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A HIGH AFFINITY, CALMODULIN-RESPONSIVE (Ca^{2+} + Mg^{2+})-ATPase IN ISOLATED BONE CELLS

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Although acute alterations in Ca^{2+} fluxes may mediate the skeletal responses to certain humoral agents, the processes subserving those fluxes are not well understood. We have sought evidence for Ca^{2+}-dependent ATPase activity in isolated osteoblast-like cells maintained in primary culture. Two Ca^{2+}-dependent ATPase components were found in a plasma membrane fraction: a high affinity component (half-saturation constant for Ca^{2+} of 280 nM, V_{max} of 13.5 nmol/mg per min) and a low affinity component, which was in reality a divalent cation ATPase, since Mg^{2+} could replace Ca^{2+} without loss of activity. The high affinity component exhibited a pH optimum of 7.2 and required Mg^{2+} for full activity. It was unaffected by potassium or sodium chloride, ouabain or sodium azide, but was inhibited by lanthanum and by the calmodulin antagonist trifluoperazine. This component was prevalent in a subcellular fraction which was also enriched in 5′-nucleotidase and adenylate cyclase activities, suggesting the plasma membrane as its principal location. Osteosarcoma cells, known to resemble osteoblasts in their biological characteristics and responses to bone-seeking hormones, contained similar ATPase activities. Inclusion of purified calmodulin in the assay system caused small non-reproducible increases in the Ca^{2+}-dependent ATPase activity of EGTA-washed membranes. Marked, consistent calmodulin stimulation was demonstrated in membranes exposed previously to trifluoperazine and then washed in trifluoperazine-free buffer. These results indicate the presence of a high affinity, calmodulin-sensitive Ca^{2+}-dependent ATPase in osteoblast-like bone cells. As one determinant of Ca^{2+} fluxes in bone cells, this enzyme may participate in the hormonal regulation of bone cell function.

Introduction

A substantial compilation of experimental results points to Ca^{2+} as a participant in the hormonal regulation of bone cell function. Parathyroid hormone rapidly increases the accumulation of Ca^{2+} by skeletal tissues and isolated bone cells, and this increase precedes the appearance of alterations in bone cell function [1–7]. Maintaining a high extracellular Ca^{2+} concentration or exposure to divalent cation ionophores (A23187, Ionomycin) replicates certain metabolic effects of parathyroid hormone on isolated bone cells and organ cultured bone segments, and ionophores in themselves stimulate bone resorption and modify skeletal collagen synthesis in vitro [8–14]. Moreover, Ca^{2+} channel blockers are known to decrease protein synthesis and to inhibit parathyroid hormone- and prostaglandin-mediated

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Abbreviations: CDTA, *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

resorption [15,16]. Recently, we showed that extracellular Ca^{2+} augments the increases in bone cell cyclic AMP caused by parathyroid hormone and prostaglandin E_2, an effect that requires the accumulation of Ca^{2+} in an intracellular or intramembranous compartment [17].

The mechanisms subserving Ca^{2+} fluxes in bone cells are not well understood. The bulk of available evidence suggests that Ca^{2+} enters by inactive mechanisms, whereas its exodus is served by an active process [18]. Studies with many other types of mammalian cells point to similar mechanisms for transmembrane Ca^{2+} transport, and indicate further that a Ca^{2+}-dependent ATPase promotes Ca^{2+} exodus (see, for example, Refs. 19 and 20). Although Ca^{2+}-dependent ATPase activity has been demonstrated in skeletal tissues [21–23], it has not been well characterized and its study in bone cells has not been reported. The results presented herein demonstrate a high-affinity Ca^{2+}-dependent ATPase in a plasma membrane-enriched fraction prepared from osteoblast-like bone cells as well as from osteogenic sarcoma cells, demonstrate certain properties of enzyme in the plasma membrane preparation, and disclose that this enzyme is stimulated by calmodulin (calcium-dependent regulator).

