

An Investigation of Functional Similarities between the Sarcoplasmic Reticulum and Platelet Calcium-Dependent Adenosinetriphosphatases with the Inhibitors Quercetin and Calmidazolium[†]

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ABSTRACT: The platelet and skeletal sarcoplasmic reticulum calcium-dependent adenosinetriphosphatases (Ca²⁺-ATPases) were functionally compared with respect to substrate activation by steady-state kinetic methods using the inhibitors quercetin and calmidazolium. Quercetin inhibited platelet and sarcoplasmic reticulum Ca²⁺-ATPase activities in a dose-dependent manner with IC₅₀ values of 25 and 10 μ M, respectively. Calmidazolium also inhibited platelet and sarcoplasmic reticulum Ca²⁺-ATPase activities, with half-maximal inhibition measured at 5 and 4 μ M, respectively. Both inhibitors also affected the calcium transport activity of intact platelet microsomes at concentrations similar to those which reduced Ca²⁺-ATPase activity. These inhibitors were then used to examine substrate ligation by the platelet and sarcoplasmic reticulum calcium pump proteins. For both Ca²⁺-ATPase proteins, quercetin has an affinity for the E-Ca₂ (fully ligated with respect to calcium at the exterior high-affinity calcium binding sites, unligated with respect to ATP) conformational state of the protein that is approximately 10-fold greater than for other conformational states in the hydrolytic cycle. Quercetin can thus be considered a competitive inhibitor of the calcium pump proteins with respect to ATP. In contrast to the effect of quercetin, calmidazolium interacts with the platelet and sarcoplasmic reticulum Ca²⁺-ATPases in an uncompetitive manner. The dissociation constants for this inhibitor for the different conformational states of the calcium pump proteins were similar, indicating that calmidazolium has equal affinity for all of the reaction intermediates probed. These observations indicate that the substrate ligation processes are similar for the two pump proteins. This supports the concept that the hydrolytic cycles of the two proteins are comparable.

Microsomal fractions from human blood platelets transport calcium in an energy-dependent manner. Influx of calcium requires adenosine 5'-triphosphate (ATP),¹ is accompanied by the release of inorganic phosphate, is inhibited by agents that inhibit calcium-pumping Ca²⁺-ATPases, and is enhanced by membrane-permeable calcium binding anions (Statland et al., 1969; Robblee et al., 1973; Enouf et al., 1984; Adunyah & Dean, 1986). A Ca²⁺-ATPase activity can be demonstrated functionally (Robblee et al., 1973; Rodan & Feinstein, 1976; Käser-Glanzmann et al., 1978; Javors et al., 1982; Dean & Sullivan, 1982) and immunologically (Dean, 1981; Fischer et al., 1985) in the microsome fraction. The Ca²⁺-ATPase and transport activities in microsomes copurify with platelet subcellular fractions that contain cyclooxygenase activity (Menashi et al., 1982; Carey et al., 1982), a marker for the dense tubular system. This observation and histochemical studies localizing a Ca²⁺-ATPase activity to the dense tubular system (Cutler et al., 1978) have led to the suggestion that the dense tubular system is a calcium-regulating organelle in platelets, like the sarcoplasmic reticulum of muscle (Ikemoto, 1982).

We have been interested in the Ca²⁺-ATPase of the platelet dense tubular system and its functional relationship to the sarcoplasmic reticulum Ca²⁺-ATPase. Both proteins have molecular weights of 103 000 as estimated by NaDodSO₄

electrophoresis (Dean, 1981; Fischer et al., 1985). The platelet Ca²⁺-ATPase cross-reacts with polyclonal antibodies to the sarcoplasmic reticulum Ca²⁺-ATPase (Dean & Sullivan, 1982; Fischer et al., 1985). The platelet and sarcoplasmic reticulum proteins are also activated by calcium and ATP over similar concentration ranges (Javors et al., 1982; Ikemoto, 1982), although a direct substrate activation comparison of the two proteins has not been performed. The platelet Ca²⁺-ATPase is reported to form a phosphoaspartyl intermediate (Le Peuch et al., 1983; Enyedi et al., 1986), as does the sarcoplasmic reticulum Ca²⁺-ATPase (Martonosi, 1969). Despite these similarities, there is evidence that the two pump proteins are structurally different. Structural differences have been noted between the two Ca²⁺-ATPases both with limited proteolysis (Fischer et al., 1985) and with anti-sarcoplasmic reticulum Ca²⁺-ATPase monoclonal antibody probe (Zubrzycka-Gaarn et al., 1984) studies.

