The Active Species of Plasma Membrane Ca\textsuperscript{2+}-ATPase Are a Dimer and a Monomer-Calmodulin Complex*

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The purified plasma membrane Ca\textsuperscript{2+}-ATPase is fully activated through the enzyme concentration-dependent self-association at physiologically relevant Ca\textsuperscript{2+} concentrations (Kosk-Kosicka, D., and Bzdaga, T. (1988) J. Biol. Chem. 263, 18184-18189; Kosk-Kosicka, D., Bzdaga, T., and Wawrzynow, A. (1989) J. Biol. Chem. 264, 19495-19499). We have previously shown that the Ca\textsuperscript{2+}-ATPase activity of the oligomeric enzyme is independent of calmodulin, in contrast to another active enzyme species, a presumable monomer, that is activated by calmodulin binding. Presently, we have succeeded in determining the molecular mass of the two active enzyme species by equilibrium ultracentrifugation. For the calmodulin-dependent species, the molecular mass is 170 ± 30 kDa, which is consistent with predominantly monomeric Ca\textsuperscript{2+}-ATPase with bound calmodulin. The molecular mass of calmodulin-independent oligomers is 260 ± 34 kDa, indicating that they are dimers. Results of experiments performed under different calcium and potassium concentrations and in the presence of dextran that causes molecular crowding verify a strict Ca\textsuperscript{2+} requirement of the dimerization process. We conclude that the active species of the Ca\textsuperscript{2+}-ATPase are a monomer-calmodulin complex and a dimer.

The erythrocyte Ca\textsuperscript{2+}-ATPase is a plasma membrane Ca\textsuperscript{2+} pump responsible for Ca\textsuperscript{2+} removal from the cytoplasm to maintain the steep Ca\textsuperscript{2+} concentration gradient. The protein exhibits an M\textsubscript{s} of 134,000 upon SDS-gel electrophoresis, in agreement with its primary sequence (for review, see Ref. 3). It has been shown recently that the erythrocyte enzyme consists of two isoforms encoded by two different genes designated PMCA1 and PMCA4 (4–6). The isoforms are expressed in all of the tissues recently tested by Western blot analysis and are believed to be housekeeping pump isoforms, whereas PMCA2 and PMCA3, which are absent from erythrocyte plasma membrane, are more specialized (7).

We have demonstrated that the Ca\textsuperscript{2+} pump isolated from human erythrocytes undergoes reversible, enzyme concentration-dependent oligomerization that reaches maximal levels at 30–40 mM enzyme (1, 2). The oligomerization process produces a highly cooperative Ca\textsuperscript{2+}-regulated activation of the enzyme at physiologically relevant calcium concentrations (K\textsubscript{Ca\textsuperscript{2+}} = 50 nM) (2, 8). Using measurements of fluorescence resonance energy transfer between appropriately labeled enzyme molecules, we were able to differentiate two active species of the enzyme, which we defined as oligomers and monomers with bound calmodulin (2). Calmodulin can activate the enzyme, apparently by binding to the monomeric form. Calmodulin does not increase the activity of enzyme oligomers, and the resonance energy transfer measurements indicate that calmodulin does not dissociate preformed oligomers (9). Instead, calmodulin-monomer complex formation appears to be competitive with a monomer to oligomer transition, and both enzyme species are fully active. The extent of oligomerization and thus the structure of the active forms of the enzyme could not be determined by the methods used previously, and it is this point that is addressed here.

Some of these data have been reported in a preliminary form (10).

MATERIALS AND METHODS

Egg yolk phosphatidylcholine (PS763) and CNBr-activated Sepharose 4B and dextran (D4751) were purchased from Sigma, and octaethylene glycol dodecyl ether (C\textsubscript{12}E\textsubscript{8}) was obtained from Nikko (Tokyo, Japan). Coupling of bovine calmodulin to Sepharose was performed in accordance with Pharmacia Biotech Inc. instructions as described earlier (1).

