Effects of Ca^{2+}, Zn^{2+} and Cd^{2+} on Uridine Diphosphate-Glucuronyltransferase and β-Glucuronidase Activities in Rat Liver Microsomes

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The effect of various metals on uridine diphosphate (UDP)-glucuronyltransferase and β-glucuronidase activities in rat liver microsomes was investigated. The presence of Mn^{2+}, Cd^{2+}, Zn^{2+}, V^{5+}, Ni^{2+}, Co^{2+}, Cu^{+} or Ca^{2+} (20 μM) in the enzyme reaction mixture did not cause a significant alteration of UDP-glucuronyltransferase activity in hepatic microsomes. Of these metals, Zn^{2+} and Cd^{2+} (20 μM) caused a remarkable increase in hepatic microsomal β-glucuronidase activity. Appreciable effects of Zn^{2+} and Cd^{2+} on β-glucuronidase activity were seen at 5.0 μM, and the effects were saturated at 50 μM. Ca^{2+} (5.0—50 μM) and/or the Ca^{2+}-binding protein regucalcin (2.0 μM) did not have an appreciable effect on UDP-glucuronyltransferase and β-glucuronidase activities in hepatic microsomes. Thus, Zn^{2+} and Cd^{2+} uniquely increased β-glucuronidase activity. The Zn^{2+}- and Cd^{2+}-induced increase in β-glucuronidase activity was completely reversed by the presence of an SH group-protecting reagent (dithiothreitol). The response of the microsomal enzyme to Zn^{2+} and Cd^{2+} (20 μM) was no longer seen after treatment with 0.2% Triton X-100 [polyoxyethylene(10) octylphenyl ether], indicating that the stimulation by these metals is dependent on membrane association. The present study suggests that, of various metals tested, Zn^{2+} and Cd^{2+} can uniquely increase hepatic microsomal β-glucuronidase activity, and that their effect is based on binding to membranous SH groups, beside the enzyme protein.

Keywords zinc; cadmium; calcium; regucalcin; uridine diphosphate-glucuronyltransferase; β-glucuronidase; rat liver microsome

Liver microsomal uridine diphosphate (UDP)-glucuronyltransferase catalyzes the glucuronidation of a wide variety of endogenous and xenobiotic compounds.[1] A significant fraction of β-glucuronidase in liver is associated with the hepatic microsomal fraction,[2,3] although the function of the microsomal enzyme remains poorly defined.[2] Since both UDP-glucuronyltransferase and β-glucuronidase are components of the endoplasmic reticulum, a conjugation–deconjugation cycle has been proposed as a determinant of net glucuronide production from a substrate generated *via* mixed-function oxidation or supplied from other sources.[4] Thus, liver microsomal UDP-glucuronyltransferase and β-glucuronidase may play a cell physiological role in glucuronidation, which is a major pathway for non-oxygenative metabolism of many drugs, steroids, and toxic and carcinogenic chemicals.[5]

In recent years, it has been demonstrated that Ca^{2+} plays an important role in liver metabolism.[6,7] Ca^{2+} and other metal ions may modulate glucuronidation in hepatic microsomes. This, however, has not been confirmed thus far. The present investigation was undertaken, therefore, to clarify the effects of various metal ions on UDP-glucuronyltransferase and β-glucuronidase activities in rat liver microsomes. It was found that Zn^{2+} and Cd^{2+} increase β-glucuronidase activity, but not UDP-glucuronyltransferase activity in the hepatic microsomes, and that Ca^{2+} does not influence either of the enzyme activities.

Materials and Methods

Animals Male Wistar rats, weighing 100—120 g, were used. They were obtained commercially from Japan SLC Inc. (Hamamatsu, Japan). The animals were fed commercial laboratory chew (solid) containing 57.5% carbohydrate, 1.1% Ca, and 1.1% P at a room temperature of 25 °C, and were allowed distilled water freely.

Reagents All reagents were purchased from Wako Pure Chemical Co. (Osaka, Japan) and Sigma Chemical Co. (St. Louis, Mo., U.S.A.), and were dissolved in distilled water then passed through an ion-exchange resin to remove metal ions.

