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Original article

Suppressive effects of O_4 coordination-type zinc complexes on *in vitro* AGE formation

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia. Persistent hyperglycemia causes various complications and vascular damage. Increased production of advanced glycation end products (AGEs) via glycation is involved in DM onset. Previous reports have demonstrated the therapeutic effects of zinc (Zn) complexes on diabetic model mice *in vivo*. In this study, we aimed to investigate the *in vitro* anti-glycation effects of the $Zn(O_4)$ type Zn complexes, $[Zn(mal)_2]$, $[Zn(emal)_2]$, $[Zn(trp)_2]$, and $[Zn(hkt)_2]$. We determined the effects of the $Zn(O_4)$ coordination type complexes on glycation via screening tests of their suppressive effects on fluorescent AGE formation and pentosidine, a fluorescent AGE. The screening test was performed using a phosphate buffer (pH 7.4) at 60 °C for 40 h. Then, pentosidine was extracted from the supernatant using MonoSpin AG and quantified using high-performance liquid chromatography-fluorescence analysis. The tested $Zn(O_4)$-type complexes significantly suppressed fluorescent AGE formation more than the positive control, aminoguanidine. Moreover, pentosidine levels were decreased by $Zn(O_4)$ complexes with a 7-member troponoide structure, $[Zn(trp)_2]$ and $[Zn(hkt)_2]$. To the best of our knowledge, this study is the first to demonstrate the suppressive effects of Zn complexes on glycation reactions *in vitro*.

***KEY WORDS*:** Zn complex, AGEs (advanced glycation end products), pentosidine, anti-glycation

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia. Approximately 537 million DM cases were reported worldwide in 2021, and this number is predicted to increase to 783 million by 2045 [1)]. Approximately 20 million DM or pre-DM cases are observed in Japan [2)]. DM is classified into two types based on its pathogenesis: type 1 and type 2 DM. Type 1 DM is caused by autoimmunity induced by viral infections, damage of pancreatic β cells by immune cells, and genetic factors. In contrast, type 2 DM is a chronic metabolic disease caused by genetic factors, such as insufficient secretion or action of insulin, and environmental factors, such as overeating and lack of exercise. Persistent hyperglycemia causes various complications, thereby reducing the patient quality of life [2)]. To prevent these complications, DM treatments involve lifestyle changes and drugs to control the blood glucose levels. Complications caused by chronic hyperglycemia in diabetes include diabetic nephropathy, diabetic retinopathy, neuropathy, and vascular disorders. Non-enzymatic glycation reactions are a major cause of these complications, and advanced glycation end products (AGEs) formed by glycation reactions are involved in the pathogenesis of such complications [3-6)]. Glycation reactions can be divided into early- and late-stage reactions. In early-stage reactions, reducing sugars combine with the amino groups of proteins to yield Schiff bases and Amadori rearrangement products. HbA1c is a compound produced in early- stage glycation reactions, and its level indicates the blood glucose status. In late-stage reactions, AGEs are generated via the crosslinking Amadori rearrangement products [7)]. Accumulation of AGEs reduces insulin secretion in patients with type 2 DM; therefore, suppression of AGE production has attracted considerable attention for DM treatment [8)]. In addition to DM, AGE-induced glycation stress causes various changes, including osteoporosis and

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Alzheimer's disease, leading to lifestyle-related diseases and aging [9,10]. Glycated insulin levels are increased in animals and patients with DM, and the glycation of insulin reduces its biological activity [11-15].

Pentosidine, a fluorescent AGE initially isolated from the human brain dural collagen in 1989 [16], is produced from the amino-terminal ends of ribose, arginine, and lysine (*Fig. 1*). Plasma pentosidine levels are elevated in patients with DM and chronic renal disease and used as early clinical markers of nephropathy. Recently, pentosidine has attracted attention as a biological marker of bone aging and is used for the diagnosis of osteoporosis [17].

