Modification of Histidine 5 in Sarcoplasmic Reticulum Ca\(^{2+}\)-ATPase by Diethyl Pyrocarbonate Causes Strong Inhibition of Formation of the Phosphoenzyme Intermediate from Inorganic Phosphate*

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Sarcoplasmic reticulum vesicles were modified with diethyl pyrocarbonate (DEPC), a histidine-modifying reagent. Phosphoenzyme formation from Pi, in the Ca\(^{2+}\)-ATPase (reversal of hydrolysis of the phosphoenzyme intermediate) was almost completely inhibited by this modification. Tight binding of F\(^{-}\) and Mg\(^{2+}\) and high affinity binding of vanadate in the presence of Mg\(^{2+}\), both of which produce transition state analogs for phosphoenzyme formation from the magnesium-enzyme-phosphate complex, were also inhibited. Formation of the phosphoenzyme from acetyl phosphate in the forward reaction was only weakly inhibited, but hydrolysis of the phosphoenzyme was strongly inhibited. The enzyme was protected by tight binding of F\(^{-}\) and Mg\(^{2+}\) or by high affinity binding of vanadate in the presence of Mg\(^{2+}\) against the DEPC-induced inhibition of phosphoenzyme formation from Pi. The enzyme was also protected by tight binding of F\(^{-}\) and Mg\(^{2+}\) against the DEPC-induced inhibition of phosphoenzyme hydrolysis. Peptide mapping of the tryptic digests, detection of peptides containing DEPC-modified histidine by UV absorption at 240 nm, amino acid analysis, sequencing, and mass spectrometry showed that His-5 was a single major residue protected by the above transition state analogs against the modification with DEPC. These results indicate that modification of His-5 with DEPC is responsible for the DEPC-induced inhibition of phosphoenzyme formation from Pi, and of phosphoenzyme hydrolysis and suggest that His-5 is located in or very close to the catalytic site in the transition state for phosphoenzyme formation from the magnesium-enzyme-phosphate complex and is likely involved in the catalytic process of this reaction step.

The SR\(^{2+}\) Ca\(^{2+}\)-ATPase is a 110-kDa membrane-bound protein, the primary structure of which has been revealed (1). This enzyme catalyzes Ca\(^{2+}\) transport coupled to ATP hydrolysis (2, 3). The enzyme is activated by high affinity Ca\(^{2+}\) binding, and then the γ-phosphoryl group of MgATP bound to the ATP-binding site is transferred to Asp-351 (1, 4–6) to form an EP intermediate (7, 8). A subsequent conformational change of the EP results in Ca\(^{2+}\) release to the lumen (9). Finally, the EP is hydrolyzed to form P\(_i\) and the dephosphoenzyme. Acetyl phosphate also serves as a substrate through formation and hydrolysis of EP (10, 11). The EP can be formed from P\(_i\) in the presence of Mg\(^{2+}\) and absence of Ca\(^{2+}\) by reversal of EP hydrolysis (12, 13). This EP formation occurs through a magnesium-enzyme-phosphate complex that is formed by random binding of Mg\(^{2+}\) and P\(_i\) to the enzyme (14, 15).

It was previously shown (16–18) that F\(^{-}\) and Mg\(^{2+}\) bind simultaneously and tightly to the catalytic site of this enzyme to form a stable transition state analog for EP formation from the magnesium-enzyme-phosphate complex. Vanadate also binds with high affinity to the enzyme in the presence of Mg\(^{2+}\) to form a transition state analog for this EP formation (19, 20). By utilizing the protection of the catalytic site by these transition state analogs against chemical modification, we have recently identified Arg-198 involved in 1,2-cyclohexanedione-induced inhibition of EP formation from P\(_i\), and suggested that this residue is located in or close to the catalytic site in the transition state (21).

It has been well established that modification of histidyl residues in the SR Ca\(^{2+}\)-ATPase by DEPC (22–25) or photooxidation (22, 26, 27) causes inhibition of the enzyme. Coan and DiCarlo (24) showed previously that EP hydrolysis in this enzyme is inhibited by modification of histidine with DEPC. However, the histidyl residue(s) involved in this inhibition has not yet been identified.

In the present study, to identify the histidyl residue(s) involved in the DEPC-induced inhibition of the SR Ca\(^{2+}\)-ATPase, we have modified the enzyme with DEPC and examined effects of the above transition state analogs on this modification. DEPC reacts with histidyl residues to yield N-carboxyhistidine derivatives (28), which can be detected spectrophotometrically in HPLC of proteolytic digests since this modification causes an increase in absorbance at 240 nm (29, 30). We have found that EP formation from P\(_i\) has been inhibited by modification with DEPC and that the enzyme has been protected by these transition state analogs against this inhibition. Peptide mapping of the tryptic digests, detection of peptides containing DEPC-modified histidine by absorption at 240 nm, amino acid analysis, sequencing, and mass spectrometry have shown that His-5 is a single major residue protected by the transition state analogs against the modification with DEPC. The results indicate that modification of His-5 is responsible for the inhibition of EP formation from P\(_i\), and suggest that His-5 is located in or very close to the catalytic site in the transition state for EP formation from the magnesium-enzyme-phosphate complex and is likely involved in the catalytic process of this reaction step.

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The abbreviations used are: SR, sarcoplasmic reticulum; EP, phosphoenzyme; DEPC, diethyl pyrocarbonate; MOPS, 3-(N-morpholino)propanesulfonic acid; MES, 2-(N-morpholinoethanesulfonic acid; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; HPLC, high performance liquid chromatography.

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EXPERIMENTAL PROCEDURES

Preparation of SR Vesicles—SR vesicles were prepared from rabbit skeletal muscle and stored at -80 °C as described previously (31). The contents of phosphorylation site in the preparations determined with γ-[32P]ATP according to Barrabín et al. (32) ranged from 3.9 to 4.4 nmol/mg.

Treatment of SR Vesicles with F- and Mg2+- Pretreatment of the SR vesicles with F- and Mg2+ was performed as described previously (18) with slight modifications. The vesicles (2 mg/ml) were incubated at 25 °C for 3 h in 1 mM KF, 10 mM MgCl2, 1 mM EGTA, 0.1 mM KCl, 20% (v/v) Me2SO, 20% (v/v) glycerol, and 40 mM imidazole HCl (pH 7.5), unless otherwise stated. The reaction was quenched by diluting the mixture twice with an ice-cold solution containing 0.1 mM CaCl2, 0.1 mM KCl, 0.3 mM sucrose, and 5 mM MOPS-Tris (pH 7.0). The resulting vesicles were washed by centrifugation once with this solution.

