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Inhibition of basal and calmodulin-activated Ca^{2+}-pump ATPase by fractionated compound 48/80

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Compound 48/80 (48/80), a mixture of polycationic compounds was fractionated using affinity chromatography on calmodulin-Sepharose. Unfractionated 48/80 and various fractions were tested for their potential inhibitory effects on ATPase activities of isolated human red blood cell membranes. ATPase activities tested included: Mg^{2+}-ATPase, the Na^+/K^+-pump ATPase, and the Ca^{2+}-pump ATPase in both its basal (calmodulin-independent) and calmodulin-activated state. Neither 48/80 nor its various fractions were very potent or efficacious inhibitors of the Mg^{2+}-ATPase or the Na^+/K^+-pump ATPase. In agreement with previous reports, 48/80 was found to be an inhibitor of the calmodulin-activated Ca^{2+}-pump ATPase. By contrast, we found that unfractionated, as well as some fractionated, material inhibited both the basal (calmodulin-independent) and calmodulin-activated Ca^{2+}-pump ATPase activity. A fraction designated as Fraction III bound to calmodulin-Sepharose in the presence of Ca^{2+} and low salt and was eluted in the absence of Ca^{2+} and 0.15 M NaCl. By gel filtration, Fraction III had an apparent average molecular weight of 2064 (1320 for unfractionated material). Fraction III was the most potent inhibitor of the Ca^{2+}-pump ATPase with IC_{50} values for the basal and calmodulin-activated forms of the enzyme of 0.6 and 1.2 μg/ml, respectively. Inhibition by Fraction III was cooperative with *n* apparent values of 2.4 and 5.7, respectively, for the basal and calmodulin-activated forms of the enzyme. Thus, binding of 48/80 constituents to calmodulin can not fully account for the observed data. Direct interaction of 48/80 constituent(s) with the enzyme and/or the lipid portion of the membrane is suggested.

Introduction

As early as the first definitive description of the plasma membrane Ca^{2+} pump of the human red blood cell [1] it was noted that there was a need for a potent specific inhibitor. Recently, Gietzen et al. [2] reported that compound 48/80 (48/80), a mixture of polycationic polymeric compounds, is a potent and selective inhibitor of the calmodulin-activated form of the Ca^{2+}-pump ATPase in isolated red blood cell membranes. However, the data of Gietzen et al. [2], and preliminary data obtained in our laboratory, [3] reveal inhibition of other ATPase activities. Considering the heterogeneous nature of 48/80, we reasoned that a more selective antagonist of calmodulin activation of the Ca^{2+}-pump ATPase would be obtained by calmodulin affinity chromatography. We achieved fractionation of 48/80 on calmodulin-Sepharose, but were surprised to find that the material which we predicted to be the most selective antagonist of calmodulin (that which binds to calmodulin in a Ca^{2+}-dependent manner) was instead a potent and selective inhibitor of both the calmodulin-activated and basal forms of the Ca^{2+}-pump ATPase. A report of some of our preliminary results has been presented [3].

Materials and Methods

Compound 48/80 (lot Nos. 45F-0017, 114F-0523 and 886-0257), the peptides (molecular weights in parentheses) angiotensin II (1049), [Arg^8]vasopressin (1084), angiotensin I (1296), bacitracin (1411), colistin (1750), insulin chain A (2530), and other chemicals of reagent grade were purchased from Sigma Chemical Co (St. Louis, MO, U.S.A.). Water used was quartz distilled and de-ionized. All other chemicals were of reagent grade.

Calmodulin was purified was described by Raess and Vincenzi [4]. Calmodulin-Sepharose-4B (calmodulin = 1 mg/ml gel) was prepared according to Klee et al. [5]. Compound 48/80 was fractionated by affinity chromatography on the calmodulin-Sepharose. A column