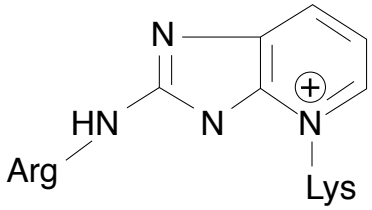

Fig. 1. **Structure of pentosidine.**

Zinc (Zn) is an essential trace element present at a concentration of approximately 2 g in the body. Many studies have reported the association of Zn with DM [18,19]. Previously, we synthesized Zn complexes with improved bioavailability of Zn^{2+} and anti-DM effects [20-25]. In 2000, bis(maltolato) zinc(II) complex ([$Zn(mal)_2$]) was reported to exhibit high insulin-like activity [24]. Our team has also focused on Zn complexes to improve their absorption in the body. We previously revealed that the enhanced activation of the insulin signaling pathway by Zn complexes exerts anti-DM effects in DM model mice [25]. These studies indicate that Zn complexes exert insulin-like and anti-DM effects, including HbA1c-lowering effects; however, the specific anti-DM mechanisms of Zn complexes remain unclear. Therefore, in this study, we focused on AGEs, factors involved in the onset of DM. Particularly, we investigated the inhibition of AGE formation by the $Zn(O_4)$ coordination type Zn complexes, [$Zn(mal)_2$], [$Zn(emal)_2$], [$Zn(trp)_2$], and [$Zn(hkt)_2$]. All these Zn complexes have been reported to exhibit insulin-like effects *in vitro* and blood glucose-lowering effects *in vivo*. Therefore, we examined their inhibitory effects on fluorescent AGE formation and determined the pentosidine levels via high-performance liquid chromatography with fluorescence detection (HPLC-FL).

Methods

Chemicals

Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), di-potassium hydrogen phosphate, sodium borohydride, sodium hydroxide (for volumetric analysis), tris(hydroxymethyl)aminomethane, acetonitrile, and acetic acid were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Lithium hydroxide monohydrate, ethanol, methanol, zinc acetate dihydrate, hinokitiol, dimethyl sulfoxide (DMSO), d-(+)-glucose, sulfuric acid, potassium dihydrogen phosphate, formic acid, 6 N hydrochloric acid, 100% w/v trichloroacetic acid solution, ammonium formate, and carboxymethyl cellulose sodium salt were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Additionally, 3-hydroxy-2-methyl-4-pyrone (maltol), 2-ethyl-3-hydroxy-4-pyrone (ethylmaltol), tropolone, and aminoguanidine (AG) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Human serum albumin (HSA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Pentosidine standard was obtained from GL Sciences (Tokyo, Japan).

Preparation of Zn complexes

[$Zn(mal)_2$] complex was prepared in deionized water by mixing $ZnSO_4$, $LiOH \cdot H_2O$, and maltol in a 1:2:2 molar ratio. The solution was stirred for 12 h at 25°C. The resulting white precipitate was collected via vacuum filtration, washed several times with a small amount of water, and dried overnight in vacuo, as previously described [24]. The prepared complex was characterized via elemental analysis and infrared (IR) absorption spectroscopy (Shimadzu FT-IR 8100A on KBr pellets; Shimadzu Co., Kyoto, Japan).

Anal. Found (%): C, 39.60; H, 4.11. Calcd. (%) for [$Zn(mal)_2] \cdot 2.7H_2O$; C, 39.57; H, 4.26. IR spectra (complex/ligand); υ C=O; 1614 cm^{-1}/1653 cm^{-1} (***Fig. 2-a***).

[$Zn(emal)_2$] complex was prepared in deionized water by mixing $ZnSO_4$, $LiOH \cdot H_2O$, and ethylmaltol in a 1:2:2 molar ratio. The solution was stirred for 12 h at 25°C. The resulting white precipitate was collected via vacuum filtration, washed several times with a small amount of water, and dried overnight in vacuo, as previously described [24]. The prepared complex was characterized via elemental analysis and IR absorption spectroscopy (Shimadzu FT-IR 8100A on KBr pellets; Shimadzu Co.).

Anal. Found (%): C, 42.98; H, 4.32. Calcd. (%) for [$Zn(emal)_2] \cdot 2.6H_2O$;C, 43.06; H, 4.96. IR (complex/ligand); υ C=O; 1595 cm^{-1}/1647 cm^{-1} (***Fig. 2-b***).

[$Zn(trp)_2$] and [$Zn(hkt)_2$] complexes were prepared as previously described [26]. For the [$Zn(trp)_2$] complex, deionized water of $(CH_3COO)_2Zn \cdot 2H_2O$ and tropolone liquid in deionized water were mixed in a 1: 2 ratio. For the [$Zn(hkt)_2$] complex, deionized waster of $(CH_3COO)_2Zn \cdot 2H_2O$ and methanol solution of hinokitiol were mixed in a 1:2 ratio. Both mixed solutions were stirred for 12 h at 25°C. The resulting white-yellow precipitate was collected via vacuum filtration, washed several times with a small amount of water, and dried overnight in vacuo [27].

Anal. Found (%): C, 54.82; H, 3.26. Calcd. (%) for [$Zn(trp)_2$]; C, 54.66; H, 3.28. IR spectra (complex/ligand); υ C=O; 1595 cm^{-1}/1607 cm^{-1} (***Fig. 2-c***).

