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The Ca^{2+}-ATPase of the Scallop Sarcoplasmic Reticulum Is of a Cold-adapted Type

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Abstract. At 0 to 20°C, the Ca^{2+}-ATPase activity of the scallop sarcoplasmic reticulum (SR) was observed to be 7–60% of the peak activity at 30°C, while the ATPase activity of the rabbit SR was 0–7% of its peak at 55°C. The relative rabbit ATPase activity (0.7–7.0%) at 7–20°C became higher (6–15 times) and lower (1/4–1/2), respectively, by the solubilization of the rabbit ATPase with a detergent, dodecyloctaethylenglycol monoether, and by the reconstitution of the ATPase with asolectin (soybean lecithin). No activity at 0°C remained irrespective of these treatments. The relative scallop ATPase activity at 0–20°C was, however, scarcely affected by such solubilization and reconstitution. In contrast to the rabbit ATPase, the scallop ATPase seems to be capable of operating independently without the help of the membrane lipid at low temperature.

Key words: Scallop — Sarcoplasmic reticulum — Ca^{2+}-ATPase — Cold-adaptation — Membrane lipid

Introduction

The Ca^{2+}-ATPase of the sarcoplasmic reticulum (SR) is a calcium pump transporting calcium ions across the SR membrane by hydrolytic coupling with ATP (Tada, Yamamoto & Tonomura, 1978; Ikemoto, 1982; de Meis & Inesi, 1982). In a previous study (Abe et al., 1992), the Ca^{2+}-ATPase activity of the scallop SR was found to be more than two times higher than that of the rabbit SR at 0–20°C; the scallop is a poikilothermal invertebrate that lives in the cold (≤20°C) sea, and the rabbit is a homoiothermal vertebrate with a rectal temperature of 39°C (Ederstrom, 1973). In the rabbit ATPase, Warren et al. (1974) have found that the structure of the lipid chain of the SR membrane is crucial for the temperature sensitivity of the ATPase activity at low temperature, and suggested the importance of the membrane fluidity in the activity. In the scallop ATPase, it has been shown that its lipid environment plays a dominant role in determining the temperature of the Arrhenius break (Kalabokis & Hardwicke, 1988). On the other hand, in cold-stenothermal fish species, evolutionary adaptation of the synaptosomal membrane lipids to low temperature has been found (Cossins & Prosser, 1978). However, as to the membrane of such an organism, which is evolutionarily adapted to low temperature, the role of the membrane lipid in the function of its membrane protein has not yet been examined. Here, we studied the role of the lipid environment of the scallop ATPase in the temperature sensitivity of ATPase activity by referring to that of the lipid environment of the rabbit ATPase. Namely, lipid environments of the scallop and rabbit ATPases were changed with a detergent, dodecyloctaethyleneglycol monoether ($C_{12}E_8$), and asolectin (soybean lecithin). We report the primary importance of the scallop ATPase protein in its own temperature sensitivity, independent of the help of the membrane lipids.

Materials and Methods

Preparations

Scallop and rabbit SR were prepared from the cross-striated adductor muscle of the scallop (*Patinopecten yessoensis*) (Abe et al.,

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1992) and the fast-twitch muscle of the rabbit (Nakamura, Endo & Konishi, 1977), respectively, as reported previously.

The scallop and rabbit Ca^{2+}-ATPases were purified from scallop and rabbit SR by washing with sodium deoxycholate (DOC) according to methods previously reported by Nagata et al. (1998) and Nakamura (1983), respectively. The contents of the ATPase protein in the deoxycholate-washed ATPase preparations (DOC-washed ATPase preparation) of the scallop and the rabbit have been estimated to be more than 90%. The phospholipid phosphorus contents of the scallop and the rabbit preparations were 23.0 and 25.0 μg P/mg of protein, respectively.

The ATPase preparations (detergent-solubilized ATPase preparations), which were solubilized with an excess amount of a nonionic detergent, $C_{12}E_8$, were obtained by solubilizing the DOC-washed ATPase preparations with an assay medium containing $C_{12}E_8$ (see "Assays" for details).