The present study was undertaken to compare the catalytic mechanisms of the platelet and sarcoplasmic reticulum calcium pump proteins. The hydrolytic cycle of the sarcoplasmic reticulum Ca²⁺-ATPase has been characterized as abbreviated in Figure 1 (De Meis & Vianna, 1979; Inesi et al., 1980; Tanford et al., 1985). Upon exposure to calcium and ATP, ligation of two calcium ions and ATP occurs in a random manner with the most favorable ligation sequence determined

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¹ Abbreviations: Ca²⁺-ATPase, calcium-dependent adenosinetriphosphatase; ATP, adenosine 5'-triphosphate; EGTA, ethylene glycol bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid; PhCH₂SO₂F, phenylmethanesulfonyl fluoride; NaDodSO₄, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; SAS, statistical analysis system.

terminated by the concentration of ATP and calcium. This type of ligation scheme has been proposed (Inesi et al., 1980; Tanford et al., 1985; Reynolds et al., 1985; Gould et al., 1986) to account for transient and steady-state kinetic measurements (Neet & Green, 1977; Inesi et al., 1980; Moller et al., 1980; Gould et al., 1986) involving substrate activation of the sarcoplasmic reticulum Ca^{2+} -ATPase. The scheme in Figure 1 includes only steps that can be probed by varying calcium and ATP concentration on a steady-state time scale. Thus, the E^* to E transition described by DeMais and Vianna (1979) and the sequence of reactions connecting the $\text{P} \sim \text{E} \cdot \text{Ca}_2$ and E states are not explicitly detailed. The step leading to ADP formation is depicted as irreversible because the ADP concentration in our system was essentially zero in the presence of the ATP regeneration system. Also, internal and external calcium concentrations were equated since leaky microsomes were used.² Finally, the effects of quercetin and calmidazolium on the Ca^{2+} -ATPases were assumed to occur through reversible binding of the inhibitors to various conformational states of the protein, yielding inactive protein-drug complexes.

The reaction sequence in Figure 1 was used to calculate values for some of the equilibrium constants and to generate functions which approximate the substrate activation curves. Using the standard expressions for the equilibrium constants in Figure 1 (K_{1a} , K_{1b} , K_{2a} , K_{2b} , K_{2c} , K_{3a} , and K_{3b} which were defined as association constants, and K_5 , K_α , K_β , K_γ , K_δ , K_ϵ , K_ζ , and K_η , which were defined as dissociation constants), we can express the total enzyme concentration $[\text{E}^0]$ as

$$[\text{E}^0] = (1 + [\text{I}]/K_\alpha)[\text{P} \sim \text{E} \cdot \text{Ca}_2] + (1 + [\text{I}]/K_\beta)[\text{E}] + (1 + [\text{I}]/K_\gamma)[\text{E} \cdot \text{Ca}] + (1 + [\text{I}]/K_\delta)[\text{E} \cdot \text{ATP}] + (1 + [\text{I}]/K_\epsilon)[\text{E} \cdot \text{Ca}_2] + (1 + [\text{I}]/K_\zeta)[\text{E} \cdot \text{ATP} \cdot \text{Ca}] + (1 + [\text{I}]/K_\eta)[\text{E} \cdot \text{Ca}_2 \cdot \text{ATP}]$$

and expressions for the velocity of ADP generation (V and V_{\max}) are

$$V = k_4[\text{E} \cdot \text{Ca}_2 \cdot \text{ATP}]$$

$$V_{\max} = k_4[\text{E}^0]$$

where k_4 is a rate constant. This yields the equation for the normalized reciprocal reaction velocity ($1/V$):

$$1/V = V_{\max}/V = (1 + [\text{I}]/K_\epsilon)/K_{3a}[\text{ATP}] + (1 + [\text{I}]/K_\zeta)/K_{3b}[\text{Ca}] + (1 + [\text{I}]/K_\gamma)/K_{2a}K_{3a}[\text{Ca}][\text{ATP}] + (1 + [\text{I}]/K_\delta)/K_{2b}K_{3b}[\text{Ca}]^2 + (1 + [\text{I}]/K_\beta)/K_{1a}K_{2a}K_{3a}[\text{Ca}]^2[\text{ATP}] + (1 + [\text{I}]/K_\alpha)/K_5K_{1a}K_{2a}K_{3a}[\text{ATP}] + (1 + [\text{I}])/K_\eta \quad (1)$$

where $[\text{Ca}]$ refers to the free Ca^{2+} concentration and $[\text{ATP}]$ refers to the concentration of Mg-ATP.

