Molecular and Cellular Biochemistry **136**: 71–76, 1994.

Activating effect of regucalcin on (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes: relation to sulfhydryl group

Hiroko Takahashi and Masayoshi Yamaguchi

Laboratory of Metabolism and Endocrinology, Graduate School of Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka City 422, Japan

Received 3 November 1993; accepted 23 March 1994

Abstract

The activating mechanism of regucalcin, a calcium-binding protein isolated from rat liver cytosol, on (Ca^{2+}-Mg^{2+})-ATPase in the plasma membranes of rat liver was investigated. (Ca^{2+}-Mg^{2+})-ATPase activity was markedly increased by a sulfhydryl (SH) group protecting reagent dithiothreitol (DTT; 2.5 and 5 mM as a final concentration), while the enzyme activity was significantly decreased by a SH group modifying reagent N-ethylmaleimide (NEM; 0.5–5 mM). The effect of DTT (5 mM) to increase the enzyme activity was clearly blocked by NEM (5 mM). Regucalcin (0.25–1.0 μM) significantly increased (Ca^{2+}-Mg^{2+})-ATPase activity. This increase was completely blocked by NEM (5 mM). Meanwhile, digitonin (0.04%), which can solubilize the membranous lipids, significantly decreased (Ca^{2+}-Mg^{2+})-ATPase activity. Digitonin did not have an effect on the DTT (5 mM)-increased enzyme activity. However, the effect of regucalcin (0.25 μM) increasing (Ca^{2+}-Mg^{2+})-ATPase activity was entirely blocked by the presence of digitonin. The present results suggest that regucalcin activates (Ca^{2+}-Mg^{2+})-ATPase by the binding to liver plasma membrane lipids, and that the activation is involved in the SH groups which are an active site of the enzyme. (Mol Cell Biochem **136**: 71–76, 1994)

Key words: regucalcin, (Ca^{2+}-Mg^{2+})-ATPase, SH group, rat liver plasma membrane

Introduction

Calcium ion (Ca^{2+}) plays an important role in liver metabolism which is regulated by increase of Ca^{2+} in the cytoplasm of liver cells due to hormonal stimulation [1–4]. Recently, it has been reported that a novel calcium-binding protein regucalcin, which is distributed in the hepatic cytosol of rats [5, 6], has a reversible effect on the activation and inhibition of various enzymes by Ca^{2+} and/or calmodulin in liver cells [7–10]. This novel protein probably plays an important role in the regulation of liver cell function related to Ca^{2+}.

On the other hand, the regulation of Ca^{2+} extrusion in liver cells is poorly understood. The high-affinity (Ca^{2+}-Mg^{2+})-ATPase is located on the plasma membranes of rat liver [11, 12]. This enzyme acts as a Ca^{2+} pump to exclude the metal ion from the cytoplasm of liver cells [12]. Recently, it has been reported that regucalcin can increase (Ca^{2+}-Mg^{2+})-ATPase activity in the plasma membranes of rat liver [13], suggesting that regucalcin plays a role in the regulation of Ca^{2+} pump activity. The mechanism by which regucalcin increases (Ca^{2+}-Mg^{2+})-ATPase activity, however, has not been clarified fully.

Previous investigations showed that regucalcin activates directly (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes [13], and that the activation is not involved in the Ca^{2+}-dependent phosphorylation of the enzyme [14] and in GTP-binding protein which modulates the receptor-mediated hormonal effect in the plasma membranes [15]. Furthermore,

Address for offprints: Masayoshi Yamaguchi, Ph.D., Laboratory of Metabolism and Endocrinology, Graduate School of Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka City 422, Japan

the present study was undertaken to clarify whether the activating mechanism of regucalcin on (Ca^{2+}-Mg^{2+})-ATPase is related to the sulfhydryl group of the enzyme. It was found that the effect of regucalcin is completely inhibited by the sulfhydryl modifying reagent, suggesting that regucalcin acts directly on the sulfhydryl groups which are an active site of (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes.

Materials and methods

Chemicals

Adenosine-5′-triphosphate (ATP), guanosine-5′-triphosphate (GTP), dithiothreitol (DTT), N-ethylmaleimide (NEM), heparin and digitonin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Calcium chloride, sodium vanadate and all other reagents were purchased from Wako Pure Chemical Co. (Osaka, Japan). The reagents were dissolved in distilled water and then passed through ion-exchange resin to remove metal ions.