Modification with DEPC—Modification was started at 25 °C in 10 mM MgCl2, 2 mM CaCl2, 0.1 mM KCl, and 100 mM MES-NaOH (pH 6.0), unless otherwise stated, by adding DEPC (dissolved in acetonitrile) or the same volume of acetonitrile to a suspension of the SR vesicles to give 10 mg of the vesicles/ml, 4.5 or 0 mM DEPC, and 2% (v/v) acetonitrile. The modification was quenched at 25 °C by diluting the mixture 10 times with a solution containing 0.1 mM KCl and 7 mM histidine HCl (pH 6.0).

Determination of Tightly Bound F- and Mg2+—The SR vesicles were treated with F- and Mg2+ as described above. In the control samples, CaCl2 was added to the incubation medium to give 0.1 mM free Ca2+ under the otherwise same conditions as above (tight binding of F- and Mg2+ is prevented by 0.1 mM Ca2+ (see Ref. 18)). The treated vesicles were centrifuged by sedimentation four times with a solution containing 2 mM EDTA, 0.1 mM KCl, 1 mM AZ3187, 10% (v/v) Me2SO, and 5 mM MOPS-NaOH (pH 7.0) and suspended in deionized water. Magnesium bound to the vesicles was extracted with 0.8 N HNO3, and the concentration of magnesium in the extract was determined by atomic absorption spectrophotometry as described previously (17). The content of tightly bound Mg2+ was obtained by subtracting the content of magnesium in the extract from the control sample. Fluoride bound to the vesicles was extracted by incubating the vesicles at 95 °C for 5 min in 5 mM HEPES-KOH (pH 8.0). The sample was then centrifuged to remove insoluble materials, and KNO3 was added to the supernatant to give 0.1 mM KCl.

Determination of Bound Vanadate—The SR vesicles (0.2 mg/ml) were incubated at 25 °C for 30 min in various concentrations of vanadate, 0.1 mM KCl, 20% (v/v) Me2SO, 30 mM MOPS-NaOH (pH 7.0), and others as described in the legend to Fig. 3. The mixture was centrifuged, and the pellet was dissolved in 2% (v/v) SDS. The concentration of vanadate was measured by the method of Goodno (23). When the SR vesicles were pretreated with F- and Mg2+, tightly bound F- and Mg2+ were entirely released by incubation at 25 °C for 30 min in 20 mM CaCl2, 0.1 mM KCl, and 100 mM MOPS-NaOH (pH 7.0) (17) before vanadate binding was determined.

Proteolysis, Peptide Mapping, Detection of Peptides Containing DEPC-modified Histidine, Amino Acid Analysis, Sequencing, and Mass Spectrometry—The DEPC-modified SR vesicles (5 mg/ml) were digested with TPKC-trypsin (1 mg/ml) at 37 °C for 3 h in 30 mM CaCl2 and 30 mM Tris-HCl (pH 7.0). After centrifugation, the supernatant was subjected to reversed phase HPLC that was performed at a flow rate of 1 ml/min as described previously (34). The absorbance of peptides containing DEPC-modified histidine was monitored at 240 nm (see Refs. 29 and 30), and the absorbance of peptides was monitored at 214 nm. It was difficult to determine the content of DEPC-modified histidine in the DEPC-modified vesicles from the absorbance at 240 nm because, in addition to mono-N-carboxyhistidine, bis-carboxyhistidine was produced at an unknown ratio to mono-N-carboxyhistidine by modification of the vesicles with DEPC (see Fig. 7, Table II, and text described under “Results”). The amino acid compositions of the isolated peptides were analyzed with a precolumn derivatization reversed phase HPLC system with a Bio-Rad Aminex HPX-87H column after acid hydrolysis of the peptides in 6 N HCl at 110 °C for 21 h. Sequencing was performed with an Applied Biosystems 477A/120A sequencer. Mass determination of the isolated peptides was made on a JEOL JMS-SX102 mass spectrometer with the frit-fast atom bombardment probe as described previously (21).

Phosphorylation of Ca2+-ATPase—Phosphorylation of the SR vesicles (0.4 mg/ml) with 1 mM [32P]P, was performed at 25 °C for 10 min in 20 mM MgCl2, 5 mM EGTA, 40% (v/v) Me2SO, and 50 mM MOPS-Tris (pH 7.0). The reaction was quenched with trichloroacetic acid containing P32P. Phosphorylation of the vesicles (1 mg/ml) with 3 mM acetyl [32P]P phosphate was performed at 25 °C in 5 mM MgCl2, 2 mM CaCl2, 50 mM KCl, and 50 mM Tris-HCl (pH 7.5), unless otherwise stated. The reaction was quenched with trichloroacetic acid containing nonradioactive acetyl phosphate. The amount of DEPC formed was determined as described previously (17). When the SR vesicles were pretreated with F- and Mg2+, tightly bound F- and Mg2+ were released as described above before phosphorylation was performed. When the SR vesicles were preincubated with vanadate and Mg2+, bound vanadate and Mg2+ were entirely released by incubation at 25 °C for 30 min in 3 mM CaCl2, 2 mM ATP, 0.1 mM KCl, and 100 mM MOPS-NaOH (pH 7.0) (35, 36) before phosphorylation was performed.

Miscellaneous Methods—DEPC, TPKC-trypsin, and Na2VO3 were purchased from Sigma. KF was from Nacalai Tesque (Kyoto, Japan). ATP was from Yamasa Biochemicals (Choshi, Japan). Acetyl phosphate was from Kojin (Tokyo, Japan). γ-[32P]ATP and [32P]P, were obtained from NEN Life Science Products. [32P]P, was purified according to Kanaizawa and Boyer (12). Acetyl [32P]P phosphate was prepared by Procedure B in the method of Stadtman (37). Vanadate solutions were prepared from Na2VO3 according to Goodno (33) just before use. Protein concentrations were determined by the method of Lowry et al. (38) with bovine serum albumin as a standard. Data were analyzed by the nonlinear least squares method as described previously (17).

