In Vitro Selection and Characterization of DNA Aptamers Specific for Phospholamban

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ABSTRACT
Calcium transport across the membrane of the sarcoplasmic reticulum (SR) plays an important role in the regulation of heart muscle contraction and relaxation. SERCA2a is a membrane protein of the cardiac SR that possesses Ca\textsuperscript{2+}-dependent ATPase activity. SERCA2a is responsible for Ca\textsuperscript{2+} uptake by this organelle and is inhibited in a reversible manner by phospholamban, another SR membrane protein. Thus, alleviation of phospholamban-mediated inhibition of SERCA2a is a potential therapeutic option for heart failure and cardiomyopathy. We have now applied the systematic evolution of ligands by exponential enrichment protocol to a library of single-stranded DNA molecules containing a randomized 40-nucleotide sequence to isolate aptamers that bind phospholamban. One of the obtained aptamers, designated Apt-9, was found to specifically bind to the cytoplasmic region of phospholamban in vitro with high affinity (dissociation constant, ~20 nM). Apt-9 increased the Ca\textsuperscript{2+}-dependent ATPase activity of cardiac SR vesicles but not that of SR vesicles from skeletal muscle in a concentration-dependent manner. It also shifted the Ca\textsuperscript{2+} concentration-response curve for this ATPase activity to the left. These effects of Apt-9 were not mimicked by an oligonucleotide with a scrambled version of the Apt-9 sequence. Thus, our results indicate that Apt-9 activates SERCA2a by alleviating the inhibitory effect of phospholamban on this ATPase, and they suggest that phospholamban-specific aptamers warrant further investigation as potential therapeutic agents for heart failure and cardiomyopathy.

Calcium transport across the membrane of cardiac SR plays an important role in the regulation of contraction and relaxation of heart muscle. SERCA2a is a membrane protein of the cardiac SR that possesses Ca\textsuperscript{2+}-dependent ATPase activity. SERCA2a is responsible for Ca\textsuperscript{2+} uptake by this organelle and is inhibited by phospholamban, another SR membrane protein (Tada and Katz, 1982; Fleisher and Inui, 1989; James et al., 1989; MacLennan and Kranias, 2003). The dephosphorylated form of phospholamban inhibits SERCA2a by reducing its apparent affinity for Ca\textsuperscript{2+} (Inui et al., 1988). Phosphorylation of phospholamban by cAMP-dependent protein kinase alleviates its inhibition of SERCA2a, with the consequent increase in the Ca\textsuperscript{2+}-pumping activity of SERCA2a resulting in acceleration of muscle relaxation and enhancement of cardiac contractility. This mechanism of SERCA2a regulation underlies the positive inotropic effect of \beta-adrenergic receptor stimulation (Tada and Inui, 1983; Sham et al., 1991; Luo et al., 1994).

Evidence suggests that the expression of SERCA2a is reduced in the failing heart, with an increase in the inhibitory effect of phospholamban on Ca\textsuperscript{2+}-pumping activity (Hoshijima et al., 2006). Excessive inhibition of SERCA2a by mutant forms of phospholamban was shown to impair cardiac contractility and relaxation in mice, resulting in heart failure and cardiomyopathy (Zhai et al., 2000; Zvaritch et al., 2000; Haghigi et al., 2001). Conversely, ablation of phospholamban rescued the impairment in cardiac function and morphological changes associated with dilated cardiomyopathy in a mouse model of this condition (Minamisawa et al., 1999), although a mutation in the human phospholamban gene that resulted in the apparent loss of phospholamban expression in the heart was associated with the development of dilated cardiomyopathy and heart failure (Haghigi et al., 2003). Several attempts have been made to reduce the phospholamban-mediated inhibition of SERCA2a in vitro and in vivo. Depletion of phospholamban or expression of a mutant form of the protein was shown to increase the contractility of cultured cardiomyocytes (He et al., 1999). Furthermore, ex-
pression either of phospholamban antisense RNA (Eizema et al., 2000) or of an antibody-derived protein targeting phospholamban (Dieterle et al., 2005) ameliorated heart failure in animal models. Thus, relief of phospholamban-mediated inhibition of SERCA2a is a potential therapeutic option for treatment of heart failure and cardiomyopathy.

