Distinct Topologies of Mono- and Decavanadate Binding and Photo-oxidative Cleavage in the Sarcoplasmic Reticulum ATPase*

Received for publication, April 14, 2000, and in revised form, July 6, 2000
Published, JBC Papers in Press, July 20, 2000, DOI 10.1074/jbc.M003218200

Suming Hua and Giuseppe Inesi‡
From the Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland 21201

Chikashi Toyoshima
From the Institute of Molecular and Cellular Biosciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

UV irradiation of the sarcoplasmic reticulum (SR) ATPase in the presence of vanadate cleaves the enzyme at either of two different sites. Under conditions favoring the presence of monovanadate, and in the presence of Ca^{2+}, ADP, and Mg^{2+}, cleavage results in two fragments of 71- and 38-kDa electrophoretic mobility. On the other hand, under conditions permitting formation of decavanadate, and in the absence of Ca^{2+} and ADP, cleavage results in two fragments of 88- and 21-kDa electrophoretic mobility. The amino terminus resulting from cleavage is blocked and resistant to Edman degradation. However, the initial photo-oxidation product can be reduced with NaB{H}_4, resulting in incorporation of radioactive ^3H label. Extensive digestion of the labeled protein with trypsin then yields labeled peptides that are specific for the each of the photo-oxidation conditions, and can be sequenced after purification. Collection of the Edman reaction fractional products reveals the radioactive label and demonstrates that Thr^{353} is the residue oxidized by monovanadate at the phosphorylation site (i.e. Asp^{351}). Correct positioning of monovanadate at the phosphorylation site requires binding of Mg^{2+} and ADP to the Ca^{2+}-dependent conformation of the enzyme. Subsequent hydrolytic cleavage is likely assisted by the neighboring Asp^{601}, and yields the 71- and 38-kDa fragments. On the other hand, Ser^{186} (and possibly the following three residues: Val^{187}, Ile^{188}, and Lys^{189}) is the residue that is photo-oxidized by decavanadate in the absence of ADP. Hydrolytic cleavage of the oxidized product at this site is likely assisted by neighboring acidic residues, and yields the 88- and 21-kDa fragments. The bound decavanadate, which we find to produce steric interference with TNP-AMP binding, must therefore extend to the A domain (i.e. small cytosolic loop) in order to oxidize Ser^{186}. This protein conformation is only obtained in the absence of Ca^{2+}.

The catalytic mechanism of the Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) includes a covalent phosphorylated enzyme intermediate which is formed by transfer of ATP terminal phosphate to an aspartyl residue (Asp^{351}) at the catalytic site (1, 2). Several kinetic studies have suggested that, in the absence of ATP, orthovanadate can bind to the SR ATPase and form a transition state analog of the phosphorylated intermediate (3–5). On the other hand, decavanadate has been very useful in stabilizing bidimensional crystals of the SR ATPase (6), thereby rendering possible electron crystallographic studies (7, 8). It is not entirely clear whether vanadate monomers and oligomers, which are known to coexist in various experimental conditions (9), occupy the phosphorylation and/or distinct sites in the SR ATPase (10). We considered that this uncertainty may be clarified by studies of vanadate-dependent ATPase cleavage, which was reported to occur following UV irradiation at different sites depending on the presence or the absence of Ca^{2+} (11, 12). However, the cleavage sites and the mechanism of cleavage could not be characterized in previous studies on the SR ATPase, due to amino terminus blockage in the cleaved fragments (11, 12).

Detailed clarification of the mechanism of vanadate-dependent cleavage was recently obtained by studies on photo-oxidation and cleavage of the vanadate-myosin complex (13–15). In analogy to the cation transport ATPases, the mechanism of myosin ATPase includes complexation of the ATP terminal phosphate, which is involved in energy transduction. Although the myosin-phosphate complex does not include a covalent bond, it is still possible to obtain its transition state analog by reaction of myosin with orthovanadate. UV irradiation of such a complex produces photo-oxidation of Ser^{186}, followed by rearrangement and peptide cleavage. Photocleavage of the vanadate complex was also obtained with the F1 mitochondrial ATPase (16, 17). In this case cleavage occurs at the level of an alanine residue (Ala^{158}) of the catalytic subunit. Taking advantage of the experience gained with these systems, we then performed a series of experiments in which we clarified the mechanism of cleavage in the SR ATPase, determined unambiguously the residues undergoing photo-oxidation and cleavage, and identified two distinct binding sites for monovanadate and decavanadate.

