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A COMPARATIVE STUDY OF PLASMA MEMBRANE Mg^{2+}-ATPase ACTIVITIES IN NORMAL, REGENERATING AND MALIGNANT CELLS

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Plasma membranes have been prepared from rat normal liver cells, regenerating liver cells and Yoshida ascites hepatoma 66 cells after intact cells were first bound to polylysine-coated polyacrylamide beads, and the membrane-associated Mg^{2+}-ATPase activity was assayed directly on beads with membrane attached. With plasma membranes from normal liver cells, K_m for ATP and V were found to be higher than those in regenerating liver cells and hepatoma cells. Vanadate caused a different sensitivity of the activity, without an effect in normal liver cells and with an inhibition in regenerating liver cells and hepatoma cells. The activity in normal and regenerating liver cells decreased with increasing temperature above 24–30°C, while the activity in hepatoma cells continued to increase linearly to 37°C. Unlike the enzyme in normal and regenerating liver cells, the hepatoma enzyme was shown to have a higher phase transition temperature and lower activation energies. In all three kinds of cells the activity was increased by the dephosphorylation of plasma membranes and unaffected by the phosphorylation. By means of histochemical Mg^{2+}-ATPase staining applied on polyacrylamide gels, at least three major bands which show the enzymic activity were visible in normal and regenerating liver and a single band was detected in hepatoma cells.

Introduction

It is well established that malignant cells exhibit profound alterations in the plasma membrane. In the previous cytochemical work on monolayer cultures, we observed the increased activity of Mg^{2+}-ATPase at the contact surface of hepatoma cells [1]. No increase in the activity was found in density-inhibited cells, such as hepatic parenchymal cells, dibutyryl cyclic AMP and theophylline-treated hepatoma cells [2], and the hepatoma cells cultured in a lipid-depleted medium [3]. The levels of K_m for ATP and V of the Mg^{2+}-ATPase increased when hepatoma cells reached confluent, and remained constant in density-inhibited cells [3,4]. It is therefore of interest to determine which specific alterations of enzyme properties associated with the malignant state, result in changes of the activity at cell-to-cell contact.

This report confirmed that the method for rapid isolation of plasma membranes from HeLa cells [5] could be applied to normal, regenerating liver cells and hepatoma cells. The studies described in this paper were designed to characterize the activity of Mg^{2+}-ATPase with respect to kinetic analysis, Arrhenius plots, sensitivity to inhibitors, effects of phosphorylation and dephosphorylation of plasma membranes, and electrophoretic mobility.

Materials and Methods

Intact cells. To prepare free normal and regenerating liver cells, as previously described [6], Donryu strain male rat liver was perfused through

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portal vein with 30 ml of 27 mM sodium citrate in Ca^{2+}-free Locke solution at 37°C. Excised liver was hand-homogenized in 10 volumes of 10% polyethylene glycol (mol. wt. 4000) with a rubber pestle with 10 strokes at room temperature. Homogenates were filtered through four layers of gauze at 4°C, centrifuged at $70 \times g$ for 5 min at 4°C, and washed twice with 217 mM sucrose/46.5 mM sodium acetate buffer, pH 5.0. Regenerating liver was used 24 h after partial hepatectomy performed according to the procedure of Higgins and Anderson [7].

Yoshida ascites hepatoma 66 cells were grown for 6–7 days in Donryu strain male rats. After adding 10 volumes of Ca^{2+}, Mg^{2+}-free phosphate buffered saline to the ascitic fluid, the cells were separated by centrifugation at $70 \times g$ for 5 min at 4°C, washed a total of three times; once with 10% polyethylene glycol, and twice with 217 mM sucrose/46.5 mM sodium acetate buffer, pH 5.0.

Isolation of plasma membranes. The intact cells were first bound to polylysine (mol. wt. 84800–91200, Miles Lab.)-coated polyacrylamide beads (Bio-Gel P2, 200–400 mesh, Bio-Rad Lab.) suspended in 217 mM sucrose/46.5 mM sodium acetate buffer, pH 5.0, and the plasma membranes were isolated according to the method of Cohen et al. [5]. Fig. 1 shows isolated liver cells, hepatoma cells, and the plasma membranes attached to beads, where the Sudan black-stained cytoplasmic surface was exposed.