Synthetic nucleic acid ligands have been shown to bind to protein targets with high specificity and affinity. Such ligands, or aptamers, have also been shown to be potent inhibitors of protein function; thus, they have been applied to the development of new drugs (Ellington and Szostak, 1990; Tuerk and Gold, 1990; Blank and Blind, 2005; Lee et al., 2006). SELEX is a method for the rapid in vitro selection of specific aptamers. We have now applied this approach to isolate DNA aptamers specific for phospholamban with the use of a fusion protein containing the cytoplasmic region of this protein. A selected aptamer was found not only to bind to phospholamban but also to increase the Ca$^{2+}$-dependent ATPase activity of cardiac SR vesicles by relieving phospholamban-mediated inhibition of SERCA2a.

**Materials and Methods**

**Materials.** A fusion protein consisting of Met1-Gln26 of human phospholamban fused to the PDZ domains of PSD-95 and a control protein lacking the phospholamban sequence (PDZ-Myc-His$_6$) were expressed in *E. coli* BL21 with the use of the pTrcHis2B vector (Invitrogen, Carlsbad, CA). The recombinant proteins were purified by Ni-NTA column chromatography as described previously (Kimura and Inui, 2002). The members of the DNA library for SELEX consisted of two 20-base primer regions and a 40-base random region (5'-ATGACATGACCTCCCTCACAC-N$_9$-TCAGACTGTGGCAGGGAAAC-3'). The forward (P1) and reverse (P2) primers for polymerase chain reaction (PCR)-mediated amplification were 5'-ATGACATGACCTCCCTCACAC-3' and 5'-GGTTCCTGCGCACGCTGCA-3', respectively. A forward (P3) or reverse (P4) primer labeled at the 5' end with biotin was used to label DNA. Mouse hybridoma cells that produce a monoclonal antibody to phospholamban (mAb-A1) were kindly provided by J. Wang (Suzuki and Wang, 1986; Kimura et al., 1991), and horseradish peroxidase-conjugated goat antibodies to mouse immunoglobulin G (IgG) were obtained from Promega (Madison, WI). SR vesicles were isolated from beagle dog (11 kg) heart or Japanese albino rabbit (3 kg) skeletal muscle as described previously (Tada et al., 1983). These experiments were approved by the Animal Ethics Committee of Yamaguchi University School of Medicine.