Isolation of Regucalcin Ca^{2+}-binding protein regucalcin in the cytosol fraction of rat liver was purified to electrophoretic homogeneity by gel filtration on Sephadex G-75 and G-50 followed by ion-exchange chromatography on diethylaminoethyl (DEAE)-cellulose, as reported previously.[8]

Preparation of Hepatic Microsomes Rats were sacrificed by cardiac puncture, and the liver was perfused with ice-cold 250 mM sucrose solution, immediately cut into small pieces, suspended 1:9 in the homogenization medium containing 250 mM sucrose, 20 mM Hepes 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1.0 mM EGTA ethylene glycol bis(2-aminoethylether) N,N,N',N'-tetraacetic acid and 1.0 mM dithiothreitol, pH 7.2 and homogenized in Potter–Elvehjem homogenizer with a Teflon pestle.[9]

The homogenate was centrifuged at $1000 \times g$ for 10 min to remove nuclei, unbroken cells and cell debris. The resultant supernate was centrifuged at $7700 \times g$ for 20 min to remove the mitochondrial fraction. The postmitochondrial supernate was then centrifuged at $110000 \times g$ for 60 min to sediment the microsomal fraction. The microsomal fraction was resuspended in 120 mM KCl, 10 mM Hepes, pH 6.8, to a final protein concentration of 20—30 mg/ml. In a separate experiment, the microsomal fraction was resuspended in 5.0 mM 2,6-pyridinedicarboxylic acid (dipicolinate) solution for 10 min at 4 °C, or in 0.2% polyoxyethylene(10) octylphenyl ether (Triton X-100) solution for 60 min at 4 °C. The suspensions were then centrifuged at $110000 \times g$ for 60 min to obtain the microsomes. The microsomal fraction was resuspended in 120 mM KCl, 10 mM Hepes, pH 6.8.

Analytical Methods The glucuronidation of 4-nitrophenol by liver microsomes was measured as described by Burchell and Weatherill.[10] A 20 mM solution of UDP-glucuronic acid triammonium salt, adjusted to pH 7.4 with KOH, was used in assays. UDP-glucuronyltransferase activity was measured by incubation for 10 min at 37 °C in the reaction mixture (final volume 0.25 ml) containing 0.5 M Tris–HCl, 1.0 mM 4-nitrophenol, 20 mM UDP–glucuronic acid, 10 mM $MgCl_2$, other metal ions, and the microsomes (0.7—0.8 mg as protein). The reaction was initiated by the addition of 20 mM UDP–glucuronic acid. After incubation, ice-cold 0.5 M trichloroacetic acid was added and the mixture was rapidly vortexed and kept in ice before removal of the protein pellet by centrifugation at $2000 \times g$ for 10 min. The supernatant solution was mixed with 2.0 M NaOH solution and then diluted by addition of distilled water. Absorbance was measured at 405 nm to assess the reduction in color caused by the formation of 4-nitrophenyl glucuronide. The enzyme activity was expressed as nmol of 4-nitrophenol glucuronidated per min per mg protein.

β-Glucuronidase activity was measured by incubation for 45 min at 37 °C in a final volume of 0.4 ml containing 75 mM Hepes buffer (pH 7.3), 5.0 mM $MgCl_2$, 125 mM KCl, 1.5 mM phenolphthalein glucuronide, metal ions, and the microsomes (0.7—0.8 mg as protein).[11] The reaction was

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stopped by the addition of 50 mM $NaHCO_3$–Na_2CO_3 buffer (4.0 ml), pH 10.3. Absorbance was measured at 555 nm to assess the increase in color caused by the formation of phenolphthalein. The enzyme activity was expressed as nmol of phenolphthalein liberated per min per mg protein.

Protein concentration was determined by the method of Lowry *et al.*[12]

Statistical Methods The significance of differences between values was estimated by using Student's *t* test; *p* values of less than 0.05 were considered to indicate statistically significant differences.

