inhibitory activity of the compounds on FFA release from isolated rat adipocytes treated with epinephrine was evaluated with respect to the IC_{50} values, that is, the 50% inhibitory concentration of the compound with respect to the FFA release during the 3-h incubation. The assay confirmed the dose-dependent concentrations of the tested compounds: 10^{-4} and 5×10^{-4}, 10^{-3} M for the Zn(II) complexes and 10^{-4}, 5×10^{-4}, and 7×10^{-4} M for $ZnSO_4$.

Measurement of Partition Coefficients of the Zn(II) Complexes The partition coefficients (log P) of the Zn(II) complexes were determined by the conventional method with the 10 mM HEPES buffer (pH 7.4)/*n*-octanol system. After shaking the mixture for 1 h at 37 °C, it was centrifuged at 8000 rpm for 10 min. The 2 resulting phases were separated. The Zn(II) concentration in each phase was measured at the characteristic wavelength of approximately 232—256 nm due to the aromatic ring.

Statistical Analysis The data are expressed as mean±S.D. The differences between the groups were tested by a one-way ANOVA followed by Tukey's multiple-comparison *post hoc* test. Pairwise associations were examined by Pearson's correlation coefficient test.

* To whom correspondence should be addressed. e-mail: naemi@suma.kobe-wu.ac.jp

Table 1. Physicochemical Properties and Insulinomimetic Activities of Zn(II) Complexes

Complexes	Elemental Analysis Found/Calcd.			IR spectra for $\nu_{C=O}$ (ligand)/cm^{-1}	IC_{50} value (μM)	EC_{50} value (μM)	log P
	C/%	H/%	N/%				
$ZnSO_4$	—	—	—	—	280±20	250±10	—
$Zn(tc)_2$	37.83	2.02		1615	270±20	150±10[a]	−1.61
	37.57	1.89		(1684)			
$Zn(ta)_2$	41.73	2.95		1559	260±20	120±10[a]	−1.55
	41.45	2.90		(1705)			
$Zn(fa)_2$	41.51	2.31		1620	310±50	230±10	−2.23
	41.77	2.10		(1686)			
$Zn(pc)_2$	36.60	3.64	8.49	1561	280±20	190±20[a]	−1.76
	36.35	3.42	8.48	(1663)			

Data are expressed as means±S.D. for 3 experiments. *a*) Significance at $p<0.05$ *vs.* $ZnSO_4$.

Results and Discussion

Structural Characteristics The Zn(II) complexes were characterized by several physicochemical methods, as summarized in Table 1. For the elemental analysis, both the calculated and empirical values of the percent concentrations of C, H, and N were identical within the estimated range of experimental error. In the IR spectra, the frequency of the $\nu_{C=O}$ band of the complex shifted with respect to that of the free ligand. These results suggest that the binding ratio of the Zn(II) complexes with the Zn(II) ligand was 1 : 2 (Fig. 1).

***In Vitro* Insulinomimetic Activity of the 4 Zn(II) Complexes** The FFA release inhibitory activity (IC_{50} values) and the glucose-uptake activity (EC_{50} values) were calculated from the experimental data (Table 1). The IC_{50} values indicated that $Zn(tc)_2$ and $Zn(ta)_2$ with the $Zn(S_2O_2)$ coordination mode had higher insulinomimetic activities than $Zn(fa)_2$ with the $Zn(O_4)$ coordination mode and $Zn(pc)_2$ with the $Zn(N_2O_2)$ coordination mode. Moreover, $Zn(ta)_2$ exhibited a higher potent insulinomimetic activity than $Zn(tc)_2$ with the same coordination mode. The EC_{50} values of Zn(II) complexes ($Zn(tc)_2$=150, $Zn(ta)_2$=120, $Zn(fa)_2$=230, and $Zn(pc)_2$=190 μM) were significantly lower than those of $ZnSO_4$ (250 μM). Especially, $Zn(ta)_2$ with $Zn(S_2O_2)$ coordination mode indicated not only the highest IC_{50} value but also the highest EC_{50} value. The partition coefficients ($C_{n\text{-octanol}}/C_{buffer}$) of the Zn(II) complexes were measured with UV spectrophotometry. Many researchers have previously reported that a simple diffusion is important for transport through the membrane.[16,17] Moreover, it is believed that the action sites of Zn(II) are thought to be primarily located in the cells.[18] The high lipophilicity is thus the most important factor for transport through the membrane. The lipophilicity of $Zn(ta)_2$ was the highest among all the 4 Zn(II) complexes (Table 1). Interestingly, a good linear correlation was observed between IC_{50} or EC_{50} and the partition coefficients (log P) of the Zn(II) complexes (Fig. 2): we found that when the Zn(II) complexes have higher lipophilicity, the insulinomimetic activity will show the highest values. It is considered that $Zn(ta)_2$ with the $Zn(S_2O_2)$ coordination mode that exhibited a high lipophilicity, accelerated the cellular uptake and consequently, the complex showed the highest FFA release inhibitory activity and glucose-uptake effect among the 4 Zn(II) complexes. In conclusion, we developed new, potent insulinomimetic Zn(II) complexes with the $Zn(S_2O_2)$ coordination mode, and $Zn(ta)_2$ showed the most highest insulinomimetic activity among all other Zn(II) complexes. The effectiveness of this complex in diabetic animals will be reported in the near future.

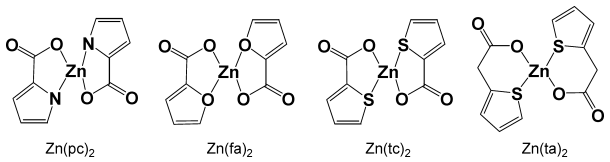

Fig. 1. Proposed Structure of Zn(II) Complexes

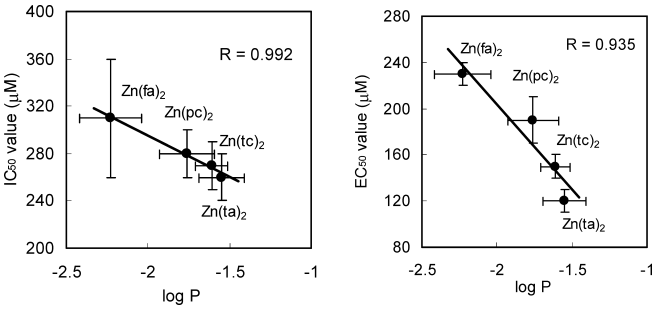

Fig. 2. Correlation between the IC_{50} or EC_{50} Values and Partition Coefficient (log P)

The IC_{50} values on FFA-release from isolated rat adipocytes treated with epinephrine and EC_{50} values on glucose-uptake. The log P ($C_{n\text{-octanol}}/C_{buffer}$) of the Zn(II) complexes were measured by using UV spectrophotometry.

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