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THE COMMON OCCURRENCE OF ATP DIPHOSPHOHYDROLASE IN MAMMALIAN PLASMA MEMBRANES *

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In the plasma membranes from several mammalian tissues (including normal and tumor tissues), a Mg^{2+} (or Ca^{2+})-dependent ATP phosphohydrolase activity is present in much greater amount than the ($Na^+ + K^+$)-ATPase. The ouabain-insensitive activity can be attributed to at least two enzymes, an ATPase (EC 3.6.1.3) and an ATP diphosphohydrolase (EC 3.6.1.5). The ATPase hydrolyzes ATP and other nucleoside triphosphates and is not inhibited by azide. The ATP diphosphohydrolase hydrolyzes both ATP and ADP (and other nucleoside tri- and diphosphates) and the hydrolysis of adenine nucleotides is strongly inhibited by 10 mM azide. The ratios of these two enzymes in the various membranes (as determined by the extent of azide inhibition) vary widely. The ATP diphosphohydrolase accounts for most of the Mg^{2+} (or Ca^{2+})-dependent ATP hydrolysis activity of the plasma membranes of liver (mouse), kidney (dog), two mouse sarcomas, and a human astrocytoma (xenograft in athymic mice). The ATPase is more dominant in the plasma membranes from mouse brain and human oat cell carcinoma. The widespread presence of the ATP diphosphohydrolase in plasma membrane from various types of tissues is demonstrated for the first time and is of particular interest in view of its relatively high activity in the plasma membranes of two sarcomas. The membrane-bound ATP diphosphohydrolase is characterized with respect to its metal ion activators, substrates, and inhibitors. These results should facilitate the distinction of this enzyme from other ATP hydrolyzing enzymes of plasma membranes in future investigations.

Plasma membranes from various mammalian tissues contain different ATP-hydrolyzing activities. Those that are best characterized are the ($Na^+ + K^+$)-ATPase of kidney [1], brain [2], and the electric organ of the eel [3], the ($Mg^{2+} + Ca^{2+}$)-ATPase of red blood cell membrane [4], and heart sarcolemma [5], and the ($H^+ + K^+$)-ATPase of gastric mucosa [6]. All of these enzymes are engaged in maintaining gradients of specific ions (Na^+, K^+, Ca^{2+}, or H^+) across the cell membranes. Although most cells would require a certain intracellular ionic condition, i.e. low concentrations of Ca^{2+}, Na^+ and high concentrations of K^+, it has been noted by us and many other investigators that the ATPase activities responsible for maintaining such conditions are not readily observable in the plasma membrane preparation in all cases. For example, it is difficult to detect the ($Na^+ + K^+$)-ATPase in liver plasma membrane,

* Dedicated to Professor Nathan O. Kaplan on the occasion of his 65th birthday.

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Abbreviations: NTP, nucleoside triphosphates; NDP, nucleoside diphosphates; Ado*PP*[NH]*P*, adenosine 5′-[β,γ-imido]triphosphate; Ado*PP*[CH_2]*P*, adenosine 5′-[β,γ-methylene]triphosphate; pCMPS, *p*-chloromercuriphenylsulfonate; DCCD, dicyclohexylcarbodiimide; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

and the high affinity Ca^+-stimulated Mg^{2+}-dependent ATPase could only be observed under special assay conditions [7,8]. The dominant ATP hydrolyzing activity in liver plasma membranes [8] as well as membranes from many other tissues [9–18] is due to a low affinity (for Ca^{2+}, or Mg^{2+}), nonspecific nucleoside triphosphatase. As a result, although the ($Na^+ + K^+$)-ATPase is generally considered a specific marker for plasma membranes, the enrichment of the Mg^{2+} (or Ca^{2+})-dependent nucleoside triphosphatase, which is usually 10–20-fold, is much easier to monitor in procedures devised for the purification of plasma membranes [9,10,12,18].

Nucleoside triphosphatase is usually assayed by following the release of inorganic phosphate. Inorganic phosphate is determined colorimetrically if a nonradioactive substrate is used, or isotopically after separation of $^{32}P_i$ from the radioactive substrate, [γ-^{32}P]ATP. We have found that the ATPase measurement with some plasma membranes yielded significantly different values depending on the procedure for the determination of phosphate. By monitoring $^{32}P_i$ release from [γ-^{32}P]ATP, the activity obtained could be as low as 50% of the activity obtained when P_i was determined colorimetrically. We found subsequently that the discrepancy could be explained by the hydrolysis of ADP by these membranes.

