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Purification and Characterization of $(Ca^{2+}+Mg^{2+})$-ATPase of Sarcoplasmic Reticulum from Rat Skeletal Muscle

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쥐 근소포체의 $(Ca^{2+}+Mg^{2+})$-ATPase의 분리정제와 그 효소특성에 관하여

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요 약

$(Ca^{2+}+Mg^{2+})$-ATPase를 쥐의 근소포체로부터 sucrose density gradient centrifugation의 방법을 사용하여 분리 정제하였다. 정제된 효소를 폴리아크릴 아마이드 젤에서 전기영동한 결과, 토끼와 닭의 경우에서와 같이 분자량 115,000인 단일 단백질 띠로 나타났다.

정제된 이 효소의 활성도는 50 μM의 Mg^{2+}, Ca^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}에 의해서는 증가되었고, 같은 농도의 Zn^{2+}, Cu^{2+}, Hg^{2+}에 의해서는 감소되었다. Quinine와 quinacrine 같은 antimalarial drug는 이 효소의 활성도에 큰 영향을 주지 않았으나, *p*-hydroxymercuric benzoate와 phenylmethylsulfonylfluoride는 이 효소의 활성을 억제하였다. 이 효소는 pH 6과 7 사이에서 가장 높은 활성을 나타내었고, ATP를 기질로 사용하였을 때 Km값은 98 μM이었다.

$(Ca^{2+}+Mg^{2+})$-ATPase는 microsomal fraction에서 선택적으로 분해되었다. ^3H-casein이나 ^{125}I-insulin같은 방사성 동위원소로 표지된 기질을 사용하여 단백질 분해에 대한 활성도를 조사해 본 결과, microsomal preparation에 metalloendoprotease가 존재하였다. 그러나 아직까지는 그 효소가 $(Ca^{2+}+Mg^{2+})$-ATPase를 분해하는지는 확실하지 않다.

INTRODUCTION

Purification of $(Ca^{2+}+Mg^{2+})$-ATPase has been reported by numerous investigators. Martonosi (1968) first succeeded in purifying the enzyme using deoxycholate (DOC) and

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salt, although a large amount of the enzyme activity was lost. MacLennan (1970) modified the procedure by first removing loosely bound and water-soluble proteins (extrinsic membrane proteins) from the membrane-bound ATPase with low levels of DOC in the presence of 1 M KCl. The intrinsic membrane protein fraction was then dissolved in a higher concentration of DOC and the ATPase was fractionated by precipitation with ammonium acetate. Another method for purifiying the ATPase was developed by Warren *et al.* (1974). This involves treating sarcoplasmic reticulum (SR) vesicles with DOC (0.4 mg/mg protein) and sucrose density gradient centrifugation. Barrabin *et al.* (1984) have also reported purification method for the ATPase based on solubilization of SR with dodecyl octaethylene glycol monoether and seperation of the enzyme by anion exchange chromatography.

Most of these investigators used rabbit or chick skeletal muscle as the source of (Ca^{2+}+Mg^{2+})-ATPase, and with the purified enzyme, a number of biochemical properties were evaluted, including optimal pH, composition of amino acids and effect of various divalent cations. Although the isolation of the ATPase from rat skeletal muscle has also been attempted, little or none is known about the physicochemical properties of the rat enzyme. Therefore, the present studies were undertaken to purify (Ca^{2+}+Mg^{2+})-ATPase from rat skeletal muscle using a modified procedure and to characterize the biochemical properties of the enzyme for the comparison with those of the (Ca^{2+}+Mg^{2+})-ATPase isolated from rabbit muscle.

Estimates of the percentage of membrane protein that make up the ATPase protein vary from 35 to 90%. The 90% estimate obtained by Yu and Masoro (1970) seems too high. The lowest estimate, 35 to 45%, made by MacLennan *et al.* (1974), may have been made using an initial SR preparation that contained impurities. The estimate made by Meissner *et al.* (1973) of 60 to 70% was based on carefully prepared SR and on phosphoenzyme production for the determination of the ATPase activity. Thus, a figure of 60 to 70% of the total SR protein as composing the ATPase protein appears the best estimate at present.