The methods used for preparation of erythrocyte ghost membranes, purification of the Ca\textsuperscript{2+}-ATPase from the membranes, and determination of protein and Ca\textsuperscript{2+} concentrations were as described previously (1, 11). Free Ca\textsuperscript{2+} concentrations were calculated from total calcium and EGTA concentrations based on the constants given by Schwartzbach et al. (12, 13). Total calcium was measured by atomic absorption.

Ca\textsuperscript{2+}-ATPase Activity—This was determined by colorimetric measurement of inorganic phosphate production as described previously (1). The standard reaction mixture contained 50 mM Tris maleate, pH 7.4, 120 mM KCl, 8 mM MgCl\textsubscript{2}, 3 mM ATP, 1 mM EGTA, and CaCl\textsubscript{2}, at a concentration yielding 17.5 µM free Ca\textsuperscript{2+}. In some experiments, KCl concentration was reduced to 20 mM and free Ca\textsuperscript{2+} was 100 nM as specified in Tables II and III. The concentration of C\textsubscript{12}E\textsubscript{8} was kept constant at 150 µM. The enzyme was either 15 or 50 nM as indicated in Table II, and calmodulin, when present, was 100 nM. Total reaction volume was 100 µl. The reaction was started with 3 mM ATP and carried out for up to 30 min at 37 or 25 °C. Aliquots were withdrawn at various times for colorimetric inorganic phosphate measurement. Steady-state velocities were obtained from plots of inorganic phosphate production that were linear with time.

Ultracentrifugation—The state of oligomerization of the enzyme was determined by equilibrium ultracentrifugation. The studies were performed using both a Beckman model E analytical ultracentrifuge and a model XLA. Both studies used absorbance optics for the analysis. The instruments are described in detail below followed by a description of the sample preparation, centrifugation protocol, and data treatment.

Bedekar Model E Ultracentrifuge—The model used for these studies was equipped with a xenon arc light source, a Beckman DU monochromator, a standard Beckman scanner, modifications to minimize background light, and a digital data collection system. All of these modifications are described in detail elsewhere (14). All runs used double sector scanner cells with elevated bases and quartz windows. Samples were usually run in a Ti AN-F 4-place rotor. Scans were performed at 280 nm, and background scans were collected at 350 nm. The slit width on this instrument is quite broad (2 mm), giving a spectral bandwidth

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of 10–20 nm, centered on the chosen wavelength.

XLA Instrument—This was a standard Beckman Ultragamma XLA analytical ultracentrifuge. Runs were performed with the 3-sample (6-hole) centerpieces with quartz windows. Cells were centrifuged in the 4-place, An-60-Ti rotor. The monochromator of the XLA provides much higher spectral resolution than is possible with the model E. The bandwidth is about 2 nm. Additionally, the XLA optics allow analysis at wavelengths as short as 190 nm. We were unable to use the lower wavelengths available due to high buffer absorbance. Scans were typically taken at 236, 280, and 350 nm. Following recording of the baseline scan at equilibrium, the rotor speed was increased to delete the meniscus region of the sample column, and the cells were rescanned to provide an estimate of the baseline absorbance value.

Example Preparation—Enzyme solutions were prepared for analysis by thawing an aliquot of the enzyme stored in the elution buffer (10 mM Tris maleate, pH 7.4, 120 mM KCl, 5 mM EGTA, 0.5 mM MgCl2, 0.75 mM C12E8, 20% glycerol, 2 mM dithiothreitol) at 25°C, but no differences were seen. The enzyme was kept in the standard reaction mixture for the indicated length of time after which 95 μl were withdrawn for the Ca2+/ATPase assay that was initiated by addition of 5 μl of ATP. The reaction mixture and assay conditions were as described under “Materials and Methods.” The Ca2+/ATPase reaction was terminated after 30 min. The whole procedure was performed at 20 and 25°C, and similar results were obtained.

| Time (h) | Activity (%) |
|---------|-------------|
| 0       | 100         |
| 0.5     | 78          |
| 1.5     | 78          |
| 3       | 68          |
| 4       | 60          |
| 6       | 55          |
| 24      | 38          |