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(1.5 × 15 cm) was equilibrated with 20 mM morpholinepropanesulfonic acid (Mops) buffer (pH 7.1) containing 0.2 mM $CaCl_2$. 10 mg of 48/80 dissolved in equilibrating buffer were applied to the column. Fractions of 48/80 were eluted stepwise in a minimum of three bed volumes of buffer with and without added salt and ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) at a flow rate of 1.3 ml/min in 5 ml collections. Fraction I was defined as material not bound to the column and eluted with buffer containing 0.2 mM $CaCl_2$. Fraction II eluted with 0.15 M NaCl and 0.2 mM Ca^{2+}. Fractions III, IV, and V eluted with 1.0 mM EGTA and 0.15, 0.5 and 1.0 M NaCl, respectively. The fractions of 48/80 were concentrated by lyophilization and were desalted by gel filtration through Sephadex G-10 prior to suspension in 10 mM Mops (pH 7.1). Osmolarity of solutions was measured by vapor pressure osmometry (Wescor 5100C). Absorbance at 280 nm (6.7 $A = 1$ mg/ml) was used to determine 48/80 concentrations. Chromaticity of 48/80 was independent of pH between 6.7 and 7.5 and was assumed to be constant for all fractions.

The apparent average molecular weights of unfractionated 48/80 and of various fractions of 48/80 were determined by gel filtration Peptides were used to calibrate the column. Void and total bed volumes of the column were determined with myoglobin and sodium azide, respectively. Samples of 48/80 (0.25–0.5 mg) or peptides (0.5–2.5 mg) in 0.5 ml of eluant were applied to a Sephadex G-25 (150 medium) column (1.0 × 45 cm). The elution buffer contained 0.02 M acetic acid buffer adjusted to pH 3.2 with HCl in 20% glycerol. The flow rate was 1 ml/min and 0.6 ml fractions were collected. Elution profiles of 48/80 and peptides were monitored at 280 nm.

The average degree of polymerization of unfractionated and fractionated 48/80 was estimated from the formula:

$$n = ((M - 422)/178) + 2$$

where n is the polymer number of the 48/80 sample (nearest whole integer). This calculation assumes that terminal monomers were comprised of $-CH_2OH$ with a molecular weight of 204 and $-CHO_2H$ of 218. It further assumes internal monomeric units of 178 molecular weight [6].

Calmodulin-deficient red blood cell membranes were prepared from outdated red blood cells by the method of Farrance and Vincenzi [7] with the modification that EGTA (0.5 mM) was included in the 20 mM imidazole buffer (pH 7.4) during hemolysis and the first wash. Activities of Mg^{2+}-ATPase, Na^+/K^+-pump ATPase (measured as ouabain-inhibitable ($Na^+ + K^+ + Mg^{2+}$)-ATPase), and Ca^{2+}-pump ATPase (measured as ($Ca^{2+} + Mg^{2+}$)-ATPase) were assayed in the presence of various concentrations of unfractionated and fractionated 48.80 by the method of Raess and Vincenzi [8] with minor modifications, as noted. Assays contained in a final volume of 0.5 ml: membrane protein (25–50 μg), histidine (18 mM), imidazole (18 mM) buffer (pH 7.1), $MgCl_2$ (3 mM), NaCl (80 mM), KCl (15 mM) EGTA (0.1 mM), and ATP (3 mM) with or without $CaCl_2$ (0.2 mM, provides free Ca^{2+} of approx. 10^{-5} M), ouabain (0.1 mM) and calmodulin (30 nM), as appropriate. The plasma membranes were diluted 1 : 10 in either water or 0.1 mg saponin/ml in water and held at 4C for 15 min prior to use. The reaction mixture with membranes was preincubated at 37°C for 10 min in the presence of 48/80 and then for an additional 10 min in the presence or absence of calmodulin prior to the addition of ATP. After 60 min at 37°C, the reaction was terminated by addition of 0.25 ml of 5% SDS and inorganic phosphate was measured [8]. Membrane protein was measured according to the method of Petersen [9] using bovine serum albumin as the standard. Cooperativity of inhibition of the ($Ca^{2+} + Mg^{2+}$) ATPase by Fraction III was estimated from the best fit linear slopes (n apparent, n_{app}) of Hill plots between 10 and 90% inhibition.