Animals and isolation of regucalcin

Male Wistar rats, weighing 100–130 g, were used. They were obtained commercially (Japan SLC, Inc., Hamamatsu, Japan). The animals were given commercial laboratory chow containing 1.1% Ca, 1.1% P and 57.4% carbohydrate (Oriental Test Diet, Tokyo, Japan) and distilled water freely. Rats were killed by bleeding. The livers perfused with Tris-HCl buffer (pH 7.4, containing 100 mM Tris, 120 mM NaCl, 4 mM KCl, cooled to 4°C). The livers were removed, cut into small pieces; suspended 1:4 in Tris-HCl buffer (pH 7.4) to isolate regucalcin, as reported previously [5]. Protein concentration was determined by the method of Lowry *et al.* [16] using albumin as a standard.

Preparation of liver plasma membranes

The livers were removed after perfusion with ice-cold 0.25 M sucrose solution and placed in ice-cold medium containing 0.25 M sucrose, 5 mM Hepes-KOH, and 1mM EGTA, pH 7.4. The liver plasma membranes were prepared according to the procedure of Prpić *et al.* [17]. Livers were minced with scissors and homogenized by 10 passes with a loose-fitting Dounce homogenizer followed by 3 passes with a tight-fitting homogenizer, then diluted to give a 6% (w/v) homogenate. The homogenate was then centrifuged at 1,464 x g for 10 min, and the resulting pellet was resuspended in the isolation medium and diluted to give a 6% (w/v) suspension. A volume (10.4 ml) of this was mixed with 1.4 ml of Percoll (Pharmacia) in 15-ml Cortex tubes and centrifuged at 34, 540 × g for 30 min. Two distinct layers close to the top of the tube were revealed. These were harvested and washed in 5 volumes of 0.25 M sucrose, 50 mM Tris-HCl, pH 8.0, and the resulting pellets were resuspended in the same medium. Assay of marker enzyme (5′-nucleotidase, succinate dehydrogenase, glucose-6-phosphatase, and RNA polymerase) showed that there was less than 5% contamination by nuclei, mitochondria, or microsomes. Especially, the activity of plasma membrane 5′-nucleotidase showed a great value (405.1 ± 10.4 nmol/min/mg protein) in comparison with the activity of other enzymes. The prepared plasma membranes were in inside-out oriented and sealed membrane vesicles.

Assay of (Ca^{2+}-Mg^{2+})-ATPase

(Ca^{2+}-Mg^{2+})-ATPase in the plasma membranes was measured under conditions described by Lotersztajn *et al.* (11), except that phosphate release was determined as described elsewhere [18]. The standard assay for (Ca^{2+}-Mg^{2+})-ATPase activity contained in a final volume of 250 µl, 200–300 µg of plasma membrane protein, 250 µM Mg-ATP, 50 mM Tris-HCl (pH 8.0), 400 µM EGTA with either no Ca^{2+} or 400 µM total Ca^{2+}, and others (various reagents and regucalcin). After 10 min at 30°C, aliquots were assayed for inorganic phosphate formed. (Ca^{2+}-Mg^{2+})-ATPase activity was calculated by substracting values obtained with chelator alone from those obtained with chelator plus Ca^{2+}. Results were expressed as nmoles of inorganic phosphate liberated per min per mg of protein.

Statistical methods

The significance of the difference between values was estimated by means of Student's *t*-test; p values less than 0.05 were considered to indicate statistically significant differences.

Results

Effect of various reagents on (Ca^{2+}-Mg^{2+})-ATPase activity

The effects of various reagents on (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes were examined first. The presence of dithiothreitol (DTT; 2.5 and 5 mM) in the enzyme reaction mixture caused a remarkable increase in (Ca^{2+}-Mg^{2+})-ATPase activity, while the enzyme activity was significantly decreased by the presence of N-ethylmaleimide (NEM; 0.5–5 mM), s sulfhydryl modifying reagent (Fig. 1).