If the purification of the ATPase is attempted from the SR vesicles containing 60 to 70% of the ATPase, the purification factor (i.e., the total amount of protein SR to that of the purified ATPase) should be less than two-fold. And yet the same investigators have obtained the purified ATPase with purification factor of 3 to 5-fold. Thus, it is likely that the ATPase is being degraded by some unknown protease during the isolation procedure, and consequently the removal of the digested product should have greatly increased the purification factor. Therefore, we have examined whether the SR fraction contains a protease that is responsible for the degradation of the (Ca^{2+}+Mg^{2})-ATPase.

MATERIALS AND METHODS

Materials

$Na^{125}I$ and ^{3}H-formaldehyde were purchased from New England Nuclear, and sodium deoxycholate from Merck. All other chemicals used were of analytical grade, and were obtained from Sigma. Radioiodination of insulin was performed by using chloramine T as described by Greenwood *et al.* (1963). ^{3}H-casein was prepared by methylating crystalline bovine α-casein with ^{3}H-formaldehyde (Rice and Means, 1971).

Assays

Proteins were assayed by the method of Lowry *et al.* (1951) or by the dye binding assay of Bradford (1976) using crystalline bovine serum albumin as a standard.

ATPase assay was carried out as follows: 200 μl of reaction mixtures containing 50 mM Tris-HCl (pH 7.4), 100 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, and 2 mM ATP were added with 10 to 20 μg of the enzyme preparations. Incubations were performed for 1 to 2 hr at 37°C, and the reaction was then stopped by addition of 1% sodium dodecyl sulfate (SDS). The amount of inorganic phosphate released from ATP was determined colorimetrically as described by Ames (1966).

The pH optimum of ATPase was determined by using acetate as a buffer for pH 4.0 and 5.0, 2-(N-morpholino)-ethanesulfonic acid (Mes) for 6.0 and 6.5, Tris for 7.0 to 8.0, and glycine for 9.0 and 10.0. These buffers were used at 50 mM.

The proteolytic activity was estimated by following the degradation of ^{3}H-casein and ^{125}I-insulin to products soluble in 10% (w/v) trichloroacetic acid (TCA). All the assays were carried out in 25 mM Tris-HCl (pH 8.0) and 2.5 mM $MgCl_2$. When assayed with casein as a substrate, the incubation mixture contained proper amounts of the enzyme samples and 10 μg of ^{3}H-casein in a final volume of 100 μl. Assays using insulin as a substrate contained 3 μg of insulin, a trace amount of ^{125}I-insulin (about 15,000 cpm) and the enzyme samples in a final volume of 100 μl. Incubations were performed for 90 min at 37°C. After the incubation, 50 μl of bovine serum albumin (1.2 mg/ml) was added as a carrier and 50 μl of 40% (w/v) TCA to precipitate the proteins. The assay tubes were kept on ice for 30 min, and after centrifuging them, 100μl of acid-soluble products were counted (Goldberg *et al.*, 1981).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis was performed essentially according to the procedure of Laemmli (1970) by using 10% polyacrylamide slab gel. After the electrophoresis, gels were stained with 0.2% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 10% (v/v) acetic acid and destained in 35% (v/v) methanol and 10% (v/v) acetic acid.

Amino Acid Analysis

Amino acid composition of the purified (Ca^{2+}+Mg^{2+})-ATPase was determined by using

the LKB 4150 alpha amino acid analyzer. The ATPase (500 μg) was hydrolyzed in vacuum sealed tubes with 1 ml of constant-boiling HCl at 110°C for periods of 48 and 72 hr. Serine and threonine were estimated by extrapolation to zero time (Spackman *et al.*, 1958).