Results

Effect of Metal Ions on UDP-Glucuronyltransferase Activity in Hepatic Microsomes The effect of metal ions on UDP-glucuronyltransferase activity in rat liver microsomes is shown in Fig. 1. Various metal ions were present at 20 μM (final concentration) in the enzyme reaction mixture. UDP-glucuronyltransferase activity was not significantly altered by addition of $MnCl_2$, $CdCl_2$, $ZnCl_2$, V_2O_5, $NiCl_2$, $CoCl_2$, CuCl or $CaCl_2$. A previous investigation showed that addition of Ca^{2+} causes a significant increase of glucose-6-phosphatase activity in rat liver microsomes, and that hepatic Ca^{2+}-binding protein regucalcin reverses the Ca^{2+} effect.[13] Then, the effect of increasing concentrations of Ca^{2+} on UDP-glucuronyltransferase activity was examined (Fig. 2). Ca^{2+} (5.0—50 μM) did not cause a significant alteration of UDP-glucuronyltransferase activity, and the Ca^{2+} effect was not modulated by regucalcin (2.0 μM). Thus, it seemed that hepatic microsomal UDP-glucuronyltransferase activity was not altered by a comparatively low concentration of metal ion.

Effect of Metal Ions on β-Glucuronidase Activity in Hepatic Microsomes The effect of metal ions on β-glucuronidase activity in rat liver microsomes is shown in Fig. 3. When metal ion at 20 μM (final concentration) was contained in the enzyme reaction mixture, β-glucuronidase activity was markedly increased by the presence of Cd^{2+} or Zn^{2+}. The enzyme activity was not significantly altered by the presence of Mn^{2+}, V^{5+}, Ni^{2+}, Co^{2+}, Cu^{+} or Ca^{2+}. As shown in Fig. 4, the effects of increasing concentrations of Cd^{2+}, Zn^{2+}, V^{5+} and Ca^{2+} were examined. None of the metals at 1.0 μM had an appreciable effect on β-glucuronidase activity. The presence of 5.0 μM Cd^{2+} or Zn^{2+} caused a significant increase in the enzyme activity. The effect of Cd^{2+} or Zn^{2+} was saturated at 50 μM. Meanwhile, V^{5+} or Ca^{2+} (1.0—100 μM) did not cause a significant alteration of β-glucuronidase activity.

It has recently been reported that Zn^{2+} can modulate the

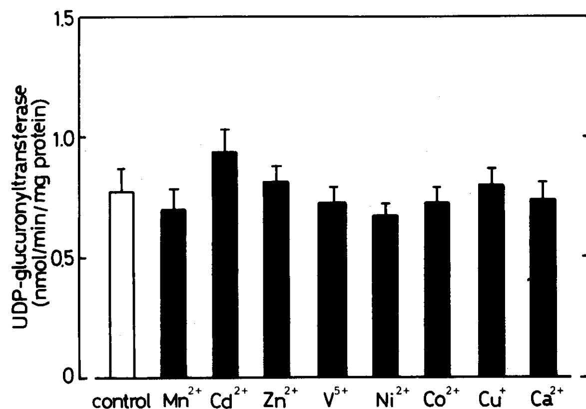

Fig. 1. Effect of Various Metals on UDP-Glucuronyltransferase Activity in the Microsomes of Rat Liver

The enzyme activity was measured in the reaction mixture containing 20 μM metal (final concentration). Each value represents the mean $\pm$ S.E.M. of 5 experiments. The differences were not significant.

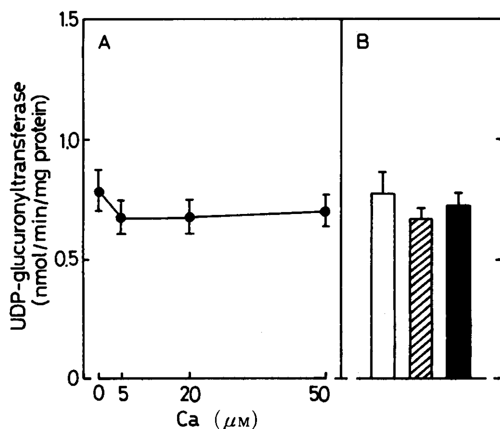

Fig. 2. Effect of Ca^{2+} and Ca^{2+}-Binding Protein Regucalcin on UDP-Glucuronyltransferase Activity in the Microsomes of Rat Liver