**In Vitro Selection of Phospholamban Aptamants (SELEX).** Protein-bound beads were prepared by first equilibrating Ni-NTA beads (Qiagen, Valencia, CA) with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T). The equilibrated beads (5 μl) were resuspended in 200 μl of PBS-T containing 10 μg of PLN$^{1-26}$-PDZ-Myc-His$_6$ or PDZ-Myc-His$_6$ and were incubated with rotation for 30 min at 4°C. The protein-bound beads were then washed three times with 1 ml of PBS-T. The single-stranded DNA (ssDNA) library (1 nmol) was denatured by incubation at 98°C for 3 min and then cooled immediately to 4°C in 100 μl of PBS-T. In the initial round of selection, the library was added to 10 ml of PBS-T containing bead-bound PDZ-Myc-His$_6$ (5 μl) and bovine serum albumin (BSA; 1 μg/ml). After incubation at room temperature for 30 min, the mixture was centrifuged, and the resulting supernatant was mixed with bead-bound PLN$^{1-26}$-PDZ-Myc-His$_6$ (6 μl) and incubated for 30 min at room temperature. The beads were then washed three times with 1 ml of PBS-T, and aptamers were eluted from the beads with 50 μl of 0.5 M imidazole-HCl, pH 7.4. The eluate was subjected to extraction with phenol-chloroform, and ssDNA was precipitated with ethanol. After washing with 70% ethanol, the ssDNA was dissolved in 100 μl of a PCR mixture containing 10 μl of 10x PCR buffer, 8 μl of deoxyribonucleosides (each at 2.5 mM), 100 pmol primers P1 and P4, 5% dimethyl sulfoxide, and 2.5 μl of Ex Taq DNA polymerase (Takara Bio, Otsu, Japan). The PCR incubation protocol for amplification of double-stranded DNA (dsDNA) included an initial denaturation for 2 min at 95°C, 20 cycles of denaturation (30 s at 95°C), annealing (30 s at 58°C), and extension (15 s at 72°C); and a final extension for 2 min at 72°C. The size of the amplification products was verified by electrophoresis on a 4% agarose gel, after which 90 μl of the PCR products were mixed with 23 μl of 5 M NaCl and 5 μl of NeutrAvidin beads (Pierce Chemical, Rockford, IL). After incubation for 10 min at room temperature, the beads were washed three times with 1 ml of PBS-T, and the nonbiotinylated DNA strand was separated from the immobilized complementary strand by incubation of the beads for 5 min at room temperature in 50 μl of 0.1 M NaOH. The released ssDNA was isolated by centrifugation, and the supernatant was neutralized by the addition of 5 μl of 1 M HCl. The ssDNA was precipitated with ethanol, washed with 70% ethanol, and dissolved in 100 μl of PBS-T. For additional rounds of selection, the amount of PLN$^{1-26}$-PDZ-Myc-His$_6$ bound to the beads was reduced to 5 μg/5 μl of beads.

After nine rounds of selection with PLN$^{1-26}$-PDZ-Myc-His$_6$, dsDNA was amplified by PCR with the primers P1 and P2, cloned into the pCR4-TOPO vector (Invitrogen), and introduced into E. coli TOP10 (Invitrogen). The plasmid inserts of 45 colonies were sequenced with the use of T7 promoter and M13 reverse primers.

**Biotin Labeling of Aptamers.** Two methods were applied for biotin labeling of aptamers. The 5' end of ssDNA was labeled with biotin by asymmetric PCR performed with dsDNA and a biotin-labeled primer. Template dsDNA (1 pmol) was dissolved in 100 μl of PCR mixture containing 10 μl of 10x PCR buffer, 8 μl of deoxyribonucleosides (each at 2.5 mM), 50 pmol primer P3, 0.5 pmol primer P2, and 2.5 μl of Ex Taq DNA polymerase. The PCR incubation protocol comprised an initial denaturation for 2 min at 95°C; 25 cycles of denaturation (30 s at 95°C), annealing (30 s at 58°C), and extension (15 s at 72°C); and a final extension for 2 min at 72°C. The ssDNA product was purified by electrophoresis on a 4% agarose gel. Chemically synthesized aptamers were labeled at the 3' end with biotin with the use of terminal transferase and biotin-16-dUTP (Roche Diagnostics, Basel, Switzerland).

**Aptamer Binding Assay.** Biotin-labeled aptamer was suspended in 100 μl of PBS-T, denatured at 98°C for 3 min, and then cooled immediately to 4°C. The aptamer was mixed with 5 μl of Ni-NTA bead-bound PLN$^{1-26}$-PDZ-Myc-His$_6$ or PDZ-Myc-His$_6$ (2 μg of protein/μl of beads) in the presence of BSA (0.1 mg/ml). After incubation at room temperature for 30 min, the beads were washed three times with 1 ml of PBS-T. The beads were then incubated at room temperature for 30 min with 100 μl of PBS-T containing a 1/100,000 dilution of horseradish peroxidase-labeled avidin (Sigma-Aldrich, St. Louis, MO) and BSA (1 mg/ml). After washing with PBS-T, the beads were exposed to TMB One Solution (Promega) and then removed by centrifugation, and the absorbance of the supernatant was measured at 650 nm.