Anal. Found (%): C, 61.31; H, 5.59. Calcd. (%) for [$Zn(hkt)_2$]; C, 61.31; H, 5.66. IR spectra (complex/ligand); υ C=O; 1589 cm^{-1}/1608 cm^{-1} (***Fig. 2-d***).

Measurement of fluorescent AGE levels in the HSA–glucose glycation model

Suppressive effects of Zn complexes on AGE formation were evaluated using the HSA–glucose model, as previously described [28,29]. AG, a glycation reaction inhibitor, was used as a positive control, and the sample buffer (50 mM phosphate buffer [pH 7.4], 8 mg/mL HSA, and 200 mM glucose) was

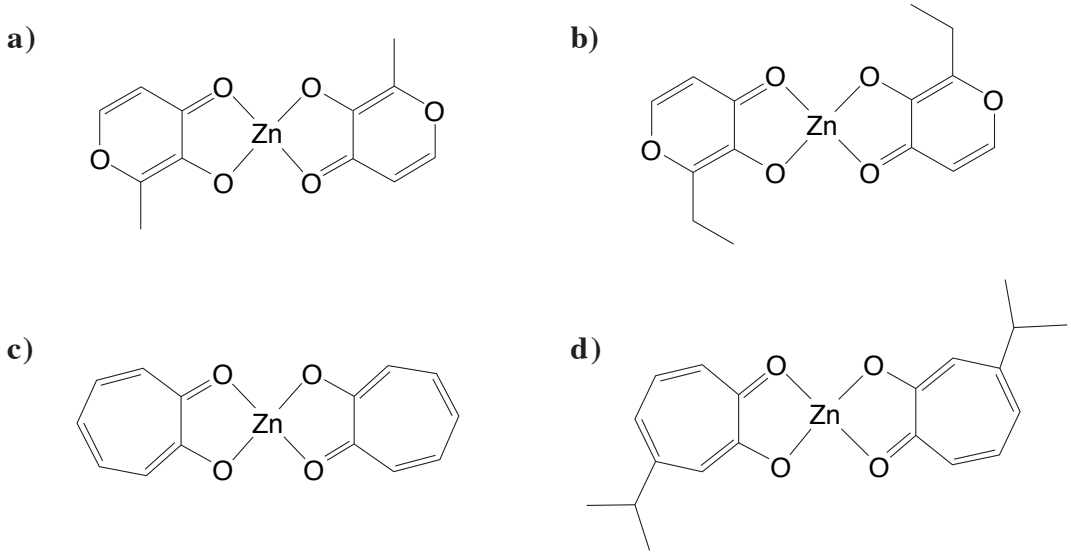

Fig. 2. **Estimated structures of Zn complexes.**
(a) [Zn(mal)$_2$]. **(b)** [Zn(emal)$_2$]. **(c)** [Zn(trp)$_2$]. **(d)** [Zn(hkt)$_2$].

prepared. Zn complexes were dissolved in 100% DMSO, added as test samples to the sample buffer in 1% DMSO, incubated at 60°C for 40 h, and centrifugated at 18,000 × g and 25°C for 10 min. Then, the supernatants were collected and the fluorescence intensity was measured to quantify the fluorescent AGEs (excitation wavelength 360 nm/fluorescent wavelength 450 nm) using a 96-well black bottom plate with Tecan infinite F200PRO (Tecan, Männedorf, Switzerland). The obtained values were calculated as AGE inhibition rates, and the fluorescence vales were adjusted using 5 μg/mL quinine sulfate solution.

Inhibition rate (%) = {1 – (A – B)/(C – D)} × 100
A: Reaction solution (Glucose (+) + tested sample)
B: Reaction solution without glucose (Glucose (–) + tested sample)
C: Without sample (Glucose (+) + sample buffer)
D: Blank (Glucose (–) + sample buffer)