MATERIALS AND METHODS

SR vesicles were obtained with the microsomal fraction of rabbit leg muscle homogenate, as described by Eletr and Inesi (18). Protein concentration was determined by the method of Lowry (19). SDS-electrophoresis was carried out according to Weber and Osborn (20), and protein staining was obtained with Coomassie Blue. TNP-AMP was fonic acid; TNP-AMP, 2-(or 3)-O-(trinitrophenyl)adenosine-5’-monophosphate, sodium salt; A, N, and P domains, small cytosolic, nucleotide binding, and phosphorylation domains, respectively.

* This work was supported by National Institutes of Health Program Project HL27867 and by grants-in-aid for scientific research and for international scientific research from the Ministry of Education, Science, Sports and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed. Tel.: 410-706-3220; Fax: 410-706-8297; E-mail: ginesi@umaryland.edu.
1 The abbreviations used are: SR, sarcoplasmic reticulum; HPLC, high pressure liquid chromatography; MOPS, 4-morpholinepropanesulfonic acid; TNP-AMP, 2-(or 3)-O-(trinitrophenyl)adenosine-5’-monophosphate, sodium salt; A, N, and P domains, small cytosolic, nucleotide binding, and phosphorylation domains, respectively.
purchased from Molecular Biospheres. Orthovanadate solutions were prepared as described by Ko et al. (16, 17), dissolving Na3VO4 (Sigma) in water, and adjusting the pH to 10 (orange color). This solution was boiled until it became clear (2 min), and the pH readjusted to 10, repeating this procedure three times. The orthovanadate concentration was determined by using an extinction coefficient of 2925 M⁻¹ cm⁻¹ at 265 nm wavelength. The stock solution used in these experiments was 20 mM, and was stored at –80 °C under aluminum foil cover. Decavanadate was obtained by acidiﬁcation of a 20 mM Na3VO4 solution to pH 2.0, cooling in ice, and then adjusting the pH to 6.5. The presence of decavanadate was revealed by a strong yellow color (21).

Vanadate-dependent photo-oxidation of ATPase was carried out at pH 8.6, in a reaction mixture containing 1 mg/ml microsomal protein, 50 mM Tris-HCl buffer, pH 8.6, 100 mM KCl, 5 mM MgCl2, 1 mM ADP, 1 mM vanadate, and either 0.1 mM CaCl2 or 1 mM EGTA. Alternatively, the reaction mixture contained 20 mM MOPS, pH 7.4, 100 mM KCl, 5 mM MgCl2, 1 mM vanadate, and either 1 mM EGTA or 0.1 mM CaCl2. The vanadate concentrations are always given as “monovanadate,” although oligovanadate is present at neutral pH. Following a 30-min preincubation, the mixture was illuminated for 15 min with long wavelength UV light, and then the protein was subjected to enzymatic or electrophoretic analysis.