RESULTS

Purification of ATPase

Microsomal fraction from rat skeletal muscle was prepared as described by MacLennan (1970) with a modification of extraction buffer which was 0.1 M Tris-HCl (pH 8.0) instead of 5 mM imidazole (pH 7.4). Rats were killed by stunning and decapitation. Skeletal muscle from the hind legs was dissected out and placed immediately in cold deionized water. The muscle was homogenized for 2 min in a Waring blender with 4 volumes of the extraction buffer containing 0.12 M NaCl and 10^{-4} M phenylmethylsulfonylfluoride (PMSF). The suspension was centrifuged at 1,600×g for 30 min to remove debris. The supernatant was filtered through 4 layers of cheesecloth previously washed with deionized water. The filtrate was centrifuged at 10,000×g for 30 min to remove mitochondria, and the supernatant was filtered again. This filtrate was centrifuged at 78,000×g for 50 min. The supernatant was discarded and the pellet, microsomal fraction, was suspended at a protein concentration of 13 mg per ml in 0.1 M Tris-HCl buffer (pH 7.4) containing 96 mM (3.3%, w/v) sucrose and 10^{-4} M PMSF.

$(Ca^{2+}+Mg^{2+})$-ATPase was purified from this microsomal fraction by sucrose density gradient centrifugation. Microsomes were treated with 10%(w/v) sodium deoxycholate

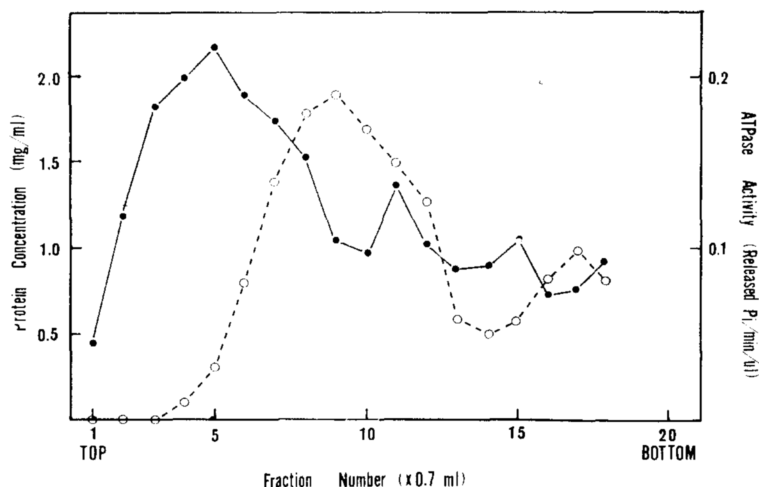

Fig. 1. Sucrose density gradient centrifugation of microsomal fraction solubilized by deoxycholate. Hydrolysis of ATP (○—○) and protein concentration (•—•) were measured as described in *MATERIALS AND METHODS.*

(DOC) at a ratio of 0.5 mg DOC per mg protein to solubilize membrane proteins. After incubating them for 30 min at 4°C, the resulting mixture was centrifuged at 180,000×g for 25 min to remove unsolubilized membrane proteins. Clear supernatant of 2 ml was overlayed on a 12 ml of 6 to 21% (w/v) sucrose gradient containing 0.1 M Tris-HCl (pH 7.4) and 10^{-4} M PMSF. After this system was centrifuged at 130,000×g for 24 hrs, fractions of 0.7 ml were collected from the top of the tube by using Beckman Universal Fraction Recovery System. As shown in Fig. 1, there were two distinct peaks of ATPase activity; the major one appeared about at the middle of the gradient and the other minor