RESULTS

DEPC-induced Inhibition of EP Formation from P1 and Protection by Pretreatment with F- and Mg2+ against the Inhibition—As shown in Fig. 1A, when the SR vesicles were preincubated with F- in the presence of Mg2+, and then treated with DEPC in the presence of 2 mM CaCl2 (3), EP formation from P1 was strongly inhibited with apparent first order kinetics at a rate of 0.11 min⁻¹ and fell to 10% of the original level in 40 min. When the vesicles were pretreated with F- and Mg2+, and then treated with DEPC (3), the enzyme was partially protected against the DEPC-induced inhibition of EP formation (33.2% maximum inhibition with a rate constant of 0.15 min⁻¹). When the vesicles were treated with DEPC in the presence of 0.2 mM CaCl2 (∆ and △) or in the absence of Ca2+ (∨ and ∨), the DEPC-induced inhibition (∨ and ∨) and the protection by the pretreatment with F- and Mg2+ (∨ and △) were somewhat less than those in the presence of 2 mM CaCl2. For this reason, in subsequent experiments, modification with DEPC was performed in the presence of 2 mM CaCl2 unless otherwise stated. Pretreatment with F- in the absence of Mg2+ provided no protection (data not shown).

Protection by Presence of Vanadate and Mg2+ against DEPC-induced Inhibition of EP Formation from P1—As shown in Fig. 1B, when the SR vesicles were treated with DEPC in the presence of 0.5 mM vanadate and 10 mM MgCl2, and absence of Ca2+ (∆), the enzyme was protected against the DEPC-induced inhibition of EP formation from P1 (49.1% maximum inhibition with a rate constant of 0.12 min⁻¹). The extent of this protection was almost the same as that of the protection afforded by the pretreatment with F- and Mg2+. In the absence of vanadate and presence of 9.7 mM Mg2+ (□ or in the presence of 0.5 mM vanadate and absence of Mg2+ (△), no protection was observed (84.6% maximum inhibition with a rate constant of 0.13 min⁻¹).

Lack of Protection by EP Formation from P1 against DEPC-induced Inhibition of EP Formation from P1—As shown in Fig. 1C, when the SR vesicles were preincubated with P1 in the presence of Mg2+ and absence of Ca2+ in 30% (v/v) Me2SO at 25 °C for 20 min (4.9 nmol/mg EP was formed by this preincubation) and then treated with DEPC, the enzyme was hardly protected against the DEPC-induced inhibition of EP formation from P1 (compare □ with □). Binding of P1 to the catalytic site in the presence of 10 mM P1 and absence of Mg2+ afforded no protection (compare △ with ∨). Binding of Mg2+ in the absence of P1 and presence of 9.7 mM Mg2+ provided some protection.
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Fig. 1. DEPC-induced inhibition of EP formation from P₀ protection by pretreatment with F⁻ and Mg²⁺ or by presence of vanadate and Mg²⁺ against the inhibition, and lack of protection by EP formation from P₁ against the inhibition. A, the SR vesicles were pretreated with (○, ●, △, ▲, and ▼) or without (□, ■, ○, ◆, ▽, ◄, ◄, ▼, and ▼) DEPC for various times in the presence of 2 mM CaCl₂ (○, ●, △, and ▲) or without (□, ■, ○, ◆, ▽, ◄, ◄, ▼, and ▼), or 5 mM EGTA without added CaCl₂ (△, ▲, ◄, ◄, ▼, and ▼) as described under “Experimental Procedures.” The vesicles were then incubated with KF in the presence of MgCl₂ otherwise as described under “Experimental Procedures.” The vesicles were then treated with KF and MgCl₂ as described under “Experimental Procedures,” and the contents of tightly bound F⁻ and Mg²⁺ were determined. Solid lines drawn under open circles and open triangles show least squares fit to single exponentials.

Fig. 2. DEPC-induced inhibition of tight binding of F⁻ and Mg²⁺. The SR vesicles were incubated with (○ and △) or without (● and ▲) DEPC for various times. The vesicles were then treated with KF and MgCl₂ as described under “Experimental Procedures,” and the contents of tightly bound F⁻ and Mg²⁺ were determined. Solid lines drawn under open circles and open triangles show least squares fit to single exponentials.

(compare □ with ▼), but higher concentrations of Mg²⁺ up to 29 mM provided no further protection (data not shown). This is in contrast to no protection afforded by 9.7 mM Mg²⁺ in the absence of Me₂SO (see Fig. 1B, ■). DEPC-induced Inhibition of Tight Binding of F⁻ and Mg²⁺—The SR vesicles were treated with or without DEPC for various times, and then tight binding of F⁻ and Mg²⁺ was determined (Fig. 2). The amounts of tightly bound F⁻ and Mg²⁺ at zero time of the treatment were 17.0 and 8.1 nmol/mg, respectively, being 4.3 and 2.0 times the content of phosphorylation site (4.0 nmol/mg) in the vesicles used. This is in agreement with our previous findings (18, 21) that the stoichiometry of tight binding of F⁻ and Mg²⁺ to the maximum level of phosphorylation is 4:2:1. The tight binding was inhibited progressively during the least squares fit to single exponentials.