Once correct activity was observed, the enzyme was terminated by incubating at high pH (8.5) for 15 min. Total time between thawing the enzyme and recording the baseline absorbance was estimated to be approximately 4 h. Standard conditions (50 mM Tris maleate, pH 7.4, 120 mM KCl, 5 mM EGTA, 0.5 mM MgCl2, 0.75 mM C12E8, 20% glycerol, 2 mM dithiothreitol) at ~80°C and diluting it appropriately in the reaction mixture with additions required for a given experiment (denoted in Tables II and III as solution conditions). The length of time between thawing and diluting the enzyme and placing the loaded rotor into the centrifuge was kept to a minimum, typically ~15 min. Total time between thawing the enzyme and recording the equilibrium concentration gradient was approximately 4 h.

Centrifugation Protocol—The loaded rotor was placed directly into the centrifuge and accelerated to about 2,000 rpm. A scan was taken at this speed to provide a base line of absorbance and to check for any rapidly sedimenting, large aggregates, which were not found. The rotor speed was then increased to 12,000–15,000 rpm, and scans were taken at approximately 45-min intervals until subtracting one scan from the previous scan gave a flat line, indicating no change during the intervening 45 min (14). Scans were taken at this point for calculation. At the end of the run, the rotor speed was increased to 35,000 rpm for 45 min to delete the meniscus. Scans were then taken to provide an estimate of the baseline absorbance (non-sedimentable absorbance due to buffer). Most runs were performed at 20°C. Some early runs were at 25°C, but no differences were seen.

There were two technical problems posed in the application of this technique to the Ca2+/ATPase system. First, the low optical density presented by 50 mM enzyme meant that detection of signal over background was difficult. Second, the stability of the enzyme upon dilution in standard buffer conditions placed limits on the possible length of experiments and therefore the available time to reach equilibrium.

The stability of the enzyme required that analysis be performed as quickly as possible after thawing the enzyme. This requirement was accomplished by using small sample volumes (40 μl) that yielded short column heights (~1 mm) and consequently rapid attainment of equilibrium (15). These small samples could be accommodated in both instruments used. About 4 h of centrifugation proved to be enough to reach equilibrium. Under these conditions, the enzyme lost about 30% activity during this time, as shown in Table I. The problem presented by low optical density was reduced by averaging multiple scans. In the case of the model E data collected at 280 nm, multiple scans of each cell were taken at equilibrium, aligned at the meniscus, and points at corresponding radial positions were averaged. In the case of the XLA data collected at 230 nm, data at each radial position were averages of several determinations, as set by the control parameters of the scans. Multiple experiments were performed in this way on both instruments.

Data Treatment—At sedimentation equilibrium, the gradient of concentration (c) of a molecule as a function of radius (r) from the center of rotation is given by

$$c_r = c_0 \exp(MBC) (r^2 - r_0^2)$$

(Eq. 1)

where $$r_0$$ is the radius of a reference point such as the base of the cell and $$c_0$$ is the concentration of the molecule at that point. M is the weight average molecular mass of the molecule, B is the buoyancy factor (discussed below), and $$C = \omega^2RT$$, where $$\omega$$ is the angular velocity, R is the gas constant, and T is the absolute temperature. The data (absorbance versus radius) will then be of the form

$$A_r = A_{\text{abs}} \exp(MBC) (r^2 - r_0^2) + e$$

(Eq. 2)

where $$A_{\text{abs}}$$ is the absorbance at radius r, $$A_{\text{abs}}$$ is the absorbance at the base of the cell due to the protein, and e is a base-line absorbance term. The buoyancy factor B is defined as

$$B = (1 - \nu p)$$

(Eq. 3)

where $$v$$ is the partial specific volume of the molecule and $$\rho$$ is the solution density. If the protein has bound detergent, an additional term must be added to correct for the buoyancy of the detergent, and B becomes

$$B = (1 - \nu p) + \delta_e (1 - \nu p)$$

(Eq. 4)

where $$\delta_e$$ is the amount (g) of detergent bound per g of protein and $$\nu_p$$ is the partial specific volume of the detergent.