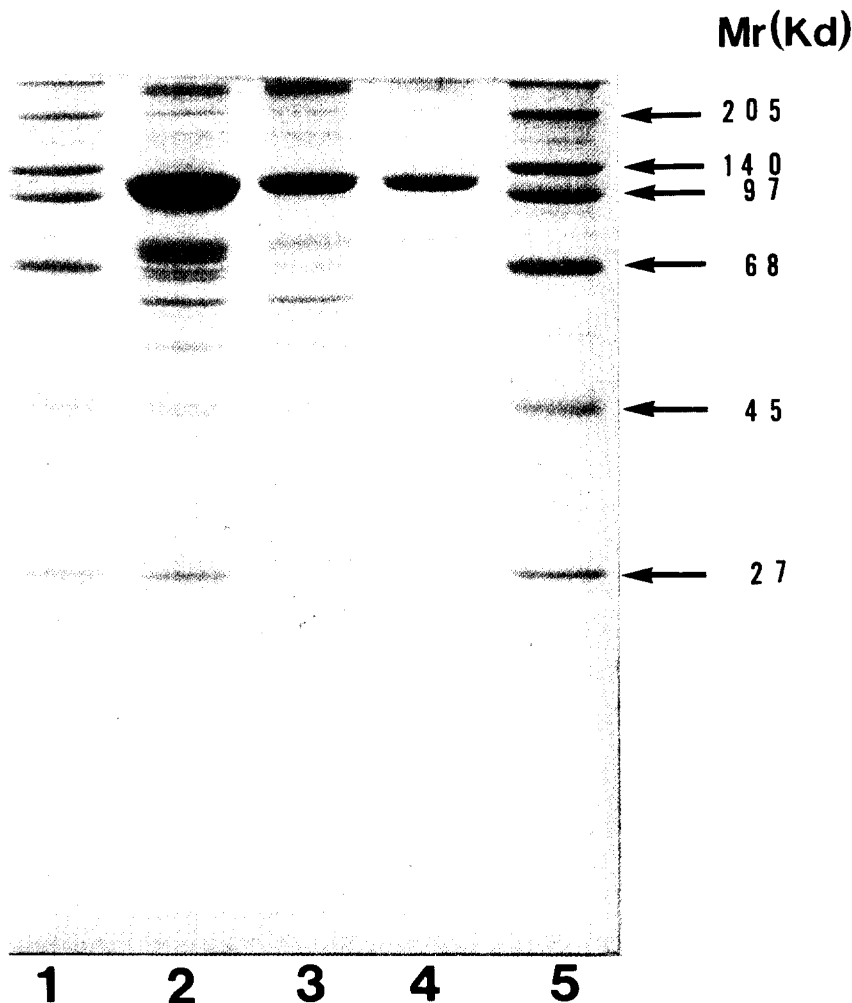

Fig. 2. SDS-PAGE of $(Ca^{2+}+Mg^{2+})$-ATPase preparations obtained from each successive purification steps. Lane 1 and 5, molecular weight marker; 2, microsomal fraction; 3, DOC-solubilized microsomal fraction obtained from the centrifugation at 160,000×g for 25 min; 4, purified ATPase which is the pooled fraction of number 8 to 10 from sucrose density gradient centrifugation.

peak nearly at the bottom of the tube. It is presently unclear whether the ATPase having higher molecular weight is aggregates or a naturally existing oligomeric form of the enzyme.

To examine the purity of (Ca^{2+} + Mg^{2+})-ATPase, aliquots of individual fraction obtained from the sucrose density gradient centrifugation were analyzed by polyacrylamide gel electrophoresis in the presence of 0.1% (w/v) SDS (data not shown). The least contaminated fractions of number 8 to 10 were pooled, and were again electrophoresed as above. As shown in Fig. 2, the purified ATPase was nearly homogeneous. The purified ATPase was added with glycerol to give a final concentration of 35% and kept frozen at −60°C. All subsequent experiments used this purified material unless otherwise mentioned.

Properties of Purified ATPase

Effects of divalent cations: The activity of (Ca^{2+}+Mg^{2+})-ATPase was measured in the presence and absence of various divalent cations (Table 1). Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+} and Fe^{2+} at 50 μM stimulated the ATPase activity by about 10 to 20%. On the other hand, Cu^{2+} and Hg^{2+} at the same concentration strongly inhibited the ATPase activity but Zn^{2+} by about 10%.

Effects of antimalarial drugs: Mego and Chung (1979) have demonstrated that antimalarial drugs, such as quinine, showed marked inhibitory effect on the activity of lysosomal membrane ATPase isolated from rat liver. In addition, it has been reported that lysosomotropic amines including antimalarial drugs inhibit myoblast fusion in chick embryonic muscle cell culture (Kent, 1982). Futhermore, it has also been reported that Ca^{2+} may play important roles in the muscle cell differentiation (Ha *et al.*, 1979). Therefore, the effects of antimalarial drugs on the purified (Ca^{2+}+Mg^{2+})-ATPase was examined if the

Table 1. Effects of divalent cations on the activity of the purified (Ca^{2+}+Mg^{2+})-ATPase.