B, the SR vesicles (10.2 mg/ml) were preincubated in 0.102 M KCl and 102 mM MES-NaOH (pH 6.0) at 25 °C for 30 min in the presence of 0.51 mM vanadate, 10.2 mM MgCl₂, and 10.2 mM EDTA (● and ◆), in the absence of vanadate and presence of 10.2 mM MgCl₂ and 10.2 mM EDTA (□ and ◄), in the presence of 0.51 mM vanadate and 10.2 mM EDTA and absence of MgCl₂ and EDTA (◂ and ▼), or in the absence of vanadate, MgCl₂, and EDTA and presence of 10.2 mM EDTA (◇ and ▼). After addition of acetonitrile with (◇, □, ◆, and ▼) or without (●, ■, ◄, and ▼) DEPC, the vesicles were incubated for various times. The final composition was 10 mg of vesicles/ml, 0 or 4.5 mM DEPC, 0 or 0.5 mM vanadate, 0 or 10 mM MgCl₂ (9.7 mM Mg²⁺), 0 mM CaCl₂, 0 or 10 mM EDTA, 0 or 10 mM EGTA, 0.1 M KCl, 2% (v/v) acetonitrile, and 100 mM MES-NaOH (pH 6.0). EP formation from ³²P₀ was determined. Solid line shows the curve generated by the mean of best fit constants of single exponentials for open circles, open triangles, and open inverted triangles. Dashed line shows least squares fit of a single exponential to open circles. C, the SR vesicles (10.2 mg/ml) were preincubated in 30.6% (v/v) Me₂SO and 102 mM MES-NaOH (pH 6.0) at 25 °C for 20 min, in the presence of 2.04 mM Pi, 10.2 mM MgCl₂, and 10.2 mM EDTA (● and ◆), in the absence of P₀, and presence of 10.2 mM MgCl₂ and 10.2 mM EDTA (□ and ◄), in the presence of 10.2 mM P₀ and 10.2 mM EDTA and absence of MgCl₂ and EDTA (◂ and ▼), or in the absence of P₀, MgCl₂, and EDTA and presence of 10.2 mM EDTA (◇ and ▼). The amount of EP formed from P₀, by the preincubation in the presence of P₀, MgCl₂, and EDTA (● and ◆), was 4.8 nmol/mg. After addition of acetonitrile with (◇, □, ◆, and ▼) or without (●, ■, ◄, and ▼) DEPC, the vesicles were incubated for various times. The final composition was 10 mg of vesicles/ml, 0 or 4.5 mM DEPC, 0, 2, or 10 mM P₀, 0 or 10 mM MgCl₂ (9.7 mM Mg²⁺), 0 mM CaCl₂, 0 or 10 mM EDTA, 0 or 10 mM EGTA, 50% (v/v) Me₂SO, 2% (v/v) acetonitrile, and 100 mM MES-NaOH (pH 6.0). EP formation from ³²P₀ was determined. Solid and dashed lines show least squares fit to single exponentials.
vanadate binding in the presence of Mg$^{2+}$ increased with increasing concentration of vanadate and was saturated with 4 μM vanadate. The maximum level of this binding was in approximate agreement with the content of phosphorylation site (4.0 nmol/mg). This binding was nearly completely inhibited by the treatment with DEPC (●). When the vesicles were pretreated with F$^-$ and Mg$^{2+}$ and then treated with DEPC (○), the enzyme was strongly protected against the DEPC-induced inhibition of vanadate binding.

When the vesicles were pretreated without F$^-$ and then treated with DEPC for various times (Fig. 3B, □), vanadate binding in the presence of Mg$^{2+}$ was inhibited with apparent first order kinetics at a rate of 0.11 min$^{-1}$ and its maximum inhibition was 80.4%. These kinetic parameters were in essential agreement with the rate (0.11 min$^{-1}$) and maximum (89.7%) of the DEPC-induced inhibition of EP formation from P$i$ under the same conditions (see Fig. 1A, □). The enzyme was protected by the pretreatment with F$^-$ and Mg$^{2+}$ against the inhibition of vanadate binding (Fig. 3B, ○) to a similar extent (44.2% maximum inhibition with a rate constant of 0.09 min$^{-1}$) as protected against the inhibition of EP formation from P$i$ (see Fig. 1A, ○).

**DEPC-induced Weak Inhibition of EP Formation from Acetyl Phosphate**—The SR vesicles were pretreated with (○ and ●) or without (□ and ■) F$^-$ in the presence of Mg$^{2+}$ (Fig. 4, A and B). In the experiments shown in Fig. 4A, the pretreated vesicles were treated with (○ and □) or without (● and ■) DEPC for various times. After tightly bound F$^-$ and Mg$^{2+}$ were removed, EP formation from acetyl phosphate was determined. DEPC caused only weak inhibition of the EP formation when the vesicles were pretreated without F$^-$ (compare □ with ■). This inhibition was unaffected by the pretreatment with F$^-$ and Mg$^{2+}$ (compare ○ with □). In contrast to the results reported by Coan and DiCarlo (24), EP formation from ATP was almost completely inhibited by the treatment with DEPC (data not shown). This discrepancy may be possibly due to the difference in the experimental conditions used.

In the experiments shown in Fig. 4B, the pretreated vesicles were treated with (○ and □) or without (● and ■) DEPC for 40 min. After tightly bound F$^-$ and Mg$^{2+}$ were removed, the vesicles were phosphorylated with acetyl phosphate for various times. When the vesicles were pretreated without F$^-$ and then treated with DEPC (□), EP formation was only slightly slower than that (■) in the vesicles that were pretreated without F$^-$ and then treated without DEPC. The pretreatment with F$^-$ and Mg$^{2+}$ had virtually no effect on the kinetics of EP formation in the DEPC-treated vesicles (compare ○ with □).

**DEPC-induced Inhibition of Hydrolysis of EP Formed from Acetyl Phosphate**—In the experiments shown in Fig. 4C, the SR vesicles were pretreated with F$^-$ and Mg$^{2+}$ (○ and ●), without F$^-$ and with Mg$^{2+}$ (□ and ■), or with F$^-$ and without Mg$^{2+}$ (△ and ▲) and then treated with (○, □, △, and ▲) or without (●, ■, and ▼) DEPC for 40 min. After tightly bound F$^-$ and Mg$^{2+}$ were removed, the vesicles were phosphorylated with acetyl [32P]phosphate at 0 °C for 4 min. EP formation was quenched by addition of nonradioactive acetyl phosphate and EGTA, and the decay of EP was followed. When the vesicles were treated without DEPC (●, ■, and ▼), the EP decay was rapid and could be described by a single exponential with a decay constant of 2.37 min$^{-1}$, the kinetics of this decay was unaffected by any above pretreatment. When the vesicles were pretreated without F$^-$ and with Mg$^{2+}$ (□ or with F$^-$ and without Mg$^{2+}$ (△) and then treated with DEPC, the EP decay was very slow and could be described by a single exponential with a decay constant of 0.17–0.21 min$^{-1}$.