In the absence of measured values for the amount of bound detergent, $$\delta_e$$, centrifugation is commonly carried out in solutions of varying density, allowing extrapolation to the condition where the solution density $$\rho = 1/A_e$$. At this point, the second term in B becomes 0, and only the protein partial specific volume, $$\nu_p$$, influences the observed buoyant molecular weight (16). The same result is obtained if the detergent used has partial specific volume equal to 1. This is the case here since the buffer is not much different from water (measuring $$\rho_0 = 1.014 \text{ g/cm}^3$$) and the detergent, C12E8, has partial specific volume, $$\nu = 0.973$$ (17). With this detergent in low salt buffer, the bound detergent makes little contribution to the buoyancy factor B, and thus the second term in B may be taken as 0 (18). Data were thus fit to Equation 2 with B defined as in Equation 3.

Data from the model E were analyzed using the non-linear least squares fitting routines of MLAB (Civilized Software, Bethesda, MD) and a logarithmic form of Equation 2, as described previously (15). Error estimates given are the standard error estimates given by MLAB. Standard error estimates are not calculated by the Beckman software with the same fitting results as obtained with MLAB. Standard error estimates are not calculated by the Beckman software with the same fitting results as obtained with MLAB. Standard error estimates are not calculated by the Beckman software with the same fitting results as obtained with MLAB.

RESULTS

We undertook the determination of the molecular mass of different forms of the erythrocyte Ca2+/ATPase, the oligomeric form with calmodulin-independent activity and the form with calmodulin-dependent activity previously termed monomer. To characterize the enzyme, we have applied analytical ultracentrifugation that is a rigorous means of determining the mass of covalent and non-covalent macromolecular complexes (20, 21). Due to the technical difficulty of obtaining data from samples containing very diluted proteins, the lowest enzyme concentra-
Ca\(^{2+}\) and K\(^+\) effects on Ca\(^{2+}\)-ATPase activity, its stimulation by calmodulin, and the effect of dextran at two enzyme concentrations. Ca\(^{2+}\)-ATPase activity assays were performed as described under "Materials and Methods." Final concentration of dextran was 10%, and it was achieved by addition of aliquots of freshly prepared solution of 30% dextran.

Table II: Ca\(^{2+}\)-ATPase activity

| Solution condition set | Enzyme concentration | Ca\(^{2+}\) | K\(^+\) | +Calmodulin | +Calmodulin |
|------------------------|----------------------|-------------|--------|-------------|-------------|
|                        | m\(\text{M}\) | m\(\text{M}\) | m\(\text{M}\) | m\(\text{M}\) | m\(\text{M}\) |
| 1                      | 50 | 17.5 | 120 | 200 | 210 |
| 2                      | 50 | 17.5 | 120 | 210 | 220 |
| 3                      | 50 | 0.1 | 20 | 40 | 40 |
| 4                      | 15 | 17.5 | 120 | 100 | 190 |
| 5                      | 15 | 0.1 | 20 | 15 | 45 |

Ca\(^{2+}\)-ATPase activity

\(\mu\text{mol Pi/mg protein/h}\)

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Ca\(^{2+}\)-ATPase requires calmodulin for activation, suggesting that it is monomeric (1, 9) (compare also with set 4 in Table II). Dextran had no effect on the 50 nM (dimeric) enzyme (Table II, set 1; for details, see also Ref. 22). Addition of dextran to the 50 nM enzyme at low K\(^+\)/Ca\(^{2+}\) concentrations condition was derived from experiments with dextran. Dextran was used because its addition to the Ca\(^{2+}\)-ATPase at 15 nM concentration (at which concentration the Ca\(^{2+}\)-ATPase requires calmodulin for activation, suggesting that it is monomeric) induced self-association of the enzyme as judged by fluorescence resonance energy transfer experiments (22), and thus led to full, calmodulin-independent activity (Table II, set 4). Dextran had no effect on the 50 nM (dimeric) enzyme (Table II, set 1; for details, see also Ref. 22). Addition of dextran to the 50 nM enzyme at low K\(^+\)/Ca\(^{2+}\) concentrations in