Additions	Concentration (μM)	Relative activity (%)	Additions	Concentration (μM)	Relative activity (%)
None	—	100	$CuSO_4$	50	33
$CaCl_2$	50	109		200	33
	200	104	$FeCl_2$	50	122
$CoCl_2$	50	110		200	118
	200	105	$MnCl_2$	50	112
$MgCl_2$	50	123		200	112
	200	108	$HgCl_2$	50	29
$ZnCl_2$	50	90		200	31
	200	70			

Reaction mixtures contained 50 mM Tris-HCl (pH 7.4), 100 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, 2 mM ATP, 12 μg of the purified enzyme and various divalent metal ions. Incubations were performed for 90 min at 37°C. The ATP-hydrolyzing activity in the absence of added divalent cations was expressed as 100%.

Table 2. Effects of antimalarial drugs and various site specific inhibitors on $(Ca^{2+}+Mg^{2+})$-ATPase activity.

Additions	Concentration (mM)	Relative activity (%)	Additions	Concentration (mM)	Relative activity (%)
None	—	100	Dithiothreitol	0.1	101
Quinine	0.1	94		1.0	112
	1.0	113	2-Mercaptoethanol	0.1	104
Quinacrine	0.1	95		1.0	111
	1.0	89	*p*-Hydroxymercuric	0.1	43
Phenylmethyl-	0.1	96	benzoate	1.0	19
sulfonylfluoride	1.0	65			
Iodoacetamide	0.1	107			
	1.0	105			

The reaction mixtures were prepared as described in Table 1, but various inhibitors were added. The ATPase activity in the absence of added inhibitors was expressed as 100% activity.

drugs also could affect the enzyme activity or if the inhibitory effect on myogenesis by antimalarials would be mediated by their reaction with the enzyme. As shown in Table 2, neither quinine nor quinacrine exhibited significant effect on the ATPase activity. Thus, the Ca^{2+}-pumping ATPase in SR membrane is distinct from the Ca^{2+}-activated ATPase of lysosomal membrane at least by their sensitivity to antimalarials. In addition, the inhibitory effects by these drugs on myogenesis appears to be independent on intracelluarly occuring Ca^{2+}-transport process catalyzed by $(Ca^{2+}+Mg^{2+})$-ATPase.

Effects of the site specific inhibitors: Table 2 shows the influences on the ATPase activity by various site specific inhibitors. *p*-Hydroxymercuric benzoate (pHMB) at 0.1 mM inhibited about 60% of the ATP hydrolyzing activity, suggesting that sulfhydryl residues play a role in the catalytic function of the enzyme. However, the other sulfhydryl blocking agent, such as iodoacetamide, and reducing agents, including dithiothreitol and 2-mercaptoethanol showed little or no effect on the activity. Thus, it is likely that the inhibitory effect of pHMB is due to its nonspecific interaction with the protein. PMSF that is known to react on many serine enzymes, showed some inhibitory effect (by about 25% at 1 mM). It is presently unclear whether the inhibition is due to specific interaction of PMSF with serine residues in the active site of the enzyme.

pH dependence: The ATPase activity at different pH was examined using the buffers listed in *MATERIALS AND METHODS* (Fig. 3). The enzyme was maximally active over a broad pH of 6.0 to 7.0. It showed no activity at pH above 10.0, but relatively active at lower pH's.

Kinetic analysis: To estimate Km value for ATP, the rates of ATP hydrolysis were measured by addition of various concentrations of ATP, and were then plotted according to the method of Lineweaver-Burk (1934). The Km value was calculated to be 98 μM.