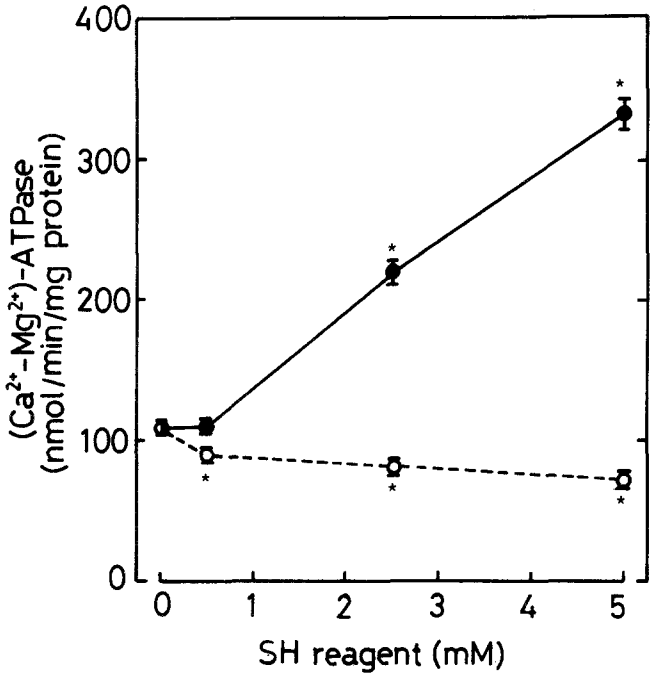

Fig. 1. Effect of sulfhydryl (SH) reagents on (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes. The enzyme activity was measured in the reaction mixture containing either dithiothreitol or N-ethylmaleimide in the range of 0.5 to 5 mM as a final concentration. Each value represents the mean ± SEM of five separate experiments with different plasma membrane preparation. * $P < 0.01$, as compared with the control (none) value. o, N-ethylmaleimide; •, dithiothreitol.

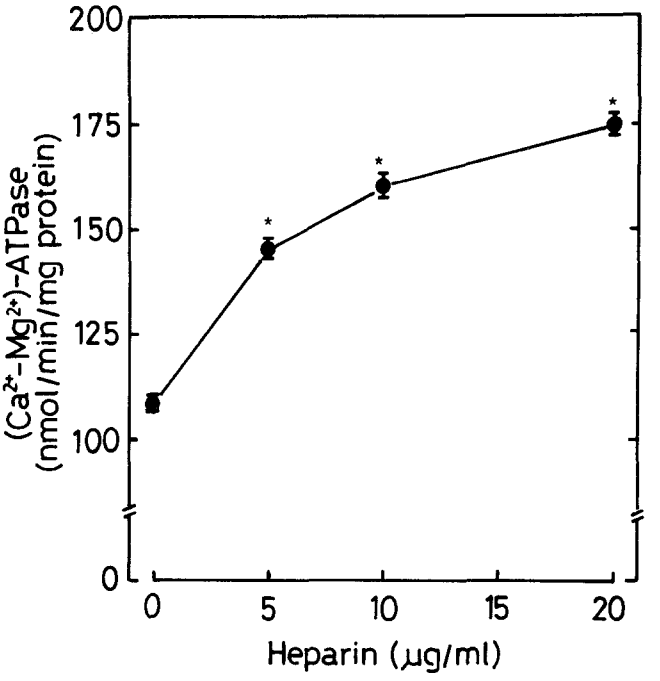

Fig. 2. Effect of heparin on (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes. The enzyme activity was measured in the reaction mixture containing heparin in the range of 5 to 20 μg/ml as a final concentration. Each value represents the mean ± SEM of five separate experiments with different plasma membrane preparation. * $P < 0.01$, as compared with the control (none) value.

Thus, (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes was activated by a sulfhydryl group protecting reagent DTT.

The effect of heparin on (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes is shown in Fig. 2. Heparin is a sulphated polysaccharide, and it can bind on liver membranes [19]. The presence of herparin (5–20 μg/ml) caused a significant increase in (Ca^{2+}-Mg^{2+})-ATPase activity.