Measurement of pentosidine levels in the HSA–glucose glycation samples via HPLC

Next, supernatants of the HSA–glucose glycation samples were pretreated as previously described [30, 31]. The supernatants were reduced with 200 mM sodium borohydride solution (pH 9.2) to prevent pentosidine formation during hydrolysis for 30 min at 25°C. Proteins were removed using 20% and 5% TCA solutions. After removing the supernatants, ultrapure water was added, and the precipitate was resolved. Then, 6 N iron-free hydrochloric acid was added, and the mixture was heated at 105°C for 18 h for hydrolysis. After hydrolysis, 1.5 M Tris was added to dilute hydrochloric acid in the solution and centrifuged using Centricut Ultra Mini (Kurabo, Osaka, Japan). The hydrolyzed samples were pretreated using the Monospin AG column (GL Sciences), and the eluates were obtained using a measurement sample. Analytical HPLC was performed using Shimadzu LC-20AB (Shimadzu Co.) fitted with the reverse-phase silica gel column Inert Sustain AG (3 μm, 100 mm × 4.6 mm ID; GL Sciences) and RF-20A (Shimadzu Co.) Fluorescence detector. The measurement conditions of HPLC were as follows: column temperature, 20°C; eluent, A solution: 0.1% formic acid (v/v) in H_2O solution, B solution: 100% acetonitrile; elution conditions as step gradient: 0% B (0–10 min), 50% B (10–12 min), 0% B (12–30 min) flow rate, 1.0 mL/min; detection, fluorescence (excitation [Ex] wavelength 325 nm, emission [Em] wavelength 385 nm); injection volume, 20 μL. In this gradient program, the latter two steps (with 50% B [10–12 min] and 0% B [12–30 min]) were programmed for column washing and conditioning, respectively.

Statistical analyses

Data are expressed as the mean ± standard deviation. Multiple comparisons with the AG group were analyzed using analysis of variance and Dunnett's post-hoc tests with Bell Curve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

Suppressive effects of Zn complexes on fluorescent AGE formation

In this study, we examined the suppressive effects of Zn complexes including Zn^{2+} on fluorescent AGE formation. We found that all Zn complexes with $ZnSO_4$ (half-maximal inhibitory concentration [IC_{50}] = 610 ± 93 μM), except [Zn(emal)$_2$] (IC_{50} = 1,531 ± 257 μM), significantly suppressed AGE formation better than AG (IC_{50} = 859 ± 70 μM). Moreover, Zn complexes exerted their suppressive effects in a concentration-dependent manner. Especially, Zn(O_4) complexes with a 7-member troponoide structure, [Zn(trp)$_2$] and [Zn(hkt)$_2$], exhibited approximately 40–100-times stronger anti-glycation effects than AG, with IC_{50} value

of 20 ± 1 and 9 ± 2 μM, respectively (*Table 1*). We also investigated the suppressive effects of their ligands. Compared with AG, only Trp and Hkt exhibited significant suppressive effects (*Table 1*).

Table 1. IC_{50} values of Zn complexes for AGE formation in glycation samples.

Sample	IC_{50} (μM)
AG	859 ± 70
$ZnSO_4 \cdot 7H_2O$	610 ± 93**
$[Zn(mal)_2]$	468 ± 33**
$[Zn(emal)_2]$	1,531 ± 257
$[Zn(trp)_2]$	20 ± 1**
$[Zn(hkt)_2]$	9 ± 3**
mal	1,093 ± 26
emal	1,823 ± 44
trp	38 ± 1**
hkt	18 ± 6**

Values are expressed as the mean ± standard deviation (SD). ** $p < 0.01$ *vs.* AG. IC_{50}, half-maximal inhibitory concentration; AGE, advanced glycation end product; AG, aminoguanidine; SD, standard deviation.

Measurement of pentosidine levels in glycated residues

Next, we set the gradient conditions for column washing and conditioning in the latter two steps: 50% B (10–12 min) and 0% B (12–30 min). A standard pentosidine-derived peak was detected with an average retention time of 9.1 min (*Fig. 3-a*). The peak area and concentration of pentosidine showed good linearity ($y = 260{,}085\,x + 1199$, $R = 1$) in the concentration range of 0.1–2.0 ng/mL. The amount of pentosidine was quantified based on the calibration curve prepared for each HPLC-FL measurement.

Suppressive effects of Zn complexes on pentosidine formation

Notably, all tested Zn complexes were found to suppress pentosidine formation in a concentration-dependent manner. Next, we determined the IC_{50} values of these complexes for pentosidine formation (*Table 2*). All $Zn(O_4)$ complexes exhibited suppressive effects. Particularly, $[Zn(trp)_2]$ and $[Zn(hkt)_2]$, $Zn(O_4)$ complexes with a 7-member troponoide structure, exhibited the strongest suppressive effects on pentosidine formation among the tested Zn complexes. Although $[Zn(emal)_2]$ did not sufficiently inhibit fluorescent