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The enzyme is dimeric at concentrations that yield full activity. Enzyme solutions were prepared at 50 nM in standard buffer (Tris maleate, pH 7.4, 120 mM KCl, 17 mM NaCl, 1 mM EGTA, 8 mM MgCl\(_2\), 150 \(\mu\text{M}\) Ca\(^{2+}\), 1 mM C\(_{12}\)E\(_8\) i.e. standard reaction mixture (condition 1 in Tables II and III), and centrifuged to equilibrium in the model E (A) or the XLA ultracentrifuge (B). Attainment of equilibrium was determined by difference scans as described under "Materials and Methods." At equilibrium, scans were taken at 280 nm (A) or 230 nm (B). The scans presented are typical of those obtained from multiple runs. The upper section of each panel presents the data and fit and the lower sections show the differences between the data and the fit. In panel A, the solid curve shows the calculated gradient of absorbance for the best fit mass of 255 kDa. In panel B, the solid curve shows the calculated gradient corresponding to the best fit mass of 288 kDa. O.D., optical density.
Ca\textsuperscript{2+} determinethesizesofthetwospeciesoftheplasmamembrane the labile protein tubulin (14, 15) made it possible to finally cations introduced previously to analyze the dimerization of the absence of calmodulin (Table II, set 3) did not increase Ca\textsuperscript{2+}-ATPase activity, indicating that the apparent molecular mass of about 180 kDa (Table III, set 3) is that of monomers that are prevalent under these conditions. Apparently at low calcium, the enzyme simply cannot self-associate. Also, at low enzyme concentration (15 nM) in the presence of low K\textsuperscript{+}/Ca\textsuperscript{2+}, the Ca\textsuperscript{2+}-ATPase activity (Table II, set 5) is not fully activated in the presence of dextran, again indicating that insufficient calcium hinders dimerization. Potassium concentration appeared to have no significant effect on enzyme dimerization since the apparent molecular mass of the 50 nM enzyme in the presence of high K\textsuperscript{+} (but also in the absence of Ca\textsuperscript{2+}) was again about 170 kDa (Table III).

### DISCUSSION

The application of analytical centrifugation with the modifications introduced previously to analyze the dimerization of the labile protein tubulin (14, 15) made it possible to finally determine the sizes of the two species of the plasma membrane Ca\textsuperscript{2+}-ATPase whose contribution to enzyme activity was demonstrated several years ago (1, 2, 8, 9, 23). We have now established that the fully active calmodulin-independent enzyme is dimeric. We have also confirmed that the species activated by calmodulin is indeed a monomer-calmodulin complex.

Previous attempts to establish the sizes were hampered by the low enzyme concentrations at which the transformation from calmodulin-dependent monomers to the calmodulin-independent dimers of Ca\textsuperscript{2+}-ATPase occur (K\textsubscript{d} = 8–15 nM enzyme, see Refs. 1 and 2).

