A Ca²⁺-stimulated adenosine triphosphatase in Golgi-enriched membranes of lactating murine mammary tissue

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A membrane fraction isolated from lactating murine mammary tissue and enriched for the Golgi membrane marker enzyme galactosyltransferase exhibited Ca^{2+}-stimulated ATPase activity (Ca-ATPase) in $20\,\mu$M-free Mg^{2+} and $10\,\mu$M-MgATP, with an apparent K_m for Ca^{2+} of $0.8\,\mu$M. Exogenous calmodulin did not enhance Ca^{2+} stimulation, nor could Ca-ATPase activities be detected in millimolar *total* Mg^{2+} and ATP. When assayed with micromolar Mg^{2+} and MgATP the Ca-ATPases of skeletal-muscle sarcoplasmic reticulum and of calmodulin-enriched red blood cell plasma membranes were half-maximally activated by $0.1\,\mu$M- and $0.6\,\mu$M-Ca^{2+} respectively. All three Ca-ATPases were inhibited by similar micromolar concentrations of tri-fluoperazine, but the Golgi activity was unaffected by quercetin in concentrations which completely inhibited both the sarcoplasmic-reticulum and red-blood-cell enzymes. The results are consistent with the hypothesis that the high-affinity Ca-ATPase is responsible for the ATP-dependent Ca^{2+} transport exhibited by Golgi-enriched vesicles derived from lactating mammary gland [Neville, Selker, Semple & Watters (1981) *J. Membr. Biol.* **61**, 97–105; West (1981) *Biochim. Biophys. Acta* **673**, 374–386].

Most animal cells maintain cytoplasmic concentrations of free Ca^{2+} at least three orders of magnitude lower than those found in their environments, using membrane-localized and ATP-dependent transport systems (Carafoli & Crompton, 1978; Marban *et al.*, 1980). Given the important functions ascribed to calcium, it is not surprising that Ca^{2+}-transport systems have received considerable attention (Ikemoto, 1982; Sakardi, 1980).

In particular, the calcium 'pumps' of skeletal-muscle SR and of RBC plasma membranes have been well characterized; they utilize MgATP, require Mg^{2+} as a co-activator and are stimulated half-maximally by Ca^{2+} at a concentration just above that which normally exists in the cytoplasm (Penniston, 1982; Sakardi, 1980; Tada *et al.*, 1978;

Vianna, 1975). They also form acid-stable and hydroxylamine-sensitive phosphorylated intermediates during the cycle of ATP hydrolysis (MacLennan, 1970; Schatzmann & Burgin, 1978). Thus Ca^{2+} pumps behave enzymically like $(Ca^{2+}+Mg^{2+})$-dependent nucleoside triphosphatases (EC 3.6.1.3) or, more commonly, high-affinity Ca-ATPases.

Lactating mammary tissue also should provide excellent material for the study of Ca^{2+} transport, since large amounts of Ca^{2+} are secreted into milk as a complex with casein (Neville & Peaker, 1979). The formation of these complexes occurs within Golgi and secretory vesicles (Wooding & Morgan, 1978) and appears to require millimolar Ca^{2+} (Thompson & Farrell, 1974). Ca^{2+} sequestration above the presumed submicromolar cytoplasmic concentration is thought to require a pump, and we (Neville *et al.*, 1981) and another (West, 1981) have found that Golgi-enriched vesicles accumulated Ca^{2+} in an ATP-dependent and ionophore-A23187-releasable manner. The presence of an ATPase (Baumrucker & Keenan, 1975) and acid-stable hydroxylamine-sensitive and Ca^{2+}-stimulated phosphorylation of a 105 kDa polypeptide (Neville

et al., 1981) have also been reported in similar Golgi fractions.

The present paper characterizes a high-affinity Ca-ATPase activity in a Golgi-enriched membrane fraction isolated from lactating murine mammary tissue. Kinetic parameters of the activity and the effects of exogenous calmodulin, trifluoperazine and quercetin are described, and a direct comparison is made between the activity in the Golgi fraction and those found in skeletal SR and the RBC plasma membrane.

A preliminary account of some of this work has already been published (Watters, 1981; Watters & Neville, 1981).