Experimental procedures

Materials. Culture media, antibiotics, glutamine and fetal calf serum were obtained from Flow Laboratories, McLean, VA, [γ-^{32}P]ATP and kits for the radioimmunoassay of calmodulin from New England Nuclear Corporation, Boston, MA, and all other chemicals (reagent grade) from Sigma Corporation or Fischer Corporation, St. Louis, MO. Purified calmodulin was provided by Calbiochem-Behring Corporation, La Jolla, CA, and trifluoperazine was a gift of Dr. Michael Landt, Washington University Medical School.

Isolation and culture of bone cells. Frontal portions of calvaria obtained from 20- to 21-day-old rat fetuses were used to isolate osteoblast-like bone cells as described before [24]. Dispersed cells were grown in bone fluid medium [24] supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml of streptomycin. All cultures were maintained in an atmosphere of 5% CO_2 in air at 37°C. Osteosarcoma cells (ROS17/2) kindly provided by Dr. G. Rodan, University of Connecticut, were cultured under the same conditions. Media were changed every 3–4 days and cells were harvested after 7 days of seeding.

Preparation of plasma membrane-enriched fractions. All subsequent procedures were performed at 4°C. After removing the medium, the culture dishes were quickly cooled on ice and washed with 20 mM Tris-HCl, pH 7.5 and 2.5 mM dithiothreitol and the cells collected with a rubber policeman. Cell suspensions were homogenized with a tight-fitting Dounce homogenizer until over 95% of the cells were disrupted. Residual free Ca^{2+} and Mg^{2+} were removed by adding EGTA and EDTA (1 mM final concentration). The homogenate was centrifuged at $10\,000 \times g$ for 45 min. The pellet was resuspended in a small volume of original buffer (20 mM Tris-HCl, 2.5 mM dithiothreitol) and enough sucrose solution in the same buffer was added to give a final concentration of 38%. The 38% sucrose solution was carefully overlayed upon a 2 ml 42% sucrose cushion in an SW 41 allomer centrifuge tube, and covered with 2 ml of 32% sucrose in the same buffer. The density gradient was centrifuged at $100\,000 \times g$ for 90 min in a Beckman centrifuge using an SW 41 rotor. The 32–38% interface pellets (plasma membrane-enriched fraction) were carefully withdrawn and diluted with 2 volumes of 20 mM Tris-HCl/2.5 mM dithiothreitol. The 38–42% interface pellet (endoplasmic reticulum (ER)-enriched fraction) and the pellet at the bottom of the tube (nuclei and mitochondria-enriched fraction) were collected similarly. After mixing, all fractions were centrifuged at $30\,000 \times g$ for 60 min and the pellet was resuspended in a small volume of 50 mM Tris-HCl and 2.5 mM dithiothreitol, pH 7.2, at 30°C. Glucose-6-phosphatase and succinate cytochrome *c* dehydrogenase (endoplasmic reticulum and mitochondrial markers) were assayed according to Dallner et al. [25] and Bachmann et al. [26] on the day of preparation. (Ca^{2+} + Mg^{2+})-ATPase [27], adenylate cyclase [28] and 5′-nucleotidase [29] (plasma membrane enzymes) were assayed using separate fractions that had been quick-frozen in small aliquots with solid CO_2/alcohol and stored at −70°C. Proteins were determined either by the

method of Lowry et al. [30] or by the BioRad dye-binding method.

(Ca^{2+} + Mg^{2+})-ATPase assay. ATPase was assayed at 30°C with 5–10 μg of protein in 50 mM Tris-HCl (pH 7.2), 1 mM ATP, 20 mM NaN_3, 0.3 mM ouabain, 0.2 μCi of [γ-^{32}P]ATP (2.5 Ci/μmol), 185 μM EGTA and enough $CaCl_2$ to yield the desired free Ca^{2+}. The free Ca^{2+} (in EGTA and *trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid (CDTA) buffer) concentrations were calculated according to the method of Pershadsingh and McDonald [20]. The Ca^{2+} contents of the reaction mixture and enzyme preparations were measured by atomic absorption [31] at about 3 to 5 μM and this amount of Ca^{2+} was taken into consideration in the calculation [20]. The reaction was run at 30°C for 30 min and released $^{32}P_i$ was measured as described by Seals et al. [27]. Ca^{2+}-dependent ATPase was determined by subtracting the activity obtained in the presence of Ca^{2+} and EGTA from that obtained with EGTA alone. In some experiments, shorter incubation times were used to ensure that less than 5% of ATP was hydrolyzed.