Results

In the absence of 48/80, specific activities of (Mg^{2+})-ATPase and of basal and calmodulin-activated Ca^{2+}-pump ATPase for a typical membrane preparation were 4.4, 16.7 and 58.5 nmol inorganic phosphate/min per mg membrane protein (nmol/min/mg), respectively (Fig. 1). Unfractionated 48/80 (lot No. 114F-0523) inhibited calmodulin-activated Ca^{2+}-pump ATPase activity with an IC_{50} of 9.8 μg/ml. However, at this concentration, basal Ca^{2+}-pump ATPase activity was inhibited by 36%; and Mg^{2+}-ATPase activity by 32%. Inhibition of basal Ca^{2+}-pump

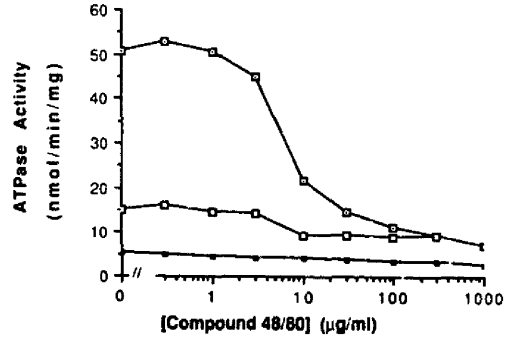

Fig. 1. Inhibition of ATPase activities by unfractionated compound 48/80 (48/80). Semi-logarithmic plot of Mg^{2+} (■) and calmodulin-activated (□) and basal (□) Ca^{2+}-pump ATPase activities in the presence of various concentrations of 48/80. The results demonstrate the relatively selective inhibition of the calmodulin-activated ATPase by 48/80, as reported by Gietzen et al. [2].

ATPase activity occurred mainly between 1 and 10 μg/ml 48.80. Above 10 μg/ml the concentration-effect relationship became less steep and at 1000 μg/ml inhibition was 49%. Mg^{2+}-ATPase and ($Na^+ + K^+ + Mg^{2+}$)-ATPase activities (data not shown) decreased steadily over the concentration range tested. The results demonstrate the relatively selective inhibition by unfractionated 48/80 of the calmodulin-activated Ca^{2+}-pump ATPase activity of isolated red blood cell membranes. However, there ws also inhibition of other ATPase activities. In particular, there was inhibition of basal Ca^{2+}-pump ATPase activity between 1 and 10 μg/ml, and some inhibition of Mg^{2+}-ATPase and Na^+/K^+-pump ATPase at these and higher concentrations.

We observed minor batch to batch differences in potency of unfractionated 48/80 (data not shown), but all three batches tested gave qualitatively similar results. These data are similar to those of Gietzen et al. (1983) [2] and were included to emphasize that 48/80 inhibited more that just calmodulin activation of the Ca^{2+}-pump ATPase and to provide results with unfractionated 48/80 to facilitate comparison with results obtained using fractionated 48/80.

Fig. 2 is a typical elution profile of calmodulin-Sepharose fractionation of 48/80. The fractions were defined as: (I) the flow through of 48/80 in 20 mM Mops with 0.2 mM $CaCl_2$, (II) the fraction eluted upon the addition of 150 mM NaCl to the Ca^{2+}-containing buffer, (III) the fraction eluted in the presence of 1 mM EGTA without added $CaCl_2$ in 150 mM NaCl buffer, (IV) the fraction eluted when NaCl was added to a concentration of 0.5 M and (V) the fraction eluted when NaCl was added to a concentration of 1.0 M. When 5 mg of 48/80 was added to the column the total amount of the fractions other than Fraction I, was reduced by half. This suggests that a 10 mg sample did not saturate the binding capacity of the column.

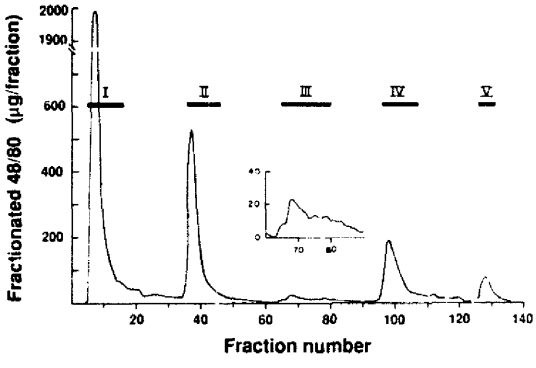

Fig. 2. Calmodulin-Sepharose fractionation of 48/80. Data are plotted as the concentration of fractionated material as a function of fraction number. The inset shows Fraction III in more detail. Fraction I failed to bind to calmodulin-Sepharose in the presence of 20 mM Mops buffer containing 0.2 mM $CaCl_2$. Fraction II was eluted by the addition of 150 mM NaCl. Fraction III was eluted by the removal of $CaCl_2$, and the addition of 1 mM EGTA. Fraction IV was eluted by increasing the NaCl concentration to 500 mM, and Fraction V eluted by increasing the NaCl concentration to 1000 mM. In the typical experiment shown here, total recovery was 93%. The average recovery in five such fractionations was 91% of the total and the average portion of recovered material was 57.5, 22.4, 3.1, 13.8 and 3.3% for Fractions I–V, respectively.