For reduction with borohydride, the photo-oxidized samples were diluted 1:25 with 10 mM MOPS, pH 7.0, and 10% sucrose. The microsomes were then centrifuged at 100,000 × g for 50 min, and the sediment obtained by centrifugation at 100,000 × g was resuspended in 0.5 ml of 50 mM Tris-HCl buffer, pH 8.6, and 100 mM NaBH4 (522 mM cm⁻¹ mol⁻¹). Following incubation with borohydride for 1 h, the samples were diluted 1:25 with 10 mM MOPS, pH 7.0, and 10% sucrose. The microsomes were then sedimented and resuspended in 0.5 ml of 50 mM Tris-Cl, pH 8.1, 0.25 mM sucrose, and trypsin (trypsin:microsomal protein = 1:10). Following a 2-h incubation at 37 °C, the mixture was centrifuged at 100,000 × g for 1 h, and the supernatant was digested with additional trypsin (trypsin:original microsomal protein = 1:50) for 4 h at 37 °C. The resulting peptide fragments were then placed in a Waters HPLC system equipped with two 510 pumps, automated gradient controller 680, and a 490E multi-wavelength detector. Separation was obtained with an acetonitrile gradient containing 0.1% trifluoroacetic acid, followed by purification with an acetonitrile gradient containing 10 mM ammonium acetate. The elution fractions were characterized by light absorption at 215 nm for peptide content, and by liquid scintillation spectrometry for determination of radioactivity. The radioactive fraction was then subjected to two further runs of HPLC purification. Sequencing of the radioactive peptide fragments was performed at Johns Hopkins University by Judie Franklin, and repeated for fractional collection of Edman degradation products at the Protein Chemistry Core Laboratory of Baylor University by Dr. Richard G. Cook.

RESULTS

Monovanadate- and Decavanadate-dependent Cleavage of SR ATPase—Consistent with previous reports (3–5), we found that vanadate affects strongly the SR ATPase, producing complete inhibition at 0.1 mM concentration (data not shown). As reported by Vegh et al. (11) and Molnar et al. (12), exposure of the ATPase-vanadate complex to UV light at neutral pH yields a pattern of peptide cleavage that is dependent on the presence or the absence of Ca2+: two fragments of 88- and 21-kDa electrophoretic mobility in the absence of Ca2+, and 71- and 38-kDa in the presence of Ca2+ (Fig. 1). On the other hand, we found that, at alkaline pH, only the Ca2+-dependent cleavage pattern (i.e. 71- and 38-kDa fragments) is obtained, whereas no cleavage is observed in the absence of Ca2+ (Fig. 1). The Ca2+-dependent cleavage proceeds more efﬁciently at pH 8.6 than at pH 7.4 (data not shown). Nevertheless, we noted that under all conditions cleavage occurs only during the initial several minutes and, when after prolonged illumination, involved a minimum of approximatively 50% of the ATPase molecules. This limit is likely due to protein unfolding upon prolonged illumination, suggesting again that cleavage occurs only when the ATPase is able to acquire native conformations, in the presence or in the absence of Ca2+.

The pH dependence of the cleavage pattern prompted us to perform spectroscopic measurements in order to clarify whether mono- or oligovanadate would be present in the reaction mixture at different pH. Fig. 2A shows the light absorption spectrum of a monovanadate solution (made initially at alkaline pH 10) and then transferred to the pH 8.6 reaction mixture. The spectrum exhibits a monophasic character that is attributed to monovanadate. However, when the same solution of monovanadate is transferred to the pH 7.4 reaction mixture, the absorption spectrum changes rapidly to reﬂect the presence of oligovanadate species, in addition to monovanadate. On the other hand, when a decavanadate solution (made originally at pH 2.0) is transferred to the pH 7.4 reaction mixture, the absorption spectrum shows a mixture of oligo- and monovanadate (Fig. 2B). Complete transformation to monovanadate is obtained if the decavanadate solution is transferred to the pH 8.6 reaction mixture (Fig. 2B). It is then apparent that the speciﬁc cleavage pattern obtained at pH 8.6 can only be related to monovanadate binding, since monovanadate is the only species present at alkaline pH. Since this cleavage requires Ca2+, the Ca2+-dependent pattern obtained at pH 7.4 must be also due to monovanadate. On the other hand, the cleavage pattern obtained in the absence of Ca2+ appears related to oligovanadate binding, since it is not observed when monovanadate is the only species present in the reaction mixture (pH 8.6).

Among the vanadate oligomers, decavanadate has been shown the species that binds to the SR ATPase, favoring formation of tubular crystals (6–8). Therefore, we assume that decavanadate is the active oligomeric species.