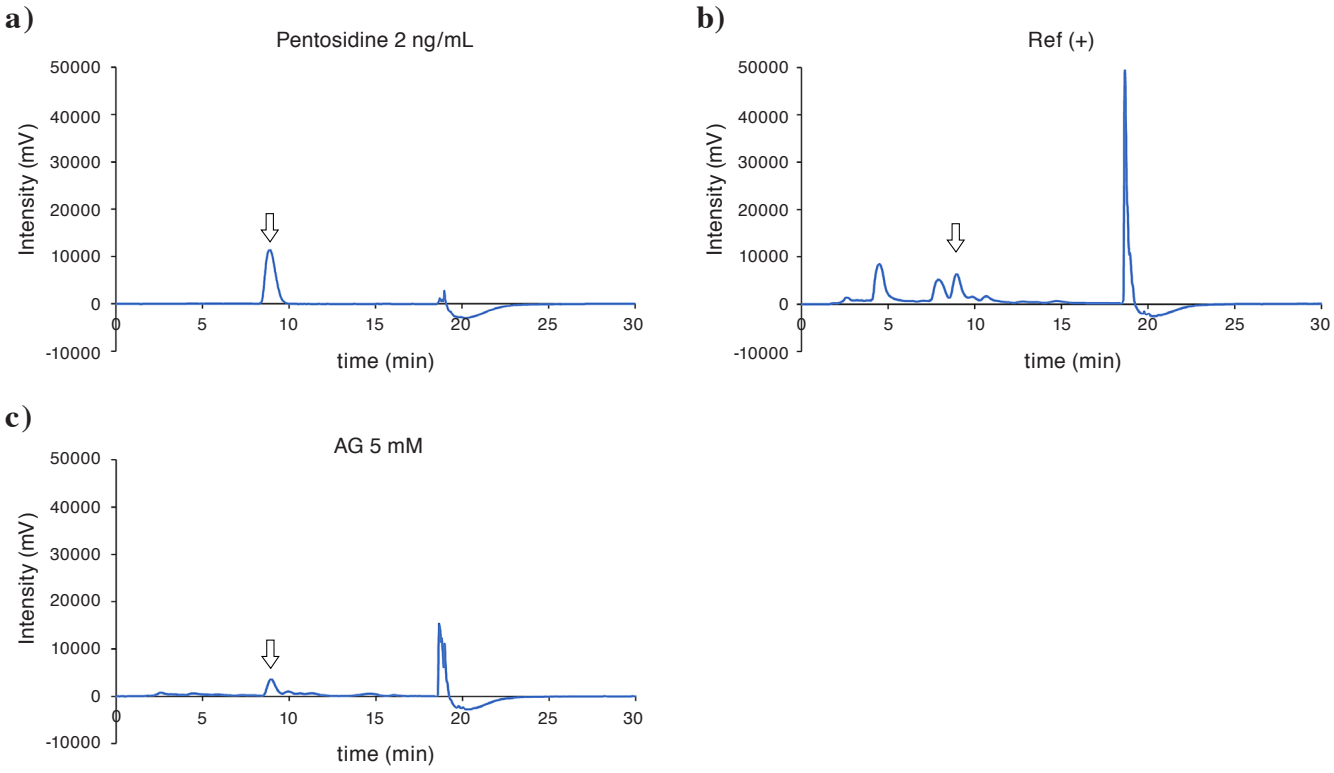

Fig. 3. **Chromatograph of glycation samples using a spin column (Monospin AG).**

(a) Pentosidine (2 ng) standard curve. **(b)** Reference (+) sample. **(c)** AG (5 mM). Glycation sample (50 μL) was hydrolyzed and prepared using a spin column (Monospin AG). Arrow indicates the pentosidine peak. High-performance liquid chromatography with fluorescence detection (HPLC-FL) was performed as follows: column, Inert Sustain AG (3 μm, 100 mm × 4.6 mm ID); column temperature, 20 °C; flow rate, 1.0 mL/min; detection, Ex 325 nm/Em 385 nm; eluent, A solution: 0.1% formic acid (v/v) in H_2O solution, B solution: 100% acetonitrile; elution conditions as step gradient: 0% B (0–10 min), 50% B (10–12 min), 0% B (12–30 min); injection volume, 20 μL. In this gradient program, the latter two steps (50% B [10–12 min] and 0% B [12–30 min]) were programmed for column washing and conditioning, respectively. AG, aminoguanidine.

Table 2. IC_{50} values of Zn complexes for pentosidine formation in glycation samples.

Sample	IC_{50} (μM)
AG	102 x 10^3 ± 73 x 10^3
$ZnSO_4 \cdot 7H_2O$	638 ± 212**
$[Zn(mal)_2]$	304 ± 84**
$[Zn(emal)_2]$	371 ± 155**
$[Zn(trp)_2]$	19 ± 6**
$[Zn(hkt)_2]$	15 ± 8**

Values are expressed as the mean ± SD. ** $p < 0.01$ vs. AG. IC_{50}, half-maximal inhibitory concentration; AG, aminoguanidine; SD, standard deviation.