ATP activation data were used to estimate the values of K_{3a} and K_ϵ . The ATP activation experiments were performed with a fixed free calcium concentration of 78 μM . At this concentration, eq 1 can be approximated as

$$1/V \approx \frac{1 + [\text{I}]/K_\epsilon}{K_{3a}[\text{ATP}]} + (1 + [\text{I}])/K_\eta \quad (2)$$

On the basis of the equilibrium constant estimated by Inesi et al. (1980), each term that has been ignored in obtaining eq 2 from eq 1 contributes less than 1% to the value of $1/V$. These approximations reflect the predominance of the pathway in Figure 1 involving K_{1a} , K_{2a} , and K_{3a} at high calcium concentrations. The first derivative of eq 2 with respect to $1/[\text{ATP}]$ gives

$$d(1/V)/d(1/[\text{ATP}]) = (1 + [\text{I}]/K_\epsilon)/K_{3a} \equiv S \quad (3)$$

Thus

$$K_{3a} = 1/S \text{ at } [\text{I}] = 0 \quad (4)$$

and

$$K_\epsilon = [\text{I}]/(K_{3a}S - 1) \quad (5)$$

Least-squares analysis was performed to measure $d(1/V)/d(1/[\text{ATP}])$ in the presence and absence of inhibitors and to obtain K_{3a} and K_ϵ .

Calcium activation data were used to measure K_{3b} , K_{2b} , K_ζ , and K_η . Free calcium concentrations were varied with an EGTA buffer system (Fabiato & Fabiato, 1979) at a fixed Mg-ATP concentration of 2 mM. At this concentration of ATP, the pathway in Figure 1 involving K_{1b} , K_{2b} , and K_{3b} is favored, and eq 1 can be approximated as

$$1/V \approx 1 + [\text{I}]/K_\eta + (1 + [\text{I}]/K_\zeta)/K_{3b}[\text{Ca}] + (1 + [\text{I}]/K_\delta)/K_{2b}K_{3b}[\text{Ca}]^2 \quad (6)$$

The equilibrium constant estimates of Inesi et al. (1980) indicate that each term that was eliminated from eq 1 contributes less than 1% to the value of $1/V$ at 2 mM ATP. The calcium activation data were expressed in terms of $1/V$ and then fit with a power function to yield numerical values in terms that were zero, first, and second order with respect to $1/[\text{Ca}]$:

$$1/V = A + B(1/[\text{Ca}]) + C(1/[\text{Ca}])^2 \quad (7)$$

Thus

$$A = (1 + [\text{I}])/K_\eta \quad (8)$$

$$B = (1 + [\text{I}]/K_\zeta)/K_{3b} \quad (9)$$

$$C = (1 + [\text{I}]/K_\delta)/K_{2b}K_{3b} \quad (10)$$

and

$$K_\eta = [\text{I}]/(A - 1) \quad (11)$$

$$K_{3b} = 1/B \text{ at } [\text{I}] = 0 \quad (12)$$

$$K_\zeta = [\text{I}]/(BK_{3b} - 1) \quad (13)$$

$$K_{2b} = 1/CK_{3b} \text{ at } [\text{I}] = 0 \quad (14)$$

K_δ was not measured because statistically significant values of C were not obtained in the presence of inhibitors.

Statistical Analysis. Statistical analysis of data, including least-squares analysis and power curve fit, was performed by using the statistical analysis system (SAS) software package (SAS Institute, Cary, NC) and an IBM 4381 mainframe computer. Errors in A , B , C , and S (ΔA , ΔB , ΔC , and ΔS , respectively) were provided by SAS and were based on 90% confidence intervals. The errors in K_{3a} , K_{2b} , K_{3b} , K_η , K_ζ , and K_ϵ (ΔK_{3a} , ΔK_{2b} , ΔK_{3b} , ΔK_η , ΔK_ζ , and ΔK_ϵ , respectively) were calculated from A , B , C , S , ΔA , ΔB , ΔC , and ΔS as follows, with total differential error propagation:

$$\Delta K_{3a} = \Delta S/S^2 \quad (15)$$

$$\Delta K_{3b} = \Delta B/B^2 \quad (16)$$

$$\Delta K_{2b} = \Delta C/C^2K_{3b} + \Delta K_{3b}/CK_{3b}^2 \quad (17)$$

$$\Delta K_\epsilon = [[\text{I}]/(K_{3a}S - 1)^2]\Delta K_{3a} + [[\text{I}]/K_{3a}]/(K_{3a}S - 1)^2\Delta S \quad (18)$$

$$\Delta K_\eta = [\text{I}]\Delta A/(A - 1)^2 \quad (19)$$

$$\Delta K_\zeta = [[\text{I}]/(K_{3b}B - 1)^2]\Delta B + [[\text{I}]/(K_{3b}B - 1)^2]\Delta K_{3b} \quad (20)$$

RESULTS

Dose-response curves for the effect of quercetin on platelet and sarcoplasmic reticulum Ca^{2+} -ATPase activity are pres-

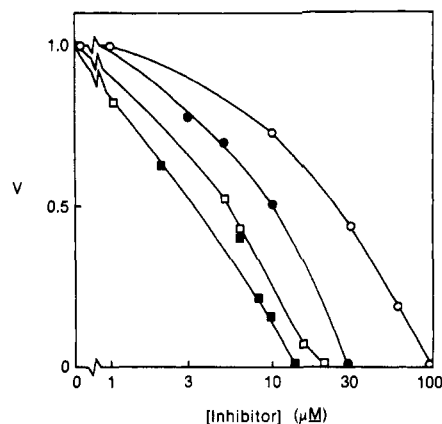


FIGURE 2: Dose-response curve for the effects of quercetin (●, ○) and calmidazolium (■, □) on platelet (○, □) and sarcoplasmic reticulum (●, ■) Ca^{2+} -ATPase activity. Ca^{2+} -ATPase activity (V) was measured as described under Materials and Methods and normalized to the maximum activity in the absence of inhibitors.