Additional and specific features of the cleavage patterns obtained in the presence or in the absence of Ca2+ are related to their ADP and vanadate concentration dependence. It is shown in Fig. 3 that the Ca2+-dependent cleavage (71–38-kDa fragments) is favored by ADP in the presence of Mg2+, whereas the cleavage obtained in the absence of Ca2+ (88–21-kDa fragments) is not. Furthermore, the 88–21-kDa cleavage requires at least 0.5 mM vanadate, whereas the 71–38-kDa cleavage occurs at a lower vanadate concentration (Fig. 4).

Identification of the Cleavage Sites—We considered that, in analogy to the mechanism proposed for myosin (13–15), vanadate-dependent photo-cleavage of the SR ATPase may be a stepwise process beginning with oxidation of a serine or threonine residue, followed by rearrangement and hydrolytic cleavage. We then proceeded to react the oxidized residues with [3H]borohydride, thereby obtaining radioactive labeled intermediates.

In order to identify the 71-38-kDa cleavage site, we then placed a [3H]radioactive label on the ATPase irradiated at pH 8.6 in the presence of vanadate, Ca2+, Mg2+, and ADP. The labeled ATPase was then digested extensively with trypsin.
The soluble peptide fragments were collected and separated by HPLC, and a single radioactive fragment was isolated. When the isolated peptide was subjected to sequencing analysis, seven productive cycles of Edman degradation were obtained, yielding Thr, Gly, Thr, Leu, Thr, Thr, and Asn. This sequence demonstrates that the isolated fragment corresponds to the ATPase segment beginning with Thr353, and cleaved by trypsin at Lys352 at one end, and either at Lys365 or at Lys371 at the other end. Most importantly, the radioactive label appeared only with the first degradation cycle, indicating that only Thr353 was radioactive. Therefore, Thr353 is the residue undergoing monovanadate-dependent photo-oxidation and cleavage. Thr353 is not within the Lys515–Asp659 predicted in previous studies (11, 12). Cleavage at Thr353, however, is consistent with the sizes (71 and 38 kDa) of the two fragments resulting from cleavage, with reference to 109 kDa for the entire ATPase. It should be pointed out that Thr353 is quite close to Asp351, the main residue undergoing photo-oxidation by decavanadate.

In order to identify the 88–21-kDa cleavage site, we placed 3H radioactive label on the ATPase irradiated at pH 7.4 in the presence of vanadate (including decavanadate) and EGTA (Fig. 5). The labeled ATPase was then digested extensively with trypsin. Following HPLC separation, we obtained a single radioactive fragment that yielded 13 productive cycles of Edman degradation, including Val, Asp, Gln, Ser, Ile, Leu, Thr, Gly, Ghu, Ser, Val, Ser, and Val. This fragment corresponds to the ATPase segment beginning to Val175 (cleaved at Arg174 and Asp180). The labeled ATPase was then subjected to sequencing analysis, including Val, Asp, Gln, Ser, Ile, Leu, Thr, Gly, Ghu, Ser, Val, Ser, and Val. This fragment corresponds to the ATPase segment beginning to Val175 (cleaved at Arg174 and Asp180). The labeled ATPase was then subjected to sequencing analysis, including Val, Asp, Gln, Ser, Ile, Leu, Thr, Gly, Ghu, Ser, Val, Ser, and Val. This fragment corresponds to the ATPase segment beginning to Val175 (cleaved at Arg174 and Asp180). The labeled ATPase was then subjected to sequencing analysis, including Val, Asp, Gln, Ser, Ile, Leu, Thr, Gly, Ghu, Ser, Val, Ser, and Val. This fragment corresponds to the ATPase segment beginning to Val175 (cleaved at Arg174 and Asp180). The labeled ATPase was then subjected to sequencing analysis, including Val, Asp, Gln, Ser, Ile, Leu, Thr, Gly, Ghu, Ser, Val, Ser, and Val.
TNP-AMP by decavanadate. On the contrary, monovanadate (at alkaline pH) actually enhances the fluorescence of bound TNP-AMP (Fig. 5B), due to a long range effect of occupancy of the phosphorylation on TNP-AMP bound at the nucleotide site (24). Steric interference between TNP-AMP and decavanadate binding is consistent with recent structural studies (22).