The effect of DTT and heparin to increase (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes was compared, and the result is shown in Table 1. The Ca^{2+}-dependent phosphorylation of the enzyme protein in the plasma membranes is inhibited by vanadate (0.1 mM) [12]. The presence of vanadate (0.1 mM) did not inhibit the DTT (5 mM)-increased (Ca^{2+}-Mg^{2+})-ATPase activity, while it caused an appreciable inhibition of the heparin (20 μg/ml)-elevated enzyme activity. Moreover, the effect of DTT increasing (Ca^{2+}-Mg^{2+})-ATPase activity was clearly blocked by the presence of NEM (5 mM), although the effect of heparin on the enzyme activity was not significantly influenced by the sulfhydryl modifying reagent.

The presence of digitonin with a comparatively lower concentration (0.005–0.04%) in the enzyme reaction mixture caused a significant decrease of (Ca^{2+}-Mg^{2+})-ATPase activity in the liver plasma membranes (Fig. 3). However, the effect of digitonin (0.04%) decreasing (Ca^{2+}-Mg^{2+})-ATPase activity was not seen in the presence of DTT (5 mM) or

Table 1. Effect of vanadate and N-ethylmaleimide on the dithiothreitol- or heparin-increased (Ca^{2+}-Mg^{2+})-ATPase activity in the plasma membranes of rat liver

Treatment	(Ca^{2+}-Mg^{2+})-ATPase (nmol/min/mg protein)
Control	105.5 ± 5.6
Dithiothreitol (5 mM)	347.6 ± 21.2*
Vanadate (0.1 mM)	74.3 ± 3.5*
Dithiothreitol + vanadate	315.4 ± 8.3*
N-ethylmaleimide (NEM; 5 mM)	77.6 ± 7.7*
Dithiothreitol + NEM	159.4 ± 9.6*,**
Heparin (20 μg/ml)	174.5 ± 11.5*
Heparin + vanadate	124.5 ± 9.6**
Heparin + NEM	167.8 ± 15.6*

The enzyme activity was measured in the reaction mixture containing either dithiothreitol, heparin, vanadate, NEM or dithiothreitol (heparin) plus NEM (vanadate). Each value represents the mean ± SEM of five separate experiments with different plasma membrane preparations. * $P < 0.01$, as compared with the control value. ** $P < 0.01$, as compared with the value of dithiothreitol or heparin alone.

heparain (20 μg/ml), as shown in Table 2. Digitonin may act on the membranous lipids, but not in the enzyme protein.

Effect of regucalcin on (Ca^{2+}-Mg^{2+})-ATPase activity in the presence of various reagents

The effect of increasing concentrations of regucalcin on

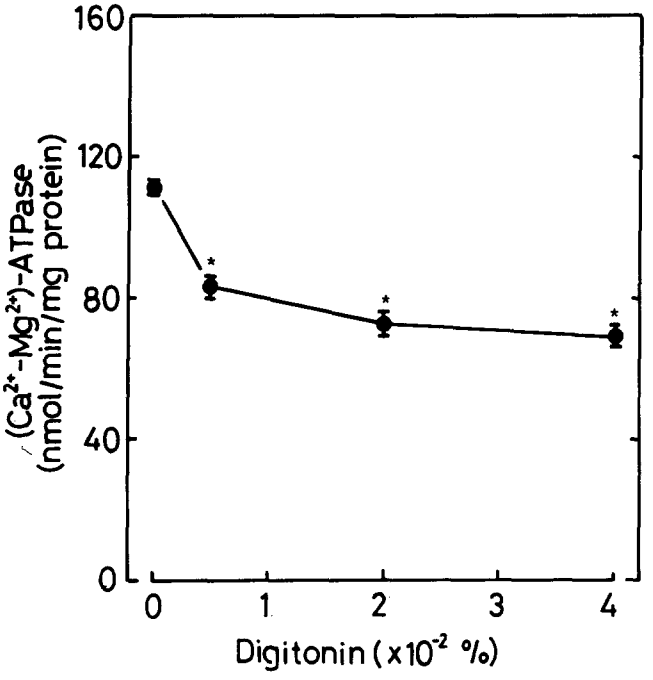

Fig. 3. Effect of digitonin on (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes. The enzyme activity was measured in the reaction mixture containing digitonin in the range of 0.005 to 0.04% as a final concentration. Each value represents the mean ± SEM of five separate experiments with different plasma membrane preparation. * $P < 0.01$, as compared with the control (none) value.

