In Vitro Alpha-glucosidase Inhibitory Effect of Zn(II) Complex with 6-Methyl-2-picolinmethylamide

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We found alpha-glucosidase inhibitory effect of Zn(II) complex with 6-methyl-2-picolinmethylamide (6mpa-ma) which showed the highest blood glucose lowering effect in Zn(II) complexes with picolinamide derivatives in KK-A^y mice. The Zn(II) complex showed strong alpha-glucosidase inhibitory activity greater by about eighty times (substrate: maltose) and forty times (substrate: sucrose) compared with acarbose.

Key words Zn(II) complex; alpha-glucosidase; 6-methyl-2-picolinmethylamide

Diabetes Mellitus is a life style related disease, and estimated that over 16 million (including pre-diagnosed) people are affected in Japan as of 2002.[1] We have reported that Zn(II) complexes, the organic compound chelate zinc, provide insulinomimetic activity and blood glucose lowering effects.[2—6] Quite recently, we have reported the insulinomimetic mechanism in rat adipocytes.[7] Alpha-glucosidase inhibiter, one kind of oral anti-diabetic medication, has been approved as not only a medicine but also a food for specified health uses (FOSHU) available without prescription.[8—10] In addition, as reported at the 57th Annual Conference of the Japanese Society of Nutrition and Food Science, the amount of zinc decreases in people with type 2 diabetes treated with insulin.[11] Therefore, it is no exaggeration to say that promoting the consumption of zinc is an important issue in Japan. Several researchers have reported the relationships between metals and enzymes,[12—17] but when it comes to the relationship between zinc and alpha-glucosidase, there is only one report that zinc accelerates the effect of alpha-glucosidase.[18] In 2003, we first found that Zn(II) complexes inhibited the disaccharide digestion in *in vivo*.[19] However, we have not examined the inhibitory effect of Zn(II) complexes on the disaccharide digestion in *in vitro*, therefore detailed activity has not been confirmed. Thus, we synthesized Zn(6mpa-ma)$_2$SO$_4$ complex which showed the highest blood glucose lowering effect in Zn(II) complexes with picolinamide derivatives in KK-A^y mice.[5] In this paper, we have investigated the alpha-glucosidase inhibitory effect of Zn(6mpa-ma)$_2$SO$_4$ in *in vitro* assay for the first time.

The proposed structure of Zn(6mpa-ma)$_2$SO$_4$ is shown in Fig. 1.[20] We improved upon the method of Dahlqvist and analyzed the alpha-glucosidase inhibitory effect.[21,22] In brief, 0.1 ml of Zn(6mpa-ma)$_2$SO$_4$ (final concentration 300, 30, 10, 3, 0.3 μM) in HEPES buffer 0.15 M (pH 6.8) and 0.1 ml of alpha-glucosidase (*Saccharomyces* sp.) 5 units/ml in HEPES buffer 0.015 M were added to 0.1 ml of substrates (0.1 M maltose or sucrose in HEPES buffer 0.15 M) and then incubated at 37 °C for 5, 30 and 60 min. After incubation, each reaction was stopped in boiling water and each glucose value was determined by glucose CII-test Wako (Tokyo Japan). For comparison, $ZnCl_2$, acarbose and 6mpa-ma were added instead of Zn(6mpa-ma)$_2$SO$_4$. The alpha-glucosidase inhibitory effect was calculated by the following formula:

$$\text{Alpha-glucosidase inhibitory effect (\%)} = [(Ac - As)/Ac] \times 100$$

Ac: production glucose concentration of control (alpha-glucosidase and substrate)
As: production glucose concentration of the subject (alpha-glucosidase, substrate and Zn(6mpa-ma)$_2$SO$_4$)

Time dependence analysis of Zn(6mpa-ma)$_2$SO$_4$ was carried out in its final concentration 300, 7.5 or 9.6 μM, the solutions were incubated for 5, 30 and 60 min (Fig. 2). From the above results, the reaction in 300 μM of Zn(6mpa-ma)$_2$SO$_4$ took almost 30 min to complete. The inhibitory rate main-

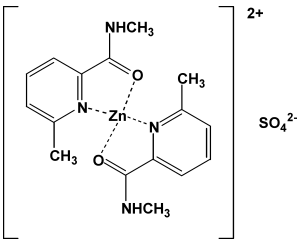

Fig. 1. Proposed Structure of Zn(6mpa-ma)$_2$SO$_4$

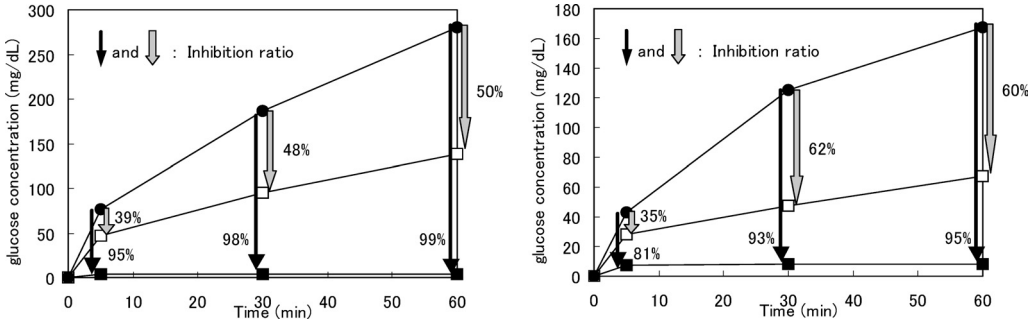

Fig. 2. Inhibitory Effect of Zn(6mpa-ma)$_2$SO$_4$ on the Activity of Alpha-glucosidase