**Measurement of Ca$^{2+}$-Dependent ATPase Activity of Cardiac SR Vesicles.** The Ca$^{2+}$-dependent ATPase activity of cardiac SR vesicles was assayed as described previously (Sasaki et al., 1992), with some modifications. The vesicles (final concentration, 50–100 μg/ml protein) were incubated for 4 min at 37°C in a final volume of 50 μl containing 20 mM imidazole-HCl, pH 6.9, 100 mM KCl, 2 mM MgCl$_2$, 5 mM Na$_2$ATP, 0.1 mM ATP, 11 to 16.3 μM free Ca$^{2+}$ (Ca$^{2+}$-EGTA buffer containing 0.5 mM CaCl$_2$ and various amounts of EGTA), 5 μM iomyoncin, and an ATP-regenerating system consisting of 2.5 mM phosphoenolpyruvate and pyruvate kinase (30 IU/ml). The reaction was initiated by the addition of ATP and stopped by the addition of 170 μl of a solution containing 0.3 mM 2,4-dinitrophenylhydrazine and 0.35 M HCl. The Ca$^{2+}$-dependent ATPase activity was then determined as described previously (Tada et al., 1983). Free
Ca\(^{2+}\) concentrations were calculated with the use of the WEBMAXC program (http://maxchelator.stanford.edu).

**Pull-Down Assay with Aptamer-9.** Cardiac SR vesicles (0.1 mg) were incubated with biotin-labeled aptamer 9 (Apt-9) (125 pmol) in 100 \(\mu\)l of binding/wash buffer (20 mM HEPES-NaOH, pH 7.4, 100 mM KCl, 2 mM 2-mercaptoethanol) containing 0.5% Tween 20 for 30 min at 4°C. After centrifugation at 16,000 g for 20 min, the supernatant was mixed with 5 \(\mu\)l of NeutrAvidin beads. After incubation for 30 min at room temperature, the beads were isolated by centrifugation, washed with the binding/wash buffer containing 0.1% Tween 20 and then with the binding/wash buffer containing 500 mM KCl and 0.1% Tween 20, and subjected to elution with SDS sample buffer. The eluted proteins were separated by SDS-polyacrylamide gel electrophoresis on a 15% gel and subjected to silver staining and to immunoblot analysis with the monoclonal antibody to phospholamban mAb-A1, horseradish peroxidase-conjugated goat antibodies to mouse IgG, and enhanced chemiluminescence reagents (GE Healthcare, Chalfont St. Giles, UK).

**Statistical Analysis.** Data are presented as means \(\pm\) S.D. and were compared by Student’s \(t\) test. A \(p\) value of <0.05 was considered statistically significant.

### Results

**In Vitro Selection of Phospholamban-Specific DNA Aptamers.** To obtain DNA aptamers specific for the cytoplasmic region of phospholamban, we performed the SELEX protocol with an aptamer library based on a random 40-nucleotide sequence and with a fusion protein consisting of Met1-Gln26 of human phospholamban fused to the PDZ domains of PSD-95 followed by Myc epitope and His\(_{6}\) tags (PLN\(^{1-26}\)-PDZ-Myc-His\(_{6}\)), which retains the ability to interact with the cytoplasmic domain of SERCA2a (Kimura and Inui, 2002). PDZ-Myc-His\(_{6}\) was used to remove aptamers that might bind nonspecifically to the fusion protein. After nine rounds of selection with PLN\(^{1-26}\)-PDZ-Myc-His\(_{6}\), the aptamer pool yielded a substantially higher level of binding to PLN\(^{1-26}\)-PDZ-Myc-His\(_{6}\) than to PDZ-Myc-His\(_{6}\) (data not shown) when analyzed with aptamers labeled at the 5’ end with biotin by asymmetric PCR. The aptamer pool was then cloned, and 45 clones were sequenced, and the sequences of the 11 identified aptamers and the frequency of their occurrence are shown in Table 1.