We report here the common occurrence of ADPase, more appropriately an ATP diphosphohydrolase, in the plasma membranes of various mammalian tissues and some of the characteristics which distinguish it from the ATPase.

Experimental Procedures

Plasma membranes

In this study, plasma membranes from the following tissues were used: mouse liver, mouse brain, dog kidney, mouse sarcomas (Li-7 (m) and T362 (m)), human astrocytoma (T24), oat cell carcinoma (T293) and melanoma (T355). All tumors (human or mouse) were maintained in athymic mice and their characteristics and some of their biochemical properties have been described [18–21]. Purified plasma membranes were obtained from all tissues (except dog kidney) by differential and sucrose gradient centrifugation as described by Knowles et al. [18]. Crude plasma membrane fractions (microsomes) from dog kidney were prepared according to Jørgenson [22] and was kindly provided by Mr. Jonathan Lee of the Biology Department at UCSD.

Enzyme assays

1. Colorimetric method. To determine nucleoside triphosphatase or nucleoside diphosphatase activity, the reaction was carried out in a 1 ml reaction mixture containing 30 mM histidine-Tris, pH 7.4, 5 mM $MgCl_2$ and 5 mM NTP or NDP with 40–400 μg membrane protein. The reaction was initiated by the addition of 5 μmol NTP or NDP. After 10 min at 37°C, the reaction was terminated by the addition of 0.1 ml 50% trichloroacetic acid. An aliquot of the deproteinized supernatant (0.3–0.8 ml) was used for phosphate determination by the method of Lohmann and Jendrassik [23] scaled down to 2.5 ml.

2. Isotopic method. Reaction mixture and conditions were exactly the same as described above except [γ-^{32}P]ATP ($2 \cdot 10^4$ cpm/μmol) was used as substrate. An aliquot of the deproteinized supernatant (0.5 ml) was mixed with 1 ml of 5% ammonium molybdate and 4 ml of 1.25 N $HClO_4$. $^{32}P_i$ was extracted by 5 ml of isobutanol/benzene (1 : 1, v/v) [24]. 1 ml of the organic phase was used for Cerenkov counting.

Cells

Human oat cell carcinoma cells in culture were kindly provided by Dr. H. Masui of the Cancer Center of UCSD. The cells were established in culture from oat cell carcinoma xenografts in nude mice. Li-7(m) cells were established in culture as described by Beattie et al. [21]. Both cell lines were maintained in DME/F12 media supplemented with 10% newborn calf serum.

For determination of ADP hydrolysis by intact cells, cells growing near to confluency in 35-mm plates were assayed in a reaction mixture containing 0.1 M NaCl, 0.01 M KCl, 25 mM Tris-HCl (pH 7.5) with 5 mM $MgCl_2$ (or $CaCl_2$) and 5 mM [β-^{32}P]ADP. The reaction was carried out at 37°C for 30 min.

Materials

Na_2ATP was purchased from Boehringer-Mannheim. It was converted to Tris-ATP by passing a

Na_2ATP solution through a column of Dowex-Tris. Other NTP and NDP (Tris or Na salts) were purchased from Sigma. [γ-^{32}P]ATP was obtained from Amersham.

[β-^{32}P]ADP was prepared from [γ-^{32}P]ATP by the following procedure. To a reaction mixture (0.5 ml) containing 70 mM triethanolamine-HCl, pH 8, 0.5 mM [γ-^{32}P]ATP (0.01–0.1 mCi), 0.5 mM AMP and 1 mM $MgSO_4$, were added 10 units rabbit muscle myokinase (grade III, Sigma). The reaction was allowed to proceed for 15 min at 37°C. The tube was then transferred to ice and the entire content was applied to a PEI-column. [β-^{32}P]ADP was eluted by 1 M LiCl as described by Magnusson et al. [25]. Fractions containing [β-^{32}P]ADP were pooled, lyophilized and then converted to the Tris form. The yield of [β-^{32}P]ATP was approximately 30%.

Results

Discrepancy between ATPase activities measured by the colorimetric method and the isotopic method

When the ATPase activities of the various plasma membranes were measured, the results obtained by the colorimetric method and the isotopic method were very different. It can be seen from Table I that ATPase activity obtained by the isotopic method was 86% (brain) to as low as 50% (sarcoma) of the activity as determined by the colorimetric method. These differences can be readily explained by the presence in these plasma membranes of a second enzyme which is capable of hydrolyzing the product of an ATPase reaction, i.e. ADP.