Figure A shows the effect of increasing concentrations of Ca^{2+} (5.0, 20 and 50 μM). Figure B shows the effect of regucalcin (2.0 μM) on UDP-glucuronyltransferase activity in the presence of 20 μM Ca^{2+}. Each value represents the mean $\pm$ S.E.M. of 5 experiments. The differences were not significant. □, control; ▨, 2.0 μM regucalcin; ■, 20 μM Ca^{2+} and 2.0 μM regucalcin.

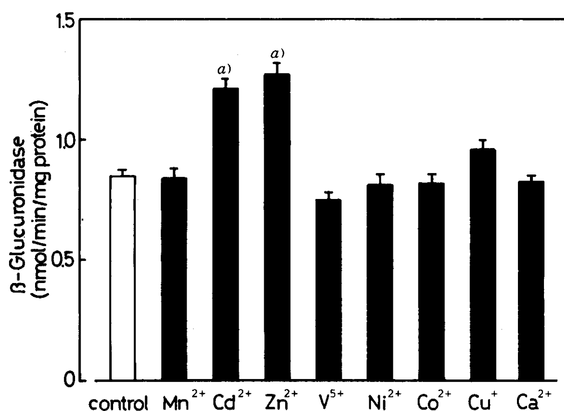

Fig. 3. Effect of Various Metals on β-Glucuronidase Activity in the Microsomes of Rat Liver

The enzyme activity was measured in the reaction mixture containing 20 μM metal (final concentration). Each value represents the mean $\pm$ S.E.M. of 5 experiments. *a)* $p<0.01$, as compared with the control value.

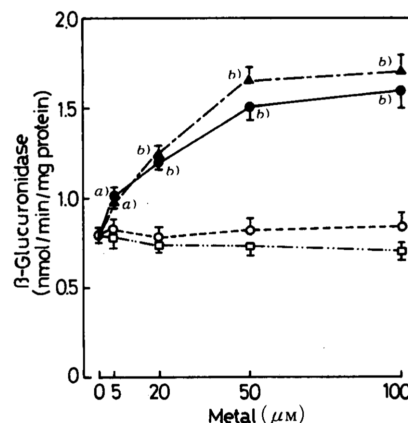

Fig. 4. Effect of Increasing Concentrations of Ca^{2+}, Zn^{2+}, Cd^{2+} and V^{5+} on β-Glucuronidase Activity in the Microsomes of Rat Liver

The enzyme activity was measured in the reaction mixture containing 1.0, 5.0, 20, 50 or 100 μM metal. Addition of 1.0 μM metal did not alter the enzyme activity. Each value represents the mean $\pm$ S.E.M. of 5 experiments. *a)* $p<0.05$, and *b)* $p<0.01$, as compared with the value without metal. ○, Ca^{2+}; ▲, Zn^{2+}; ●, Cd^{2+}; □, V^{5+}.

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Ca^{2+} effect on protein kinase C[14] and Ca^{2+}-binding protein.[15] Thus, the effect of Zn^{2+} on hepatic microsomal β-glucuronidase activity was examined in the presence of Ca^{2+}. The effect of Zn^{2+} (5.0 and 20 μM) to increase β-glucuronidase activity was not enhanced by the presence of 5.0 or 20 μM Ca^{2+} (Fig. 5). Moreover, hepatic Ca^{2+}-binding protein regucalcin (2.0 μM) did not modulate the effects of Cd^{2+} (20 μM) and Zn^{2+} (20 μM) to increase hepatic microsomal β-glucuronidase activity (Fig. 6). The presence of both Ca^{2+} (20 μM) and regucalcin (2.0 μM) had no effect on the enzyme activity. Thus, hepatic microsomal β-glucuronidase activity was uniquely increased by Zn^{2+} and Cd^{2+}, among the various metal ions tested.