Experimental

Materials

Lactating CD1 mice were obtained from the breeding colony of the mammary-gland research group. The vanadate-free Tris salt of ATP, as well as sucrose, imidazole, Mops, Tris, EGTA, oligomycin, quercetin and the Fiske–SubbaRow reagent were obtained from Sigma. [γ-^{32}P]ATP was obtained from New England Nuclear Corp. Calmodulin and trifluoperazine were generously given by, respectively, Smith, Kline and French Laboratories and Dr. T. C. Vanaman. The standard $CaCl_2$ solution was purchased from Orion Research, and all other chemicals were of reagent grade. All solutions were prepared in de-ionized glass-distilled water, and pH was adjusted at the temperatures at which the solutions were used.

Preparation of membrane fractions

The fourth and fifth mammary glands were removed between days 11 and 15 of lactation. Membranes enriched in the Golgi marker galactosyl-transferase (lactose synthetase protein A; Kuhn & White, 1977) were prepared from Polytron homogenates of these glands by differential and isopycnic centrifugation as previously described (Neville *et al.*, 1981). The Golgi fraction was washed twice and resuspended in 1 mM-EGTA and 10 mM-imidazole or Mops, pH 7.0. Portions were then frozen rapidly over a solid-CO_2/methanol mixture and stored at $-73°C$ until needed. A membrane fraction prepared in this manner was enriched 28-fold in the activity of galactosyltransferase, a finding similar to our earlier one (Neville *et al.*, 1981). The activity of succinate dehydrogenase was 0.2 times that present in the homogenate.

SR membranes were obtained from diced thigh muscle of a lactating mouse essentially by the method of Meissner & Fleischer (1971). Contamination by myosin was reduced by overnight incubation

(4°C) in a solution containing 0.6 M-KCl/0.25 M-sucrose/10 mM-Hepes, pH 7.4. Stripped membranes were resuspended in 0.3 M-sucrose/10 mM-Hepes, pH 7.0, and stored as described above. Murine blood was obtained by cardiac puncture and washed three times in 0.11 M-NaCl/0.04 M-Mops, pH 7.0. Membranes were prepared by slowly adding freshly washed packed RBC to 20 vol. of a chilled solution containing 1 mM-EGTA/10 mM-imidazole, pH 7.0. The pellet was collected and washed in the same solution, three times or until the supernatant was colourless. The pink pellet was frozen as described above, slowly thawed, washed two additional times, refrozen and stored until needed at $-73°C$.

Characterization of ATPase activities

Membrane fractions were assayed for ATPase activity at 37°C in 0.3 ml of a standard solution containing 100 mM-KCl, 50 mM-imidazole or -Mops, 1 mM-EGTA and 2.7 μg of oligomycin (pH 7.0). After preincubation for 2 min at 37°C, the assay was begun by the addition of Tris/ATP (containing approx. 0.1 μCi of [γ-^{32}P]ATP as the tetraethylammonium salt); it was terminated by adding 0.1 ml of a solution ('STOP') containing 8% SDS and 0.1 mM-P_i. An equal volume of Fiske–SubbaRow reagent (1% ammonium molybdate in 1.32 M-HCl) was then added to each sample and the resulting phosphomolybdate complex was partitioned immediately into 3.5 ml of xylene/butan-2-ol (13 : 7, v/v) (Seals *et al.*, 1978). A portion of the organic phase was assayed for radioactivity in a liquid-scintillation spectrometer.

Total concentrations of Mg^{2+}, Ca^{2+} and ATP for given concentrations of free Mg^{2+}, free Ca^{2+} and MgATP were obtained from a calculator program by using the constants listed in Table 1. The composition of a representative assay mixture is presented in Table 2. All assays were run for periods no longer than 15 min and with levels of hydrolysis

Table 1. *Association constants*
Values for the association of EGTA and the indicated cations were adjusted for I0.15 (for ATP and its ligands, I0.1). All values were determined at, or corrected for, 37°C (Scharff, 1979; Taqui Khan & Martell, 1966).

| | | $\log K$ | |
Cation	Ligand	EGTA	ATP
H^+	L^{4-}	9.29	6.52
	HL^{3-}	8.69	3.92
Ca^{2+}	L^{4-}	10.51	3.95
	HL^{3-}	5.32	2.13
Mg^{2+}	L^{4-}	5.21	4.27
	HL^{3-}	3.36	2.28

equivalent to no more than 10% of the total ATP; these conditions ensured the measurement of initial rates of hydrolysis. Blanks, to which 'STOP' was added *before* ATP, were used to correct all samples for the spontaneous hydrolysis of ATP. Oligomycin was included at a concentration that completely inhibited the ATPase of rat liver mitochondria (Lardy *et al.*, 1958).