ATPase assay. Incubations for the Mg^{2+}-ATPase activity were performed in nitrocellulose tubes at 37°C. The assay was initiated by the addition of plasma membranes attached to beads to an incubation medium containing 75 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, and 3 mM Na_2-ATP in a total of 0.5 ml. The reaction was terminated after 10 or 30 min by the addition of 0.15 ml of 30% trichloroacetic acid. The hydrolyzed inorganic phosphate was measured by the method of Martin and Doty [8]. Controls containing no plasma membrane were used to correct for non-enzymic hydrolysis. For enzyme kinetic studies 0.067–0.5

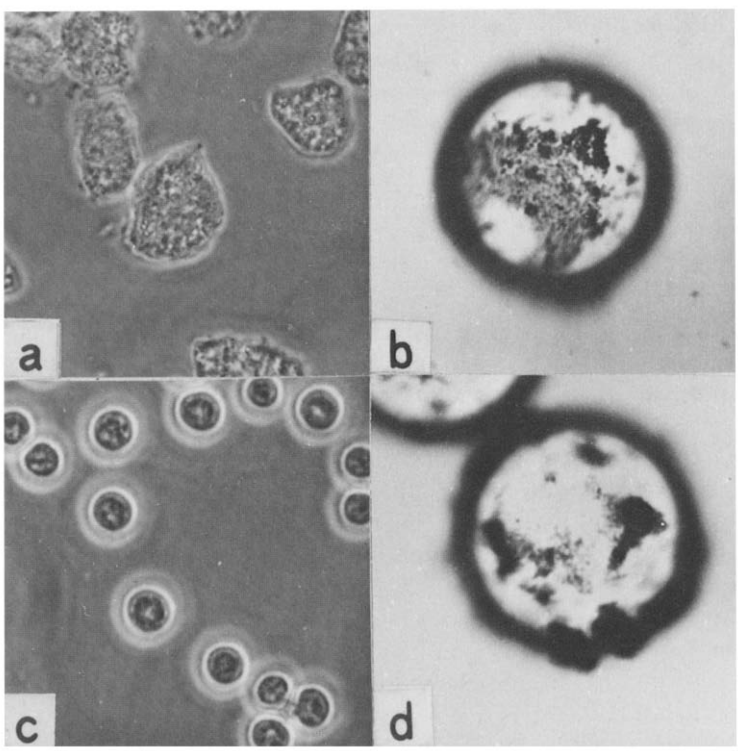

Fig. 1. (a) Isolated liver cells, (b) liver plasma membrane on bead, (c) hepatoma cells, (d) hepatoma plasma membrane on bead. Magnification: ×400.

mM [γ-^{32}P]ATP (Radiochemical Centre, Amersham) (spec. act. 0.67 μCi/μmol) was used as a substrate, and the hydrolyzed inorganic phosphate was measured by the method of Seals et al. [9]. To determine the amount of plasma membrane protein the modification of the method of Lowry et al., as described in Ref. 5, was used with bovine serum albumin as a standard.

Phosphorylation and dephosphorylation Plasma membranes on beads were phosphorylated by the method of Ziegelhoffer et al. [10] by incubating at 25°C in the presence of 5 mM Na_2-ATP, 5 mM $MgCl_2$, 1 μM cyclic AMP (sodium salt, Sigma), 20 μg/ml of freshly dialyzed protein kinase (cyclic AMP-dependent, bovine heart muscle, Sigma) and 40 mM histidine buffer, pH 6.8 in a nitrocellulose tube. After 5 min of reaction, the plasma membranes were washed with 10 mM Tris-HCl, pH 7.4.

Dephosphorylation was carried out by incubating plasma membranes on beads for 1 h at 20°C in the presence of 0.4 mg/ml of *Escherichia coli* alkaline phosphatase (10 U/mg protein, Sigma) [11] or 2 mg/ml of calf intestine alkaline phosphatase (1.1 U/mg solid, Sigma) [12], 20 mM Tris-HCl, pH 8.5, 2 mM $MgCl_2$ and 0.2 mg/ml of bovine serum albumin. Then the plasma membranes were washed with 10 mM Tris-HCl, pH 7.4.