Effect of calmodulin. Endogenous calmodulin associated with the plasma membrane-enriched fraction was estimated by radioimmunoassay, as described by Chafouleas et al. [32]. The effect of exogenous calmodulin was estimated by adding the protein directly to the assay mixture, using membranes prepared as described above. In separate experiments we used membranes previously exposed to trifluoperazine, according to the method of Kotagal et al. [33]. Frozen and thawed membranes were sonicated for 10 s, then incubated for 30 min at 4°C in the presence of 50 mM Tris-HCl (pH 7.2), 20 μM free Ca^{2+}, and 100 μM trifluoperazine. The preparation was centrifuged at 150000 × *g* for 60 min in a Beckman preparative ultracentrifuge using an SW 50.1 rotor. The pellet was then resuspended in 50 mM Tris-HCl and used in the (Ca^{2+} + Mg^{2+})-ATPase assay.

Results

Identification and characterization of the Ca^{2+}-dependent ATPase

Initial experiments failed to disclose Ca^{2+}-dependent ATPase activity in the presence of greater than 1 mM $MgCl_2$. By contrast, two discrete Ca^{2+}-dependent ATPase activities were evident in the presence of low Mg^{2+} levels (less than 1 mM) and in the absence of added Mg^{2+} (Fig. 1). One Ca^{2+}-dependent ATPase, termed the high-affinity component, had a half-saturation constant for Ca^{2+} of 280 nM and a V_{max} of 13.5 nmol/mg per min. A second Ca^{2+}-dependent ATPase, which exhibited low affinity, was in reality a cation-dependent ATPase system in which Ca^{2+} could be replaced by Mg^{2+}. These ATPase activities differed in their pH optima: the high affinity ATPase had a pH optimum of 7.2 and the low affinity component an optimum of 7.8 (Fig. 2).

Other characteristics of the high affinity Ca^{2+}-dependent ATPase

The high affinity Ca^{2+}-dependent ATPase activity was not affected by the addition of 20 mM KCl, 20 mM NaCl, 0.3 mM ouabain or 20 mM NaN_3 but was almost totally inhibited by 2 mM lanthanum chloride (Table I). Trifluoperazine, an

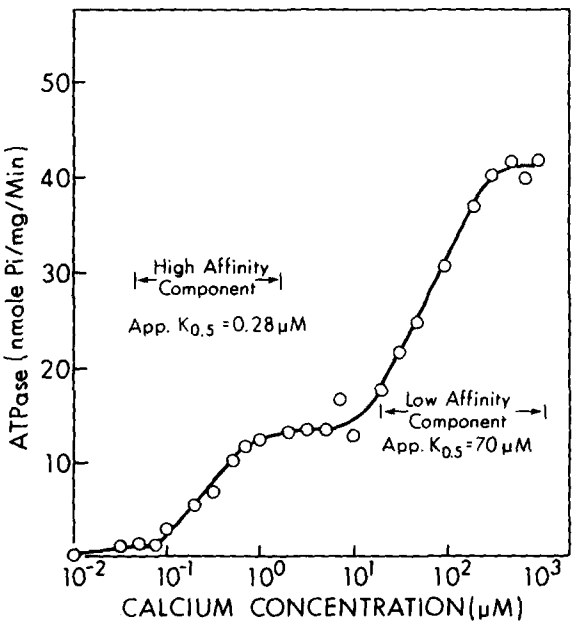

Fig. 1. Dependence of Ca^{2+}-stimulated ATPase activity on free Ca^{2+} concentration in plasma membrane-enriched fraction of primary osteoblast cells. Net Ca^{2+}-stimulated ATPase is shown on the ordinate and was determined at free Ca^{2+} concentrations between 0.01 and 1000 μM. The apparent one-half saturation constants for low and high affinity components are shown.