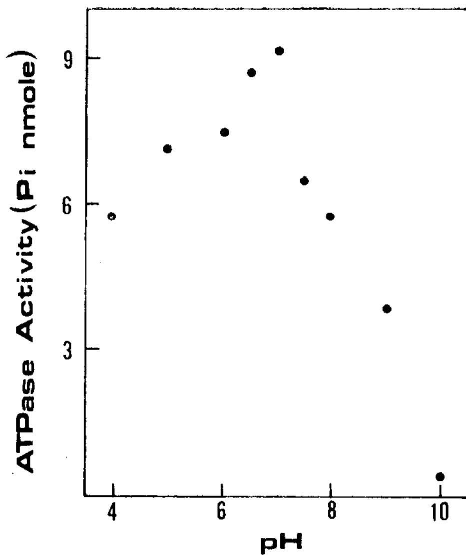

Fig. 3. pH dependence of $(Ca^{2+} + Mg^{2+})$-ATPase activity. Reaction mixtures were prepared as described in Table 1, and were incubated for 90 min at 37°C. Buffers used were acetate for pH 4.0 and 5.0, Mes for 6.0 and 6.5, Tris for 7.0 to 8.0 and glycine for 9.0 and 10.0.

Amino acid analysis: Amino acid composition of the purified $(Ca^{2+}+Mg^{2+})$-ATPase was analyzed as described in *MATERIALS AND METHODS* (Table 3).

From the data of amino acid analysis, the minimal molecular weight of the ATPase was calculated to be 115,000. This value is well in accord with that obtained from SDS-PAGE (Fig. 2).

Degradation of ATPase: Martonosi *et al.* (1980) have reported that microsomes obtained from rabbit skeletal muscle contain $(Ca^{2+}+Mg^{2+})$-ATPase by about 70~90% of total microsomal proteins, and similar data were obtained in this study using microsomes prepared from rat skeletal muscle (Fig. 2). However, it was noticed that as the purification

Table 3. The amino acid composition of the purified $(Ca^{2+}+Mg^{2+})$-ATPase.

Amino acid	Residues/ Monomer	Mol %	Amino acid	Residues/ Monomer	Mol %
Lys	65	6	Ala	98	9
His	28	3	Val	96	9
Arg	42	4	Met	7	1
Ser	103	10	Half-Cys	ND*	—
Asp	107	10	Ile	63	6
Thr	48	4.5	Leu	112	11
Trp	ND*	—	Tyr	5	0.5
Glu	136	13			
Pro	62	6	Total	1055	100
Gly	83	8			

* ND; Not determined.

procedures (i.e., DOC-treatment and sucrose density gradient centrifugation) proceeded to obtain the purified ATPase from microsomes, not only the amount of the ATPase decreased but also the amount of proteins having molecular weight less than 100,000 accumulated when analyzed by SDS-PAGE (data not shown). These results strongly suggest that the ATPase is being degraded during the purification period by some unknown protease(s) in microsomal preparation.