Fresh stocks of quercetin and trifluoperazine were prepared for each assay. Trifluoperazine solutions were stored briefly in a chilled brown bottle, and all assays with this inhibitor were run in a darkened room, lighted indirectly by an incandescent bulb. The stock concentration of trifluoperazine was determined spectrophotometrically at a wavelength of 256 nm by using $\varepsilon 3.26 \times 10^4 \, M^{-1} \cdot cm^{-1}$ (Hinds *et al.*, 1981).

Other assays

Galactosyltransferase was measured by a column-chromatographic method, using UDP-[^{14}C]-galactose and N-acetylglucosamine as substrates (Ebner *et al.*, 1972; Kuhn & White, 1977). Succinate dehydrogenase activity was assayed spectrophotometrically (King, 1967), using dichlorophenol indophenol to accept hydrogen and 0.1% Triton X-100 to expose cryptic sites. Protein was determined by a modification of the Lowry method (Peterson, 1977), using bovine serum albumin as standard. The activity of calmodulin was assessed by measuring its effect on the RBC Ca-ATPase.

Determination of ionized Ca^{2+} and Mg^{2+} and of MgATP concentrations

Ca^{2+} and Mg^{2+} bind to both EGTA and ATP and the complex equilibria are affected by pH and to a lesser extent, by temperature and ionic strength (Scharff, 1979; Tsien & Rink, 1980). The

effect of all three parameters on the association of Ca^{2+} and EGTA has been studied systematically (Harafuji & Ogawa, 1980; Scharff, 1979), and the apparent association constants independently derived are in remarkable agreement. The constants also agree favourably with those determined empirically with the aequorin assay (Allen *et al.*, 1977). For this reason Scharff's (1979) constants were used in the present study (Table 1).

The binding of Mg^{2+} and Ca^{2+} with ATP has been corrected for the effect of temperature, but not ionic strength (Scharff, 1979; Taqui Khan & Martell, 1966), and the constants in Table 1 are the ones reported for 0.1 *I*. Assays were performed at the more physiological 0.15 *I*, since variations in the binding of Mg^{2+} and ATP in the range 0.1–0.2 have been reported to be minimal (Storer & Cornish-Bowden, 1976).

Results

Kinetic parameters of the Golgi ATPase

ATPase activities with high affinity for MgATP were observed in both freshly isolated and stored membrane fractions enriched for Golgi membranes. Significantly greater activity was obtained in micromolar Ca^{2+} (Fig. 1, ●), and the Ca^{2+}-
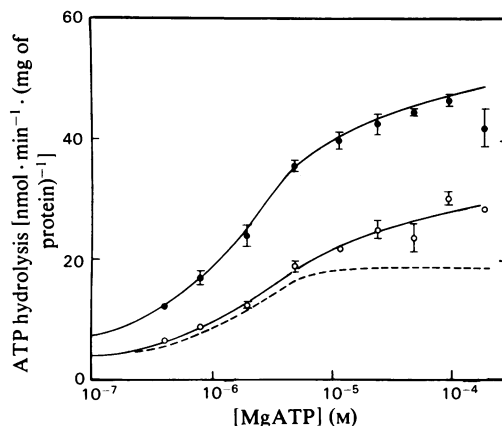

Fig. 1. *The MgATP-dependence of ATPase activities in Golgi-enriched fractions*
All assays contained 20 μM free Mg^{2+} and those involving 0.45 μM-MgATP were run for 5 min, 0.9 and 1.8 μM for 10 min, and the remainder for 15 min. ●, 64 μM free Ca^{2+} (1.06–1.09 mM total Ca^{2+}); ○, 10 nM free Ca^{2+} (0.030–0.032 mM total Ca^{2+}). –––– represents the Ca^{2+}-activated component of ATPase activity. In this and subsequent Figures, each data point represents the average ± S.E.M. of three determinations. The standard error of any point lacking error bars is less than the vertical dimension of the point.

Table 2. *Representative assay conditions*
The indicated total concentrations of CaCl$_2$, MgCl$_2$, and ATP generated 20 μM free Mg^{2+}, 10 μM-MgATP and the designated concentrations of free Ca^{2+}. All samples contained, in addition, 100 mM-KCl, 50 mM-Mops and 1 mM-EGTA, and the assay was run at 37°C and at a pH of 7.0.