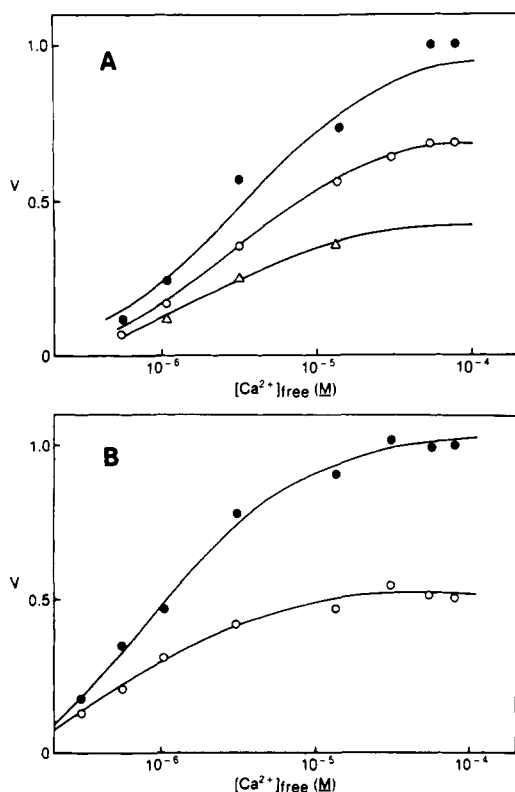


FIGURE 3: (Panel A) Calcium activation curve for the platelet Ca^{2+} -ATPase in the presence of 0 (●), 10 (○), and 30 μM (Δ) quercetin. (Panel B) Calcium activation curve for the sarcoplasmic reticulum Ca^{2+} -ATPase in the presence of 0 (●) and 10 μM (○) quercetin. Ca^{2+} -ATPase activity (V , normalized to the maximal activity in the absence of inhibitor) was measured as detailed under Materials and Methods. The solid curve represents a fit of the data to eq 6 with the parameters in Table I. Free calcium concentration was varied with an EGTA buffer system as detailed under Materials and Methods.

ented in Figure 2. Quercetin inhibited both calcium pump proteins in a dose-dependent manner, with IC_{50} values of 10 and 25 μM , respectively, for the sarcoplasmic reticulum and platelet calcium pump proteins. Figure 2 depicts the effect of increasing concentrations of calmidazolium on the platelet and sarcoplasmic reticulum Ca^{2+} -ATPase activities. As with quercetin, calmidazolium inhibited the calcium pump proteins in a dose-dependent manner with IC_{50} values of 4 and 5 μM for the sarcoplasmic reticulum and platelet Ca^{2+} -ATPases, respectively. In both fractions, the inhibition of Ca^{2+} -ATPase

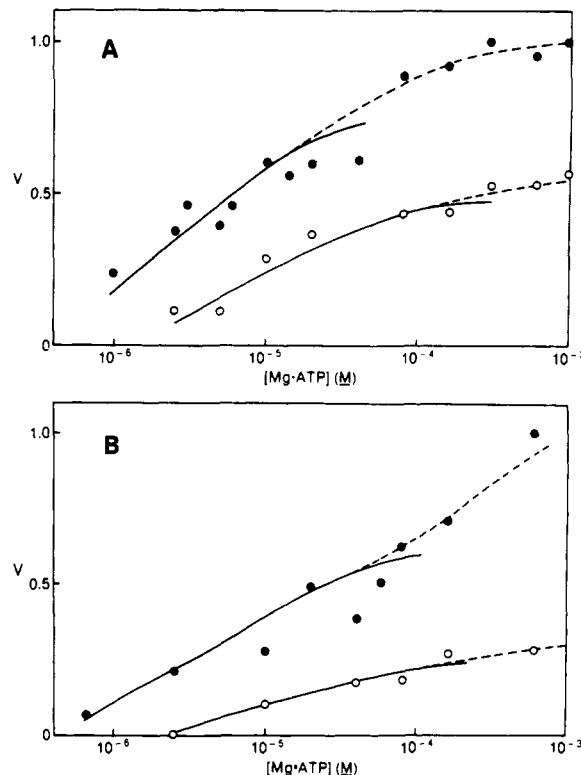


FIGURE 4: (Panel A) Mg-ATP activation curve for the platelet Ca^{2+} -ATPase in the presence of 0 (●) and 10 μM (○) quercetin. (Panel B) Mg-ATP activation curve for the sarcoplasmic reticulum Ca^{2+} -ATPase in the presence of 0 (●) and 10 μM (○) quercetin. Ca^{2+} -ATPase activity (V , normalized to the maximal activity in the absence of inhibitor) was measured as described under Materials and Methods. The solid line represents a fit of the data to eq 2 with the parameters in Table I. The dashed line represents the deviation of data from the fit to eq 2. $\text{Mg}\cdot\text{ATP}$ concentrations were calculated as detailed under Materials and Methods.