Demonstration of ADP hydrolysis activity in the plasma membranes

When ADP was used as a substrate instead of ATP, ADP hydrolysis activities were found in all plasma membranes. These data are also presented in Table I. In membranes where there was greater discrepancy of ATP phosphohydrolase activities obtained by the two P_i determination methods, the ADPase activity was also very much higher (60–80% of the ATP phosphohydrolase activity) as seen in the plasma membranes of liver, kidney, astrocytoma and the two sarcomas.

TABLE I

ATP AND ADP PHOSPHOHYDROLASE ACTIVITIES OF VARIOUS PLASMA MEMBRANES

ATP phosphohydrolase reaction was carried out as described in Experimental Procedures with [γ-^{32}P]ATP (Tris). After deproteinization, an aliquot (0.3 ml) of the supernatant was taken for colorimetric determination of P_i. Another aliquot (0.5 ml) was used for extraction and $^{32}P_i$ was counted in a liquid scintillation counter. ADP phosphohydrolase activity was determined colorimetrically. The activities are presented in μmol P_i liberated per min per mg protein.

Plasma membranes from	ATP phosphohydrolase		ADP phosphohydrolase
	Colorimetric	Isotopic	
Normal tissues			
Liver (mouse)	0.33	0.19	0.28
Kidney (dog)	0.97	0.56	0.59
Brain (mouse)	0.29	0.21	0.10
Human tumors			
Astrocytoma (T24)	0.66	0.46	0.39
Melanoma (T355)	0.66	0.41	0.35
Oat cell carcinoma (T293)	1.05	0.71	0.39
Mouse tumors			
Sarcoma (Li-7 (m))	1.55	0.83	1.14
Sarcoma (T362 (m))	2.65	1.43	1.97

ATP phosphohydrolase and ADP phosphohydrolase are two different enzymes

It has been reported that high concentrations of azide inhibited the microsomal ADPase of rat liver [26] and pig pancreas [27]. Fig. 1 shows azide inhibition curves of ATPase and ADPase of the plasma membranes of oat cell carcinoma (T293) and sarcoma (Li-7(m)). It can be seen that the ADPase of both membranes were strongly inhibited by 10 mM azide (70–90% inhibition). There was considerable inhibition (50–60%) of the ATPase (measured by either P_i determination method) of the plasma membranes of sarcoma (Li-7(m)). Azide inhibition of the oat cell carcinoma plasma membrane ATPase was very much weaker especially when the activity was determined by the isotopic method (20% inhibition at 10 mM azide). Table II shows that while 10 mM azide inhibited the ADP hydrolysis in all plasma

TABLE II

AZIDE INHIBITION OF ADP AND ATP PHOSPHOHYDROLASE OF VARIOUS PLASMA MEMBRANES

Azide inhibition data presented here were results from the second part of the experiment described in Table I. Azide concentration was 10 mM. ATP phosphohydrolase was determined by the isotopic method. The ratios of ADP hydrolysis to ATP hydrolysis (determined by the isotopic method) were calculated from data of Table I.

Plasma membranes from	Percent inhibition		ADP hydrolysis
	ADP phosphohydrolase	ATP phosphohydrolase	ATP hydrolysis
Normal tissues			
Liver (mouse)	71.4	52.6	1.47
Kidney (dog)	76.3	42.9	1.05
Brain (mouse)	55.0	16.0	0.4
Human tumors			
Astrocytoma (T24	69.6	54.3	0.85
Melanoma (T355)	74.3	24.4	0.85
Oat cell carcinoma (T293)	61.3	25.0	0.43
Mouse tumors			
Sarcoma (Li-7(m))	79.9	50.6	1.37
Sarcoma (T362(m))	79.7	67.2	1.47

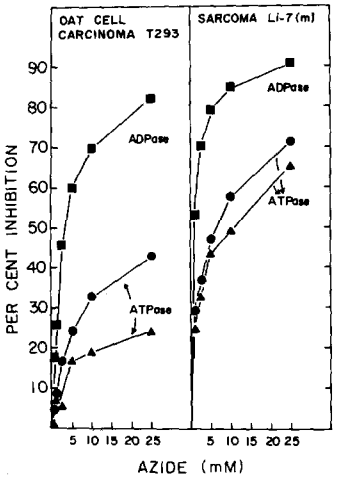

Fig. 1. Azide inhibition curves of the ATP phosphohydrolase and ADP phosphohydrolase of the plasma membranes of oat cell carcinoma (T293) and sarcoma (Li-7 (m)). Purified plasma membranes (50–200 μg) were preincubated in 30 mM histidine-Tris (pH 7.5), 5 mM $MgCl_2$ and various concentrations of NaN_3 for 10 min at room temperature before the initiation of the reaction by the addition of 5 μmol ATP. Because of the stimulating effect of Na^+ on the plasma membrane ATPase of oat cell carcinoma [20], Na^+ concentration was maintained at 25 mM by NaCl when less than 25 mM NaN_3 was used. ■, ADPase activity; ●, ATPase activity determined colorimetrically; ▲, ATPase activity determined isotopically.