To further select aptamers with the potential to modulate the function of phospholamban in cardiac SR, we examined the effects of the 11 identified aptamers on the Ca\(^{2+}\)-dependent ATPase activity of isolated cardiac SR vesicles at a free Ca\(^{2+}\) concentration of 0.42 \(\mu\)M. None of the 11 aptamers had a significant effect on Ca\(^{2+}\)-dependent ATPase activity of SR vesicles isolated from skeletal muscle, which lacks phospholamban. In contrast, nine of the 11 aptamers significantly increased the Ca\(^{2+}\)-dependent ATPase activity of cardiac SR vesicles (Fig. 1A), indicating that the effect of these aptamers was dependent on phospholamban. This effect was most pronounced with Apt-9, the theoretical structure (Murphy et al., 2003), of which is shown in Fig. 1B. Thus, subsequent experiments were performed with chemically synthesized 40-base ssDNA of Apt-9 as a phospholamban-specific aptamer and with an oligonucleotide (scrambled Apt-9) whose sequence (5’-GTAGAGATTAGTATTTTGGGAGAGCGGCGG−3’) is a scrambled version of that of Apt-9.

**Binding of Apt-9 to the Cytoplasmic Region of Phospholamban.** We examined the binding of Apt-9 to the cytoplasmic region of phospholamban with the use of PLN\(^{1-26}\)-PDZ-Myc-His\(_{6}\). Such binding was dependent on the concentration of Apt-9, with a dissociation constant (\(K_d\)) of \(~20\) nM, and was sequence specific, given that no significant binding was observed with scrambled Apt-9 (Fig. 2).

The monoclonal antibody mAb-A1 binds to the cytoplasmic domain of phospholamban and thereby attenuates its inhibitory effect on SERCA2a (Suzuki and Wang, 1986; Kimura et al., 1991). We found that Apt-9 had no significant effect on the binding of mAb-A1 to PLN\(^{1-26}\)-PDZ-Myc-His\(_{6}\) at concentrations up to 20 \(\mu\)M (Fig. 3A), even though the maximal binding of Apt-9 to PLN\(^{1-26}\)-PDZ-Myc-His\(_{6}\) (Fig. 2) and the maximal stimulation of SERCA2a activity in cardiac SR vesicles by Apt-9 (see below) were observed at this aptamer concentration. Conversely, mAb-A1 did not interfere with the binding of Apt-9 to PLN\(^{1-26}\)-PDZ-Myc-His\(_{6}\) at concentrations up to 50 \(\mu\)g/ml (Fig. 3B), even though the antibody at this concentration almost completely inhibited the interaction between PLN\(^{1-26}\)-PDZ-Myc-His\(_{6}\) and the cytoplasmic domain of SERCA2a (Kimura and Inui, 2002) and maximally stimulated SERCA2a activity in cardiac SR vesicles (Kimura et al., 1991).

**Specificity of the Apt-9 Binding.** To determine the specificity of Apt-9, cardiac SR vesicles were solubilized and incubated with biotinylated Apt-9. Proteins bound to Apt-9 were recovered by NeutrAvidin beads. Silver staining of the bound proteins after SDS-polyacrylamide gel electrophoresis revealed that Apt-9 but not scrambled Apt-9 specifically bound phospholamban (Fig. 4). The specificity was the same as that of the monoclonal antibody to phospholamban, mAb-A1 (Fig. 4).