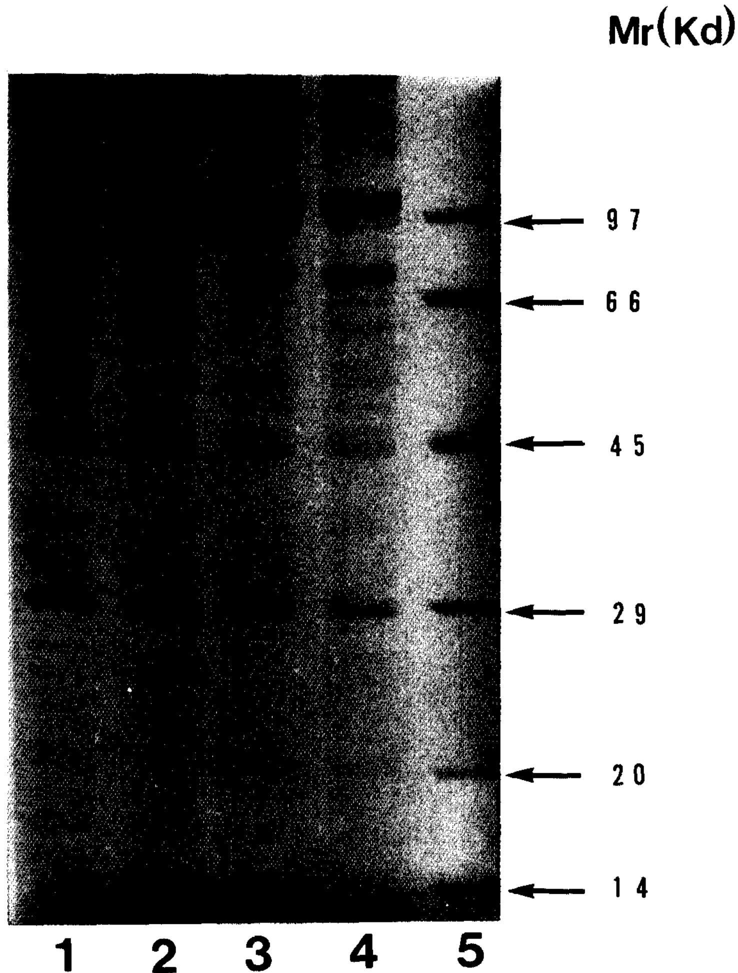

Fig. 4. Degradation of the ATPase in microsomes. Microsomes prepared from rat skeletal muscle as described in the text were incubated at 4°C for 1 week. After the incubation, same amount of proteins (50 μg) were treated with 2% (w/v) SDS and electrophoresed by the method of Laemmli (1970). Controls (Lane 1 and 2) were kept frozen at −60°C.
Lane 1, microsome; 2, DOC-treated microsome; 3, microsome after the incubation for 1 week; 4, DOC-treated microsome after the incubation for 1 week; 5, molecular weight marker.

To test this possibility, microsomes and DOC-treated microsomes were incubated at 4°C for 1 week, and were then analyzed by SDS-PAGE to compare the protein patterns of the microsomes with those of controls which were kept at −60°C without incubations. The incubation period and temperature were chosen as above, because the purification of the enzyme was performed at 4°C and took about a week. As shown in Fig. 4, the amount of ATPase (115,000 dalton band) was decreased by the incubation whether DOC was treated or not. By contrast, the amount of other proteins such as myosin and the proteins having molecular weight of 29,000 and 97,000 remained fairly constant. Thus, there appears to exist a certain protease(s) that specifically degrades $(Ca^{2+}+Mg^{2+})$-ATPase.

To confirm the presence of proteolytic activity, aliquots of microsomal preparation were incubated with radioactively labeled protein substrates, such as ^{3}H-casein and ^{125}I-insulin. As shown in Table 4, both proteins were rapidly hydrolyzed by the microsomes. PMSF, that is known to inhibit serine enzymes, showed little or no inhibitory effect on the proteolytic activity. Since the addition of *o*-phenanthroline inhibited the proteolytic activities, the microsomal protease(s) appears to be a metalloprotease(s). However, it is presently unclear whether the metalloprotease is responsible for the degradation of the $(Ca^{2+}+Mg^{2+})$-ATPase.

Table 4. Proteolytic activity of microsomal fractions.

Additions	% Casein hydrolysis	Relative activity (% of control)	% Insulin hydrolysis	Relative activity (% of control)
None	11.6	100	43.0	100
PMSF	10.7	92.2	39.0	90.7
o-Phenanthroline	0.0	0.0	0.3	0.7

Reaction mixtures contained 25 mM Tris-HCl (pH 8.0), 2.5 mM $MgCl_2$, 400 μg of microsomal fraction, 10 μg of ^{3}H-casein or 3 μg of insulin with trace amount of ^{125}I-insulin as substrate, and 1.0 mM of PMSF or *o*-phenanthroline. Incubations were performed for 90 min at 37°C. The activity in the absence of inhibitors was expressed as control.

DISCUSSION

$(Ca^{2+}+Mg^{2+})$-ATPase was purified homogeneously from rat skeletal muscle. Microsomes were prepared as described by MacLennan (1970) and the sucrose density gradient centrifugation of DOC-treated microsomes by the method of Warren *et al.* (1974). By the combination of these procedures, highly purified ATPase could easily be obtained within a short period of time.