membranes, up to 60–80%, the azide inhibition of the hydrolysis of the γ-phosphate of ATP varied more widely. The greater inhibition of ATP hydrolysis by azide was observed in membranes where there was more active hydrolysis of ADP. Three conclusions can be drawn from these experiments. (1) Azide is a more specific inhibitor of the ADPase

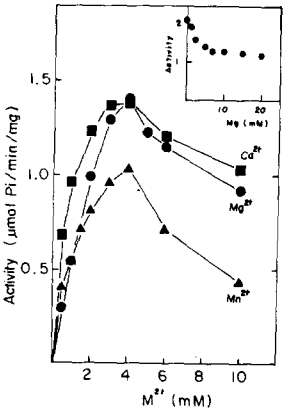

Fig. 2. Activation of ATP phosphohydrolase activity in the plasma membranes of sarcoma (Li-7 (m)) by divalent metal ions. Plasma membranes from sarcoma Li-7 (m) (60 μg) were assayed for its ATPase activity with various concentrations of $CaCl_2$ (■), $MgCl_2$ (●), or $MnCl_2$ (▲). In the inset, 5 mM $CaCl_2$ was present in all tubes in addition to $MgCl_2$. ATPase activity was determined colorimetrically.

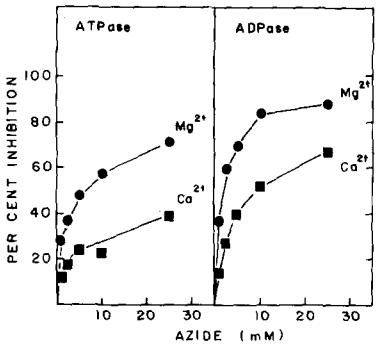

Fig. 3. Inhibition of ATPase and ADPase by azide in the presence of Mg^{2+} or Ca^{2+}. Plasma membranes from sarcoma Li-7 (m) were assayed for its ATPase and ADPase activity with either 5 mM $MgCl_2$ (●), or 5 mM $CaCl_2$ (■), with various concentration of sodium azide. Both ATPase and ADPase were determined colorimetrically.

in the concentration range employed. (2) Since ATP hydrolysis is also inhibited to a certain extent by azide, the simplest explanation is that the AD-Pase is also capable of hydrolyzing ATP, thus it is an ATP diphosphohydrolase. (3) The dominant ATP hydrolysis activity in the plasma membranes of the sarcoma is the ATP diphosphohydrolase whereas the plasma membranes of the oat cell carcinoma have a much greater ATPase activity. Experiments described in the remainder of the paper were carried out with these two membranes representing two different prototypes.

TABLE III

HYDROLYSIS OF NTP AND NDP BY THE PLASMA MEMBRANES OF SARCOMA (Li-7(m))

In these experiments, inorganic phosphate was determined colorimetrically. Specific activity of ATPase was 1.63 μmol P_i/min per mg protein with Mg^{2+} and was 1.69 μmol P_i/min per mg protein with Ca^{2+}. Rel. act., relative activity.

Substrates	Mg^{2+}			Ca^{2+}
	Rel. act.	Percent inhibition		Rel. act.
		10 mM NaN_3	20 mM NaF	
ATP	1.0	64	74	1.0
ITP	1.1	41	47	1.11
GTP	1.08	35	41	0.93
CTP	1.12	16	47	1.46
UTP	0.98	25	65	1.11
ADP	0.57	80	95	0.64
IDP	0.55	62	92	0.65
GDP	0.27	38	92	0.37
CDP	0.23	59	96	0.38
UDP	0.33	37	98	0.51

Some properties of the membrane-bound ATP diphosphohydrolase

The ATP diphosphohydrolase activity of the plasma membranes of Li-7(m) was characterized with respect to its metal ion activators, substrates and inhibitors.