Gel electrophoresis. Plasma membranes on beads were treated for 20 min with a medium containing 1% digitonin, 75 mM Tris-HCl, pH 7.6 and 5 mM $MgCl_2$. After centrifugation at 100000 $\times g$ for 60 min at 4°C the supernatant was concentrated with an Amicon filtration apparatus (UM-2 filter). Disc gel electrophoresis in the absence of detergents was carried out in 4% polyacrylamide gel at 2 mA/gel as described by Davis [13]. Detection of ATPase activity was performed as described by White and Ralston [14]; in brief, the gels were washed in cold 20 mM Tris-maleate, pH 8.0, incubated overnight at room temperature in 20 mM Tris-maleate, pH 8.0 containing 1 mM $Pb(NO_3)_2$, 1 mM $MgCl_2$ and 1 mM Na_2-ATP, and stained with 2% ammonium sulfide. To investigate electrophoretic mobility as a function of molecular weight, electrophoresis in a continuous 4–25% polyacrylamide gel gradient [15] was carried out at 1.5 mA/gel for 20 h. After electrophoresis one portion of gel was stained for Mg^{2+}-ATPase activity and the remainder was stained with Coomassie blue.

All experimental determinations were performed in duplicate and each experiment was repeated three times, and mean results (ATPase activity) or typical results (gel electrophoresis) are presented.

Results

Membranes on beads were assayed for 5′-nucleotidase activity, ouabain-sensitive ($Na^+ + K^+$)-ATPase activity [16], and for Mg^{2+}-ATPase activity. A 4-10 fold enrichment was achieved for these enzymes (Table I). Recoveries of enzyme activities on beads ranged from 2.6 to 5.6%.

In the presence of 3 mM ATP very little hydrolytic activity was observed unless Mg^{2+} was added (Fig. 2a). The Mg^{2+} concentration at which the activity was half activated was 0.15 mM, and an optimum concentration of Mg^{2+} was reached at 5 mM. The Mg^{2+}-ATPase activity as a function of ATP concentration is shown in Fig. 2b. Kinetic analysis by double-reciprocal plots gave an apparent K_m for ATP of 1.1 mM for normal liver cells, 0.27 mM for regenerating liver cells and 0.24 mM for hepatoma cells. V (μmol P_i/mg protein per h) was 37 for normal liver cells, 12 for regenerating liver cells and 11 for hepatoma cells, respectively. The activity was linear in time during the 120 min of incubation when at least 2 mM ATP was added as a substrate in the presence of 5 mM Mg^{2+}. At a fixed 5 mM Mg^{2+} concentration, Ca^{2+} caused a slight inhibition at 0.2 mM or 5 mM and a slight additive increase in the activity at 0.5–2 mM in normal liver cells, however, the addition of Ca^{2+} was always slightly inhibitory in the case of regenerating liver cells and hepatoma cells. As shown in Fig. 2c, the activities showed similar pH dependency with a relatively broad pH optimum around pH 8.

Compounds, known for their inhibitory effects on ATP-splitting enzymes, were tested. *N*-Ethylmaleimide did not substantially modify the activity of Mg^{2+}-ATPase at concentrations from 0.01 mM to 1 mM. Olygomycin (0.1–1 mg/ml), quercetin (4–20 μg/ml), sodium metaperiodate