AGE formation in HSA–glucose screening test, it exerted stronger suppressive effect on pentosidine formation than AG or $ZnSO_4$.

Discussion

AGEs are associated with various DM complications and aging by increasing and accelerating glycation reactions in the body under hyperglycemic conditions. Pentosidine is an AGE that accumulates in human tissues with aging, and its accumulation associated with many diseases, including DM complications [32]. Anti-glycation effects of various substances, such as flavonoids and L-carnitine, have been evaluated [33-36]. Such pathological conditions need to be further investigated to develop new treatments.

Zn complexes exert insulin-like and hypoglycemic effects. In this study, we investigated the anti-glycation effects of Zn complexes from a novel perspective. We evaluated the effects of $Zn(O_4)$ coordination type Zn complexes on the inhibition of AGE formation. Determination of the association between AGEs and Zn complexes, in addition to the anti-DM effects of Zn complexes, will aid in their long-term administration for the prevention and treatment of various diseases, including DM complications.

Here, we examined the anti-glycation effects of Zn complexes by determining their effects on glycation reactions using the HSA–glucose glycation model. In this glycation model, glucose was used as the reducing sugar, and the model assumed a DM state. The influence of Zn complexes on protein glycation was evaluated based on the inhibition rate of fluorescent AGEs. We found that Zn compounds, including Zn^{2+} and Zn complexes, suppressed fluorescent AGEs in the screening test. These ligands exhibited suppressive effects in the screening test (*Table 1*). A previous study revealed that bis(ascrobinato)Zn(II) complex ($[Zn(Vc)_2]$), with weak coordinating strength between ascorbic acid and Zn ($\log\beta = 1$) [37], exists as a Zn complex in both 0.015 M HEPES-phosphate buffer (pH 7.4) and 0.015 M HEPES buffer (pH 7.4) using cyclic voltammograms data [38]. As $[Zn(Vc)_2]$ maintains its complex structure in both buffer solutions with and without phosphoric acid, we hypothesized that Zn complexes inhibit fluorescent AGE formation in the present study. Furthermore, for detailed evaluation of AGEs, quantitative analysis of pentosidine against Zn complexes was also conducted using HPLC-FL.

Pentosidine is a type of fluorescent AGE. Blood pentosidine concentration is associated with various diseases, such as DM and renal failure, and used as a biomarker. To evaluate the HPLC-FL method, a calibration curve was plotted using standard pentosidine, and the sample solution was prepared from a previously glycated sample solution in the HSA–glucose model and pentosidine extraction process. A pentosidine peak was observed at approximately 9.1 min, and the calibration curve showed good linearity (*Fig. 3*). Here, samples were analyzed in two ways: one sample was subjected to HPLC measurement after undergoing pentosidine extraction without refrigeration, whereas the other was subjected to HPLC measurement after refrigeration for one-week post-pentosidine extraction. No significant differences were observed in the results of the two samples, suggesting that the samples could be refrigerated for one week after pentosidine extraction (data not shown). As AGEs are produced in a non-enzymatic manner, their long-term storage under heat and light is not desirable.

In this study, we found that $Zn(O_4)$ type complexes, especially $[Zn(trp)_2]$ and $[Zn(hkt)_2]$ complexes with a 7-member troponoide structure, exhibited significantly greater suppressive effects on pentosidine formation than AG. Although $[Zn(emal)_2]$ suppressed pentosidine formation, it did not sufficiently suppress fluorescent AGE formation in the screening test (*Table 2*). $[Zn(hkt)_2]$ exhibited the strongest inhibitory effect on pentosidine formation among all tested complexes. We previously reported that $[Zn(hkt)_2]$ activates the insulin signaling pathway and ameliorates insulin resistance in peripheral tissues via islet-protecting effects in type 2 DM model KK-A^y mice [23, 39].

To the best of our knowledge, this study is the first to demonstrate the anti-glycation effects of Zn complexes *in vitro*. Specifically, $[Zn(trp)_2]$ and $[Zn(hkt)_2]$, $Zn(O_4)$ type complexes with a 7-member troponoide structure, exerted strong anti-glycation effects, suppressing both fluorescent AGE and pentosidine formation. Furthermore, they inhibited AGE formation and accumulation, thereby preventing DM complications. Notably, the anti-glycation effects differed depending on the type of Zn coordination. Therefore, further studies are necessary to investigate the mechanism of the anti-glycation effects based on the structure–activity relationships of Zn complexes. With regard to glycative stress and the development of type 2 DM, it has recently been suggested that postprandial hyperglycemia is followed by a chain reaction of the formation of multiple aldehydes [40], and that these aldehydes non-physiologically modify intravascular and intracellular proteins and are involved in both insulin biosynthesis and insulin resistance in β cells [41]. The aldehyde scavenging activity of Zn complexes to prevent these series of reactions will also be investigated in the future.