activity by the inhibitors was an inverse function of microsome concentration. The higher the microsome concentration, the greater the IC_{50} for inhibition of the Ca^{2+} -ATPase activity. Similar effects have been reported by other investigators with these inhibitors and are probably related to the ability of these hydrophobic drugs to partition into the microsomal membranes at high drug:protein ratios.

The effect of quercetin and calmidazolium on calcium transport by the platelet Ca^{2+} -ATPase was also examined. Both inhibitors reduced the ability of the platelet calcium pump protein to translocate calcium into the interior of the microsomes in a dose-dependent manner with IC_{50} values similar to those for the inhibition of Ca^{2+} -ATPase activity (data not shown).

The effect of quercetin on the activation of the platelet and sarcoplasmic reticulum Ca^{2+} -ATPases by calcium is presented in Figure 3. With both calcium pump proteins, quercetin was a slightly better inhibitor at saturating free calcium concentrations, this effect being more predominant with the sarcoplasmic reticulum Ca^{2+} -ATPase. Figure 4 shows the effect of quercetin on the activation of Ca^{2+} -ATPases with ATP. The calcium pump protein from both platelet and sarcoplasmic reticulum sources was inhibited to a much greater extent at low ATP concentrations than at higher ATP concentrations. A Lineweaver-Burk plot of the data in Figure 4A is shown in Figure 5 and indicates that quercetin inhibition was somewhat competitive with respect to ATP. However, quercetin cannot be considered a purely competitive inhibitor, since inhibition was not eliminated at very high ATP concentrations.

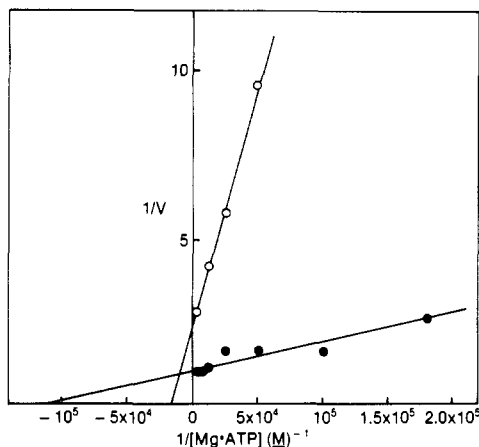


FIGURE 5: Lineweaver-Burk plot for the effect of quercetin on Mg-ATP activation of the platelet Ca^{2+} -ATPase at 0 (●) and 30 μM (○) quercetin. Reciprocal activity ($1/V$) is the inverse of the Ca^{2+} -ATPase activity normalized to the maximal value obtained in the absence of inhibitor. Other details are as described in Figure 4.

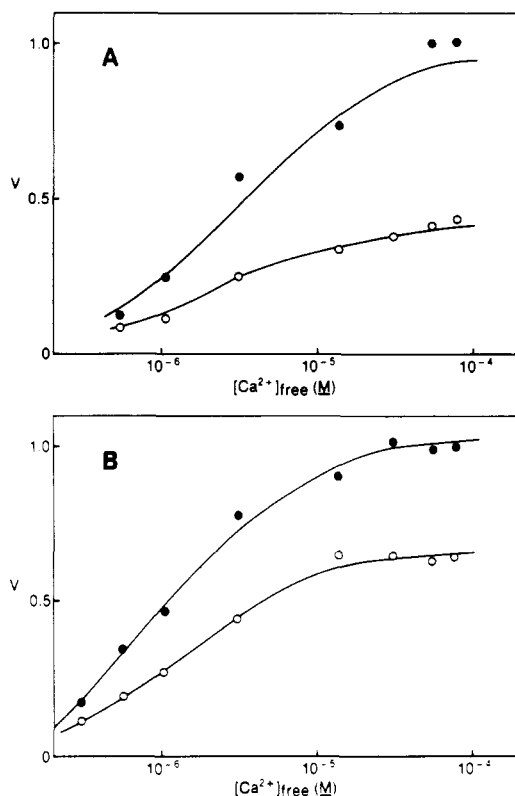


FIGURE 6: (Panel A) Calcium activation curve for the platelet Ca^{2+} -ATPase in the presence of 0 (●) and 6 μM (○) calmidazolium. (Panel B) Calcium activation curve for the sarcoplasmic reticulum Ca^{2+} -ATPase in the presence of 0 (●) and 2 μM (○) calmidazolium. Other details are as in Figure 3.