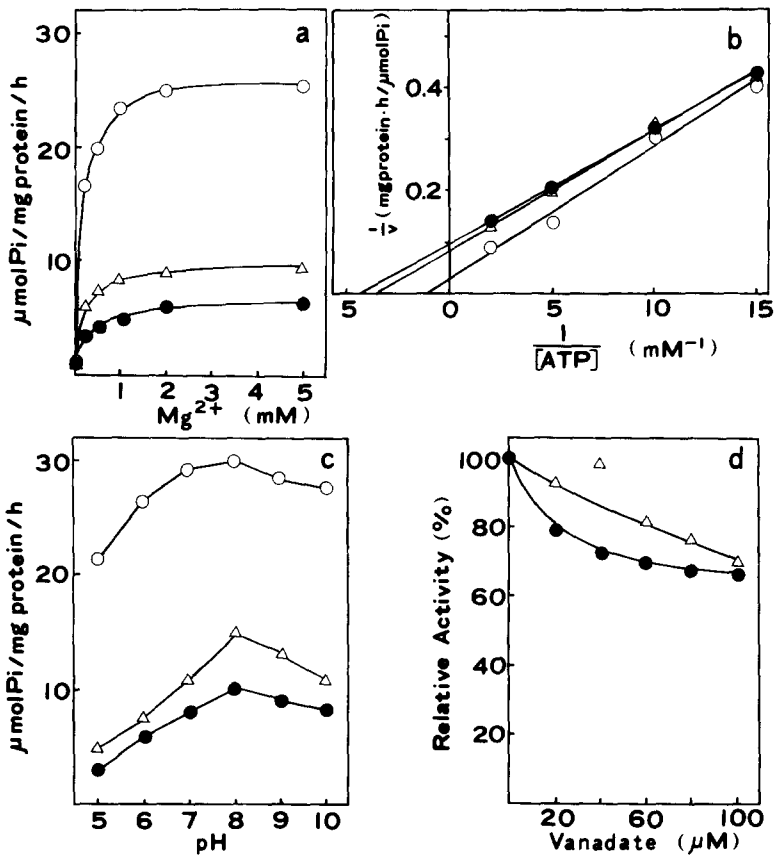

Fig. 2. (a) Effect of Mg^{2+} concentration, (b) Lineweaver-Burk plots of activity, (c) pH dependence of activity, (d) effect of vanadate. ○———○, normal liver plasma membrane; △———△, regenerating liver plasma membrane; ●———●, hepatoma plasma membrane. Activity was measured after 10 min (a, b, c), 30 min (d) incubation in the presence of 3 mM Na_2-ATP (a, c, d) or 0.067–0.5 mM [γ-^{32}P]ATP (specific activity, 0.67 μCi/μmol) (b), 5 mM $MgCl_2$ (b, c, d) and ammonium metavanadate (d). The reaction medium was buffered with 75 mM Tris-HCl, pH 7.6 (a, b, d) or with 75 mM Tris-glycine-maleic acid (c). V is expressed in μmol P_i/mg protein per h (b). Results were corrected for the interference by vanadate in the inorganic phosphate determination, and a percentage of control activity versus vanadate concentrations is shown (d).

TABLE I

MEMBRANE-ASSOCIATED ENZYME ACTIVITIES IN WHOLE CELLS AND PLASMA MEMBRANES

Specific activity is expressed as μmol P_i per mg of protein per hour. Relative activity of membranes on beads is given as ratio of specific activity to that in whole cells. Values in parentheses indicate corrected ones for the measured inhibition or stimulation given in column 4. To measure the effects of beads on enzyme activities, whole cell homogenates were assayed in the presence or absence of beads.

	Assay	Specific activity of whole cell homogenate	Relative activity of membranes on beads	Total activity recovered on beads (%)	Measured inhibition (−) or stimulation (+) of enzyme by beads (%)
Normal liver cells	Mg^{2+}-ATPase	2.3	5.2 (4.9)	3.5 (3.3)	+6
	$(Na^+ + K^+)$-ATPase	0.88	10.0	3.3	±0
	5′-Nucleotidase	2.4	4.9 (8.3)	3.3 (5.6)	−41
Hepatoma cells	Mg^{2+}-ATPase	1.6	5.5 (6.0)	3.3 (3.6)	−9
	$(Na^+ + K^+)$-ATPase	1.5	4.2 (4.3)	2.5 (2.6)	−3
	5′-Nucleotidase	0.37	3.2 (5.6)	1.9 (3.3)	−43