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Conflict of Interest

The authors declare no conflicts of interest.

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References

1) Chapter 1: What is diabetes? In: IDF Diabetes Atlas, 10th Ed, 2021; 11-19.
2) Ministry of Health, Labour and Welfare, Summary of the 2016 National Health Survey and Nutrition Survey Results.
3) Koyama H. AGE/RAGE: Potential therapeutic target for diabetic vascular disease. *J Jpn Diabetes Soc*. 2010; 53: 227-230.
4) Yamagishi S. Role of AGEs-RAGE system in vascular complications in diabetes. *J Jpn Coll Angiol*. 2010; 50: 555-559.
5) Paneni F, Beckman JA, Creager MA, et al. Diabetes and vascular disease: Pathophysiology, clinical consequences, and medical therapy: Part I. *Eur Heart J*. 2013; 34: 2436-2446.
6) Vistoli G, De Maddis D, Gasparovic AC, et al. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): An overview of their mechanisms of formation. *Free Radic Res*. 2013; 47: 3-27.
7) Meerwaldt R, Links T, Zeebregts C, et al. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. *Cardiovasc Diabetol*. 2008; 7: 29.
8) Okura T, Ueta E, Nakamura R, et al. High serum advanced glycation end products are associated with decreased insulin secretion in patients with type 2 diabetes: A brief report. *J Diabetes Res*. 2017; 2017: 5139750.
9) Eller-Vainicher C, Cairoli E, Grassi G, et al. Pathophysiology and management of type 2 diabetes mellitus bone fragility. *J Diabetes Res*. 2020; 2020: 7608964.
10) Srikanth V, Maczurek A, Phan T, et al. Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. *Neurobiol Aging*. 2011; 32: 763-777.
11) Abdel-Wahab YH, O'Harte FP, Boyd AC, et al. Glycation of insulin results in reduced biological activity in mice. *Acta Diabetol*. 1997; 34: 265-270.
12) Boyd AC, Abdel-Wahab YH, McKillop AM, et al. Impaired ability of glycated insulin to regulate plasma glucose and stimulate glucose transport and metabolism in mouse abdominal muscle. *Biochim Biophys Acta*. 2000; 1523: 128-134.
13) Hunter SJ, Boyd AC, O'Harte FP, et al. Demonstration of glycated insulin in human diabetic plasma and decreased biological activity assessed by euglycemic-hyperinsulinemic clamp technique in humans. *Diabetes*. 2003; 52: 492-498.
14) Lindsay JR, McKillop AM, Mooney MH, et al. Effects of nateglinide on the secretion of glycated insulin and glucose tolerance in type 2 diabetes. *Diabetes Res Clin Pract*. 2003; 61: 167-173.
15) Walke PB, Bansode SB, More NP, et al. Molecular investigation of glycated insulin-induced insulin resistance via insulin signaling and AGE-RAGE axis. *Biochim Biophys Acta Mol Basis Dis*. 2021; 1867: 166029.
16) Mikulikova K, Eckhardt A, Kunes J, et al. Advanced glycation end-product pentosidine accumulates in various tissues of rats with high fructose intake. *Physiol Res*. 2008; 57: 89-94.
17) Yagi M, Takabe W, Ishizaki K, et al. The evaluation of glycative stress and anti-glycation effect. *J Oleo Sci*. 2018; 18: 17-23. (in Japanese)
18) Fukunaka A, Fujitani Y. Role of zinc homeostasis in the pathogenesis of diabetes and obesity. *Int J Mol Sci*. 2018; 19: 476.
19) Tamaki M, Fujitani Y. Role of zinc in type 2 diabetes. *Jpn J Hyg*. 2014; 69: 15-23. (in Japanese)
20) Coulston L, Dandona P. Insulin-like effect of zinc on adipocytes. *Diabetes*. 1980; 29: 665-667.
21) Chen MD, Liou SJ, Lin PY, et al. Effects of zinc supplementation on the plasma glucose level and insulin activity in genetically obese (ob/ob) mice. *Biol Trace Elem Res*. 1998; 61: 303-311.
22) Yoshikawa Y, Yasui H. Zinc complexes developed as metallopharmaceutics for treating diabetes mellitus based on the bio-medicinal inorganic chemistry. *Curr Top Med Chem*. 2012; 12: 210-218.
23) Naito Y, Yoshikawa Y, Masuda K, et al. Bis(hinokitiolato) zinc complex ([Zn(hkt)$_2$]) activates Akt/protein kinase B independent of insulin signal transduction. *J Biol Inorg Chem*. 2016; 21: 537-54s8.
24) Yoshikawa Y, Ueda E, Kawabe K, et al. New inslin-mimetic zinc (II) complexes; bis-maltolato zinc (II) and bis-2-hydroxypyridine-N-oxido zinc (II) with Zn(O$_4$) coordination mode. *Chem Lett*. 2000; 29: 874-875.
25) Yoshikawa Y, Adachi Y, Yasui H, et al. Oral administration of bis(aspirinato)zinc(II) complex ameliorates hyperglycemia and metabolic syndrome-like disorders in spontaneously diabetic KK-A^y mice: Structure-activity relationship on zinc-salicylate complexes. *Chem Pharm Bull*. 2011; 59: 972-977.