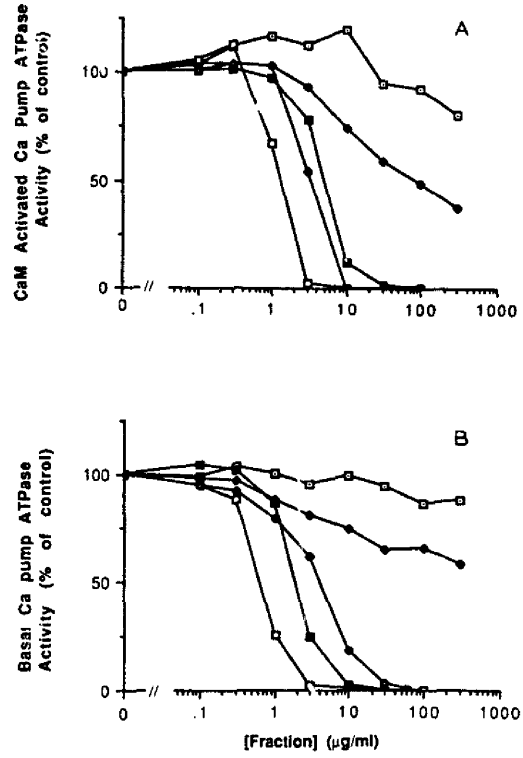

Fig. 3. Inhibition of calmodulin-activated (A) and basal (B) Ca^{2+}-pump ATPase by fractionated 48/80. Semi-logarithmic plot of calmodulin-activated ATPase activity in the presence of various concentrations of Fractions I (□), II (◆), III (□), IV (◇), and V (■). The results demonstrate significant differences in the potency and efficacy of the various fractions, with Fraction III being the most potent. The results also demonstrate that fractionated 48/80 inhibited both forms of the enzyme more or less equally over a wide range of concentrations.

Fig. 3 presents concentration-effect data for fractionated 48/80 acting upon the calmodulin activated and basal Ca^{2+}-pump ATPase. The various fractions of 48/80 showed differences from each other, and from unfractionated 48/80 (Fig. 1), in inhibition of the ATPase activities. As expected, Fraction III produced complete inhibition of the calmodulin activated Ca^{2+}-pump ATPase. But in an unexpected result, Fraction III also completely inhibited the basal Ca^{2+}-pump ATPase, and Fractions IV and V also inhibited both the calmodulin activated and basal Ca^{2+}-pump ATPase. With minor exceptions the respective fractions were more or less equipotent in inhibition of the calmodulin-activated and basal enzyme activities. Fractions III, IV, and V were up to 3-fold more potent than unfractionated 48/80 in inhibition of both calmodulin-

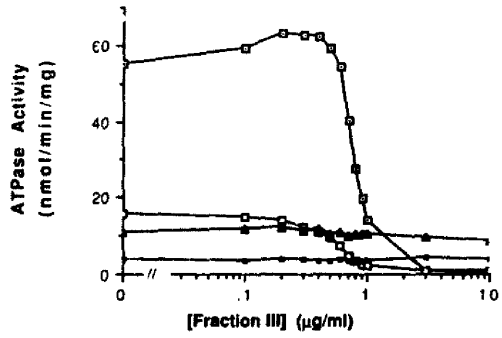

Fig. 4. Inhibition of ATPase activities by Fraction III. Semi-logarithmic plot of Mg^{2+}-ATPase (■), Na^+/K^+-pump ATPase (▲) and calmodulin-activated (□) and basal (□) Ca^{2+}-pump ATPase activities in the presence of various concentrations of 48.80. The results demonstrate relatively selective inhibition of the Ca^{2+}-pump ATPase by Fraction III, both in its basal and calmodulin-activated states. The Mg^{2+}-ATPase and Na^+/K^+-pump ATPase activities were resistant to inhibition by Fraction III. At low concentrations, Fraction III produced slight activation of the calmodulin-activated Ca^{2+}-pump ATPase activity.