The fact that the centrifuge data were collected on two different instruments, which was a necessity due to a coincidence in timing, proved to be an advantage allowing us to compare two independently obtained data sets. The model E and the model XLA are quite different instruments, but they yielded comparable results. The model E has a very wide spectral bandpass and was essentially limited to 280 nm for these experiments. This presented a problem of low absorbance at this wavelength of the Ca\textsuperscript{2+}-ATPase at 50 nM. The XLA, in contrast, has a narrow spectral bandpass and allowed wavelengths as low as 190 nm to be used for analysis. Additionally, the XLA data acquisition system is considerably simpler to use and much more powerful than the one we had on the model E. Lower wavelengths presented a significantly higher sample absorbance but also presented a significantly increased buffer absorbance background. A compromise of 230 nm was chosen. This allowed a reasonable sample absorbance to be recorded but introduced significant noise in the baseline determination, requiring a different approach to error estimation. Despite these differences and the problems common to both, experiments with the two instruments provided results that indicate the same biological result. The molecular mass of the enzyme in the absence of calmodulin is consistent with the predominance of dimers (Fig. 1), while the molecular mass of the enzyme in the presence of calmodulin is consistent with monomer-calmodulin complexes (Fig. 2). Within the 4 h required for equilibration, the molecular mass was never significantly higher than that expected for enzyme dimers, thus indicating absence of a meaningful amount of oligomers of a higher size than of dimers. In addition, the analytical centrifugation confirmed two previous findings that have been made using fluorescence spectroscopy methods (fluorescence energy transfer and polarization measurements). First, activation by enzyme dimerization is a Ca\textsuperscript{2+}-dependent process (2). Second, our data indicated that the equilibrium between enzyme monomers and dimers and the availability of calmodulin to the two enzyme forms determine the activation pathway of the purified Ca\textsuperscript{2+}-ATPase (9). Accordingly, the distinctly lowered molecular mass of 50 nM enzyme that was centrifuged to equilibrium at insufficient Ca\textsuperscript{2+} indicates a predominantly monomeric population in agreement with the extent of dimerization that is limited by the availability of Ca\textsuperscript{2+}. Second, in the presence of superstoechiometric calmodulin at optimal Ca\textsuperscript{2+}, the enzyme at 50 nM concentration after 4 h equilibrated to yield molecular mass that is consistent with monomer-calmodulin species rather than with dimers (Fig. 2). This finding could be plausibly explained by the differences in the affinities of the enzyme-calmodulin and the enzyme-enzyme interactions. The apparent affinity of enzyme monomer for calmodulin is higher (K\textsubscript{d} = 1.6–3.5 nM) (1, 24) than its affinity for another monomer (K\textsubscript{d} = 8–15 nM enzyme) (2). While the affinity for calmodulin binding to the dimer is not known, there are multiple experimental

### Table III

| Solution condition set | Model E data | Model XLA data | Combined data |
|------------------------|--------------|----------------|---------------|
| 1                       | Standard     |                |               |
| 2                       | K\textsuperscript{+}/Ca\textsuperscript{2+} |                |               |
| 3                       | K\textsuperscript{+}/no Ca\textsuperscript{2+} |                |               |
| 4                       | High K\textsuperscript{+}/Ca\textsuperscript{2+} |                |               |

a. Solution compositions were as described under "Materials and Methods" for Ca\textsuperscript{2+}-ATPase assay, except that ATP was omitted. Conditions for set numbers 1–3 were as in Table II, respectively. Enzyme was 50 nM in all samples.

b. Data are best fit weight average masses, ± S.D. The numbers in parentheses are the number of complete experiments.

**FIG. 2.** Calmodulin forms a complex with monomeric Ca\textsuperscript{2+}-ATPase. Enzyme solutions were prepared exactly as in Fig. 1 except that calmodulin was added at a concentration (100 nM) superstoichiometric to that of the enzyme (50 nM), i.e. condition 2, Table II. Panel A presents a typical scan taken at 280 nm with the model E, and the solid curve shows the calculated gradient for the best fit mass of 190 kDa. Panel B presents a typical scan taken at 230 nm with the XLA. The solid curve here shows the gradient for the best fit mass of 208 kDa. In both panels, the lower sections show the residuals from the fits. O.D., optical density.
demonstrations of such binding (1, 23). In fluorescence spectrophotometry measurements of 5-min duration, an addition of calmodulin to the dimers formed between donor-labeled and acceptor-labeled enzyme molecules did not decrease energy transfer, suggesting that calmodulin did not dissociate them. However, calmodulin prevented increased energy transfer resulting from dimerization if it was added to 28 nM donor-labeled enzyme before the addition of 28 nM acceptor-labeled enzyme (9).