The effect of calmidazolium on calcium activation of the platelet and sarcoplasmic reticulum Ca^{2+} -ATPases is presented in Figure 6. The degree of inhibition of both calcium pump proteins with calmidazolium was approximately constant over the investigated range of calcium concentrations. Figure 7 depicts the effect of calmidazolium on activation of the Ca^{2+} -ATPases by ATP. At ATP concentrations below approximately 50 μM , the degree of inhibition of both calcium pump proteins was not highly dependent on ATP concentration. As the ATP concentration was increased above 50 μM , the additional activation of the Ca^{2+} -ATPase activity that was measured in the absence of inhibitor was largely eliminated by the calmidazolium.

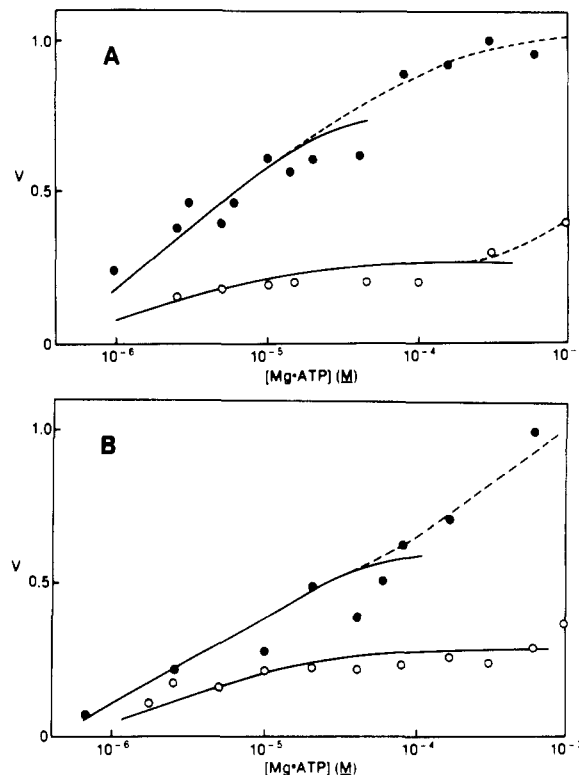


FIGURE 7: (Panel A) Mg-ATP activation curve for the platelet Ca^{2+} -ATPase in the presence of 0 (●) and 6 μM (○) calmidazolium. (Panel B) Mg-ATP activation curve for the sarcoplasmic reticulum Ca^{2+} -ATPase in the presence of 0 (●) and 6 μM (○) calmidazolium. Other details are as in Figure 4.

Table I

parameter	inhibitor	platelet	muscle
K_{ib} (M^{-1})	none	$(6.2 \pm 3.5) \times 10^6$	$(1.8 \pm 1.3) \times 10^7$
K_{3b} (M^{-1})	none	$(3.2 \pm 0.2) \times 10^5$	$(9.2 \pm 0.4) \times 10^5$
K_{3a} (M^{-1})	none	$(2.4 \pm 0.1) \times 10^5$	$(1.4 \pm 0.1) \times 10^5$
IC_{50} (μM)	quercetin	25 ± 3	10 ± 1
K_{η} (μM)	quercetin	27 ± 5	11 ± 2
K_{ϵ} (μM)	quercetin	2.4 ± 0.2	1.3 ± 0.2
K_{ζ} (μM)	quercetin	31 ± 3	14 ± 3
IC_{50} (μM)	calmidazolium	5.0 ± 0.6	4.0 ± 0.3
K_{η} (μM)	calmidazolium	4.7 ± 1.3	3.6 ± 0.5
K_{ϵ} (μM)	calmidazolium	5.3 ± 1.0	3.9 ± 0.3
K_{ζ} (μM)	calmidazolium	7.4 ± 2.6	2.2 ± 1.2

The effect of quercetin and calmidazolium on the Ca^{2+} -ATPases is examined in terms of the reaction scheme presented in Figure 1. This analysis leads to an estimation of apparent dissociation constants for the inhibitors and the Ca^{2+} -ATPases in various conformational states as outlined under Materials and Methods. The analysis using the reaction scheme in Figure 1 also yields functions which approximate the shape of the experimentally determined substrate activation curves (graphs of these curves are presented in Figures 3, 4, 6, and 7). The values of the equilibrium constants in Figure 1 for the platelet and sarcoplasmic reticulum calcium pump proteins that are deduced from the data are presented in Table I.