26) Naito Y, Yoshikawa Y, Yasui H. Cellular mechanism of Zinc-hinokitiol complexes in diabetes mellitus. *Bull Chem Soc Jpn*. 2011; 84: 298-305.
27) Yamane M, Adachi Y, Yoshikawa Y, et al. A new anti-diabetic Zn(II)-hinokitiol (β-thujaplicin) complex with $Zn(O_4)$ coordination mode. *Chem Lett*. 2005; 34: 1694-169s5.
28) Hori M, Yagi M, Nomoto K, et al. Experimental models for advanced glycation end product formation using albumin, collagen, elastin, keratin and proteoglycan. *Anti Aging Med*. 2012, 9, 125-134.
29) Matsuura N, Aradate T, Sasaki C, et al. Screening system for the maillard reaction inhibitor from natural product extracts. *Chem Abstr*. 2002; 48: 520-526.
30) Sakiyama C, Yagi M, Miyazaki S, et al. A rsapid pretreatment method for the determination of pentosidine in human plasma by high performance liquid chromatography. *Glycative Stress Res*. 2020; 7: 211-219.
31) Chu H, Hu X, Yao J, et al. One-pot preparation of hydrophilic citric acid-magnetic nanoparticles for identification of glycopeptides in human saliva. *Talanta*. 2020; 206: 120178.
32) Morales S, Gracia-Salcedo JA, Munoz-Torres M. Pentosidine: A new biomarker in diabetes mellitus complications. *Med Clin*. 2011; 136: 298-302.
33) Yousof Ali M, Zaib S, Mizanur Rahman M, et al. Poncirin, an orally active flavonoid exerts antidiabetic complications and improves glucose uptake activating PI3K/Akt signaling pathway in insulin resistant C2C12 cells with anti-glycation capacities. *Bioorg Chem*. 2020; 102: 104061.
34) Gao Q, Ma R, Shi L, et al. Anti-glycation and anti-inflammatory activities of anthocyanins from purple vegetables. *Food Funct*. 2023; 14: 2034-2044.
35) Swamy-Mruthinti S, Carter AL. Acetyl-L-carnitine decreases glycation of lens proteins: *In vitro* studies. *Exp Eye Res*. 1999; 69: 109-115.
36) Fukami K, Yamagishi S, Sakai K, et al. Potential inhibitory effects of L-carnitine supplementation on tissue advanced glycation end products in patients with hemodialysis. *Rejuvenation Res*. 2013; 16: 460-466.
37) Martell AE, Smith RM. Critical stability constants. In: Other organic ligands, Vol 3, Plenum Press, *New York*, 1977; 264-265.
38) Kojima Y, Yoshikawa Y, Ikura N, et al. Improvement of glucose tolerance by vitamin C/Zinc(II) complexes. *Vitamins*. 2003; 77: 571-576.
39) Naito Y, Yoshikawa Y, Shintani M, et al. Anti-hyperglycemic effect of long-term bis(hinokitiolato)zinc complex ([$Zn(hkt)_2$]) ingestion on insulin resistance and pancreatic islet cells protection in type 2 diabetic KK-A^y mice. *Biol Pharm Bull*. 2017; 40: 318-326.
40) Maessen DE, Hanssen NM, Scheijen JL, et al. Post-glucose load plasma α-dicarbonyl concentrations are increased in individuals with impaired glucose metabolism and type 2 diabetes: The CODAM study. *Diabetes Care*. 2015; 38: 913-920.
41) Yonei Y, Yagi M, Sato K, et al. Glycative stress: Molecular impacts and defense mechanisms. *Glycative Stress Res*. 2023; 10: 145-158.