(0.01–1 mM), and ouabain (0.1–1 mM) also had no influence on the activity (data not shown). With increasing concentrations of ammonium metavanadate (0.02–0.1 mM) a gradually increasing inhibition of the activity was observed in regenerating liver cells and in hepatoma cells (Fig. 2d). However, no such an inhibition of the activity was observed in normal liver cells. The Mg^{2+}-ATPase activity was measured for various periods of time in the presence of 5 μg/ml of proteolytic enzyme, such as trypsin, pronase, papain and cathepsin D. Addition of a low concentration of proteolytic enzyme to plasma membranes caused no activation of the Mg^{2+}-ATPase. Pretreatment of plasma membranes with 80 μg/ml of trypsin resulted in a marked inhibition of the activity. The activity was as well inhibited when plasma membranes were pretreated with trypsin and Mg-ATP (data not shown). This is in contrast with *Neurospora* plasma membrane ATPase, where the activity is unaffected by trypsin in the presence of Mg-ATP [17].

The temperature affected the activities differently, and the enzyme presented a biphasic behavior in the Arrhenius plots. As shown in Fig. 3 the

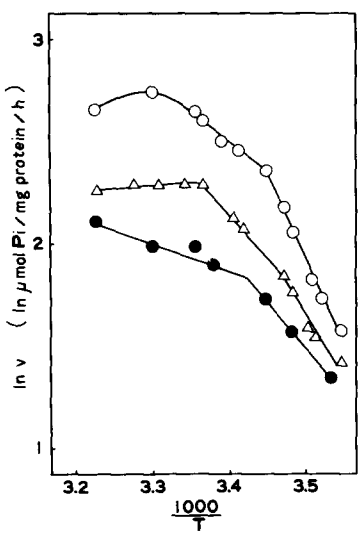

Fig. 3. Arrhenius plots of activity as a function of temperature. ○——○, normal liver plasma membrane; △——△, regenerating liver plasma membrane; ●——●, hepatoma plasma membrane. Activity was measured after 30 min incubation in the presence of 3 mM Na_2-ATP, 5 mM $MgCl_2$ and 75 mM Tris-HCl, pH 7.6.

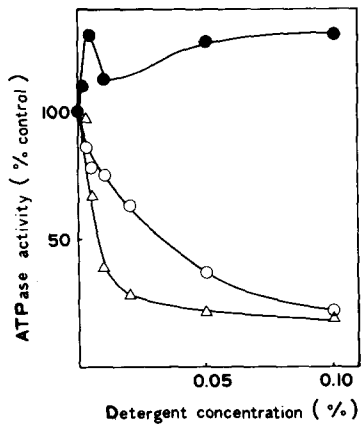

Fig. 4. Effects of detergents on Mg^{2+}-ATPase activity. Results are a percentage of control activity versus detergent concentrations. ●——●, digitonin; ○——○, Tween 20; △——△, Triton X-100.

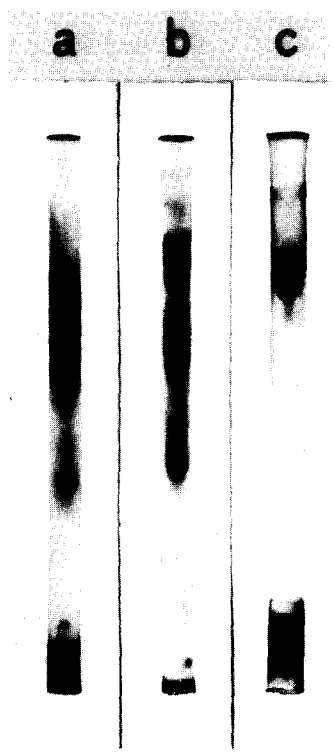

Fig. 5. Mg^{2+}-ATPase activity staining pattern on gel electrophoresis. (a) normal liver plasma membrane, (b) regenerating liver plasma membrane, (c) hepatoma plasma membrane. After overnight incubation in the presence of 1 mM Na_2-ATP, 1 mM $MgCl_2$ and 1 mM lead nitrate in 20 mM Tris-maleate, pH 8.0 at room temperature, the gels were washed and stained with 2% ammonium sulfide.