[Ca^{2+}]$_{free}$ (M)	Total concentration (mM)		
	CaCl$_2$	MgCl$_2$	ATP
1×10^{-8}	0.032	0.031	0.067
1×10^{-7}	0.249	0.031	0.067
4×10^{-7}	0.570	0.031	0.067
1.6×10^{-6}	0.843	0.030	0.068
3.2×10^{-6}	0.918	0.030	0.068
6.4×10^{-6}	0.963	0.030	0.069
1.3×10^{-5}	0.993	0.030	0.070
2.5×10^{-5}	1.02	0.030	0.073
5×10^{-5}	1.06	0.030	0.079

stimulated component exhibited maximal specific activity at approx. $10\,\mu$M-MgATP and half-maximal activity (apparent K_m) at approx. $2\,\mu$M (Fig. 1, ----).

The stimulatory effect of Ca^{2+} was examined at $20\,\mu$M free Mg^{2+} and either saturating or nearly half-saturating concentrations of MgATP. At $10\,\mu$M-MgATP, Ca^{2+} stimulated enzyme activity in a biphasic manner (Fig. 2, ----). A high-affinity component was apparent between 0.2 and $5\,\mu$M free Ca^{2+}, with an apparent K_m of approx. $0.8\,\mu$M. This high-affinity Ca-ATPase was observed in all membrane preparations studied, and maximal specific activities varied from 8 to 28 nmol of P_i/min per mg of protein, depending on preparation and length of storage. It was also observed at $1.4\,\mu$M-MgATP (Fig. 2, ——). A second, apparently low-affinity component of stimulation was observed above $8\,\mu$M free Ca^{2+} (Fig. 2, ----), only in $10\,\mu$M-MgATP (and total ATP increasing from 69 to $79\,\mu$M; see Table 2). Ca^{2+} stimulation in the presence of millimolar concentrations of total magnesium and ATP was obscured by a very high basal ATPase activity (results not shown), apparently similar to the behaviour of other Ca-ATPases when assayed in millimolar Mg^{2+} (Lambert & Christophe, 1978; Pershadsingh & McDonald, 1980; Verma & Penniston, 1981).

Effect of calmodulin on the Golgi Ca-ATPase

Data from a representative experiment examining the effects of calmodulin at three different concentrations of Ca^{2+} are presented in Table 3.
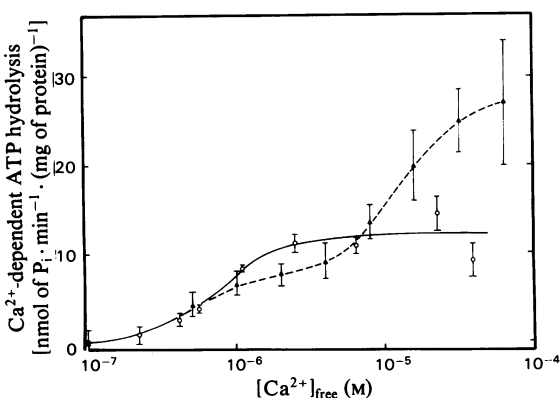

Fig. 2. *Ca^{2+}-activation of ATPase activity in Golgi-enriched fractions*

Basal ATPase activities (at 10 nM free Ca^{2+}) have been subtracted from all data points. ○, $21\,\mu$M free Mg^{2+} and $1.4\,\mu$M-MgATP; ▲, $20\,\mu$M free Mg^{2+} and $10\,\mu$M-MgATP. The total concentrations of ATP, Mg^{2+} and Ca^{2+} in the latter assay are presented in Table 2.

Neither the high-affinity nor the low-affinity component of Golgi Ca-ATPase activities was stimulated significantly by exogenous calmodulin. The only effect observed, even at concentrations that greatly stimulated the murine RBC Ca-ATPase (see below), was an occasional and slight inhibition of the basal ATPase activity.