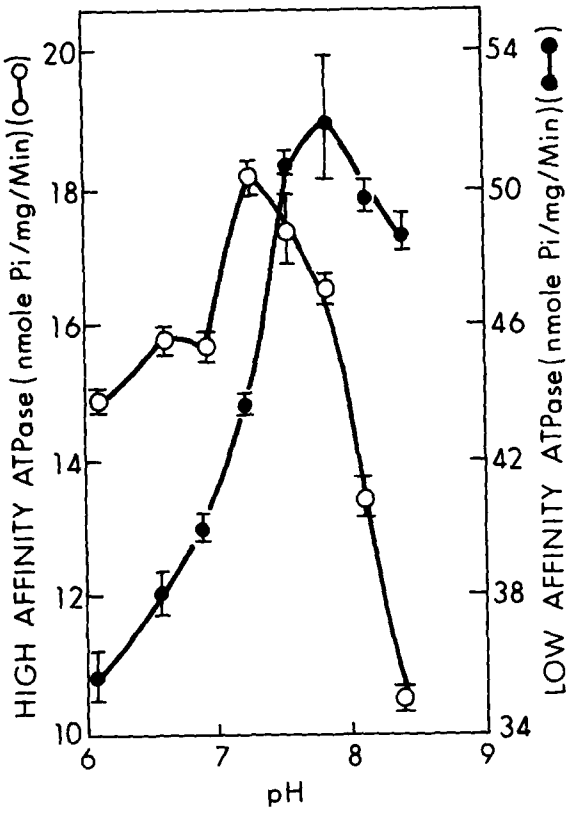

Fig. 2. pH dependency of high- and low-affinity Ca^{2+}-dependent ATPase. EGTA was omitted from the standard incubation medium because its ability to complex calcium ions is highly pH-dependent. The high affinity component was assayed at a total calcium concentration of 7 μM and low affinity component at 2 mM. The high-affinity ATPase was the net activity of 7 μM total Ca^{2+} over 185 μM EGTA alone. The low affinity ATPase was the net activity of 2 mM total Ca^{2+} over 7 μM total Ca^{2+}. Each point represents the mean ± S.E. of four determinations.

TABLE I

CHARACTERIZATION OF HIGH-AFFINITY (Ca^{2+} + Mg^{2+})-ATPase

(Ca^{2+} + Mg^{2+})-ATPase activity was assayed under standard conditions at a free calcium concentration of 2 μM using Ca^{2+}-EGTA buffer (except where indicated).

	(Ca^{2+} + Mg^{2+})-ATPase [a]	Percentage of control
No addition	6.86 ± 0.01	100
20 mM KCl	7.78 ± 0.15	114
20 nM NaCl	7.14 ± 0.03	104
0.3 mM ouabain	7.34 ± 0.08	107
20 mM NaN_3	6.61 ± 0.06	96
20 mM NaCl + 20 mM KCl	7.35 ± 0.07	107
0.3 mM ouabain + 20 mM NaN_3	7.23 ± 0.38	105
No addition [b]	7.27 ± 0.12	100
+ 0.2 mM lanthanum [b]	0.17 ± 0.31	2
No addition	10.25 ± 0.01	100
30 μM TFP	9.50 ± 0.11	93
60 μM TFP	7.80 ± 0.45	76
120 μM TFT	7.65 ± 0.51	75
240 μM TFP	1.95 ± 0.04	19
CDTA alone [c]	4.59 ± 0.01	100
CDTA + 0.7 μM free Ca^{2+}	3.96 ± 0.06	86
EGTA alone	5.11 ± 0.02	100
EGTA + 0.7 μM free Ca^{2+}	8.72 ± 0.05	162

[a] Results are expressed as nmol P_i/mg per min and are represented by the mean ± S.E. of triplicate determinations.

[b] EGTA was omitted in studies with lanthanum since it has a much higher affinity for the chelator than does calcium. Total calcium concentration was 7 μM.