In conclusion, our experiments demonstrate unambiguously that under conditions permitting the 71–38-kDa or the 88–21-kDa cleavage, photo-oxidation is catalyzed by vanadate bound as monovanadate to the phosphorylation site, or as decavanadate bound to the nucleotide (TNP-AMP) site, respectively.

**DISCUSSION**

Several enzymes undergo UV-induced oxidation or cleavage when vanadate is bound in place of phosphate at the catalytic site (reviewed in Ref. 25). With regard to the mechanism of cleavage, it was reported that irradiation of the Mg ADP-orthovanadate complex with myosin ATPase produces oxidation of a serine side chain to yield an aldehyde. Medium oxygen is then incorporated through a free radical formed on the serine α-carbon, followed by Criegee type rearrangement and final hydrolytic cleavage with assistance of a neighboring acidic residue (13–15). On the other hand, the mitochondrial F1 ATP synthase is cleaved by UV irradiation in the presence of vanadate with no need for ADP. In this case, cleavage involves an alanine residue (16, 17).

Cleavage of SR ATPase by UV irradiation in the presence of vanadate was previously reported by Vegh et al. (11) and Molnar et al. (12), who, at neutral pH, observed different cleavage sites in the absence as opposed to the presence of Ca$^{2+}$. We find that experimentation at neutral pH produces rapid equilibration of mono- and oligovanadate species, and demonstration of a selective monovanadate effect requires alkaline pH. When irradiation is performed at alkaline pH (i.e., under conditions favoring the vanadate monomer), only the Ca$^{2+}$-dependent cleavage is observed. On the other hand, at neutral pH (i.e.,

---

**Fig. 5.** Effects of decavanadate and monovanadate on the fluorescence emission of bound TNP-AMP. A, 20 mM MOPS, pH 7.4, 5 mM MgCl$_2$, 0.2 mg/ml SR protein, 20 μM TNP-AMP, and 1.0 mM EGTA. B, 50 mM TRIS-Cl buffer, pH 8.6, 5 mM MgCl$_2$, 0.2 mg/ml SR protein, 20 μM TNP-AMP, and 0.1 mM CaCl$_2$. Excitation wavelength = 410 nm. ○, controls in the absence of vanadate; □, 1 mM vanadate. Note that at pH 7.4 (A) the reaction mixture contains mono- as well as decavanadate, whereas at pH 8.6 (B) it contains only monovanadate (see Fig. 2).

**Fig. 6.** Structural relationships in the monovanadate (top) and decavanadate (bottom) photo-oxidation sites. Top, this diagram is derived from an atomic model of the phosphorylation (P) domain obtained by diffraction studies of three-dimensional crystals in the presence of Ca$^{2+}$ (22). Thr$^{353}$ (undergoing photo-oxidation in the presence of monovanadate) is near Asp$^{351}$ (undergoing phosphorylation upon utilization of ATP) and Asp$^{601}$ (likely assisting hydrolytic cleavage of the photo-oxidation product). The two segments shown in the diagram, although widely separated in the linear sequence, are both components of the P domain in the folded structure. Bottom, this diagram is derived from a fit of the high resolution model obtained in the presence of Ca$^{2+}$, to a lower resolution map obtained in the absence of Ca$^{2+}$ and in the presence of decavanadate (22). The net in pink shows a high density peak in the map that presumably represents decavanadate (purple sphere). Ser$^{186}$ (the amino acid undergoing photo-oxidation) is located in domain A and near decavanadate. The P and N domains also contribute to the decavanadate binding; side chains of the residues that are likely to contribute to the binding are shown. One of the neighboring acidic residues (Asp$^{176}$ is located just below Ser$^{186}$) is likely to assist hydrolytic cleavage of the photo-oxidation product.
permitting coexistence of mono- and decavanadate), the two cleavage patterns requiring the presence or the removal of Ca$^{2+}$ are produced by monovanadate or decavanadate, respectively.