Ca^{2+}-activation of ATPases from skeletal SR and RBC membranes

Ca-ATPase activities were evident in both SR and RBC plasma membranes (Figs. 3a and 3b). At a saturating concentration of Ca^{2+} and in the presence of $450\,\mu$M free Mg^{2+} and $800\,\mu$M-MgATP, the SR enzyme exhibited a maximum specific activity of 1500 nmol of P_i/min per mg of protein, similar to that of other SR enzymes (Meissner, 1974). The apparent K_m for calcium under these conditions was approx. $1\,\mu$M (Fig. 3a, ●). This value is approx. 5-fold higher than others reported in the literature (e.g. Yamamoto & Tonomura, 1967), a difference which probably reflects the lower Ca–EGTA association constant used in the present study. The maximum specific activity of the well-washed RBC enzyme was only 0.6-fold higher than the basal activity and its affinity for calcium was quite low (Fig. 3b, ○). Exogenous calmodulin almost trebled the enzyme's specific activity and decreased its apparent K_m for Ca^{2+} to $3\,\mu$M (Fig. 3b, ●), a finding consonant with other reports (Scharff, 1979; Vincenzi et al., 1980).

The conditions used for assaying the Golgi Ca-ATPase affected both the SR and calmodulin-enriched RBC enzymes in a similar fashion, increasing their apparent affinities for Ca^{2+} and decreasing their maximum specific activities. The K_m values for Ca^{2+} of the SR and RBC enzymes

Table 3. *Effect of exogenous calmodulin on the Golgi Ca-ATPase*

Enzyme activities are expressed as nmol of P_i/min per mg of protein (mean ± s.e.m., $n = 3$). Assay conditions: $20\,\mu$M free Mg^{2+}, $10\,\mu$M-MgATP and the indicated concentrations of free Ca^{2+} and calmodulin.

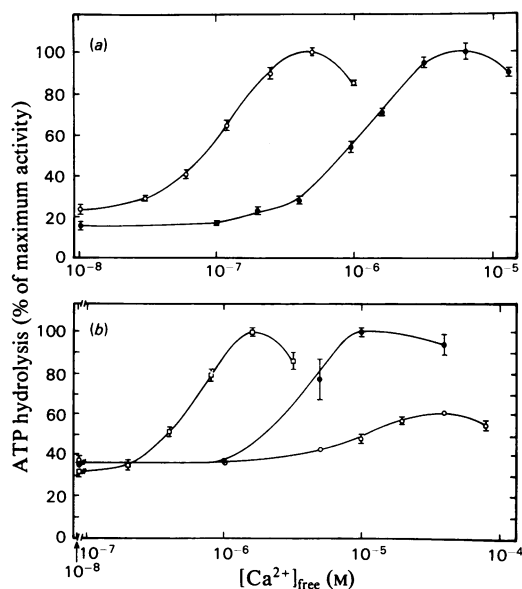| Assay conditions		ATP hydrolysis	
$[Ca^{2+}]_{free}$ (M)	Calmodulin (μg/ml)	Specific activity	Ca^{2+} activation
1×10^{-8}	0.0	20 ± 0.7	
	0.3	16 ± 1.5	
	3.3	16 ± 0.7	
2×10^{-6}	0.0	23 ± 1.5	3 ± 1.7
	0.3	22 ± 0.4	6 ± 1.6
	3.3	20 ± 0.8	4 ± 1.1
6.4×10^{-5}	0.0	34 ± 1.1	11 ± 1.8
	0.3	30 ± 0.7	8 ± 0.9
	3.3	29 ± 1.1	9 ± 1.4

Fig. 3. *Ca²⁺ activation of (a) the skeletal SR Ca-ATPase and (b) the RBC Ca-ATPase*

(a) Values are reported as the percentages of activity evident at maximum Ca²⁺ activation, which were: 154 nmol of P_i/min per mg of protein at 20 μM free Mg²⁺ and 10 μM-MgATP (○); 1800 nmol of P_i/min per mg of protein at 450 μM free Mg²⁺ and 800 μM-MgATP (●). Basal activities (at 10 nM free Ca²⁺) were 37 and 300 nmol of P_i/min per mg of protein respectively. The second assay (●) contained 1.25 mM total Mg²⁺ and 1.0 mM total ATP. (b) Values are reported as the percentage of maximum activity which at 20 μM free Mg²⁺, 10 μM-MgATP and 3.3 μM of added calmodulin/ml (□) was 3.4 nmol of P_i/min per mg of protein. The basal activity for this condition was 1.1 nmol of P_i/min per mg of protein. At 450 μM free Mg²⁺ and 800 μM MgATP (1.25 and 1.0 mM total Mg²⁺ and ATP respectively) plus (●) or minus (○) 3.3 μM of added calmodulin/ml, maximum activity was 55 nmol of P_i/min per mg of protein. Basal activities were 20 and 21 nmol of P_i/min per mg of protein respectively.