Table 2. Effect of digitonin on the dithiothreitol or heparin-increased (Ca^{2+}-Mg^{2+})-ATPase activity in the plasma membranes of rat liver

Treatment	(Ca^{2+}-Mg^{2+})-ATPase (nmol/min/mg protein)
Control	110.9 ± 4.3
Digitonin (0.04%)	74.4 ± 2.9*
Dithiothreitol (DTT; 5 mM)	344.4 ± 13.0*
Digitonin + DTT	396.1 ± 33.2*
Heparin (20 μg/ml)	174.5 ± 11.5*
Digitonin + heparin	204.9 ± 19.0*

The enzyme activity was measured in the reaction mixture containing either digitonin, various reagents (DTT and heparin) or digitonin plus various reagents. Each value represents the mean ± SEM of five separate experiments with different plasma membrane preparations. * $P < 0.01$, as compared with the control value.

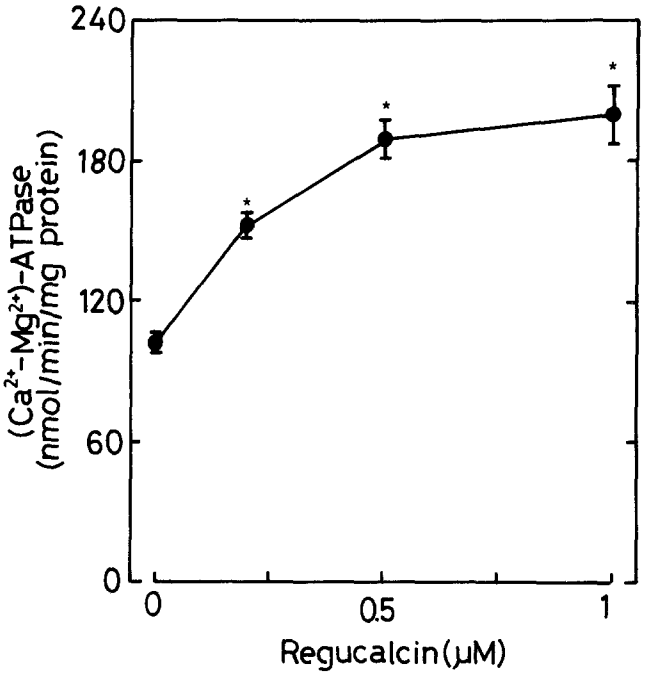

Fig. 4. Effect of regucalcin on (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes. The enzyme activity was measured in the reaction mixture containing regucalcin in the range of 0.25 to 1.0 μM as a final concentration. Each value represents the mean ± SEM of five separate experiments with different plasma membrane preparation. * $P < 0.01$, as compared with the control (none) value.

(Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes is shown in Fig. 4. The presence of regucalcin (0.25–1.0 μM) with a cell physiological concentration in the enzyme reaction mixture caused a significant increase in (Ca^{2+}-Mg^{2+})-ATPase activity in the liver plasma membranes.

The effect of regucalcin (0.25 μM) to increase (Ca^{2+}-Mg^{2+})-ATPase activity in the liver plasma membranes was not fairly

Table 3. Effect of N-ethylmaleimide, dithiothreitol and heparin on the regucalcin-increased (Ca^{2+}-Mg^{2+})-ATPase activity in the plasma membranes of rat liver

Treatment	(Ca^{2+}-Mg^{2+})-ATPase (nmol/min/mg protein)
Control	110.4 ± 4.5
Regucalcin (0.25 μM)	151.8 ± 4.8*
N-Ethylmaleimide (NEM; 5 mM)	70.4 ± 8.7*
Regucalcin + NEM	78.4 ± 13.7*,**
Dithiothreitol (DTT; 5 mM)	371.6 ± 37.1*
Regucalcin + DTT	419.4 ± 44.5*
Heparin (20 μg/ml)	169.3 ± 4.5*
Regucalcin + heparin	171.2 ± 5.9*,**

The enzyme activity was measured in the reaction mixture containing either regucalcin, various reagents (NEM, DTT or heparin) or regucalcin plus various reagents. Each value represents the mean ± SEM of five separate experiments with different plasma membrane preparations. * $P < 0.01$, as compared with the control value. ** $P < 0.01$, as compared with the value of regucalcin alone.

seen in the presence of NEM (5 mM), as shown in Table 3. Regucalcin (0.25 μM) did not additively enhance the effect of DTT on the enzyme activity. Also, the effect of regucalcin increasing (Ca^{2+}-Mg^{2+})-ATPase activity was not significantly altered by the presence of heparin (20 μg/ml).