[c] The value obtained with EGTA alone was subtracted from all the data except in the experiment with CDTA.

inhibitor of calmodulin-mediated reactions, decreased Ca^{2+}-dependent ATPase activity at concentrations of 60 μM and above.

Varying the ATP concentration in the assay yielded two components (Fig. 3), a saturable high-affinity component and a second component, of apparent low affinity, which was not tested for saturation kinetics.

The high-affinity Ca^{2+}-dependent ATPase could be detected without addition of Mg^{2+} to the assay system. To determine whether the enzyme had an absolute requirement for Mg^{2+}, perhaps satisfied by Mg^{2+} associated with the plasma membrane fraction, we used a Ca^{2+}-CDTA buffer instead of Ca^{2+}-EGTA. Since CDTA has the greater affinity for Mg^{2+} [20], under the assay conditions employed in these experiments the bulk of free Mg^{2+} would be expected to complex with CDTA. We detected no Ca^{2+}-dependent ATPase activity in the Ca^{2+}-CDTA buffer system as compared to Ca^{2+}-EGTA buffer system (see Table I); hence, a low concentration of Mg^{2+} appears to be essential for Ca^{2+}-dependent ATPase activity (referred to as (Ca^{2+} + Mg^{2+})-ATPase).

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

The specific activities of the enzyme markers

TABLE II

DISTRIBUTION OF HIGH-AFFINITY (Ca^{2+} + Mg^{2+})-ATPase AND OTHER ENZYME MARKERS IN SUBCELLULAR FRACTIONS

Subcellular fractions	(Ca^{2+} + Mg^{2+})-ATPase		Plasma membrane marker 5'-nucleotidase		Plasma membrane marker adenylate cyclase		Endoplasmic reticulum marker glucose-6-phosphatase		Mitochondria marker succinate cytochrome *c* reductase	
	Spec. act. (nmol/mg per min)	%	Spec. act. (nmol/mg per min)	%	Spec. act. (nmol/mg per min)	%	Spec. act. (arbitrary unit)	%	Spec. act. (arbitrary unit)	%
Plasma membrane	40.19 ± 1.59		158.81 ± 2.47		1.03 ± 0.10		0.081 ± 0.002		0.019 ± 0.002	
Endoplasmic reticulum	22.21 ± 4.08	55	110.74 ± 4.73	70	0.45 ± 0.06	44	0.124 ± 0.001	123	0.027 ± 0.002	142
Mitochondria	3.01 ± 0.72	7	22.64 ± 0.47	14	0.10 ± 0.03	10	0.037 ± 0.004	46	0.115 ± 0.004	605