Due to amino terminus blockage of the fragments resulting from cleavage, previous studies (11, 12) were unable to identify the cleavage sites by amino acid sequencing. Nevertheless, it was suggested that cleavage in the presence of Ca$^{2+}$ occurs "between Lys$_{515}$ and Asp$_{659}$", whereas cleavage in the absence of Ca$^{2+}$ occurs "near the T2 cleavage site" (Arg$^{198}$).

Our experiments reveal a sequential mechanism of oxidation and cleavage that matches closely that of myosin, confirming that the amino terminus of the cleavage product is blocked and resistant to Edman degradation. The initial oxidation product is demonstrated by its reduction with borohydride and incorporation of radioactive $^3$H. We were then able to identify the labeled amino acid by subjecting the protein to extensive digestion, isolating the radioactive fragment, sequencing, and demonstrating the radioactive label in the fractional product of Edman degradation. We found that the Ca$^{2+}$-dependent cleavage is preceded by photo-oxidation of Thr$^{353}$. This is quite removed from the region (between Lys$_{515}$ and Asp$_{659}$) suggested previously (11, 12), but is consistent with the sizes (71 and 38 kDa) of the two fragments resulting from the related cleavage (Fig. 1). Thr$^{353}$ is quite close to Asp$_{351}$, the residue undergoing phosphorylation as an intermediate step of the ATPase catalytic cycle. Photo-oxidation and cleavage by monovanadate at the phosphorylation site requires binding of ADP and Mg$^{2+}$ to the Ca$^{2+}$-dependent conformation of the enzyme. Hydrolytic cleavage of the photo-oxidation product is assisted by an acidic residue, likely to be Asp$_{601}$, whose side chain resides within 6 Å from Thr$^{353}$ within the Ca$^{2+}$-dependent conformation of the cytosolic P domain (Ref. 22; see diagram in Fig. 6). It is intriguing that a conformation-dependent displacement of the corresponding aspartate residue has been proposed for the Na$^+$-,K$^+$-ATPase, to approach the vanadate-magnesium complex at the catalytic site (26).

The cleavage occurring in the absence of Ca$^{2+}$ and yielding the 88- and 21-kDa fragments, is evidently related to decavanadate binding, since it does not occur when decavanadate is absent (alkaline pH). It occurs only in the presence of decavanadate and in the absence of Ca$^{2+}$ binding. The decavanadate-dependent cleavage does not require ADP.

Consistent with structural studies (22) indicating that decavanadate resides in a positively charged groove formed by the A domain, including the extreme amino-terminal sequence between Met$^1$ and Glu$^{38}$, and the following loop between Trp$^{177}$ and Ser$^{261}$. Photo-oxidation of Ser$^{186}$ by decavanadate then raises the question of its proximity to the decavanadate site (P and N domains). In fact, the A, P, and N domains are quite separated in the Ca$^{2+}$-dependent structure of the ATPase, but are approximated by the conformational change produced by removal of Ca$^{2+}$ (22). This is in agreement with our observation that oxidation by decavanadate occurs only in the absence of Ca$^{2+}$. As depicted in Fig. 6 in the model for the enzyme without Ca$^{2+}$ but with decavanadate, there is a strong density peak lined by positively charged residues in the groove formed by three cytoplasmic domains (A, N, and P). This peak has been assigned to decavanadate and some of the lining residues are likely to be responsible also for TNP-AMP binding (27). Their involvement will explain the decrease of TNP-AMP fluorescence. The locations of decavanadate and Ser$^{186}$ in the model agree very well with the present results. The neighboring Asp$^{176}$ and Glu$^{183}$ are likely to assist hydrolytic cleavage of the initial photo-oxidation product.