**Effect of Apt-9 on the Ca\(^{2+}\)-Dependent ATPase Activity of Cardiac SR.** As shown in Fig. 1A, Apt-9 increased the Ca\(^{2+}\)-dependent ATPase activity of cardiac SR vesicles. This

### Table 1

<table>
<thead>
<tr>
<th>Aptamer</th>
<th>Sequence (5’−3’)</th>
<th>Frequency</th>
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<td>Apt-1</td>
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<tr>
<td>Apt-2</td>
<td>ACACTCCTCCCCGGATTGTTTGCTGACCTCTTTTCTCGAGCTGT</td>
<td>11/45</td>
</tr>
<tr>
<td>Apt-3</td>
<td>ATGCCAGCCTGGCTCCTTTTAACCCGATCACCTTATCCCTGCTT</td>
<td>18/45</td>
</tr>
<tr>
<td>Apt-4</td>
<td>GCCCCTATGGGACCTGGGCGACACAGACTCTCGGCTCTCTGTA</td>
<td>2/45</td>
</tr>
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<td>1/45</td>
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<td>6/45</td>
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<td>Apt-11</td>
<td>GTAGTGTTGGGGCGAATTGGGACACAGAGCTGAGTGA</td>
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Fig. 2. Binding of Apt-9 to a fusion protein containing the cytoplasmic region of phospholamban. Various concentrations of biotin-labeled Apt-9 (closed circles) or scrambled Apt-9 (open circles) were incubated with bead-bound PLN<sup>1-26</sup>-PDZ-Myc-His<sub>6</sub> or PDZ-Myc-His<sub>6</sub>, and the amount of aptamer bound to the beads was subsequently determined with avidin-peroxidase as described under Materials and Methods. Specific binding to phospholamban was determined by subtraction of the binding to PDZ-Myc-His<sub>6</sub> from that to PLN<sup>1-26</sup>-PDZ-Myc-His<sub>6</sub>. Data are expressed as difference in absorbance at 650 nm (ΔA<sub>650</sub>) and are means ± S.D. of three independent determinations. The K<sub>d</sub> value for specific binding of Apt-9 determined from the regression curve was 21.4 nM.

Discussion

With the use of the SELEX method, we selected ssDNA aptamers with the ability to bind to the cytoplasmic region of phospholamban. One of these aptamers, Apt-9, was shown to increase the Ca<sup>2+</sup>-dependent ATPase activity of cardiac SR vesicles, but not that of SR vesicles from skeletal muscle, in a concentration-dependent manner. This aptamer also shifted the Ca<sup>2+</sup>-requirement of such activity in cardiac SR to lower concentrations, suggesting that the binding of Apt-9 to phospholamban relieves the inhibitory effect of this protein on SERCA2a activity. Given that activation of SERCA2a facilitates the relaxation and subsequently increases the contractility of the myocardium (Tada and Inui, 1983; MacLennan and Kranias, 2003), our results suggest that phospholamban aptamers are a potential new therapeutic tool for the treatment of heart failure.

We obtained 11 different aptamer sequences after nine rounds of SELEX. These aptamers did not share a common shorter sequence. Although all 11 aptamers bound to the cytoplasmic domain of phospholamban, the extent by which they increased the Ca<sup>2+</sup>-dependent ATPase activity of cardiac SR vesicles varied, probably reflecting different affinities of the aptamers for phospholamban or different binding sites. The binding affinity of Apt-9 for the cytoplasmic region of phospholamban was high (K<sub>d</sub>, 20 nM). Consistent with the high-affinity binding, Apt-9 specifically bound to phospholamban (Fig. 4). In contrast, much higher concentrations of the aptamer were necessary for stimulation of the Ca<sup>2+</sup>-dependent ATPase activity of cardiac SR vesicles, as well as the ability to bind to the cytoplasmic region of phospholamban (Fig. 5). If this effect represents attenuation of phospholamban-mediated inhibition of SERCA2a by Apt-9, then Apt-9 would be expected to shift the Ca<sup>2+</sup>-dependence of SERCA2a activity to lower concentrations. We found that the Ca<sup>2+</sup>-concentration-response curve for SERCA2a was shifted to the left in the presence of Apt-9, with the median effective Ca<sup>2+</sup>-concentration decreasing from 1.10 to 0.35 μM (Fig. 6). Scrambled Apt-9 had no such effect.