DISCUSSION

The principal result of this study is that the platelet and sarcoplasmic reticulum Ca^{2+} -ATPases are activated by substrates in a manner that can be modeled by the same catalytic cycle, as depicted in Figure 1. This result is evident by comparing the measurements presented in Table I. The parameters contained in Table I were used in eq 2 and 6 to obtain the solid curves presented in Figures 3, 4, 6, and 7. A good correlation

was obtained between the curves generated from eq 6 and the calcium activation data in Figures 3 and 6. This shows that the sequential addition of calcium to high-affinity binding sites was a reasonable assumption. Upon fitting the calcium activation data to a power series that was zero, first, and second order in $1/[\text{Ca}]$, it was found that the second-order terms contributed substantially to the calcium activation curves only at very low free calcium concentrations (less than $1 \mu\text{M}$ for the platelet Ca^{2+} -ATPase and less than $0.5 \mu\text{M}$ for the sarcoplasmic reticulum Ca^{2+} -ATPase). The experimental data obtained at free calcium concentrations lower than these values were not accurate enough to obtain precise values for K_{2b} . It was thus not worthwhile to use the K_{2b} values obtained in the presence and absence of inhibitors to calculate K_δ . A statistically significant fit between the ATP activation data and eq 2 was obtained only at low ATP concentrations. This deviation potentially reflects the contribution of ATP ligation of E and E-Ca states at higher ATP concentrations, which is not accounted for in eq 2.

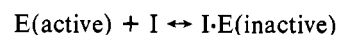
A comparison of the association constants for the platelet and sarcoplasmic reticulum Ca^{2+} -ATPases with the first calcium (K_{2b}), the second calcium (K_{3b}), and ATP (K_{3a}) in the absence of inhibitors shows that the sarcoplasmic reticulum Ca^{2+} -ATPase has a higher affinity for calcium and a lower affinity for ATP than the platelet calcium pump protein. This reflects the displacement of the sarcoplasmic reticulum Ca^{2+} -ATPase calcium activation curve to lower free calcium concentrations than the platelet Ca^{2+} -ATPase calcium activation curve, and the displacement of the sarcoplasmic reticulum Ca^{2+} -ATPase ATP activation curve. Since the scheme in Figure 1 does not contain the E^* to E transition (De Meis & Vianna, 1979), the measured values of K_{2b} , K_{3b} , and K_{3a} could vary by a factor related to $[\text{E}^*]/[\text{E}]$ from the values estimated with non-steady-state methods.

The dissociation constants in Table I determined for quercetin show that this inhibitor has an approximately 10-fold higher affinity for the E-Ca₂ state of both the platelet and sarcoplasmic reticulum calcium pump proteins than the other states. In the E-Ca₂ state, the protein has a high-affinity, unoccupied ATP binding site. This is consistent with the experimental observation that quercetin is a mixed competitive inhibitor with respect to ATP activation. The finding that quercetin does interact with other conformational states of the calcium pump proteins, although with an approximately 10-fold lower affinity, explains the "mixed" nature of the inhibition that results in the fourth-quadrant intersection of $1/V$ vs $1/[\text{ATP}]$ lines in Figure 5. The higher affinity of quercetin for the E-Ca₂ state might also explain the tendency for the drug to be a better inhibitor at higher free calcium concentrations (see Figure 3), which favor the E-Ca₂ state.

The nucleoside antagonist effects of quercetin on the platelet and sarcoplasmic reticulum calcium pump proteins reported in this paper are similar to the effect of this inhibitor on the sarcoplasmic reticulum Ca^{2+} -ATPase detailed by Shoshan et al. (1980) and Shoshan and MacLennan (1981). These investigators reported inhibition of Ca^{2+} -ATPase activity with quercetin over a concentration range similar to that obtained in this paper. Also, Shoshan and MacLennan (1981) showed that quercetin competitively inhibited the binding of ATP to the sarcoplasmic reticulum Ca^{2+} -ATPase. That result is consistent with the result presented in this paper that quercetin selectively binds to the protein state that contains a high-affinity, unoccupied ATP site (E-Ca₂). Since Shoshan and MacLennan reported that quercetin did not reduce the steady-state level of phosphoenzyme over the drug concen-

tration range that inhibits ATP hydrolysis, it is also possible that quercetin binds to phosphorylated forms of the calcium pump protein which have an unoccupied nucleoside site, as in the step described by K_ϵ in Figure 1.

In contrast to quercetin, the dissociation constants in Table I for calmidazolium show that this inhibitor does not preferentially interact with a single conformational state of the platelet or sarcoplasmic reticulum Ca^{2+} -ATPase. The dissociation constants obtained for the interaction of calmidazolium with the various conformational states of the two Ca^{2+} -ATPases were not statistically different, and hypothetical reaction schemes in which there was preferential interaction of calmidazolium with E-Ca₂, E-ATP-Ca, or E-Ca₂-ATP conformational states of the calcium pump proteins did not yield substrate activation curves that fit the data in Figures 6 and 7. Since calmidazolium interacted with similar affinity with each conformation of the Ca^{2+} -ATPases (uncompetitive inhibition), calmidazolium inhibition can be modeled by the reaction:



which is equivalent to the scheme in Figure 1 when $K_\alpha \cong K_\gamma \cong K_\delta \cong K_\epsilon \cong K_\zeta \cong K_\eta$.