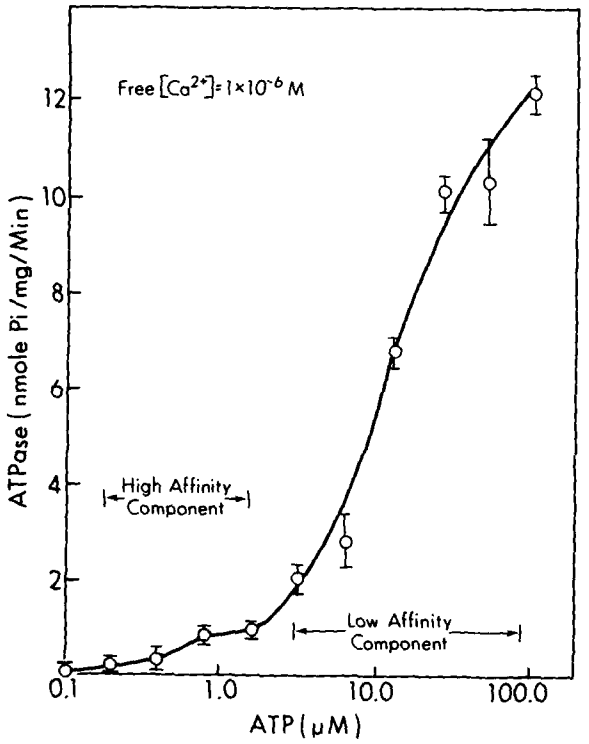

Fig. 3. Dependence of the high affinity (Ca^{2+} + Mg^{2+})-ATPase on ATP concentration. Activity was assayed in the standard incubation medium (free Ca^{2+} concentration 1 μM) except that the total ATP concentration was varied between 0.1 and 100 μM. Reaction time varied between 2 min (at low ATP concentration) and 30 min (at high ATP concentration) to ensure less than 10% hydrolysis. Net (Ca^{2+} + Mg^{2+})-ATPase activity, shown on the ordinate, was obtained by subtracting the activity in the absence of calcium (but with EGTA) at all ATP concentrations. ATP concentrations used in these experiments were calculated to have minimal impact on the free Ca^{2+} concentration. Each point represents mean ± S.E. of triplicate determinations.

for the various subcellular fractions are shown in Table II. The (Ca^{2+} + Mg^{2+})-ATPase specific activity was the highest in the bone cell fraction that was enriched in adenylate cyclase and 5'-nucleotidase activities. It was this fraction that was used for all studies described herein.

Effects of exogenous calmodulin

In initial experiments, we examined the response of the (Ca^{2+} + Mg^{2+})-ATPase to exogenous calmodulin using EGTA-washed membranes. Although calmodulin was often stimulatory when added at concentrations ranging from 5 to 15 μg/ml in the presence of 100 nM to 10 μM Ca^{2+}, the stimulation was usually small in degree (10–20%) and occasionally not observed (data not shown). In the absence of added calmodulin, the ATPase-enriched plasma membrane fraction was found to retain substantial endogenous calmodulin, despite repeated washing with EGTA buffer (data not shown). Addition of calmodulin to membranes previously exposed to trifluoperazine and then washed extensively produced marked (Fig. 4) and reproducible enhancement of (Ca^{2+} + Mg^{2+})-ATPase activity. Calmodulin lowered the concentration of Ca^{2+} required for one-half saturation of the high affinity enzyme from 200–300 nM to 50 nM, in the presence of 200 nM Mg^{2+}. Calmodulin did not appear to influence the low affinity enzyme. Prior exposure to trifluoperazine did not modify ATPase activity in calmodulin-untreated membranes (Fig. 4).

High affinity (Ca^{2+} + Mg^{2+})-ATPase in osteosarcoma cells

The bone cell population employed in the present experiments is highly enriched in osteoblast-

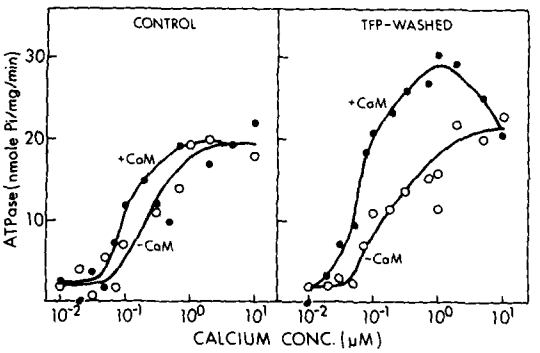

Fig. 4. Effect of calmodulin on (Ca^{2+} + Mg^{2+})-ATPase activity in control and trifluoperazine-treated membrane preparations. The plasma membrane-enriched fractions were separated and one-half were pretreated (30 min, 4°C) with 100 μM trifluoperazine in 50 mM Tris-HCl, 20 μM of free Ca^{2+} after 10 s sonication. Fractions were centrifuged at 100000 × g for 60 min and resultant pellets were resuspended in trifluoperazine-free 50 mM Tris-HCl, pH 7.2 at 30°C. Both fractions were assayed in the presence of 200 μM Mg^{2+}, in the presence or the absence of 5 μg/ml of calmodulin at various free Ca^{2+} concentrations. Left panel: control. Right panel: trifluoperazine-washed.