Effect of SH Group-Protecting Reagent on Zn^{2+} and Cd^{2+}-Increased β-Glucuronidase Activity in Hepatic Microsomes The effect of dithiothreitol, an SH group-protecting reagent, on the Zn^{2+} and Cd^{2+}-induced increase of β-glucuronidase activity in rat liver microsomes is shown in Fig. 7. Dithiothreitol (5.0—100 μM) was contained in the enzyme reaction mixture. The presence of 20 μM dithiothreitol clearly prevented the effect of Cd^{2+} (20 μM) to increase β-glucuronidase activity. At 100 μM dithiothreitol, the effects of Cd^{2+} and Zn^{2+} were completely blocked. Thus, the effects of Zn^{2+} and Cd^{2+} to increase hepatic microsomal β-glucuronidase activity were related to SH groups. If the binding sites of Zn^{2+} and Cd^{2+} on the microsomal membranes were identical, Zn^{2+} and Cd^{2+} at sub-maximum concentration (20 μM) would be expected to cause an additive increase of β-glucuronidase activity. This was examined, and the result is shown in Fig. 8. Zn^{2+} and Cd^{2+} did cause a additive increase in the enzyme activity.

Effect of Dipicolinate, a Zn^{2+}-Chelating Reagent, on Hepatic Microsomal β-Glucuronidase Activity Zn^{2+}, but not Cd^{2+}, is an essential trace metal in mammals. The role of endogenous Zn^{2+} on β-glucuronidase activity in rat liver microsomes was examined by using dipicolinate, a Zn^{2+}-chelating reagent (Fig. 9). The hepatic microsomes were suspended in 5.0 mM dipicolinate solution at 4 °C for 10 min, and recentrifuged at 110000 × ***g*** for 60 min. The hepatic microsomal β-glucuronidase activity was decreased (about 10%, $p<0.01$) by washing with dipicolinate in comparison with that of control (not washed). The presence

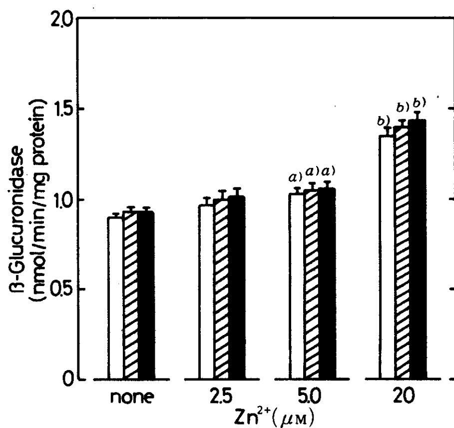

Fig. 5. Effect of Ca^{2+} on the Zn^{2+}-Induced Increase of β-Glucuronidase Activity in the Microsomes of Rat Liver

The enzyme activity was measured in the reaction mixture containing 5.0 or 20 μM Ca^{2+} (final concentration) in the presence of 2.5, 5.0 and 20 μM Zn^{2+}. Each value represents the mean ± S.E.M. of 5 experiments. *a)* $p<0.05$, and *b)* $p<0.01$, as compared with the control value without metal. □, none; ▨, 5.0 μM Ca^{2+}, ■, 20 μM Ca^{2+}

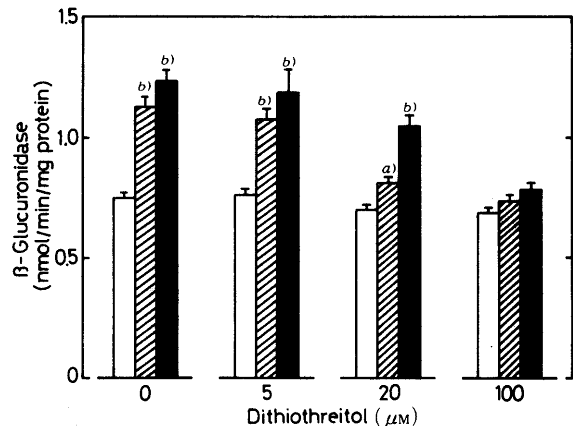

Fig. 7. Effect of Dithiothreitol on the Zn^{2+} or Cd^{2+}-Induced Increase of β-Glucuronidase Activity in the Microsomes of Rat Liver