Fig. 4. *Effect of TFP on Golgi, SR and RBC Ca-ATPase activities*

All assays were run in 20 μM free Mg²⁺ and 10 μM-MgATP. For the Golgi preparation (△), Ca-ATPase activities represent the difference between enzyme activities observed in 10 nM and 64 μM free Ca²⁺, for the RBC membranes (□), between 10 nM and 32 μM free Ca²⁺, and for the SR preparation (○), between 10 nM and 6.4 μM free Ca²⁺. Values are expressed as the percentage (mean ± S.E.M., n = 3) of the activities observed in the absence of TFP.

Table 4. *Effect of TFP on the Golgi Ca-ATPase*
Enzyme activities are expressed as nmol of P_i/min per mg of protein (mean ± S.E.M., n = 3). Assay conditions: 20 μM free Mg²⁺, 10 μM-MgATP and the indicated concentrations of free Ca²⁺ and trifluoperazine.

Assay conditions		ATP hydrolysis	
$[Ca^{2+}]_{free}$ (M)	TFP (M)	Specific activity	Ca²⁺ activation
1 × 10⁻⁷	0	18 ± 0.5	
	5 × 10⁻⁶	16 ± 0.9	
	5 × 10⁻⁵	8 ± 0.4	
2 × 10⁻⁶	0	27 ± 1.7	10 ± 1.8
	5 × 10⁻⁶	22 ± 0.7	5 ± 1.2
	5 × 10⁻⁵	11 ± 0.7	3 ± 0.8
6.4 × 10⁻⁵	0	31 ± 1.0	4 ± 2.0
	5 × 10⁻⁶	26 ± 0.3	5 ± 0.8
	5 × 10⁻⁵	12 ± 0.4	2 ± 0.4

were respectively 0.1 μM and 0.6 μM (Figs. 3a and 3b; ○, □). Similar increments in Ca²⁺ affinity have been reported for both enzymes and ascribed to decreased Mg²⁺ competition at the Ca²⁺-activation sites (Vianna, 1975; Roufogalis et al., 1982).

Effect of inhibitors on the three Ca-ATPases

Both the SR and the RBC Ca-ATPases were inhibited by TFP, with 50% inhibition occurring at approx. 17 μM (Fig. 4). The Golgi activity exhibited comparable sensitivity, although maximum inhibition was not detected at a concentration (100 μM) that completely inhibit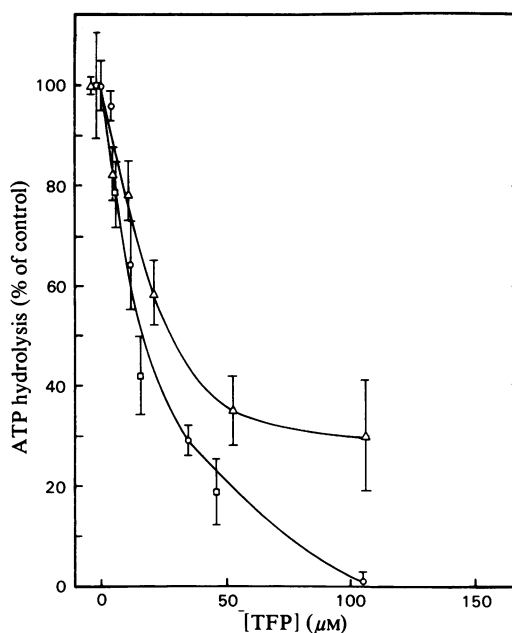ed both the SR and RBC enzymes. Examination of the Golgi preparation indicated that the basal ATPase activity and both high- and low-affinity Ca-ATPase activities were inhibited by the drug (Table 4).

The Golgi enzyme differed rather markedly from the other Ca-ATPases in its relative insensitivity to quercetin (results not shown). The activity of both the SR enzyme and the calmodulin-stimulated component of erythrocyte Ca-ATPase was inhibited 50% at approx. 5 μM. In contrast, no significant inhibition of the Golgi enzyme was observed at concentrations up to 50 μM.