Left: substrate is maltose, right: substrate is sucrose. Alpha-glucosidase inhibitory effect was examined by increasing the concentration of glucose produced during degradation reaction of maltose or sucrose. ●: control, □: Zn(6mpa-ma)$_2$SO$_4$ concentration of 7.5 μM (left) and 9.6 μM (right), ■: Zn(6mpa-ma)$_2$SO$_4$ concentration of 300 μM.

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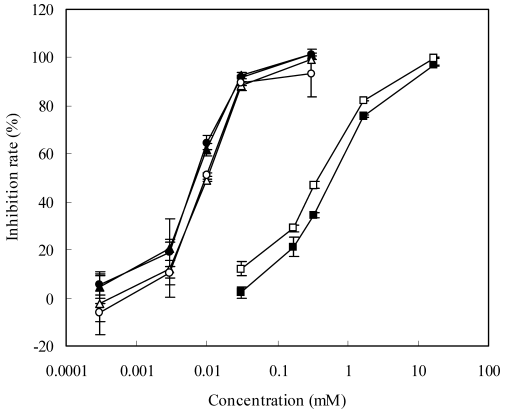

Fig. 3. Concentration Dependence of Alpha-glucosidase Inhibitory Rate

Incubation time: 60 min. ●: $Zn(6mpa\text{-}ma)_2SO_4$, ▲: $ZnCl_2$, ■: acarbose, (substrate; maltose). ○: $Zn(6mpa\text{-}ma)_2SO_4$, △: $ZnCl_2$, □: acarbose, (substrate; sucrose).

Table 1. Estimated IC_{50} Values of $Zn(6mpa\text{-}ma)_2SO_4$, $ZnCl_2$, Acarbose and 6mpa-ma

Compound	IC_{50} (μM)	
	Maltose	Sucrose
$Zn(6mpa\text{-}ma)_2SO_4$	7.5±0.4*	9.6±0.5*
$ZnCl_2$	7.3±1.0*	9.3±1.0*
Acarbose	584±39	404±16
6mpa-ma	None	None

* Significane at $p<0.001$ *vs.* acarbose.

tained from 30 to 60 min. This data suggested that Zn(II) compound showed a competitive inhibition against alpha-glucosidase. A similar tendency was also observed in $ZnCl_2$ (data not shown). The effects of $Zn(6mpa\text{-}ma)_2SO_4$, $ZnCl_2$ and acarbose (positive control) were dose dependent as shown in Fig. 3. From these results, the apparent IC_{50} values of $Zn(6mpa\text{-}ma)_2SO_4$, a 50% inhibitory concentration of glucose produced during digestion reaction of substrate, were estimated to be 7.5±0.4 μM for maltose and 9.6±0.5 μM for sucrose (Table 1). In $ZnCl_2$ and acarbose, the IC_{50} values were estimated to be 7.3±1.0, 584±39 μM for maltose and 9.3±1.0, 404±16 μM for sucrose, respectively (Table 1). However, 6mpa-ma didn't show the alpha-glucosidase inhibitory effect.

On the basis of the results, $Zn(6mpa\text{-}ma)_2SO_4$ and $ZnCl_2$ showed higher alpha-glucosidase inhibitory effect than acarbose which is used as an alpha-glucosidase inhibitory medicine. It has been reported that the active site of alpha-glucosidase most closely related to the imidazole and carboxy groups.[23] Zinc is classified into one of lewis acids, which has high affinity for an electron donor.[24] It is possible that $Zn(6mpa\text{-}ma)_2SO_4$ and $ZnCl_2$, chelate an electron donor like imidazole or carboxy group in alpha-glucosidase and inhibits its function. From these results, the alpha-glucosidase inhibitory effects of $Zn(6mpa\text{-}ma)_2SO_4$ were not different from one of $ZnCl_2$. However, we have reported that the toxicity of zinc ion decreased by chelating ligands.[25] Thus, we propose that $Zn(6mpa\text{-}ma)_2SO_4$ is better than $ZnCl_2$, considering the development of anti-diabetic medicine. In conclusion, we found that $Zn(6mpa\text{-}ma)_2SO_4$ inhibits the activity of alpha-glucosidase and then disturbs the digestion of maltose and sucrose; consequently, it seems appears effective for the treatment of diabetes. We shall continue our investigation focusing on the detailed mechanism of this Zn(II) complex related alpha-glucosidase inhibitory effects.

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