The enzyme activity was measured in the reaction mixture containing Zn^{2+} and Cd^{2+} (20 μM) in the presence of 5.0, 20 and 100 μM dithiothreitol. Each value represents the mean ± S.E.M. of 5 experiments. *a)* $p<0.05$, and *b)* $p<0.01$, as compared with the value without metal. □, control; ▨, Cd^{2+}; ■, Zn^{2+}

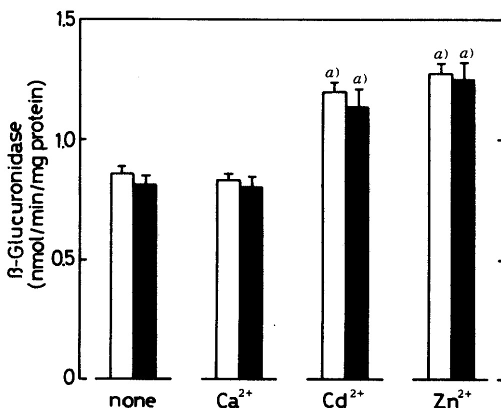

Fig. 6. Effect of the Ca^{2+}-Binding Protein Regucalcin on the Metal-Induced Increase of β-Glucuronidase Activity in the Microsomes of Rat Liver

The enzyme activity was measured in the reaction mixture containing Ca^{2+}, Zn^{2+} or Cd^{2+} (20 μM) in the presence or absence of 2.0 μM regucalcin. Each value represents the mean ± S.E.M. of 5 experiments. *a)* $p<0.01$, as compared with the control (none) value. □, control; ■, regucalcin.

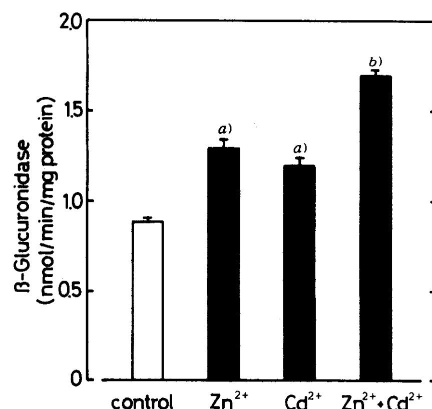

Fig. 8. Effect of Cd^{2+} on the Zn^{2+}-Induced Increase of β-Glucuronidase Activity in the Microsomes of Rat Liver

The enzyme activity was measured in the reaction mixture containing either Zn^{2+}, Cd^{2+}, or Zn^{2+} plus Cd^{2+} (20 μM). Each value represents the mean ± S.E.M. of 5 experiments. *a)* $p<0.01$, as compared with the control value. *b)* $p<0.01$, as compared with the value of Zn^{2+} or Cd^{2+} alone.

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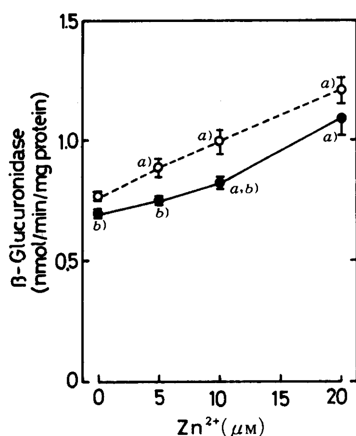

Fig. 9. Effect of Dipicolinate Treatment of β-Glucuronidase Activity in the Microsomes of Rat Liver

The microsomes were suspended in 5.0 mM dipicolinate solution at 4 °C for 10 min, and then recentrifuged at 110000 × g for 60 min. The enzyme activity was measured in the reaction mixture containing Zn^{2+} (5.0, 10 and 20 μM) and the microsomes washed with or without dipicolinate. Each value represents the mean ± S.E.M. of 5 experiments. *a)* $p<0.01$, as compared with the value without Zn^{2+}. *b)* $p<0.01$, as compared with the value without dipicolinate treatment. ○, control; ●, dipicolinate treatment.