TABLE II

EFFECT OF PHOSPHORYLATION ON ACTIVITY

Membranes were preincubated for 5 min at 25°C in the presence of 5 mM Na_2-ATP, 5 mM $MgCl_2$, 1 μM cyclic AMP, 20 μg/ml of protein kinase and 40 mM histidine buffer, pH 6.8. After washings, activity was measured after 30 min incubation in the presence of 3 mM Na_2-ATP, 5 mM $MgCl_2$ and 75 mM Tris-HCl, pH 7.6.

	Mg^{2+}-ATPase activity (μmol P_i/mg protein per h)								
	Normal liver cells			Regenerating liver cells			Hepatoma cells		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
No addition	11.2	17.1	15.2	11.1	7.1	14.5	9.1	10.3	9.6
Cyclic AMP, protein kinase, Mg^{2+}, ATP	12.0	16.3	14.7	9.7	7.1	15.9	8.5	9.8	9.2

Arrhenius plot of the activity presents a discontinuity at 17°C for normal liver cells, 15°C for regenerating liver cells and 19.4°C for hepatoma cells. The activation energies (kcal/mol) were 16.02 (below the break point) and 5.61 (above the break point) in normal liver cells, 13.03 and 8.42 in regenerating liver cells, and 9.11 and 2.78 in hepatoma cells, respectively. The hepatoma Mg^{2+}-ATPase has a higher phase transition temperature and lower activation energies. Although the activity decreased from 30°C in normal liver cells and from 24°C in regenerating liver cells, the activity increased linearly with temperature between 10°C and 37°C in hepatoma cells.

The effect of plasma membrane phosphorylation and dephosphorylation on Mg^{2+}-ATPase activity was examined. Phosphorylated membranes were obtained by incubating in the presence of cyclic AMP, ATP, Mg^{2+} and protein kinase. As can be seen in Table II the activity was unaffected by the phosphorylation. However, exposure of plasma membranes to exogenous alkaline phosphatase resulted in a marked stimulation of the activity (Table III). There was no significant difference in the stimulation rate between normal, regenerating liver cells and hepatoma cells.

Fig. 4 illustrates the effect of three detergents on the activity. Tween 20 and Triton X-100 were inhibitory, while digitonin exerted moderate stimulatory effects on Mg^{2+}-ATPase. The Mg^{2+}-ATPase solubilized by digitonin from plasma membranes was demonstrated by means of histochemical activity-staining in polyacrylamide gels electrophoresed in the absence of detergents (Fig. 5). At least three major bands were present in normal and regenerating liver cells and a single band was visible in hepatoma cells. No stained band appeared when *p*-nitrophenyl phosphate was

TABLE III

EFFECT OF DEPHOSPHORYLATION ON ACTIVITY

Membranes were preincubated for 1 h at 20°C in the presence of 0.4 mg/ml of *E. coli* alkaline phosphatase (10 U/mg) or 2 mg/ml of calf intestine alkaline phosphatase (1.1 U/mg), 2 mM $MgCl_2$, 0.2 mg/ml of bovine serum albumin and 20 mM Tris-HCl, pH 8.5. After washings, activity was measured after 30 min incubation in the presence of 3 mM Na_2-ATP, 5 mM $MgCl_2$ and 75 mM Tris-HCl, pH 7.6.

	Mg^{2+}-ATPase activity (μmol P_i/mg protein per h)								
	Normal liver cells			Regenerating liver cells			Hepatoma cells		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
No addition	9.3	10.2	10.1	11.2	6.8	6.0	6.0	6.0	7.9
E. coli alkaline phosphatase	15.0	16.0	15.8	16.3	10.4	8.9	10.9	9.7	13.3
Calf intestine alkaline phosphatase	13.7	12.9	14.0	14.9	9.9	8.7	10.3	9.6	13.0

used as a substrate or Mg^{2+} was removed from the reaction medium. Staining was not affected by the addition of ouabain. Solubilized enzyme and a series of proteins of known molecular weights, including apo-ferritin, γ-globulin, albumin and ovalbumin were examined on a continuous polyacrylamide gel gradient. From the calibration curve of molecular size as a function of migration distance, three major bands were present in normal and regenerating liver cells at the positions corresponding to a molecular weight of approx. 200000, 500000 and 1000000. On the other hand, an apparent molecular weight was approx. 500000 in hepatoma cells.