Fig. 2. shows that the enzyme could be activated by either Ca^{2+}, Mg^{2+}, or Mn^{2+}, although Mn^{2+} inhibited at higher concentrations. at 5 mM Ca^{2+}, increasing Mg^{2+} concentration inhibited the ATP diphosphohydrolase (Fig. 2, inset). Similar results were obtained with ADP as the substrate. Although both Mg^{2+} and Ca^{2+} could support the activity of the enzyme, azide inhibition was much greater with Mg^{2+} than with Ca^{2+} (Fig. 3).

Table III shows that there was little specificity towards nucleoside triphosphates as substrates with either Mg^{2+} or Ca^{2+}. However, inhibition by 10 mM azide varied widely depending on the NTP used. There was greater selectivity toward nucleoside diphosphates in the presence of Mg^{2+}, the selectivity diminished somewhat with Ca^{2+}. Again, inhibition by azide varied with the NDP used. The variability in azide inhibition did not correlate with the nature of the base of the nucleotides. Fluoride, another strong but less specific inhibitor of the ATP diphosphohydrolase, inhibited hydrolysis of the five NDP's tested more than 90% at 20 mM.

Two ATP analogs, Ado*PP*[NH]*P* and Ado*PP*[CH_2]*P*, were not hydrolyzed. At a con-

TABLE IV

KINETIC PARAMETERS OF THE MEMBRANE-BOUND ATP DIPHOSPHOHYDROLASE

Kinetic constants were calculated from data in Fig. 4. K_m values expressed in mM, V_{max} values expressed in μmol P_i/min per mg protein.

Substrate	K_{m_1}	V_{max_1}	K_{m_2}	V_{max_2}
ATP	0.0865	0.584	1.61	1.39
ADP	0.044	0.464	1.10	0.878

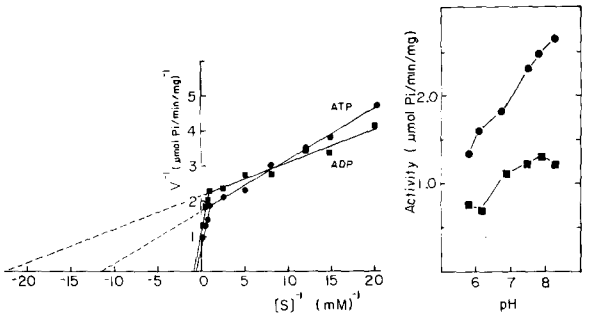

Fig. 4. Lineweaver-Burk plots of ATP and ADP hydrolysis by membrane-bound ATP diphosphohydrolase. Initial velocities were determined using [γ-^{32}P]ATP (●) and [β-^{32}P]ADP (■). The reaction was carried out for 2.5 min at 37°C. Plasma membranes (50–70 μg) from sarcoma Li-7(m) were used for these experiments.

Fig. 5. pH curves of ATPase and ADPase. For pH values below 7, 30 mM Mes-Tris buffer was used. For pH values above 7, 30 mM Tris-HCl was used. Plasma membranes (70 μg) from sarcoma Li-7(m) were used in measuring ATPase (●) and ADPase (■) activities.

centration of 0.5 mM, Ado*PP*[NH]*P* inhibited ATP hydrolysis by 30% whereas Ado*PP*[CH_2]*P* was not inhibitory.

Lineweaver-Burk plots of activities obtained at 5 mM $MgCl_2$ and varying concentrations of ATP or ADP are shown in Fig. 4. Two K_m and V_{max} values for ATP and ADP could be calculated (Table IV).

The ATP diphosphohydrolase activity remained rather constant between pH 7 and 8 with ADP as the substrate. ATP hydrolysis was greater at more alkaline pH values (Fig. 5).

The enzyme activity in the membrane was very stable. Activities were unchanged for several weeks if the membranes were frozen at −20°C. In a temperature inactivation study, 60% of the activity still remained after heating the membranes for 10 min at 75°C. However, the activity was very sensitive to detergent. There was 80% loss of activity in the presence of 0.02% Triton X-100. Other detergents, such as octyl glucoside, Lubrol and bile salts also inactivated the enzyme at concentrations necessary for solubilization.