While the ATPases purified from rabbit and rat skeletal muscle share a number of common physicochemical properties such as that both enzymes have the same subunit size of 115,000 daltons, a variety of differences in biochemical properties were observed which are as follows: (1) By contrast to the rabbit enzyme that is reported to be inhibited by

Co^{2+} (MacLennan, 1970), the ATPase from rat was stimulated by the same metal ion (Fig. 1). (2) While the rat enzyme showed significant ATP-hydrolyzing activity at lower pH's (Fig. 3), the enzyme from rabbit is known to have negligible activity at pH below 6.0 (Hidalgo *et al.*, 1983). (3) Km values for ATP were 98 μM and 2.2 mM for the rat and rabbit enzymes, respectively (Ha, 1977), suggesting that the former enzyme has a much higher affinity to ATP than the latter.

A number of reports were published about that the time course of increase in the concentration of $(Ca^{2+}+Mg^{2+})$-ATPase is coincided with that of the fusion of myoblasts into multinucleated myotubes *in vivo* and in tissuse culture (Boland *et al.*, 1974; Ha *et al.*, 1979). Furthermore, conditions inhibiting the fusion, such as the depletion of Ca^{2+} from media, also inhibit the synthesis of $(Ca^{2+}+Mg^{2+})$-ATPase (Ha *et al.*, 1979). By some unknown reason, antimalarial drugs (e.g., chloroquine) have also been reported to inhibit fusion in chick embryonic myoblast culture (Kent, 1982). Since the present study shows that quinine and quinacrine have no effect on the ATP hydrolysis at the concentrations that inhibit the fusion, it is certain that the inhibitory effect by the drugs on the fusion is neither related to intracellular level of $(Ca^{2+}+Mg^{2+})$-ATPase.

It is of particular interest that $(Ca^{2+}+Mg^{2+})$-ATPase is selectively degraded in the isolated microsomal preparation (Fig. 4). In addition, by using ^{3}H-casein or ^{125}I-insulin as substrate, proteolytic activity was demonstrated to exist in the mirosomal fractions (Table 4). These data suggest that the *o*-phenanthroline sensitive metalloendoprotease is likely to be a candidate that is responsible for the degradation of the ATPase. Since all assays measuring the proteolytic activity were carried out at pH above 7.0, degradation of ATPase is not due to any contaminants of the lysosomal protease. Recently, the protease from bovine heart muscle have been purified (Waxman, 1981). It hydrolyzed troponin and tropomyosin, but has not been determined whether or not the enzyme also degraded $(Ca^{2+}+Mg^{2+})$-ATPase. In any case, studies on the isolation of the protease is necessary to ascertain that the purified enzyme degrades selectively the ATPase. If such a selective degradation of the ATPase occurs *in vivo*, the protease should play an important physiological role in the regulation of intracellular Ca^{2+} level by controlling the amount of $(Ca^{2+}+Mg^{2+})$-ATPase.

SUMMARY

The $(Ca^{2+}+Mg^{2+})$-ATPase has been purified homogeneously from sarcoplasmic reticulum of rat skeletal muscle by sucrose density gradient centrifugation. The purified enzyme has a molecular weight of 115,000 as judged by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, and therefore has the same size of the enzyme in rabbit and chick skeletal muscle.

Ca^{2+}, Mg^{2+}, Fe^{2+}, Co^{2+}, and Mn^{2+} at 50 μM show stimulatory effect on the ATPase,

while Zn^{2+}, Cu^{2+}, and Hg^{2+} inhibit it at the same concentration. The ATPase activity is insensitive to antimalarial drugs such as quinine and quinacrine, but is sensitive to inhibition by *p*-hydroxymecuric benzoate and phenylmethylsulfonylfluoride. The enzyme has optimum pH of 6 to 7 and Km value for ATP is estimated to be 98 μM. Thus, a number of biochemical properties of this enzyme appear to be different from those of the enzyme that have been isolated from rabbit skeletal muscle.

The $(Ca^{2+}+Mg^{2+})$-ATPase appears to be selectively degraded in microsomal fraction. The activity of metalloendoprotease is evident in the microsomal preparation when assayed by radioactively labeled protein substrate, such as ^{3}H-casein and ^{125}I-insulin. However, it is presently unclear whether the metalloendoprotease is responsible for the degradation of the $(Ca^{2+}+Mg^{2+})$-ATPase.

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