Discussion

The presence of Mg^{2+}-ATPase has been reported in the plasma membrane isolated from human and bovine erythrocytes [14,18,19], human granulocytes [20], rabbit kidney cortex [21], rat pancreas [22], rat liver [23], chicken petoralis [24], and yeast [25]. In rat synaptic vesicles the vesicle Mg^{2+}-ATPase activity induces a pH gradient across the vesicle, which is the major driving force for norepinephrine uptake and storage [26]. However, the functional significance of Mg^{2+}-ATPase, often referred to as a basic ATPase, has not been established in the plasma membrane. In prior publications we have presented some evidence that plasma membrane Mg^{2+}-ATPase activity increases at cell-to-cell contact in the primary culture of monolayered hepatoma cells, and remains constant in the cells which show density-dependent inhibition of growth [1–4]. This suggests that specific alterations associated with the malignant state result in altered levels of Mg^{2+}-ATPase activity at cell-to-cell contact.

An aim of the work presented in this paper was to examine the properties of Mg^{2+}-ATPase in the plasma membranes isolated from normal, regenerating liver cells and hepatoma cells. In this experiment the procedure of Cohen and co-workers [5], in which HeLa cells are attached to polyacrylamide beads coated with polylysine, has proved to be useful for the purification of plasma membranes of liver cells and hepatoma cells. The insensitivity of Mg^{2+}-ATPase in isolated plasma membranes to oligomycin and quercetin, inhibitors of the mitochondrial and microsomal ATPases, rules out the possibility that the Mg^{2+}-ATPase activity derives from cytoplasmic organells contaminating the plasma membrane preparation. The insensitivity to meta-periodate suggests that the Mg^{2+}-ATPase might not be a glycoprotein. Furthermore, the insensitivity to a SH group reagent, an inhibitor of myosin ATPase, suggests that the Mg^{2+}-ATPase activity is not associated to subplasmalemmal contractile structures.

Several differences were observed between normal and hepatoma plasma membranes. Low K_m, low V, and decreased activity by meta-vanadate were found in hepatoma cells. However, these alterations were also found in regenerating liver cells. In our previous experiments, in which primary cell cultures were assayed biochemically in situ for ecto-Mg^{2+}-ATPase, low K_m and low V were obtained in hepatoma cells. However, in the similar experiment Karasaki et al. [27] have demonstrated high K_m and high V for ecto-Ca^{2+}, Mg^{2+}-ATPase in oncogenic variant RL34HT, a clonal line isolated from the outgrowth of a hepatocarcinoma-like tumor.

On the other hand, the two major changes which were entirely hepatoma specific were in the activities as a function of temperature, and the molecular size. The activity increases linearly with increasing temperature in hepatoma cells. In contrast, the activity of normal, and regenerating liver cells is especially temperature sensitive, activity falling off above 24–30°C. Furthermore, the Mg^{2+}-ATPase of hepatoma cells has a higher phase transition temperature and lower activation energies. The discontinuities in the Arrhenius plots of membrane bound enzyme activities have been reported to correlate with lipid phase changes [28,29]. It is therefore proposed that the hepatoma Mg^{2+}-ATPase has a different conformation possibly as a result of a modification of the membrane lipid phase. In human erythrocyte membranes, onset temperature for lipid phase separation increases with decreasing ratio of cholesterol to phospholipid [30]. The ratio is lower in hepatoma cells than in normal liver cells [31]. These results suggest that onset temperature for lipid phase separation is higher in hepatoma cells than in normal liver cells. However, we cannot rule out the possibility that the hepatoma enzyme has a differ-

ent rotational motion of the protein or a different state of aggregation of the protein, as suggested by the discontinuity in the Arrhenius plots [32].