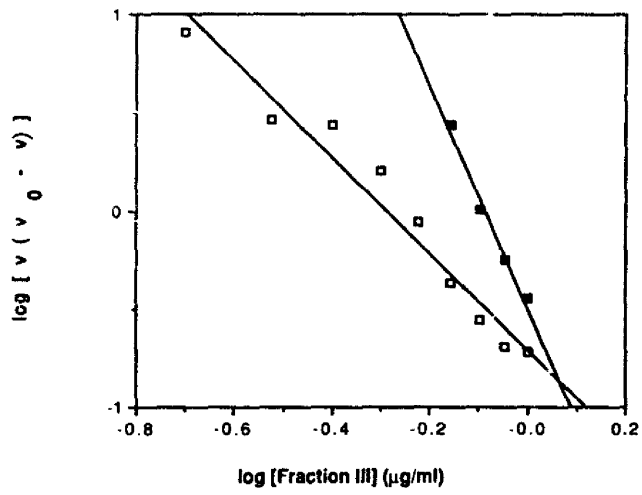

Fig. 5. Hill plot of data shown in Fig. 4, for the calmodulin-activated (■) and basal (□) Ca^{2+}-pump ATPase activities. Data points plotted here include only those for the 10% to 90% inhibition range. Hill slope values were obtained by linear best fit to these data points. The apparent Hill coefficients were 5.7 and 2.4 for the calmodulin-activated and basal Ca^{2+}-pump ATPase activities, respectively.

activated and basal Ca^{2+}-pump ATPase activities. By contrast, Fraction II was 8-fold less potent than unfractionated 48/80. Fraction I was the least effective inhibitor. The results show that Fractions I and II produced only limited inhibition of the enzyme over the range of concentrations tested.

Considering the effectiveness of Fraction III on the calmodulin-activated Ca^{2+}-pump ATPase, specificity was tested by assaying all ATPases in the presence of various concentrations of Fraction III. As shown in Fig. 4, Fraction III produced little or no inhibition of the Mg^{2+}-ATPase or the Na^+/K^+-pump ATPase. And, as already shown in Fig. 3, both the basal and calmodulin-activated forms of the Ca^{2+}-pump ATPase were completely inhibited. However, the concentration-effect relationship for calmodulin activated Ca^{2+}-pump ATPase was more complex than that of the basal enzyme. Between 0.1 and 0.5 µg/ml the activity of calmodulin activated enzyme was slightly higher than drug free control. Maximal augmentation (17%) was observed at 0.3 µg/ml. A decrease in basal enzyme activity occurred over the corresponding concentration range. Above 0.5 µg/ml, both basal and calmodulin-activated activities decreased precipitously. Fraction IV was approximately equipotent in inhibition of the two forms of the enzyme. Both basal and calmodulin-activated activities were inhibited essentially 100% at 10 µg/ml. At this concentration, inhibition of Mg^{2+}-ATPase was less than 8% and inhibition of Na^+/K^+-pump ATPase was about 20%.

As shown in Figs. 3 and 4, inhibition of ATPase activity by Fraction III occurred over a narrow concentration range. Hill plots of inhibition data for both the basal and calmodulin-activated Ca^{2+}-pump ATPase activities were prepared and are presented in Fig. 5. The IC_{50} values taken from Hill plots of basal and calmodulin-induced activity were 0.52 and 0.82 µg/ml, respectively for the data shown in Fig. 4. Whether in the absence or presence of added calmodulin, the enzyme exhibited apparent positive cooperativity (n = 2.4 without and 5.7 with added calmodulin) for inhibition by Fraction III. In a typical experiment, fractions which bound to calmodulin-Sepharose in a Ca^{2+}- and/or salt-dependent manner showed higher n_{app} values (5.7, 3.6, and 6.0, respectively, for Fractions III, IV, and V) than Fraction II (0.56) which did not bind, or unfractionated 48/80 (0.59).

It was of interest to learn some characteristics of the materials which had been separated by calmodulin-Sepharose affinity chromatography, and which were potent

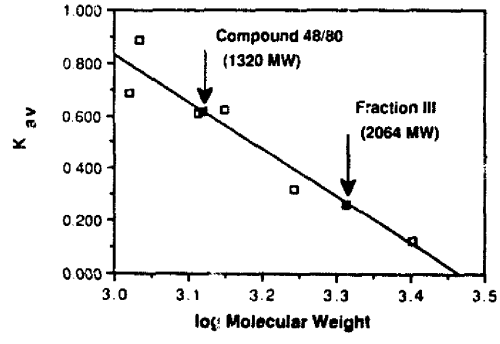

Fig. 6. Gel filtration determination of 48/80 molecular weight. Gel filtration on Sephadex G-25, as described in Methods. The peptide standards (□) and two examples of 48/80 (■) are plotted here. The average apparent molecular weight of Fraction III was much higher than the average molecular weight of the unfractionated 48/80. Values for all fractions are presented in Table I.