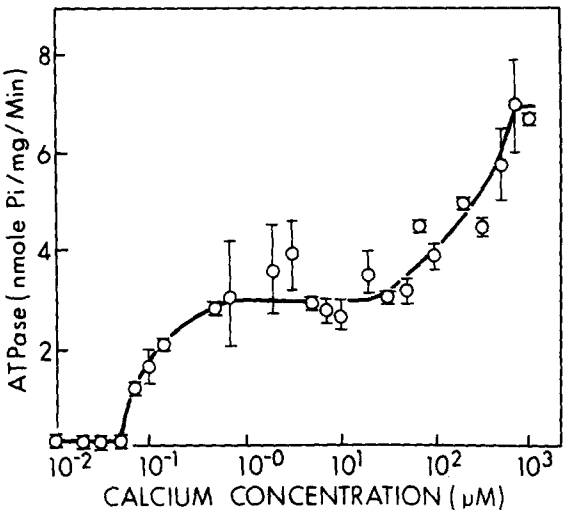

Fig. 5. Ca^{2+}-dependent ATPase activity in osteosarcoma cells. The experiment was performed the same way as described in the legend to Fig. 1, except that the plasma membrane-enriched fraction was obtained from malignant osteosarcoma cells rather than cultured osteoblasts. Each point represents the mean ± S.E. of three separate assays.

like cells, but contamination by non-osteoblast elements which could contribute ATPase activity to the subcellular test preparations cannot be excluded. Therefore, we examined ATPase activity in a plasma membrane-enriched fraction derived from a homogeneous population of osteosarcoma cells, known to have multiple properties in common with osteoblast [34,35]. The osteosarcoma cells, like their non-malignant counterparts, exhibited two Ca^{2+}-dependent ATPase activities (Fig. 5). The high affinity (Ca^{2+} + Mg^{2+})-ATPase showed an apparent half-saturation constant for Ca^{2+} of 100 nM and a V_{max} at 3.4 nmol/mg per min.

Discussion

Although we have not undertaken the purificatoin of bone cell plasma membranes to homogeneity, strong indirect evidence suggests a plasma membrane location for the bone cell high-affinity (Ca^{2+} + Mg^{2+})-ATPase. Enzyme activity was found in the same subcellular fraction that contained other known plasma membrane-located enzymes, adenylate cyclase and 5'-nucleotidase, but not a mitochondrial marker (succinate-cytochrome *c* reductase). The plasma membrane fraction did contain modest glucose-6-phosphatase activity, suggesting some contamination by endoplasmic reticulum. At least in non-skeletal cell systems, however, the high-affinity (Ca^{2+} + Mg^{2+})-ATPase of endoplasmic reticulum requires relatively high concentrations of Mg^{2+} and K^+, conditions that were avoided in studying the bone cell enzyme. However, there is a very real possibility that (Ca^{2+} + Mg^{2+})-ATPase activity may reside in endoplasmic reticulum.

In assaying the high affinity enzyme, it was expedient to exclude contributions to ATP hydrolysis by a 'non-specific' low-affinity (Ca^{2+} + Mg^{2+})-ATPase and by Ca^{2+}-independent ATPases. Interference by the low-affinity species was minimized by limiting the concentration of Mg^{2+} in the assay mixture, a tactic used in other studies to achieve the same result. The absolute requirement for Mg^{2+} that typifies the high affinity enzyme may well have been satisfied by residual Mg^{2+} in the plasma membrane preparation, and was eliminated by prior exposure of the preparation to CDTA. Resolution of the (Ca^{2+} + Mg^{2+})-ATPase from other ATPases was also accomplished by appropriate manipulations of the assay conditions. Mitochondrial ATPase activity was inhibited with sodium azide, and (Na^+ + K^+)-dependent ATPase with ouabain. Consequently, we were able to detect ATP hydrolytic activity in the presence of micromolar Ca^{2+} concentrations that was not altered by K^+, azide or ouabain, alone and in combination.