Table I. Effects of Ca^{2+}, Zn^{2+} and Cd^{2+} on the Activity of β-Glucuronidase Solubilized from Rat Liver Microsomes by Treatment with Triton X-100

Treatment[a]	β-Glucuronidase[b] (noml/min/mg protein)		
	None	10 μM	20 μM
Supernatant			
Control	3.18 ± 0.58		
$CaCl_2$		3.00 ± 0.54	2.99 ± 0.53
$ZnCl_2$		2.99 ± 0.53	2.95 ± 0.54
$CdCl_2$		3.05 ± 0.55	3.01 ± 0.54
Pellet			
Control	0.42 ± 0.10		
$CaCl_2$		0.40 ± 0.10	0.39 ± 0.11
$ZnCl_2$		0.49 ± 0.11	0.59 ± 0.14
$CdCl_2$		0.45 ± 0.12	0.49 ± 0.12

a) Samples of a microsomal suspension containing 10—15 mg/ml were treated with 0.2% Triton X-100 at 4 °C for 60 min. After treatment, aliquots were removed for assay and the samples were centrifuged at 110000 × g for 60 min. The resultant pellets and supernatants were assayed for β-glucuronidase. *b)* Values are mean ± S.E.M. of the samples from the liver microsomes of 5 rats. The differences were not significant.

of 5.0 μM Zn^{2+} in the reaction mixture with dipicolinate-washed microsomes did not cause a significant increase in β-glucuronidase activity, although the metal ions significantly raised the enzyme activity in unwashed microsomes. With greater concentrations of Zn^{2+} (10 and 20 μM), the enzyme activity was increased significantly. Thus, endogenous Zn^{2+} has a role in the preservation of hepatic microsomal β-glucuronidase activity.

Effect of Triton X-100 Treatment on Zn^{2+} or Cd^{2+}-Increased Hepatic Microsomal β-Glucuronidase Activity The β-glucuronidase is membrane-bound in microsomes. About 90% of the enzyme associated with the microsomes from rat liver was solubilized by treatment with 0.2% Triton X-100 at 4 °C for 60 min (Table I). To test whether Zn^{2+} and Cd^{2+} stimulation is dependent on membrane association, we examined the response of the microsomal enzyme to metal ions after treatment with 0.2% Triton X-100 (Table I). The solubilized form of β-glucuronidase showed a greatly reduced sensitivity to Zn^{2+} or Cd^{2+} (20 μM). After treatment with 0.2% Triton X-100, the enzyme activity remaining associated with microsomal membranes was not significantly increased by Zn^{2+} or Cd^{2+} (20 μM). Addition of Ca^{2+} (20 μM) also did not cause a significant increase in β-glucuronidase activity in either the supernatant or pellet fraction after treatment with 0.2% Triton X-100.

Discussion

The effect of various metals on the enzymes which catalyze glucuronidation was investigated in microsomes isolated from rat liver. Glucuronidation is a major pathway for nonoxygenative metabolism of many drugs, steroids, and toxic and carcinogenic chemicals.[5] Liver microsomal UDP-glucuronyltransferase is a major enzyme related to glucuronidation. It has been reported that liver microsomal UDP-glucuronyltransferase activity is increased by addition of $MnCl_2$ (1.0 and 2.0 mM).[16,17] The effect of other metals, however, was not examined. We used a comparatively low concentration of metals. Liver microsomal UDP-glucuronyltransferase activity was not significantly altered by addition of Mn^{2+}, Cd^{2+}, Zn^{2+}, V^{5+}, Ni^{2+}, Co^{2+}, Cu^{+} or Ca^{2+} (20 μM) to the enzyme reaction mixture containing 10 mM $MgCl_2$ (optimum condition). UDP-glucuronyltransferase and β-glucuronidase in hepatic microsomes both catalyze a conjugation–deconjugation cycle which was proposed to be a determinant of net glucuronide production from substrates generated *via* mixed-function oxidation or supplied from other sources, although the enzymes are present in multiple forms.[4] Liver microsomal β-glucuronidase activity was markedly increased by addition of Zn^{2+} and Cd^{2+} (20 μM) to enzyme reaction mixture containing 5 mM $MgCl_2$ (optimum condition), while Mn^{2+}, V^{5+}, Ni^{2+}, Co^{2+}, Cu^{+} and Ca^{2+} (20 μM) did not have an appreciable effect. The present finding, that Zn^{2+} and Cd^{2+} can uniquely increase hepatic microsomal β-glucuronidase activity, suggests that the metals influence glucuronide production from substrates.