We also compared the maximal effect of Apt-9 on the Ca<sup>2+</sup>-dependent ATPase activity of cardiac SR vesicles with that of mAb-A1 at a free Ca<sup>2+</sup>-concentration of 0.35 μM. The stimulatory effects of excess Apt-9 (40 μM) or excess mAb-A1 (25 μg/ml) were virtually identical (Fig. 7). Furthermore, the presence of both Apt-9 and mAb-A1 increased ATPase activity to a significantly greater extent than did that of either agent alone, although the effects of the two agents were not additive (Fig. 7).
phospholamban (mAb-A1, 1D11, and 2D12) have been shown to increase the Ca\^{2+}\/-H\^{+}-dependent ATPase activity of cardiac SR vesicles by relieving phospholamban-mediated inhibition of SERCA2a (Suzuki and Wang, 1986; Kimura et al., 1991; Briggs et al., 1992; Mayer et al., 1996). The action of Apt-9 in this regard seems similar to that of these monoclonal antibodies. Thus, Apt-9 was as effective as mAb-A1 in activation of SERCA2a in cardiac SR vesicles. The effect of the combination of Apt-9 and mAb-A1 was greater than that of either agent alone but was less than additive (Fig. 7). The monoclonal antibody 2D12 has been proposed to completely alleviate phospholamban inhibition of SERCA2a (Chen et al., 2007). Our present results indicate that some inhibition remains unrelieved by mAb-A1 and that this residual inhibition of SERCA2a is alleviated by Apt-9. Our binding assays showed that Apt-9 did not decrease the amount of mAb-A1 bound to the cytoplasmic region of phospholamban and vice versa, indicating that Apt-9 and mAb-A1 interact with different binding sites.

Several approaches that target the SERCA-phospholamban...
The Ca\(^{2+}\) dependence of the Ca\(^{2+}\)-dependent ATPase activity of cardiac SR vesicles was measured at various free Ca\(^{2+}\) concentrations in the absence (open circles) or presence (closed circles) of 20 μM Apt-9 or scrambled Apt-9 (closed triangles). Data are expressed as a percentage of the maximal activity observed and are means ± S.D. from four independent determinations. **, p < 0.001; *, p < 0.01; +, p < 0.05 versus the corresponding value without aptamer. The median effective Ca\(^{2+}\) concentrations determined from the regression curves were 1.10, 0.35, and 0.93 μM for control, Apt-9, and scrambled Apt-9, respectively.

Fig. 6. Effect of Apt-9 on the Ca\(^{2+}\) dependence of the Ca\(^{2+}\)-dependent ATPase activity of cardiac SR. The Ca\(^{2+}\)-dependent ATPase activity of cardiac SR vesicles was measured at various free Ca\(^{2+}\) concentrations in the absence (open circles) or presence (closed circles) of 20 μM Apt-9 or scrambled Apt-9 (closed triangles). Data are expressed as a percentage of the maximal activity observed and are means ± S.D. from three independent determinations. ***, p < 0.001; **, p < 0.01; *, p < 0.05 versus the corresponding value without aptamer.

Fig. 7. Comparison of the effects of Apt-9 and mAb-A1 on the Ca\(^{2+}\)-dependent ATPase activity of cardiac SR. The Ca\(^{2+}\)-dependent ATPase activity of cardiac SR vesicles was measured at a fixed Ca\(^{2+}\) concentration of 0.35 μM in the absence or presence of 40 μM Apt-9 or mAb-A1 (25 μg/ml), as indicated. Data are expressed as fold increase and are means ± S.D. from four independent determinations. ***, p < 0.001; **, p < 0.01 for the indicated comparisons.

References


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