Discussion

The existence of a Ca-ATPase activity with apparently high affinities for both MgATP (Fig. 1) and Ca^{2+} (Fig. 2) is consistent with the presence, in the Golgi-enriched fraction, of an active Ca^{2+} pump (Schuurmans Stekhoven & Bonting, 1981). Given the difficulties surrounding its characterization and the sensitivity of the apparent K_m for Ca^{2+} to assay conditions (see, e.g., Fig. 3), it was important to compare the high-affinity Golgi Ca-ATPase directly with the more thoroughly studied transport enzymes of the SR and RBC plasma membrane. These findings are summarized in Table 5. The Golgi activity more closely resembled the calmodulin-enriched RBC enzyme with respect to its affinity for Ca^{2+} and in its relative insensitivity to K$^+$. It resembled both enzymes in its sensitivity to trifluoperazine, but its apparent M_r of 105000 suggested a greater similarity to the SR Ca-ATPase than to the 150000-M_r enzyme found in plasma membranes (Caroni & Carafoli, 1981). Finally, the Golgi activity was unique in its relative insensitivity to quercetin. These latter properties suggest the high-affinity Ca-ATPase activity is not the result of contamination by a plasma-membrane enzyme, although a similar Golgi preparation contained small amounts of the plasma-membrane marker, (Na$^+$ + K$^+$)-stimulated ATPase (Neville et al., 1981).

It is not clear why the Golgi Ca-ATPase was not detected in millimolar total Mg^{2+} and ATP, conditions which more closely resemble those found in cytoplasm (Burton, 1979). The anomaly may reflect the difficulty inherent in any assay involving a low Ca-ATPase activity and a considerably larger basal activity. Possibly also, Ca^{2+} inhibited the basal activity in a manner that hindered the detection of any Ca^{2+}-stimulated component, similar to the difficulties encountered in a brain microsomal preparation (Saermark & Vilhardt, 1979). In any event, the micromolar Mg^{2+} and ATP employed were higher than the K_m values reported for other Ca^{2+}-transport ATPases (McDonald et al., 1980; Michaelis et al., 1983; Roufogalis et al., 1982; Tada et al., 1978), and it is not unreasonable to suggest that in lowering the considerable basal ATPase activity, these conditions allowed the Golgi enzyme to be detected.

It also was not clear whether the Golgi-enriched fraction contained both low-affinity and high-affinity Ca-ATPases. Systematic investigation was hampered by correlated increments in ATP, CaATP and Ca^{2+} in assays containing more than 5 μM-free Ca^{2+} (see Table 2), and it was difficult to decide whether the low-affinity activity reflected a second Ca-ATPase or, alternatively, the substrate dependence of a non-specific phosphatase. The latter alternative seemed more likely, because only the high-affinity activity was evident at 1.4 μM-MgATP (Fig. 2, ——), a concentration that required a low and constant amount of total ATP (and generated no significant CaATP). It is unlikely the low-affinity activity was produced by the mitochondrial ATPase, because all assays were performed routinely in the presence of the F$_1$-ATPase inhibitor oligomycin.

Finally, these results support the hypothesis that the active transport of Ca^{2+} into Golgi-derived vesicles and, ultimately, into milk, is mediated by a high-affinity Ca-ATPase (Neville et al., 1981). A critical test of the hypothesis awaits a correlative investigation of the kinetic properties of, and effects of inhibitors on, both the purified high-affinity Ca-ATPase and the ATP-dependent Ca^{2+}-transport system of Golgi-enriched membranes.

Table 5. *Comparison of the Golgi, SR and RBC plasma-membrane (PM) Ca-ATPases*

References: (A) Watters & Neville (1981); (B) Shigekawa et al. (1978); (C) Scharff (1978); (D) Neville et al. (1981); (E) MacLennan (1970); (F) Schatzmann & Burgin (1978). Abbreviations used: ND, not determined for Golgi conditions; NE, no effect.

Property	Golgi	SR	RBC-PM
1. Kinetic parameters			
(a) K_m (Ca^{2+}) (μM)			
— Calmodulin	0.8	0.1	ND
+ Calmodulin	NE	ND	0.6
(b) Activation (%) by 100 mM-K$^+$	40 (A)	300 (B)	74 (C)
2. Inhibition (%) by:			
(a) 50 μM-TFP	70	80	80
(b) 50 μM-Quercetin	0	65	65
3. Molecular mass (kDa)	105 (D)	105 (E)	150 (F)

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