The results generated by analytical ultracentrifugation are consistent not only with fluorescence spectroscopy measurements but also with the results produced by another approach, induction of enzyme self-association by molecular crowding in the presence of dextran. We have previously demonstrated that dextran induces oligomerization of enzyme molecules at concentrations that are too low (beginning with 6 nM enzyme) for significant self-association under standard conditions (22). At present, we have shown that at insufficient Ca$^{2+}$ even dextran cannot force the enzyme to dimerize.

In conclusion, the two active species of erythrocyte Ca$^{2+}$-ATPase identified 7 years ago can now be recognized as a dimer and a monomer-calmodulin complex.

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REFERENCES
1. Kosk-Kosicka, D., and Bzdega, T. (1988) J. Biol. Chem. 263, 18184–18189
2. Kosk-Kosicka, D., Bzdega, T., and Wawrzynow, A. (1989) J. Biol. Chem. 264, 19495–19499
3. Carafoli, E. (1994) FASEB J. 8, 993–1002
4. Shull, G. E., and Grieb, J. (1988) J. Biol. Chem. 263, 8646–8657
5. Verma, A. K., Filoteo, A. G., Stanford, D. R., Wieben, E. D., Penniston, J. T., Strehler, E. E., Fisher, R., Heim, R., Vogel, G., Mathews, S., Strehler-Page, M. A., James, P., Vorherr, T., Krebs, J., and Carafoli, E. (1988) J. Biol. Chem. 263, 14152–14159
6. Strehler, E. E., James, P., Fisher, R., Heim, R., Vogel, G., Filoteo, A. G., Penniston, J. T., and Carafoli, E. (1990) J. Biol. Chem. 265, 2835–2842
7. Stauffer, T. P., Guerini, D., and Carafoli, E. (1995) J. Biol. Chem. 270, 12184–12190
8. Kosk-Kosicka, D., Bzdega, T., and Johnson, J. D. (1990) Biochemistry 29, 1875–1879
9. Kosk-Kosicka, D., and Bzdega, T. (1990) Biochemistry 29, 3771–3777
10. Sackett, D. L., and Kosk-Kosicka, D. (1993) Biophys. J. 64, A334
11. Kosk-Kosicka, D., Scaillet, S., and Inesi, G. (1986) J. Biol. Chem. 261, 3333–3338
12. Fabiato, A., and Fabiato, F. (1979) J. Physiol. (Paris) 75, 463–505
13. Schwartz, G. A., Zenn, H., and Anderf, G. (1957) Helv. Chim. Acta 40, 1886–1900
14. Sackett, D. L., Lippoldt, R. E., Gibson, C., and Lewis, M. (1989) Anal. Biochem. 180, 319–325
15. Sackett, D. L., and Lippoldt, R. E. (1991) Biochemistry 30, 3511–3517
16. Reynolds, J. A., and McCaslin, D. R. (1983) Methods Enzymol. 117, 41–53
17. Tanford, C., Notoki, Y., and Rohde, M. F. (1977) J. Phys. Chem. 81, 1553–1560
18. McCaslin, D. R., and Tanford, C. (1981) Biochemistry 20, 5212–5221
19. Cohn, E. J., and Edsall, J. T. (1943) Proteins, Amino Acids, and Peptides, pp. 370–381, Van Nostrand Reinhold, New York
20. Harding, S. E., Rowe, A. J., and Horton, J. C. (eds) (1992) Analytical Ultracentrifugation in Biochemistry and Polymer Science, Royal Society of Chemistry, Cambridge, UK
21. Schuster, T. M., and Laue, T. M. (eds) (1994) Modern Analytical Ultracentrifugation, Birkhauser, Cambridge, MA
22. Kosk-Kosicka, D., Lopez, M. M., Filoteo, A. G., and Lew, V. L. (1995) FEBS Lett. 371, 57–60
23. Kosk-Kosicka, D., Bzdega, T., Wawrzynow, A., Scaillet, S., Nemec, K., Johnson, J. D. (1990) Adv. Exp. Med. Biol. 269, 169–171
24. Graf, E., and Penniston, J. T. (1981) Arch. Biochem. Biophys. 210, 257–262
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