In conclusion, we have clarified the mechanism of vanadate-dependent cleavage of SR ATPase, demonstrating that it includes sequential photo-oxidation and cleavage of the oxidation product. A cleavage pattern that requires Ca$^{2+}$, ADP, and Mg$^{2+}$ is produced by monovanadate binding at the phosphorylation site, and involves Thr$^{353}$. An alternative cleavage pattern that requires removal of Ca$^{2+}$ is produced by decavanadate binding at or near the nucleotide site, and involves Ser$^{186}$. The structural requirements for the specific cleavage patterns produced by monovanadate and decavanadate are consistent with current models of the ATPase conformation in the presence and in the absence of Ca$^{2+}$ (22).

Acknowledgment—Manuscript and figures were edited by Jerry Domanico.

REFERENCES
1. Bastide, F., Meissner, G., Fleischer, S., and Post, R. L. (1973) J. Biol. Chem. 248, 8385–8391
2. Degani, C., and Boyer, P. D. (1973) J. Biol. Chem. 248, 8222–8226
3. Dupont, Y., and Bennett, N. (1982) FEBS Lett. 139, 237–240
4. Inesi, G., Kurzmaczk, M., Nakamoto, R. K., de Meis, L., and Bernhard, S. (1980) J. Biol. Chem. 255, 6040–6043
5. Pick, I. (1982) J. Biol. Chem. 257, 6111–6119
6. Dux, L., and Martonosi, A. (1983) J. Biol. Chem. 258, 2599–2603
7. Taylor, K. A., Dux, L., and Martonosi, A. (1986) J. Mol. Biol. 187, 417–427
8. Toyoshima, C., Sasabe, H., and Stokes, D. L. (1993) Nature 362, 469–471
9. Aureliano, M., and Madeira, V. M. C. (1994) Biochim. Biophys. Acta 1221, 259–271
10. Hua, S., Fabris, D., and Inesi, G. (1999) Biophys. J. 77, 2217–2225
11. Vech, M., Molnar, E., and Martonosi, A. (1999) Biochim. Biophys. Acta Bioenerg. 1472, 168–181
12. Molnar, E., Varga, S., and Martonosi, A. (1991) Biochim. Biophys. Acta 1023, 17–26
13. Grammer, J. C., Loo, J. A., Edmonds, C. G., Creme, C. R., and Yount, R. G. (1996) Biochemistry 35, 15582–15592
14. Creme, C. R., Grammer, J. C., and Yount, R. G. (1989) J. Biol. Chem. 264, 6608–6611
15. Grammer, J. C., Creme, C. R., and Yount, R. G. (1988) Biochemistry 27, 8408–8415
16. Ko, Y. H., Blanchet, M., Anzel, L. M., and Pedersen, P. L. (1997) J. Biol. Chem. 272, 18575–18581
17. Ko, Y. H., Hong, S., and Pedersen, P. L. (1999) J. Biol. Chem. 274, 28585–28594
18. Kletz, S., and Inesi, G. (1972) Biochim. Biophys. Acta 282, 174–179
19. Lowry, O. H., Roseborough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–270
20. Weber, K., and Osborn, M. (1962) J. Biol. Chem. 244, 4406–4417
21. Young, H. S., Rigaud, J. L., Lacapere, J. J., Reddy, L. G., and Stokes, D. L. (1997) Biophys. J. 72, 2545–2559
22. Toyoshima, C., Nakasako, M., Nomura, H., and Ogawa, H. (2000) Nature 405, 647–655
23. Watanabe, T., and Inesi, G. (1982) J. Biol. Chem. 257, 11510–11516
24. Nakamoto, R. K., and Inesi, G. (1984) J. Biol. Chem. 259, 2961–2970
25. Mulholland, A., and Ringel, I. (1995) Mol. Membr. Biol. 12, 211–220
26. Goldshleger, R., and Karlish, S. J. (1999) J. Biol. Chem. 274, 16213–16221
27. McIntosh, D. B., Woolley, D. G., and Berman, M. C. (1992) J. Biol. Chem. 267, 5301–5309