The presence of GTP with an effective concentration (10^{-5} M) in the enzyme reaction mixture caused a significant increase of (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes (Table 4). This increase was not significantly inhibited by the presence of NEM (5 mM). Now, the effect of DTT (5 mM) to increase the enzyme activity was clearly enhanced by the presence of GTP (10^{-5} M). This result indicates that the effective site of GTP is not involved in the sulfhydryl group of (Ca^{2+}-Mg^{2+})-ATPase in liver plasma membranes. Now, the effect of regucalcin (0.25 μM) increasing the enzyme activity was weakened by the presence of GTP (10^{-5} M). Moreover, regucalcin (0.25 μM) did not significantly increase (Ca^{2+}-Mg^{2+})-ATPase activity in the presence of digitonin (0.04%), which can decrease the enzyme activity. This finding indicates that the effect of regucalcin is disappeared by the solubilization of the membranous lipids.

Table 4. Effect of GTP and digitonin on the regucalcin-increased (Ca^{2+}-Mg^{2+})-ATPase activity in the plasma membranes of rat liver

Treatment	(Ca^{2+}-Mg^{2+})-ATPase (nmol/min/mg protein)
Control	108.2 ± 3.8
GTP (10^{-5} M)	140.9 ± 3.4*
Dithiothreitol (5 mM)	344.4 ± 13.0*
GTP + dithiothreitol	464.0 ± 22.2*,**
N-Ethylmaleimide (5 mM)	78.0 ± 6.9*
GTP + N-ethylmaleimide	128.2 ± 4.7*
Regucalcin (0.25 μM)	148.1 ± 5.1*
GTP + regucalcin	125.7 ± 4.1*,**
Digitonin (0.04%)	75.6 ± 3.1*
Digitonin + regucalcin	76.1 ± 7.7*,**

The enzyme activity was measured in the reaction mixture containing either regucalcin, various reagents (GTP, dithiothreitol, N-ethylmaleimide and digitonin) or regucalcin plus various reagents. Each value represents the mean ± SEM of five separate experiments with different plasma membrane preparations. * $P < 0.01$, as compared with the control value. ** $P < 0.01$, as compared with the value without GTP or digitonin.

Discussion

The high-affinity (Ca^{2+}-Mg^{2+})-ATPase, which is located on the plasma membranes of rat liver, acts as a Ca^{2+} pump to extrude the metal ion from the cytoplasm of liver cells [11, 12]. This enzyme is regulated by various factors. The enzyme activity is inhibited by vanadate [12], vasopressin [20] and glucagon [21]. Liver plasma membrane (Ca^{2+}-Mg^{2+})-ATPase activity is not regulated by calmodulin and its antagonist [11]. Meanwhile, divalent metal ions (Fe^{2+}, Mn^{2+} and Co^{2+}) increase the (Ca^{2+}-Mg^{2+})-ATPase activity, but these metal ions uncouple the Ca2+ transport in rat liver plasma membrane [22]. Recently, it has been found that regucalcin, a calcium-binding protein isolated from rat liver cytosol, can increase (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes [13]. This effect of regucalcin was not blocked by vanadate, which inhibits Ca^{2+}-dependent phosphorylation of (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes [12], although vanadate has an inhibitory effect on the Mn^{2+} and Co-increased enzyme activity [14]. Moreover, it has been demonstrated that the activating mechanism of regucalcin on (Ca^{2+}-Mg^{2+})-ATPase is not involved in GTP-binding protein which modulates the receptor-mediated hormonal effect in rat liver plasma membranes [15], and that the effect of hormones (epinephrine, phenylephrine and insulin) to increase the enzyme activity may be related to the phosphorylation of the enzyme [15]. Thus, the activating mechanism of regucalcin was not based on the Ca^{2+}-dependent phosphorylation of (Ca^{2+}-Mg^{2+})-ATPase.