Effectors of ATPase and ATP diphosphohydrolase

We have reported previously [20] that the ATP phosphohydrolase activity of astrocytoma and oat cell carcinoma plasma membranes were stimulated by Na^+ (and other monovalent cations) and concanavalin A. These effects were reexamined in the light of the presence of both an ATPase and ATP diphosphohydrolase in most of the plasma membranes tested. The results, summarized in Table V, show that Na^+ and concanavalin A, which significantly stimulated the ATPase activity of oat cell carcinoma plasma membranes, had no effect on the ADPase in the same membranes. Similarly, the sarcoma plasma membrane ADPase was not affected by these compounds. The effect of Na^+ and concanavalin A on the sarcoma plasma membrane

TABLE V

EFFECT OF VARIOUS LIGANDS ON THE ATPase AND ADPase

Preincubation with the effectors and reaction conditions were the same as that described in the legend of Fig. 1. Either 5 mM TrisATP or 5 mM TrisADP was used as substrate. P_i was determined colorimetrically. ConA, concanavalin A.

Additions	Activity (μmol P_i/min per mg protein)			
	Oat cell carcinoma		Sarcoma (Li-7(m))	
	ATPase	ADPase	ATPase	ADPase
I None	1.02	0.4	1.46	0.90
NaCl (100 mM)	1.57	0.42	1.52	0.95
ConA (25 μg)	1.96	0.39	1.79	0.94
pCMPS (0.1 mM)	0.69	0.38	1.52	0.89
II None	1.05	0.35	2.07	1.21
Ethanol (0.02%)	0.91	0.32	2.01	1.1
DCCD (0.2 mM in 0.02% EtOH)	0.43	0.13	0.60	0.27

ATPase was very small, further supporting the notion that the dominant ATP hydrolyzing activity in these membranes is the ATP diphosphohydrolase.

There was only partial inhibition of the oat cell carcinoma plasma membrane ATPase by pCMPS, but this sulfhydryl reagent was not inhibitory to the ATP diphosphohydrolase of either membranes. DCCD was an inhibitor of both enzymes. P^1, P^5-Di(adenosine-5')pentaphosphate (AdoP_5 Ado), a potent inhibitor of adenylate kinase, had no effect on either ATP or ADP hydrolysis.

Location of the ATP diphosphohydrolase in plasma membranes

To determine if the ATP diphosphohydrolase is possibly an ectoenzyme [28,29], we had to resort to cultured cells established from the tumor tissues. From the information obtained with the isolated plasma membranes, we operationally defined the ATP diphosphohydrolase as an enzyme which hydrolyzes ADP in the presence of Mg^{2+} or Ca^{2+} and its hydrolysis of ADP is inhibited by azide with Mg^{2+}. We found that the Ca^{2+}-ADPase activity was less than the Mg^{2+}-ADPase activity in either intact cells or isolated membranes of both cell lines. Furthermore, the Mg^{2+}-ADPase activity was not decreased in the presence of 20 mM azide (data not shown).

Discussion

In our study of the plasma membrane ATPase of normal and tumor tissues, it became apparent that the major ATPase activity was due to an oligomycin- and ouabain-insensitive, low-affinity Mg^{2+} (or Ca^{2+})-dependent nucleoside triphosphatase [18,20]. The widespread occurrence of this activity in the plasma membranes of various tissues has been amply documented [9–17]. With the exception of a study carried out by Gantzer and Grisham [30], who achieved partial purification of a Mg^{2+}-ATPase with a rather low specific activity from sheep kidney medulla, this activity has been studied only in its membrane bound form. ADP hydrolysis was also determined in some of the studies [9–12], and the activity was attributed to an ADPase, the relationship to ATPase was not discussed. An earlier study by Wattiaux-De Coninck and Wattiaux [31] with the ADPase of rat liver plasma membranes as the primary subject of investigation showed that the enzyme was more active with Ca^{2+} than with Mg^{2+} and that IDP was the preferred substrate over other nucleoside diphosphates.

We show in this paper that there are two components to the Mg^{2+}-ATPase activity of all plasma membranes examined, one being insensitive and the other being inhibited by 10 mM azide. At the

TABLE VI

ATP HYDROLYZING ENZYMES IN THE PLASMA MEMBRANES OF OAT CELL CARDINOMA

Concentrations of the various effectors are: ouabain, 1 mM; pCMPS, 50 μM; concanavalin A (ConA), 25 μg/ml; azide, 10 mM; fluoride, 20 mM. ($M^+ + Mg^{2+}$)-ATPase is an ATPase activity found in the presence of Mg^{2+} and a monovalent cation (see Ref. 20).