The vesicles were pretreated with F$^-$ and Mg$^{2+}$ and then treated with DEPC (○), the EP

### Notes

- **Experimental Procedures.** A, the pretreated vesicles were incubated with (○, □, ■, and ▼) or without (△ and ▲) DEPC for 40 min. Vanadate binding to the vesicles was determined at various concentrations of vanadate in the presence of 5 mM MgCl$_2$ and 2 mM EGTA (○, □, and △) or in the presence of 5 mM EDTA (●, ■, and ▲), otherwise as described under “Experimental Procedures.” Lines drawn under open triangles, open circles, and open squares show least squares fit to a Michaelis equation, in which the dissociation constants and maximum extents of vanadate binding were 0.06 μM and 5.01 nmol/mg, 0.08 μM and 3.52 nmol/mg, and 0.78 μM and 0.62 nmol/mg, respectively. B, the pretreated vesicles were incubated with (○ and □) or without (● and ■) DEPC for various times. Vanadate binding was determined at 10 μM vanadate in the presence of 5 mM MgCl$_2$ and 2 mM EGTA, otherwise as in A. Lines drawn under open squares and open circles show least squares fit to single exponentials.
The DEPC-modified SR vesicles were pretreated with or without KF in the presence of MgCl₂, otherwise as described under "Experimental Procedures," and then incubated with or without DEPC for 12 min. The vesicles were further incubated in 20 mM CaCl₂ and others as in Fig. 1A. The resulting vesicles (0.417 mg/ml) were incubated with 0.5 mM hydroxylamine (pH 7.0), with 0.5 mM NaCl, or with neither hydroxylamine nor NaCl, at 25°C for 30 min in 16.7 mM CaCl₂, 83.3 mM KCl, and 83.3 mM MOPS-NaOH (pH 7.0). The vesicles were washed by centrifugation with a solution containing 0.1 mM KCl and 20 mM MOPS-NaOH (pH 7.0), and then EP formation from ³²P, was determined. Values are the means for two separate experiments.

| Pretreatment | DEPC | EP formation |
|--------------|------|--------------|
| None         | |               |
| NaCl         | |               |
| NH₄OH        | |               |
| MgCl₂        | 0    | 4.31 4.59 4.33 |
| MgCl₂        | 4.5  | 1.37 1.30 2.30  |
| KF and MgCl₂ | 0    | 4.21 4.31 4.21  |
| KF and MgCl₂ | 4.5  | 2.77 2.80 3.17  |

Decay could be described by a double exponential. The fast decaying component comprised 63.6% of total EP with a decay constant of 2.12 min⁻¹. This constant was in substantial agreement with the decay constant (2.37 min⁻¹) for EP (● and ▲) that was formed with the vesicles treated without DEPC. The slow decaying component comprised 36.4% of total EP with a decay constant of 0.20 min⁻¹. This constant was in good agreement with the decay constant (0.17–0.21 min⁻¹) for EP (●) and ▲ that was formed with the vesicles without MgCl₂, or with F⁻ and without MgCl₂, and then treated with DEPC.

Partial Restoration of EP Formation from P, by the Treatment of DEPC-modified SR Vesicles with Hydroxylamine—The SR vesicles were pretreated with or without F⁻ in the presence of MgCl₂ and then treated with or without DEPC for 12 min. After tightly bound F⁻ and MgCl₂ were removed, the vesicles were treated with 0.5 mM hydroxylamine (pH 7.0) or with 0.5 mM NaCl, and then EP formation from P, was determined (Table I). When the vesicles were pretreated without F⁻, EP formation from P, was markedly inhibited by the treatment with DEPC. The EP formation was partially restored by the subsequent treatment with hydroxylamine. The treatment with NaCl substituted for hydroxylamine caused no restoration of EP formation. When the vesicles were pretreated with F⁻ and MgCl₂, the DEPC-induced inhibition of EP formation was suppressed considerably. The subsequent treatment with hydroxylamine again caused a partial restoration of EP formation.

Peptide Mapping of Tryptic Digests of DEPC-modified SR Vesicles—In the first series of experiments (Fig. 5, A–C), the SR vesicles were pretreated without F⁻ and with MgCl₂ (A), with F⁻ and without MgCl₂ (B), or with F⁻ and MgCl₂ (C), and then treated with DEPC. The vesicles were digested with TPCK-trypsin and subjected to reversed phase HPLC. The peptide maps at 214 nm (lower traces of A–C) agreed closely with each other. Partial restoration of EP formation from P, by the treatment of DEPC-modified SR vesicles with hydroxylamine

**FIG. 4.** Formation and hydrolysis of EP from acetyl phosphate with DEPC-modified SR vesicles and effects of pretreatment with F⁻ and MgCl₂. The SR vesicles were pretreated with KF and MgCl₂ (○ and ●), without KF and with MgCl₂ (□ and ■), or with KF and without MgCl₂ in the presence of 5 mM EDTA and absence of EGTA (◆ and ▲), otherwise as described under "Experimental Procedures." A, the pretreated vesicles were incubated with (○ and □) or without (● and ■) DEPC for various times. The vesicles were phosphorylated with acetyl [³²P]phosphate for 5 min as described under "Experimental Procedures." The amount of EP formed was determined. B, the pretreated vesicles were incubated with (○ and □) or without (● and ■) DEPC for 40 min. The vesicles were phosphorylated with acetyl [³²P]phosphate for various times, otherwise as in A. The amount of EP formed was determined. C, the pretreated vesicles were incubated with (◆, □, and ▲) or without (●, ■, and ▲) DEPC for 40 min. The vesicles were phosphorylated with acetyl [³²P]phosphate in the presence of 0.1 mM KCl, 5 mM MgCl₂, and others as in A at 0°C for 4 min. EP formation was quenched at the indicated zero time by diluting the reaction mixture 10 times with a solution containing 3 mM nonradioactive acetyl phosphate, 5 mM MgCl₂, 4 mM EGTA, 0.1 mM KCl, 5 mM A23187, and 50 mM Tris-HCl (pH 7.5). The vesicles were further incubated at 0°C. The reaction was stopped at the indicated times by adding trichloroacetic acid, and the amount of EP was determined. 100% EP represents 1.31 (○), 0.93 (□), 1.16 (◆), 3.68 (●), 3.53 (■), and 3.26 (▲) nmol/mg. Solid line drawn under open squares and dash-dotted line drawn under open triangles show least squares fit to single exponentials. Dotted line was generated by the mean of best fit constants of single exponentials for solid circles, solid squares, and solid triangles. Dashed line drawn under open circles shows least squares fit to a double exponential.

**TABLE I**

Partial restoration of EP formation from P, by the treatment of DEPC-modified SR vesicles with hydroxylamine.
Fig. 5. Peptide mapping of tryptic digests of DEPC-modified SR vesicles and effects of pretreatment with $F^-$ and $Mg^{2+}$, of presence of vanadate and $Mg^{2+}$, or of $EP$ formation from $P_i$ on the modification with DEPC. In the first series of experiments (A–C), the
other. The absorbances at 240 nm of peptides containing DEPC-modified histidine in the peaks indicated by arrows I–III were appreciably reduced by the pretreatment with F– and Mg2+ (compare upper trace of C with upper trace of A) but not by the pretreatment with F– and without Mg2+ (compare upper trace of B with upper trace of A).