In the present study, it has been demonstrated that (Ca^{2+}-Mg^{2+})-ATPase activity in liver plasma membranes is markedly increased by the presence of dithiothreitol (DTT), a sulfhydryl (SH) group protecting reagent. The effect of DTT was clearly weakened by the presence of N-ethylmaleimide (NEM), a SH modifying reagent, although the DTT effect was not altered by vanadate. This result suggests that the active site of (Ca^{2+}-Mg^{2+})-ATPase is related to the SH groups of the enzyme. The effect of regucalcin increasing (Ca^{2+}-Mg^{2+})-ATPase was completely blocked by the presence of NEM. This finding suggests that regucalcin effects on the SH groups which are the active site of (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes. Now, heparin had the activating effect on liver plasma membrane (Ca^{2+}-Mg^{2+})-ATPase. This effect was inhibited by vanadate, but not by NEM, suggesting that the mode of heparin action differs from that of regucalcin.

Digitonin may be able to solubilize the lipids of liver plasma membranes [23]. Liver plasma membrane (Ca^{2+}-Mg^{2+})-ATPase activity was clearly decreased by the presence of digitonin. This effect, however, was not seen in the presence of DTT and heparin, suggesting that digitonin does not have a direct inhibitory effect on the enzyme. Presumably, the effect of digitonin decreasing (Ca^{2+}-Mg^{2+})-ATPase activity is involved in the detergent effect on the lipids of liver plasma membranes. The activating effect of regucalcin on the enzyme was seen in the present of digitonin. This finding suggests that the effect of regucalcin is also related to the lipids of plasma membranes. Moreover, it has been demonstrated that the radioiodinated regucalcin can bind to the plasma membranes of rat liver [13]. From these observations, it is assumed that regucalcin binds to the lipids at the close site of (Ca^{2+}-Mg^{2+})-ATPase in liver plasma membranes, and

that regucalcin acts on the SH group which is the active site of the enzyme. This does not exclude from the possibility that the effect of regucalcin on the enzyme is not, at least partly, the active site.

Interestingly, the effect of DTT increasing (Ca^{2+}-Mg^{2+})-ATPase activity in liver plasma membranes was fairly enhanced by the presence of GTP, although the effect of GTP increasing the enzyme activity was not significantly decreased by NEM, suggesting that GTP does not influence on the SH group of the enzyme. Now, the effect of GTP was slightly weakened by the presence of regucalcin. The activating site of regucalcin on the enzyme may differ from that of GTP.

The Ca^{2+}-dependent phosphorylation of (Ca^{2+}-Mg^{2+})-ATPase in liver plasma membranes is regulated by various reagents [14, 15, 20–22]. Also, the process of phosphorylation of (Ca^{2+}-Mg^{2+})-ATPase may be regulated through GTP-binding protein which modulates the receptor-mediated hormonal effect in liver plasma membranes [15, 20]. However, the mechanism of regucalcin activiting (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes may be based on the action to the SH groups of enzyme due to binding on the membranous lipids which is near close to (Ca^{2+}-Mg^{2+})-ATPase in liver plasma membranes. Regucalcin may be a novel cytosolic activating factor for (Ca^{2+}-Mg^{2+})-ATPase in liver plasma membranes. Presumably, regucalcin plays a role in the regulation of Ca^{2+} levels in the cytoplasm of liver cells by activating the plasma membranous (Ca^{2+}-Mg^{2+})-ATPase.

Acknowledgement

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (No. 04671362) from the Ministry of Education, Japan.