Enzymes	Percentage of total activity		Properties				
	Assayed without 10 mM Na^+ 10 mM K^+	Assayed with 100 mM Na^+ 10 mM K^+	Inhibition by ouabain	Inhibition by pCMPS	Stimulation by ConA	Inhibition by azide	Inhibition by fluoride
($Na^+ + K^+$)-ATPase		20	+	+	−	−	+
Mg^{2+}-ATPase	60	30	−	−	+	−	−
($M^+ + Mg^{2+}$)-ATPase		30	−	+	−	−	−
ATP diphosphohydrolase	40	20	−	−	−	+	+
($Ca^{2+} + Mg^{2+}$)-ATPase		?	−	+	−	−	?

same time, the ADPase activity of the plasma membranes can be completely inhibited by azide. Based on the similarity of the azide inhibition curves of ATP and ADP hydrolysis and other characteristics, we concluded that the azide sensitive enzyme could hydrolyze both ADP and ATP and thus was an ATP diphosphohydrolase. In several of the plasma membranes we have examined, this enzyme was the main ATP hydrolyzing component.

The occurrence of an ATP diphosphohydrolase in the plasma membranes of several smooth muscle tissues was recently reported by Kwan and Ramlal [32]. We concur with these authors that this activity, which contributed greatly to ATP hydrolysis in some membranes, must be taken into account in interpreting data on membrane ATP hydrolysis. As an example, we summarize in Table VI the enzymatic composition for ATP hydrolysis in the plasma membranes of oat cell carcinoma. The relative contribution of the various enzyme activities was calculated from data presented in this paper (Table II and Table V) and in a previous study [20]. We emphasize that precise results can only be obtained after careful kinetic analyses of the individual enzymes. We have not demonstrated the $(M^+ + Mg^{2+})$-ATPase to be a separate enzyme. It is so listed in Table VI because it can be distinguished from the basal Mg^{2+} (or Ca^{2+})-ATPase. The M^+, Mg^{2+}-stimulated activity was inhibited by 50 μM pCMPS whereas the Mg^{2+} (or Ca^{2+})-ATPase was not [20]. We have not presented any data on the high-affinity $(Mg^{2+} + Ca^{2+})$-ATPase since we were not able to demonstrate this activity using the assay conditions of Loterstajn et al. [8]. It is apparent that contributions of the various enzymes to the total ATP hydrolysis activity will vary with different membranes.

An ATP diphosphohydrolase of molecular weight of 65 000 has recently been solubilized and purified from pig pancreas zymogen granule membranes by Triton X-100, and its kinetic behavior characterized [27,33]. The pancreas membrane enzyme hydrolyzes ADP as well as ATP and could be inhibited 54% by 20 mM azide. The ADPase activity in the sarcoma plasma membrane differed from the pancreas ATP diphosphohydrolase in several aspects: pH optima, thermal stability, relative velocity of the hydrolysis of the different substrates, and K_m values for ATP and ADP. The most marked difference was the sensitivity of the sarcoma plasma membrane ATP diphosphohydrolase to detergent. Under condition where pancreas enzyme was solubilized by Triton X-100, the sarcoma membrane ATP diphosphohydrolase was almost completely inactivated. This extraordinary sensitivity could not be attributed to the possible differences between the lipid composition of normal and neoplastic cells since the ATP diphosphohydrolase in plasma membranes from normal tissues (liver and kidney) was also inactivated at low concentrations of detergent (data not shown). The sensitivity of the plasma membrane ATP diphosphohydrolase to detergent would make the purification of the enzyme a difficult task. However, such a goal must be achieved to determine if the ATP diphosphohydrolases from the plasma membranes and the zymogen granule membranes have a common origin. Ultimately, a purified enzyme is necessary to address the question of the role of the plasma membrane ATP diphosphohydrolase.

There is some evidence that the ATP diphosphohydrolase of the smooth muscle cells is an ecto enzyme [34]. It has been speculated that this enzyme acts in combination with the ecto ATPase in producing adenosine, a vasodilator [29]. At the present, the effect of adenosine on other types of cells is not known, but an extracellular orientation of the ATP diphosphohydrolase would certainly implicate the enzyme in extracellular adenine nucleotide metabolism. This question could only be approached by using intact cultured cells. However, the results with the cultured Li-7(m) cells showed very little of this activity anywhere in the cells. This is most surprising since the major ATP hydrolyzing activity in the membranes isolated from tumor tissues seemed to have disappeared upon the passage of the cells in culture. Furthermore, the activity reappeared in the tumors grown up from these cells after they were injected in the nude mice. These results would imply that the expression of this particular enzyme is under rigid environmental regulation. Further study is necessary to determine if the activity truly diminishes under the conditions of cell culture or if it is radically altered in enzymatic properties.