An effect of calmidazolium that is not reflected in the parameters in Table I is the ability of the inhibitor to largely eliminate ATP activation of the Ca^{2+} -ATPases at nucleoside concentrations above $100 \mu\text{M}$. This effect occurred with both the platelet and sarcoplasmic reticulum calcium pump proteins and is not reflected in the values of K_γ in Table I because the ATP activation curves could only be fitted to the form of eq 2 at ATP concentrations lower than $100 \mu\text{M}$. The elimination of ATP activation above approximately $100 \mu\text{M}$ in the presence of calmidazolium could result from a drug-induced shift in equilibria K_{1b} and K_{2c} through either a direct effect or distal-linked function (Tanford et al., 1985) effects. Even in the absence of calmidazolium, eq 2 fails to account for ATP activation data above $100 \mu\text{M}$. This probably arises from the contribution of steps described by K_{1b} and K_{2c} in Figure 1 at higher ATP concentrations.

The results of the experiments reported in this paper show that there are functional similarities between the platelet and sarcoplasmic reticulum Ca^{2+} -ATPases. The two calcium pump proteins were activated by substrates in a similar way, and the activation processes were affected by two inhibitors in an equivalent manner. The part of the platelet Ca^{2+} -ATPase hydrolytic cycle that describes substrate ligation can thus be modeled after the extensively studied sarcoplasmic reticulum Ca^{2+} -ATPase hydrolytic cycle. The differences that were noted in this study between the platelet and sarcoplasmic reticulum Ca^{2+} -ATPases in the values of the association constants for protein-substrate interactions could be due to structural differences in the two proteins that have been elucidated with limited proteolytic digestion studies (Fischer et al., 1985). In a more general sense, differences in the affinities of the calcium pump proteins for substrates could reflect differences in the physiological role of the protein in the source tissue, the sarcoplasmic reticulum Ca^{2+} -ATPase being responsible for a rapid repetitive lowering of cytoplasmic calcium concentrations, while the primary function of the platelet Ca^{2+} -ATPase is probably to maintain the cell in a resting state in the absence of activation.

ACKNOWLEDGMENTS

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Registry No. ATPase, 9000-83-3; Mg-ATP, 1476-84-2; Ca, 7440-70-2; quercetin, 117-39-5; calmidazolium, 57265-65-3.

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CORRECTIONS

¹³C NMR Studies of Porphobilinogen Synthase: Observation of Intermediates Bound to a 280 000-Dalton Protein, by Eileen K. Jaffe* and George D. Markham, Volume 26, Number 14, July 14, 1987, pages 4258-4264.

Page 4264. The following reference was inadvertently omitted from the reference list: Smith, D. J., Maggio, E. T., & Kenyon, G. L. (1975) *Biochemistry* 14, 766-771.

Equilibrium and Dynamic Structure of Large, Unilamellar, Unsaturated Acyl Chain Phosphatidylcholine Vesicles. Higher Order Analysis of 1,6-Diphenyl-1,3,5-hexatriene and 1-[4-(Trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene Anisotropy Decay, by Martin Straume and Burton J. Litman*, Volume 26, Number 16, August 11, 1987, pages 5113-5120.

Page 5114. In column 1, the sentence beginning on line 40 should read as follows: The model thus restricts TMA-DPH molecules to an orientation parallel to the bilayer normal.

Characterization of Alternate Reductant Binding and Electron Transfer in the Dopamine β -Monooxygenase Reaction, by Leslie C. Stewart and Judith Pollock Klinman*, Volume 26, Number 17, August 25, 1987, pages 5302-5309.

Page 5304. In Table I, the column headings should read as follows: column 2, $(V_{\max})_H$ (s^{-1}); column 3, $(V_{\max})_D$ (s^{-1}); column 4, $(V/K_{DA})_H$ ($M^{-1} s^{-1}$); column 5, $(V/K_{DA})_D$ ($M^{-1} s^{-1}$); column 6, $(V/K_{O_2})_H$ ($M^{-1} s^{-1}$); column 7, $(V/K_{O_2})_D$ ($M^{-1} s^{-1}$).

Page 5308. In column 1, line 3, Table II should read Table I.

Thermodynamics of the Quenching of Tyrosyl Fluorescence by Dithiothreitol, by Joel K. Swadesh, Philip W. Mui, and Harold A. Scheraga*, Volume 26, Number 18, September 8, 1987, pages 5761-5769.

Page 5763. On the right-hand side of eq 1, the word "log" before the term in square brackets should be deleted.