References

1. Rasmussen J: Cell communication, calcium ion, and cyclic adenosime monophosphate. Science 170: 404–412, 1970
2. Cheung WY: Calmodulin plays a pivotal role in cellular regulation. Science 202: 19–27, 1984
3. Williamson JR, Cooper RH, Hoek JB: Role of calcium in the hormonal regulation of liver metabolism. Biochim Biophys Acta 639: 243–295, 1981
4. Reinhart PH, Taylor WM, Bygrave FL: The role of calcium ions in the mechanisms of action of α-adrenergic agonists in rat liver. Biochem J 223: 1–13, 1984
5. Yamaguchi M, Yamamoto T: Purification of calcium binding substance from soluble fraction of normal rat liver. Chem Pharm Bull 26: 1915–1918, 1978
6. Shimokawa N, Yamaguchi M: Molecular cloning and sequencing of the cDNA coding from a calcium-binding protein regucalcin from rat liver. FEBS Lett 327: 251–255, 1993
7. Yamaguchi M, Mori S: Inhibitory effect of calcium-binding protein regucalcin on protein kinase C activity in rat liver cytosol. Biochem Med Metab Biol 43: 140–146, 1990
8. Yamaguchi M, Tai H: Inhibitory effect of calcium-binding protein regucalcin on Ca^{2+}/calmodulin-dependent cyclic nucleotide phophodiesterase activity in rat liver cytosol. Mol Cell Biochem 106: 25–30, 1991
9. Yamaguchi M, Sakurai T: Inhibitory effect of calcium-binding protein regucalcin on Ca^{2+}-activated DNA fragmentation in rat liver nuclei. FEBS Lett 279: 281–284, 1991
10. Yamaguchi M, Sakurai T: Reversible effect of calcium-binding protein regucalcin on Ca^{2+}-induced inhibition of deoxyuridine 5'-triphosphatase activity in rat liver cytosol. Mol Cell Biochem 110: 25–29, 1992
11. Lotersztjn S, Hanoune J, Pecker F: A high affinity calcium-stimulated magnesium-dependent ATPase in rat liver plasma membranes. Dependence on an endogenous protein activator distinct from calmodulin. J Biol Chem 256: 11209–11215, 1981
12. Chen K-M, Junger KD: Calcium transport and phosphorylated intermediate of (Ca^{2+}-Mg^{2+})-ATPase in plasma membranes of rat liver. J Biol Chem 258: 4404–4410, 1983
13. Yamaguchi M, Mori S, Kato S: Calcium-binding protein regucalcin is an activator of (Ca^{2+}-Mg^{2+})-adenosine triphosphatase in the plasma membranes of rat liver. Chem Pharm Bull 36: 3532–3539, 1988
14. Takahashi H, Yamaguchi M: Regulatory effect of regucalcin on (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes: comparison with the activiation by Mn^{2+} and Co^{2+}. Mol Cell Biochem 124: 169–174, 1993
15. Takahasahi H, Yamaguchi M: Regucalcin modulates hormonal effect on (Ca^{2+}-Mg^{2+})-ATPase activity in rat liver plasma membranes. Mol Cell Biochem 125: 171–177, 1993
16. Lowry OH, Rosebrough NH, Farr AL, Randall RF: Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–273, 1951
17. Prpić V, Green KC, Blackmore PF, Exton JH: Vassopressin-, angiotensin II-, and α_1-adrenergic-induced inhibition of Ca^{2+} transport by rat liver plasma membrane vesicles. J Biol Chem 259: 1382–1385, 1984
18. Nakamura M, Mori K: Colorimetric determination of inorganic phosphorus in the presence of glucose-1-phosphate and adenosine triphosphate. Nature 182: 1441–1442, 1958
19. Cullen PJ, Comerford JG, Dawson AP: Heparin inhibits the inositol 1,4,5-triphosphate-induced Ca^{2+} release from rat liver microsomes. FEBS Lett 228: 57–59, 1988
20. Lin S-H, Wallace MA, Fain JN: Regulation of (Ca^{2+}-Mg^{2+})-ATPase activity in hepatocyte plasma membranes by vasopressin and phenylephrine. Endocrinology 113: 2268–2275, 1983
21. Lotersztajn S, Epand RM, Mallat A, Pecker F: Inhibition by glucagon of the calcium pump in liver plasma membranes. J Biol Chem 259: 8195–8201, 1984
22. Pecker F, Lotersztajn S: Fe^{2+} and other divalent metal ions uncouple Ca^{2+} transport from (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes. J Biol Chem 260: 731–735, 1985
23. Murphy EK, Coll TL, Rich TL, Williamson JR: Hormonal effects on calcium homeostasis in isolated hepatocytes. J Biol Chem 255: 6600–6608, 1980