In the second series of experiments (Fig. 5, D–G), the SR vesicles were treated with DEPC in the absence of vanadate and Mg2+ (D), in the absence of vanadate and presence of Mg2+ (E), in the presence of vanadate and absence of Mg2+ (F), or in the presence of vanadate and Mg2+ (G). In reversed phase HPLC of the tryptic digests, the peaks indicated by arrows I–III were appreciably reduced only when both vanadate and Mg2+ were present during the treatment with DEPC (compare upper trace of G with upper traces of D–F).

In the third series of experiments (Fig. 5, H–K), the SR vesicles were preincubated in 30.6% (v/v) Me2SO at 25 °C for 20 min in the absence of P1 and Mg2+ (H), in the presence of P1, and presence of 10.2 mM MgCl2 (I), in the presence of 10.2 mM P1, and absence of Mg2+ (J), or in the presence of 2.04 mM P1 and 10.2 mM MgCl2 (K), and then treated with DEPC. In reversed phase HPLC of the tryptic digests, the peaks indicated by arrows I–III were reduced only when Mg2+ was present during the treatment with DEPC (compare upper traces of I and K with upper traces of H and J). The presence of P1 in the absence of Mg2+ had no effect on these peaks (compare upper trace of J with upper trace of H).

It is clear from the chromatographic profiles that the peaks reduced by the presence of vanadate and Mg2+ (Fig. 5, D–G, arrows) or by the presence of Mg2+ and Me6SO (Fig. 5, H–K, arrows) corresponded to those reduced by the pretreatment with F– and Mg2+ (Fig. 5, A–C, arrows).

Although the changes of peak III in different conditions were very small (but reproducible) in these experiments, the extent of the reduction in this peak varied with different preparations of SR vesicles used. In fact, in the experiments shown in Fig. 6 (A and B), the reduction in peak III was substantial.

**Purification of DEPC-modified Peptides**—The SR vesicles were pretreated without (Fig. 6A) or with (Fig. 6B) F– in the presence of Mg2+ and then treated with DEPC. The vesicles were digested with TPK-trypsin and subjected to the first reversed phase HPLC.

**Fractions I–III** in A and B were pooled separately and subjected to the second reversed phase HPLC (data not shown). **Fraction I** from A gave a peak at 240 nm that was strongly reduced in the second HPLC of fraction I from B. **Fraction II** from A gave a major peak at 240 nm that was also greatly reduced in the second HPLC of fraction II from B. **Fraction III** from A gave a peak at 240 nm that was again greatly reduced in the second HPLC of fraction III from B. **Fractions IV** from A and B showed no difference in the second HPLC. The peaks sensitive to the pretreatment with F– and Mg2+ were further purified by the third reversed phase HPLC (data not shown). Purified peptides, **Peptide 1** (from fraction I in A), **Peptide 2** (from fraction II in A), and **Peptide 3** (from fraction III in A), were obtained by final reversed phase HPLC (Fig. 6, C–E).

The peak with retention time of about 47 min in Fig. 6A was also reduced by the pretreatment with F– and Mg2+ (Fig. 6B). However, this peak was not analyzed since the reduction in this peak was not reproducible.

**Hydroxylamine Sensitivity of Isolated DEPC-modified Peptides**—The peptides isolated as above were analyzed by reversed phase HPLC before and after treatment with 0.5 μM hydroxylamine (pH 7.0). Hydroxylamine-treated **Peptide 1** and 2 had the same shorter retention time and a much smaller absorbance at 240 nm/214 nm ratio than untreated **Peptides 1** and 2 (data not shown). The absorption spectra of untreated Peptides 1 and 2 showed shoulders at 240 nm (Fig. 7, A and B, solid lines), which are characteristic of mono-N-carbethoxyhistidine (28). These shoulders disappeared when the peptides were treated with hydroxylamine (Fig. 7, A and B, dashed lines). These results suggest the presence of mono-N-carbethoxyhistidine in **Peptides 1 and 2**. In contrast, treatment of **Peptide 3** with hydroxylamine caused no change in the reversed phase HPLC profile and in the absorption spectrum (Fig. 7C, solid and dashed lines). **Peptide 3** had a 1.8 times higher absorbance at 240 nm/214 nm ratio than **Peptides 1 and 2**, but its absorption spectrum showed no definite shoulder at 240 nm. These results suggest the presence of bis-carbethoxyhistidine in **Peptide 3** (see Ref. 28).

**Amino Acid Analysis, Sequencing, and Mass Analysis of Isolated DEPC-modified Peptides**—**Peptides 1 and 2** had the same amino acid composition (Table II) that exactly matched the amino-terminal 7-amino acid sequence (Met-Glu-Ala-Ala-His-Ser-Lys) in the SR Ca2+-ATPase. **Peptide 3** also had the same amino acid composition except that histidine was missing. This missing of histidine is consistent with the above idea that histidine of **Peptide 3** is bis-carbethoxylated, because it is known (28) that acid hydrolysis in amino acid analysis does not regenerate histidine from bis-carbethoxyhistidine although it regenerates histidine from mono-N-carbethoxyhistidine. Attempts to sequence these three peptides were unsuccessful, indicating that they were blocked on their amino termini. This again suggests that these peptides are the amino-terminal peptides of the SR Ca2+-ATPase, because the amino terminus of this enzyme is blocked by an acetyl group (39). Masses of **Peptides 1 and 2** were both 887.5, being in good agreement with the monoisotopic mass (887.4) of the above acetylated amino-terminal peptide calculated on the assumption that His-5 is mono-N-carbethoxylated. Mass of **Peptide 3** was 949.4, being in exact agreement with the monoisotopic mass (949.4) of the above acetylated peptide calculated on the assumption that His-5 is bis-carbethoxylated. These results lead to the conclusion that His-5 in the SR Ca2+-ATPase was modified with DEPC and that this modification was specifically inhibited by the pretreatment with F– and Mg2+ (Fig. 5, A–C, arrows), by the presence of vanadate and Mg2+ (Fig. 5, D–G, arrows), or by the presence of Mg2+ and Me6SO (Fig. 5, H–K, arrows). The reason why peptides with the same sequence and same modification gave different retention times in HPLC remains obscure.
DISCUSSION