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References

1 Kyte, J. (1975) J. Biol. Chem. 250, 7443–7449
2 Ursugi, S., Dulak, N.C., Dixon, J.F., Hexum, T.D., Dahl, J.L., Perdue, J.R. and Hokin, L.E. (1971) J. Biol. Chem. 246, 531–543
3 Dixon, J.F. and Hokin, L.E. (1974) Arch. Biophys. Biochem. 163, 749–758
4 Schatzmann, H.J. and Vincenzi, F.F. (1969) J. Physiol. (London) 201, 369–395
5 Niggli, V., Penniston, J.T. and Carafoli, E. (1979) J. Biol. Chem. 254, 9955–9958
6 Rabon, E., Saccomani, G., Kasbekar, D.K. and Sachs, G. (1978) Biochim. Biophys. Acta 551, 432–447
7 Pershadsingh, H.A. and McDonald, J.M. (1980) J. Biol. Chem. 255, 4087–4093
8 Loterstajn, S., Hanoune, J. and Pecker, F. (1981) J. Biol. Chem. 256, 11209–11215
9 Wallach, D.F.H. and Ullrey, D. (1962) Biochim. Biophys. Acta 64, 526–539
10 Emmelot, P.E. and Bos, C.J. (1969) Int. J. Cancer 4, 723–734
11 Emmelot, P., Bos, C.J., Benedetti, E.L. and Rümke, P. (1964) Biochim. Biophys. Acta 90, 126–145
12 Perdue, J.G. and Sneider, J. (1970) Biochim. Biophys. Acta 196, 125–140
13 Parkinson, D.K. and Radde, I.C. (1971) Biochim. Biophys. Acta 242, 238–246
14 Lambert, M. and Christophe, J. (1978) Eur. J. Biochem. 91, 485–492
15 Gennaro, R., Mottola, C., Schneider, C. and Romeo, D. (1979) Biochim. Biophys. Acta 567, 238–246
16 Novogrodsky, A. (1972) Biochim. Biophys. Acta 266, 343–349
17 Shami, Y. and Radde, I.C. (1971) Biochim. Biophys. Acta 249, 345–352
18 Knowles, A.F., Leis, J.F. and Kaplan, N.O. (1981) Cancer Res. 41, 4031–4038
19 Knowles, A.F. and Kaplan, N.O. (1980) Biochim. Biophys. Acta 590, 171–180
20 Knowles, A.F. and Kaplan, N.O. (1981) Biophys. Biochem. Res. Commun. 99, 1443–1448
21 Beattie, G.M., Knowles, A.F., Jensen, J.C., Baird, S.M. and Kaplan, N.O. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 3033–3036
22 Jørgensen, P.L. (1974) Biochim. Biophys. Acta 356, 36–52
23 Lohmann, K. and Jendrassik, L. (1926) Biochem. Z. 178, 419–426
24 Pullman, M.E. (1967) in Methods in Enzymology (Estabrook, R.W. and Pullman, M.E., eds), Vol. 10, pp. 57–60, Academic Press, New York
25 Magnusson, R.T., Portis, A.R., jr. and McCarty, R.E. (1976) Anal. Biochem. 72, 653–657
26 Ernster, L. and Jones, L.C. (1962) J. Cell Biol. 15, 563–578
27 LeBel, D., Poirier, G.G., Phaneuf, S., St.-Jean, P., Laliberté, J.F. and Beaudoin, A.R. (1980) J. Biol. Chem. 255, 1227–1233
28 Trams, E.G. and Lauter, C.J. (1974) Biochim. Biophys. Acta 345, 180–197
29 Stanley, K.K., Newby, A.C. and Luzion, J.P. (1982) Trends Biochem. Sci. 7, 145–147
30 Gantzer, M.L. and Grisham, C.M. (1979) Arch. Biochem. Biophys. 198, 263–267
31 Wattiaux-De Coninck, S. and Wattiaux, R. (1969) Biochim. Biophys. Acta 183, 118–128
32 Kwan, C.Y. and Ramlal, T. (1982) Biochem. Int. 4, 439–449
33 Laliberté, J.F., St.-Jean, P. and Beaudoin, A.R. (1982) J. Biol. Chem. 257, 3869–3874
34 Pearson, J.D., Carleton, J.S. and Gordon, J.L. (1980) Biochem. J. 190, 421–429