Recently, it has been established that Ca^{2+} plays an important role in the regulation of liver metabolism.[6,7] Hepatic microsomal UDP-glucuronyltransferase and β-glucuronidase activities were not significantly altered by addition of Ca^{2+} (5.0—50 μM) to the enzyme reaction mixture. Moreover, the hepatic Ca^{2+}-binding protein regucalcin, which can regulate the Ca^{2+} effect on enzymes,[18-20] did not have an appreciable effect on either of the enzyme activities. Calmodulin also had no effect on hepatic microsomal UDP-glucuronyltransferase and β-glucuronidase activities (data not shown). Thus, Ca^{2+} and Ca^{2+}-binding protein may not regulate hepatic microsomal glucuronidation.

The effect of Zn^{2+} and Cd^{2+} to increase hepatic microsomal β-glucuronidase activity was seen at 5.0 μM metals, and was saturated at 50 μM. β-Glucuronidase activity was additively increased by addition of both Zn^{2+} and Cd^{2+} (20 μM) to enzyme reaction mixture, while Ca^{2+} (20 μM) did not enhance the effect of Zn^{2+} (20 μM) on the enzyme activity. It is assumed that Zn^{2+} and Cd^{2+} bind to identical sites on hepatic microsomes. Dithiothreitol is an SH group-protecting reagent. The presence of dithiothreitol (20 μM)

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significantly prevented the Cd^{2+} (20 μM)-induced increase in the microsomal β-glucuronidase activity, while the Zn^{2+} (20 μM) effect was not blocked significantly. At 100 μM dithiothreitol, however, the effects of Zn^{2+} and Cd^{2+} (20 μM) were completely blocked. Thus, Zn^{2+} and Cd^{2+} may bind to SH groups of microsomal membranes.

Dipicolinate can bind Zn^{2+} in biological systems.[21] Rat liver microsomes contain endogenous Zn^{2+}.[22] When hepatic microsomes were washed with 5.0 mM dipicolinate solution, the microsomal β-glucuronidase activity was significantly decreased in comparison with that obtained from unwashed microsomes. This finding suggests that the microsomal endogenous Zn^{2+} can preserve β-glucuronidase activity.

About 90% of the enzyme associated with the microsomes from rat liver was solubilized by treatment with 0.2% Triton X-100. The solubilized form of β-glucuronidase showed a greatly reduced sensitivity to Zn^{2+} and Cd^{2+} (20 μM). This indicates that Zn^{2+} and Cd^{2+} stimulation is dependent on membrane association, and that the metals do not activate the enzyme directly. As mentioned above, Zn^{2+} and Cd^{2+} may bind to SH groups of hepatic microsomal membranes to stimulate microsomal β-glucuronidase activity. Cd^{2+} is a toxic metal and may bind to the binding sites (SH groups) for Zn^{2+}, since the Zn^{2+} and Cd^{2+} effects on the enzyme activity were both completely blocked by dithiothreitol. Although the liver cytosolic free concentration of Zn^{2+} is unknown, the total cytosolic concentration of Zn^{2+} has been estimated at about 200 μM.[23,24] Addition of more than 5.0 μM Zn^{2+} to the enzyme reaction mixture caused a remarkable increase in hepatic microsomal β-glucuronidase activity. Zn^{2+} may have a physiological significance to preserve and to stimulate hepatic microsomal β-glucuronidase activity.

In conclusion, it has been demonstrated that, of various metals tested, Zn^{2+} and Cd^{2+} could uniquely increase hepatic microsomal β-glucuronidase activity, and that the metals stimulation is dependent on membrane association.

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