In Mg^{2+}-ATPase staining applied on detergent -free disc gels, a single band was visible at a position corresponding to a molecular weight of approx. 500000 in hepatoma cells. However, at least three molecules which have Mg^{2+}-ATPase activity were found in normal and regenerating cells; these bands were present at the positions corresponding to a molecular weight of approx. 200000, 500000 and 1000000, respectively. The 200000- and the 500000-dalton protein in normal and regenerating liver cells may represent the subunit dissociated in solubilization and/or electrophoresis. Although further investigations are necessary to conclude definitely, it seems likely that the plasma membrane of normal and regenerating liver cells has a large molecule of Mg^{2+}-ATPase, presumably comprised of several subunits, while the plasma membrane of hepatoma cells has a small molecule of the enzyme.

References

1 Ohnishi, T. and Kimura, S. (1976) Histochemistry 49, 107–112
2 Yamaguchi, K. and Ohnishi, T. (1977) Histochemistry 54, 191–199
3 Manabe, H. and Ohnishi, T. (1980) Exp. Cell Res. 128, 110–120
4 Ohnishi, T. and Yamaguchi, K. (1978) Exp. Cell Res. 116, 261–268
5 Cohen, C.M., Kalish, D.I., Jacobson, B.S. and Branton, D. (1977) J. Cell Biol. 75, 119–134
6 Ohnishi, T., Ohshima, E. and Ohtsuka, M. (1975) Exp. Cell Res. 93, 136–142
7 Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186–202
8 Martin, J.B. and Doty, D.M. (1949) Anal. Chem. 21, 965–967
9 Seals, J.R., McDonald, J.M., Bruns, D. and Jarett, L. (1978) Anal. Biochem. 90, 785–795
10 Ziegelhoffer, A., Anand-Srivastava, M.B., Khandelwal, R.L. and Dhalla, N.S. (1979) Biochem. Biophys. Res. Commun. 89, 1073–1081
11 Chiu, Y.S. and Tao, M. (1978) J. Biol. Chem. 253, 7145–7148
12 Cooley, L.B., Johnson, W.H. and Krause, S. (1979) J. Biol. Chem. 254, 2195–2198
13 Davis, B.J. (1964) Ann. NY Acad. Sci. 121, 404–427
14 White, M.D. and Ralston, G.B. (1976) Biochim. Biophys. Acta 436, 567–576
15 Lorentz, K. (1976) Anal. Biochem. 76, 214–220
16 Toda, G., Oka, H., Oda, T. and Ikeda, Y. (1975) Biochim. Biophys. Acta 413, 52–64
17 Dame, J.B. and Scarborough, G.A. (1980) Biochemistry 19, 2931–2937
18 White, M.D. and Ralston, G.B. (1980) Biochim. Biophys. Acta 599, 569–579
19 Niggli, V., Penniston, J.T. and Carafoli, E. (1979) J. Biol. Chem. 254, 9955–9958
20 Smolen, J.E. and Weissmann, G. (1978) Biochim. Biophys. Acta 512, 525–538
21 Busse, D., Pohl, B., Bartel, H. and Buschmann, F. (1980) Arch. Biochem. Biophys. 201, 147–159
22 Lambert, M. and Christophe, J. (1978) Eur. J. Biochem. 91, 485–492
23 Riordan, J.R., Slavik, M. and Kartner, N. (1977) 252, 5449–5455
24 Malouf, N.N. and Meissner, G. (1979) 12, 233–250
25 Willsky, G.R. (1979) J. Biol. Chem. 254, 3326–3332
26 Toll, L. and Howard, B.D. (1978) Biochemistry 17, 2517–2523
27 Karasaki, S., Suh, M.H., Salas, M. and Raymond, J. (1980) Cancer Res. 40, 1318–1328
28Madden, T.D. and Quinn, P.J. (1979) FEBS Lett. 107, 110–112
29 Wynn-Williams, A.T. (1976) Biochem. J. 157, 279–281
30 Hui, S.W., Stewart, C.M., Carpenter, M.P. and Stewart, T.P. (1980) J. Cell Biol. 85, 283–291
31 Cheng, S. and Levy, D. (1979) Arch. Biochem. Biophys. 196, 424–429
32 Wisniesky, B.J., Parkers, J.G., Huang, Y.O. and Fox, F. (1974) Proc. Natl. Acad. Sci. USA 71, 4381–4385