TABLE I

Apparent molecular weights of fractionated 48/80

	K_{av} [a]	M_r [b]	n [c]
Unfractionated	0.165	1320	7
Fraction I	0.593	1357	7
Fraction II	0.540	1452	8
Fraction III	0.265	2064	11
Fraction IV	0.249	2106	12
Fraction V	0.265	2064	11

[a] Determined from gel filtration as shown in Fig. 6.
[b] Apparent average molecular weight.
[c] Apparent average polymer number.

inhibitors of the Ca^{2+}-pump ATPase. Some fundamental information is presented in Fig. 6 and in Table I. Fig. 6 shows an example of gel filtration data which were used to obtain estimates of the average molecular weights of unfractionated and fractionated 48/80. Unfractionated 48/80 and Fractions I–V, when chromatographed by gel filtration, eluted as single broad peaks (data not shown). Trailing beyond the bed volume was observed for each preparation in preliminary work, but for the results presented here this trailing was reduced in the presence of 20% glycerol. As shown in Fig. 6, the elution volume at peak height for unfractionated 48/80 corresponded to an apparent average molecular weight of 1320. Likewise that of Fraction III corresponded to an apparent average molecular weight of 2064. Fractions I–V had apparent average molecular weights which ranged between 1357 and 2106 (filtration data not shown). Molecular weight data for all fractions and the unfractionated material are presented in Table I. From the apparent molecular weight of unfractionated 48/80, the average degree of polymerization (n) was estimated to be 7. While the precise chemical nature of the fractionated materials was not defined it is clear that the average molecular weights of the materials which were potent inhibitors of the Ca^{2+}-pump ATPase were considerably higher than the average molecular weight of the unfractionated 48/80.

Discussion

A wide variety of amphipathic cationic compounds are able to exert anti-calmodulin effects in a variety of systems [10–12]. Anti-calmodulin agents act by binding to the calmodulin-$(Ca^{2+})_n$ complex, through surface hydrophobicity [13]. Thus, anti-calmodulin agents are viewed as competing with the calmodulin binding protein for binding to the activator and/or by virtue of binding to the calmodulin-$(Ca^{2+})_n$ complex.

Compound 48/80 is produced by reaction of equimolar concentrations of formaldehyde and *p*-methoxy-*N*-methylphenylethylamine [14]. This results in a mixture of compounds which are both hydrophobic and polycationic. Thus, 48/80 appears to be a typical anti-calmodulin agent. It was suggested by Gietzen et al. [2] that 48/80 is more selective than other anti-calmodulin drugs (for example, trifluoperazine) because its polycationic nature limits its entry into the phospholipid phase of the membrane. Presumably, this also limits non-specific inhibition of the basal Ca^{2+}-pump ATPase, and the Na^+/K^+-pump ATPase and the Mg^{2+}-ATPase. While this view may be valid, the present data seem to cast the findings with 48/80 in a new light.

In the present study, and a preliminary report of related work [3], unfractionated 48/80 was found to be a potent antagonist of calmodulin-activated Ca^{2+}-pump ATPase and a partial inhibitor of other ATPases. We obtained several fractions of 48/80 which bound to calmodulin-Sepharose in a Ca^{2+}-dependent manner. These fractions, Fraction III, IV and V, were each more potent than unfractionated 48/80 in inhibiting the calmodulin-activated Ca^{2+}-pump ATPase. However, instead of the predicted decrease in inhibition of basal Ca^{2+}-pump ATPase activity, these fractions were potent and efficacious inhibitors of the presumably calmodulin-free basal enzyme. In further discussion we will consider mainly Fraction III, the most potent of these fractions.

It seems unlikely that endogenous, or otherwise tightly bound calmodulin, could account for inhibition by Fraction III of the basal Ca^{2+}-pump ATPase. Early work on calmodulin activation of the Ca^{2+}-pump ATPase and transport demonstrated that membranes as we prepare them have very little calmodulin [7,15] and that the basal activity of the Ca^{2+} pump in the membrane is an inherent property of the enzyme entirely independent of calmodulin [16,17].