The observed protection by tight binding of F$^-$ and Mg$^{2+}$ (Fig. 1A) or by high affinity binding of vanadate in the presence of Mg$^{2+}$ (Fig. 1B) against the DEPC-induced inhibition of EP formation from P$_i$ suggests that a histidyl residue(s) protected by these transition state analogs contributes toward formation of the transition state for EP formation from the magnesium-enzyme-phosphate complex or, alternatively, that the protected histidyl residue(s) is located very close to the essential components (bound phosphate, bound Mg$^{2+}$, or other functional groups) of this transition state. This view is consistent with the findings that binding of these transition state analogs is inhibited by the modification with DEPC (Figs. 2 and 3) and that the enzyme is protected by tight binding of F$^-$ and Mg$^{2+}$ against the DEPC-induced inhibition of high affinity vanadate binding (Fig. 3). It is also in harmony with the observed lack of protection by formation of EP from P$_i$ or by formation of the enzyme-phosphate complex (Fig. 1C), because it is likely that the struc-

Fig. 6. Purification of DEPC-modified peptides that are sensitive to pretreatment with F$^-$ and Mg$^{2+}$. The SR vesicles were pretreated without (A) or with (B) KF in the presence of MgCl$_2$, otherwise as described under “Experimental Procedures.” The vesicles were then incubated with DEPC, digested with TPCK-trypsin, and subjected to the first reversed phase HPLC (A and B), as in Fig. 5 (A–C). Fractions indicated by horizontal bars (I–IV) were pooled separately. Fractions I, II, and III from A and B were subjected to the second reversed phase HPLC. Elution was performed with linear gradients of acetonitrile in 20 mM Na$_2$SO$_4$ and 5 mM sodium phosphate (pH 6.4). Fractions sensitive to the pretreatment with F$^-$ and Mg$^{2+}$ were pooled separately and subjected to the third reversed phase HPLC. Elution was performed with a linear gradient of acetonitrile in 0.1% ammonium trifluoroacetate (pH 6.4). Fractions sensitive to the pretreatment with F$^-$ and Mg$^{2+}$ were further subjected to final reversed phase HPLC (C–E). Elution was performed with the following linear gradients of acetonitrile in 0.1% ammonium trifluoroacetate (pH 6.4): C, 0% from 0 to 30 min, 9% at 35 min, 13.5% at 75 min, and 90% at 80 min; D, 0% from 0 to 30 min, 9% at 35 min, 18% at 75 min, and 90% at 80 min; E, 0% from 0 to 30 min, 11.3% at 35 min, 20.3% at 75 min, and 90% at 80 min. Peptides 1, 2, and 3 thus isolated were subjected to amino acid analysis, sequencing, and mass spectrometry. A–E, the absorbance at 214 nm (lower traces) and the absorbance at 240 nm (upper traces) were monitored.
The data from peptide purification (Fig. 6), amino acid analysis (Table II), sequencing, and mass spectrometry show that His-5 is a single major histidyl residue protected by these analogs against the modification. These findings indicate that modification of His-5 with DEPC is responsible for the DEPC-induced inhibition of EP formation from P1 (Fig. 1C) and against the modification of His-5 with DEPC (Fig. 5E, arrows) suggests that His-5 is located close to the Mg2+-binding site in the magnesium-enzyme complex. The lack of protection by Mg2+ in the absence of Me3SO (Fig. 1B and Fig. 5E, arrows) may be possibly due to lower affinity for Mg2+ in the absence of Me3SO (40). The lack of protection by the presence of 10 mM Pi in the absence of Mg2+ against the DEPC-induced inhibition of EP formation from P1, (Fig. 1C) and against the modification of His-5 with DEPC (Fig. 5J, arrows) indicates that His-5 is not located at the P7-binding site in the enzyme-phosphate complex.

The weak inhibition of EP formation from acetyl phosphate by the modification with DEPC (Fig. 4A), the lack of protection by tight binding of F- and Mg2+ against this inhibition (Fig. 4A), and the lack of an appreciable effect of the modification with DEPC on the kinetics of EP formation from acetyl phosphate (Fig. 4B) indicate that phosphotransfer from acetyl phosphate to the phosphorylation site in the forward reaction is not substantially affected by the modification of His-5.

The DEPC-induced inhibition of hydrolysis of EP formed from acetyl phosphate (Fig. 4C) is consistent with the inhibition of EP formation from P1, (Fig. 1, A and B), because EP hydrolysis is reversal of EP formation from P1. However, we cannot exclude the possibility that the conformational change of EP immediately preceding its hydrolytic cleavage is also inhibited by this modification.

Biphasic kinetics of the EP decay shown in Fig. 4C (dashed line drawn under open circles) is interpreted in terms of the presence of the fast and slow decaying populations that comprise EP having unmodified His-5 and DEPC-modified His-5, respectively. This interpretation is supported by the findings that the decay constants for the fast and slow decaying components are in good agreement with the decay constants for EP formed with the unmodified vesicles (Fig. 4C, dotted line drawn under solid symbols) and with the unprotected DEPC-modified vesicles (Fig. 4C, solid and dash-dotted lines drawn under open squares and open triangles), respectively. The results indicate that the rate of EP hydrolysis is reduced to less than 10% by the modification of His-5 with DEPC and further suggests that the observed inhibition of EP hydrolysis and of its reversal is almost exclusively due to the modification of His-5 with DEPC.

His-5 is conserved in sarco(endoplasmic reticulum Ca2+-ATPases (1, 41–43) but not in plasma membrane Ca2+-ATPase (44). Therefore, we cannot entirely exclude the possibility that the observed inhibition is due to steric hindrance induced by DEPC-modified His-5.

The secondary structural model for the Ca2+-ATPase suggests that the enzyme is composed of 10 transmembrane alpha-helices (M1 to M10) and a cytoplasmic globular fraction, which is divided into two main domains, a small cytoplasmic loop between M2 and M8 and a large cytoplasmic loop between M1 and M5 (1). The large cytoplasmic loop contains the phosphorylation site (1, 4–6) and the ATP-binding site (34, 45–49). The functional role of the small cytoplasmic loop is less clear, but we
have recently suggested that Arg-198 in this loop is located in or close to the catalytic site in the transition state for EP formation from the magnesium-enzyme-phosphate complex (21). The amino-terminal region preceding the first transmembrane helix (M1) is known to be exposed to the cytoplasm (50). Its functional role is unknown, although it was previously shown (51) that deletion of the amino-terminal 30 amino acids inhibits the stable insertion of the enzyme into the membrane and inactivates the enzyme. Our present results suggest that the amino-terminal domain including His-5 contributes to the catalytic site.