Our findings indicate that the high affinity bone cell (Ca^{2+} + Mg^{2+})-ATPase is similar in its biochemical characteristics to enzymes prepared from a variety of non-skeletal cells, including erythrocytes, adipocytes, macrophages, liver cells and corpus luteum [20,36–39]. These enzymes have congruent pH optima, a high affinity for Ca^{2+}, and ATP and Mg^{2+} dependency. On the other hand, previous studies have provided little information concerning skeletal Ca^{2+}-dependent ATPase activity. Rodan and Bourret [23] found such activity in microsomal preparations from rat calvaria, but that activity was not characterized. Messer et al. [22] described a cation-dependent ATPase from chick femur in which Ca^{2+} and

Mg^{2+} were interchangeable in promoting enzyme activity. In that study 0.3 mM Ca^{2+} activated the enzyme, but lower Ca^{2+} concentrations were apparently not tested. Whether these enzymes are representative of the bone cell enzyme described in our studies remains to be determined.

Calmodulin is known to be a proximate mediator of many Ca^{2+}-regulated intracellular events, such as Ca^{2+}-dependent cyclic AMP phosphodiesterase, adenylate cyclase, protein kinase, and in some systems (Ca^{2+} + Mg^{2+})-ATPase (see Ref. 40 for review). Efforts to establish a role for calmodulin have included the use of trifluoperazine, a phenothiazine which binds to and inactivates calmodulin [41]. However, trifluoperazine is known to modify calmodulin-independent cell functions which may be important in determining Ca^{2+} access and binding [42]. Consequently, inhibition by trifluoperazine per se is not proof of calmodulin involvement. For this reason, we examined the effects of added calmodulin on bone cell ATPase activity. Exogenous calmodulin caused only modest stimulation of ATPase activity which was not enhanced by extensive prior washing of the membrane fraction with EGTA. Because the membranes contained appreciable calmodulin despite exposure to EGTA, we examined the effect of exogenous calmodulin on trifluoperazine-treated membranes, as described by Kotagal et al. [33]. In fact, it was possible to demonstrate substantial and consistent stimulation by calmodulin under those conditions. Neither our findings nor those of Kotagal et al. [33] can be attributed definitely to the dissociation of endogenous calmodulin, since actual decreases in membrane calmodulin have not been shown. More importantly, the low concentration of trifluoperazine used in preparing the membranes did not decrease basal (Ca^{2+} + Mg^{2+})-ATPase activity. In addition, removal of calmodulin alone would not explain the alterations in kinetic parameters caused by exogenous calmodulin in the trifluoperazine-washed membranes. Hence, phenothiazine treatment may have increased calmodulin responsivity by modifying membrane structure in an as yet unknown fashion.

Calmodulin-mediated stimulation of ATPase activity requires the presence of a greater concentration of Mg^{2+} than was needed to demonstrate the high-affinity component (vide supra). Consequently, we cannot exclude an effect of calmodulin on the 'low-affinity' component which is evident at high Mg^{2+} levels. Two observations make this possibility unlikely: first, our studies disclose a distinct low affinity component that did not appear to be altered by calmodulin; and second, marked dependency on low Ca^{2+} concentrations is clearly evident, and indeed magnified in the presence of calmodulin.

An actual contribution of the high affinity (Ca^{2+} + Mg^{2+})-ATPase to bone cell physiology remains to be established. It is possible to extrapolate from other systems in speculating that the plasma membrane species of this enzyme provides the impetus for active Ca^{2+} extrusion from the cell. By the same token, an endoplasmic reticulum enzyme, if present, could mediate the intracellular scavenging of cytosolic Ca^{2+}, perhaps in association with cellular contraction. There is evidence that an ATP-dependent mechanism in bone cells is more important than Ca^{2+}-Na^{+} exchange in mediating Ca^{2+} exodus from bone cells [43]. The present results provide a background for elucidating the role of the enzyme in regulating bone cell function. The fact that cultured osteogenic sarcoma cells, which resemble osteoblasts in many properties, also contain a high affinity (Ca^{2+} + Mg^{2+})-ATPase suggests that these cells will be useful in studying the biological significance of the enzyme and its role in mediating hormone effects.

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