Adamczyk-Engelmann and Gietzen [18,19] reported calmodulin-Sepharose chromatography of 48/80 under similar conditions to yield a fraction, which bound in a Ca^{2+}-dependent manner and which eluted in high salt. Their fraction so obtained was more potent in antagonizing calmodulin than was the unfractionated material. However, they did not report inhibition of basal Ca^{2+}-pump ATPase activity by the same fraction. We have no explanation of our differing result. Presumably, some subtle difference in methodologies resulted in a different fractionation of material in the two laboratories. Differing initial starting materials (which are obviously heterogeneous) could also be responsible, but we think that unlikely since we have obtained similar results with at least three different lot numbers of 48/80.

Mechanism(s) of inhibition of $(Ca^{2+} + Mg^{2+})$-ATPase by Fractions III–V remain to be determined. Unfractionated 48/80 only partially inhibited the basal activity (Fig. 1), whereas the fractionated material completely inhibited it (Fig. 3). It appears that 'protection'

from inhibition of the basal ATPase may be present in the unfractionated material. It might be suggested that the unfractionated 48/80 is a mixture of 'partial agonist' and 'full agonist' inhibitors of the ATPase. Such a mixture, and its separation, could account for the data. In any event, Fraction III contains material which inhibits the Ca^{2+}-pump ATPase irrespective of the presence of calmodulin. Thus, simple binding to calmodulin is not the mechanism of action of inhibition. Fraction III produced complete inhibition of the enzyme at 3 μg/ml. If it is assumed that the average molecular weight of Fraction III is 2064 (Fig. 6), then this is equivalent to 1.45 μM Fraction III; an approximately 50-fold excess over the calmodulin used to activate the enzyme. Even if Fraction III were found to exhibit anti-calmodulin properties in other enzyme systems, that mechanism of action could not account for the data shown in Figs. 3, 4 and 5. Inhibition of the Ca^{2+}-pump ATPase by Fraction III must be by some other, as yet undetermined, mechanism. We are tempted to suggest a direct interaction of at least some component of Fraction III with the enzyme or nearby phospholipids. This is similar to suggestions that drugs such as trifluoperazine may influence the enzyme not only by binding to calmodulin [20,21]. The slight activation of calmodulin activated Ca^{2+}-pump ATPase by Fraction III at low concentrations (Fig. 4) could be a reflection of the action of Fraction III at some non-calmodulin site.

Synthesis of 48/80 yields various sized oligomers with terminal monomers having different functional groups (H, CH_2OH, or CH_2Cl, for example) [14]. Fractions of 48/80 may have separated to some extent based on functional group properties which might account for differing potencies and efficacies of Fractions III–V, as compared to Fractions I and II or unfractionated 48/80, for example. Separation of Fraction I and II from Fractions III–V appears to have been largely size dependent. Differences in potency and efficacy of 48/80 fractions appeared to be related to polymer size. Fractions III–V with an average polymer length approximately 50% longer than Fractions I and II (Table I) were more potent inhibitors of the Ca^{2+}-pump ATPase.

The Ca^{2+}-pump ATPase appears to exist as a dimer [22], each subunit containing a single binding site for calmodulin [23]. Calmodulin is readily dissociated in the presence of EGTA. The apparent positive cooperativity of Fraction III in inhibiting the basal (Hill plot $n = 2.4$) and calmodulin activated (Hill plot $n = 5.7$) activities suggests to us that Fraction III (or some components of Fraction III) might interact with multiple sites on the enzyme, possibly enhanced by calmodulin. Other interpretations are possible, including effects on the lipid portions of the membrane. Experiments with the isolated enzyme might help to resolve these speculations.

It seems unlikely that non-specific perturbation of the lipid environment is the basis of Ca^{2+}-pump ATPase inhibition by Fraction III. First, as noted by Gietzen et al. [2] 48/80 is not likely to enter the membrane. Second, if perturbation of the lipid phase of the membrane were the basis of the profound effects of Fraction III, then we might have expected other ATPase activities to have been affected. While unfractionated 48/80 did display some inhibition of other ATPases, Fraction III produced only minimal inhibition of the Mg^{2+}-ATPase or Na^+/K^+-pump ATPase.

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