REFERENCES
1. Brandl, C. J., Green, N. M., Korczak, B., and MacLennan, D. H. (1986) Cell 44, 597–607
2. Hasselbach, W., and Makinose, M. (1961) Biochem. Z. 333, 518–528
3. Ebashi, S., and Lipmann, F. (1962) J. Cell Biol. 14, 389–400
4. Degani, C., and Boyer, P. D. (1973) J. Biol. Chem. 248, 8222–8226
5. Bastide, F., Meisner, G., Fleischer, S., and Post, R. L. (1973) J. Biol. Chem. 248, 8385–8391
6. Allen, G., and Green, N. M. (1976) FEBS Lett. 63, 188–192
7. Makinose, M. (1967) Pflegers Arch. Gesamte Physiol. Menschen Tiere 294, R82–R83
8. Yamamoto, T., and Tomomura, Y. (1967) J. Biochem. (Tokyo) 62, 558–575
9. Champell, P., and Guilani, P. (1986) Biochemistry 25, 7625–7633
10. Friedman, Z., and Makinose, M. (1970) FEBS Lett. 11, 69–72
11. Pucell, A., and Martinson, A. (1971) J. Biol. Chem. 246, 3389–3397
12. Kanazawa, T., and Boyer, P. D. (1973) J. Biol. Chem. 248, 3163–3172
13. Masuda, H., and de Meis, L. (1978) Biochemistry 17, 2489–2496
14. Punzengruber, C., Prager, R., Kolassa, N., Winkler, F., and Suko, J. (1978) Eur. J. Biochem. 92, 349–358
15. Kolassa, N., Punzengruber, C., Suko, J., and Makinose, M. (1979) FEBS Lett. 108, 495–500
16. Murphy, A. J., and Coll, R. J. (1992) J. Biol. Chem. 267, 5229–5235
17. Kubota, T., Daito, T., and Kanazawa, T. (1993) Biochem. Biophys. Res. Commun. 193, 131–134
18. Daiho, T., Kubota, T., and Kanazawa, T. (1993) Biochim. Biophys. Acta 1163, 1021–1026
19. Dupont, Y., and Bennett, N. (1982) FEBS Lett. 139, 237–240
20. Inesi, G., Lewis, D., and Murphy, A. J. (1984) J. Biol. Chem. 259, 996–1003
21. Saino, T., Daito, T., and Kanazawa, T. (1997) J. Biol. Chem. 272, 21142–21150
22. Coffey, R. L., Lagwinska, E., Oliver, M., and Martinson, A. (1975) Arch. Biochem. Biophys. 170, 37–45
23. Tenu, J.-P., Ghéis, C., Leger, D. S., and Carrette, J. (1976) J. Biol. Chem. 251, 4322–4329
24. Coan, C., and DiCarlo, R. (1990) J. Biol. Chem. 265, 5376–5384
25. Martínez-Azorín, F., Soler, F., Gomez-Fernandez, J. C., and Fernandez-Belda, F. (1996) Biochem. J. 309, 499–505
26. Martinsoni, A., Boland, R., and Halpin, R. A. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 455–468
27. Yu, B. P., Masoro, E. J., and Bertrand, H. A. (1974) Biochemistry 13, 5083–5087
28. Miles, E. W. (1977) Methods Enzymol. 47, 431–442
29. Lundblad, R. L. (1995) Techniques in Protein Modification, CRC Press, Inc., Boca Raton, FL
30. Christendat, D., and Turnbull, J. (1996) Biochemistry 35, 4468–4479
31. Nakamura, S., Suzuki, H., and Kanazawa, T. (1994) J. Biol. Chem. 269, 16015–16019
32. Barraclough, H., Scofano, H. M., and Inesi, G. (1984) Biochemistry 23, 1542–1548
33. Goodno, C. C. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2620–2624
34. Yamagata, K., Daito, T., and Kanazawa, T. (1993) J. Biol. Chem. 268, 20900–20906
35. Medda, P., and Hasselbach, W. (1983) Eur. J. Biochem. 137, 7–14
36. Yamashita, K., and Yamamoto, T. (1991) J. Biochem. (Tokyo) 110, 915–921
37. Stadmiller, E. R. (1957) Methods Enzymol. 3, 228–231
38. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
39. Tong, S. W. (1977) Biochem. Biophys. Res. Commun. 74, 1242–1248
40. de Meis, L., de Souza Oters, A., Martins, O. B., Alves, E. W., Inesi, G., and Nakamoto, R. (1982) J. Biol. Chem. 257, 4995–4998
41. MacLennan, D. H., Brandl, C. J., Korczak, B., and Green, N. M. (1985) Nature 316, 696–700
42. Lytton, J., and MacLennan, D. H. (1988) J. Biol. Chem. 263, 15024–15031
43. Burk, S. E., Lytton, J., MacLennan, D. H., and Shull, G. F. (1989) J. Biol. Chem. 264, 18561–18568
44. Verma, A. K., Piloten, A. G., Stanford, D. R., Wieser, E. D., Penniston, J. T., Strehler, E. E., Fischer, K., 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
45. Mitchinson, C., Wilderspin, A. F., Trinnaman, B. J., and Green, N. M. (1982) FEBS Lett. 146, 87–92
46. Yamamoto, H., Imamura, Y., Tagaya, M., Fukui, T., and Kawakita, M. (1989) J. Biochem. (Tokyo) 106, 1121–1125
47. Yamashita, K., Daito, T., and Kanazawa, T. (1994) J. Biol. Chem. 269, 4129–4134
48. McIntosh, D. B., Woulfe, D. G., Vilsen, B., and Andersen, J. P. (1996) J. Biol. Chem. 271, 25778–25789
49. Kimura, K., Suzuki, H., Daito, T., Yamashita, K., and Kanazawa, T. (1996) J. Biol. Chem. 271, 28933–28941
50. Reithmeier, R. A. F., and MacLennan, D. H. (1993) J. Biol. Chem. 268, 5087–5091
51. Skerjanc, I. S., Clarke, D. M., Loo, T. W., and MacLennan, D. H. (1993) FEBS Lett. 336, 168–170