The ATPase preparations (asolectin-reconstituted ATPase preparations) that were reconstituted with an excess amount of asolectin (Wako Pure Chemicals Industries, Osaka, Japan) were obtained as follows: The DOC-washed ATPase preparation (2 mg protein) was solubilized in 1 ml of 20 mM Tris-maleate (pH 7.5) buffer solution containing 0.12 M KCl, 5 mM $CaCl_2$, 5 mg/ml of $C_{12}E_8$ and 20% glycerol in ice. The solubilization medium was centrifuged at 100,000 × *g* for 30 min at 4°C to remove the insoluble residue. The resulting supernatant was mixed with 50 μl of the asolectin suspension (100 mg/ml); the asolectin suspension was obtained by sonicating the asolectin in 20 mM Tris-maleate (pH 7.5) buffer solution containing 0.12 M KC1 and 5 mM $CaCl_2$ at room temperature. This was followed by the addition of 1 ml of the asolectin suspension (100 mg) containing 20 mM Tris-maleate (pH 7.5), 0.12 M KCl, 5 mM $CaCl_2$, 100 mg of $C_{12}E_8$ and 20% glycerol, and the mixture was incubated by gentle shaking for 30 min at room temperature. After the incubation, 2.5 g of Bio-Beads SM-2 (20–50 mesh, Bio-Rad, Hercules, CA) was added to the mixture. About 1 h after the addition of the Bio-Beads, the mixture was filtrated with a cellulose filter (No. 2, Advantec Toyo, Tokyo, Japan) to remove the Bio-Beads SM-2. The filtrate was diluted with 3–4 volumes of 20 mM Tris-maleate (pH 7.5) buffer solution containing 0.12 M KCl and 5 mM $CaCl_2$. The diluted filtrate was centrifuged at 100,000 × *g* for 30 min at 4°C. The upper layer (the soft, yellow portion) of the precipitate was slid off the dense lower portion. This upper portion was homogenized in 20 mM Tris-maleate (pH 7.0) buffer containing 0.12 M KCl and 0.3 M sucrose, and used as the reconstituted ATPase preparation.

Assays

In the detergent-solubilized ATPase preparation of the scallop, it was found that a high concentration (1 mM) of $CaCl_2$ is required to maintain its Ca^{2+}-ATPase activity for 4 min; at more than 4 min, the ATPase activity gradually decreased. In the solubilized rabbit preparation, the activity has been found to be fully maintained at a lower calcium concentration of 0.1 mM (Møller, Lind & Andersen, 1980). The ATPase reactions of both the scallop and rabbit ATPase preparations were, therefore, carried out in the presence of 1 mM $CaCl_2$. Total ATPase activities of the DOC-washed (0.05 mg of protein/ml), the detergent-solubilized (0.25 mg of protein/ml) and the asolectin-reconstituted (0.05 mg of protein/ml) ATPase preparations were assayed in 1 ml of the medium containing 20 mM Tris-maleate (pH 7.0), 5 mM ATP, 0.12 M KCl, 1 mM $CaCl_2$ and 5 mM $MgCl_2$ at 0–55°C; for the assays of the solubilized and the reconstituted preparations, 12.5 mg/ml of $C_{12}E_8$ and 20% (v/v) glycerol, and 5 μM A23187 (a calcium-ionophore) were, respectively, added to their assay mediums. Mg^{2+}-ATPase activity of each ATPase preparation was determined under conditions identical to those for the assay of the total ATPase activity, except that 5 mM EGTA was added instead of $CaCl_2$. The ATPase reactions were initiated by the addition of ATP and terminated by the addition of 1 ml of 10% trichloroacetic acid 20 s to 5 min after the start. Ca^{2+}-ATPase activity was obtained by subtracting the Mg^{2+}-ATPase activity from the total ATPase activity. At 5 mM ATP, which was employed for the assay of the ATPase activity, the velocities of the ATPase activity of the native scallop (Abe et al., 1992) and rabbit (Møller et al., 1980) SR have been shown to be close (more than 95% of the V_{max}) to their V_{max} conditions. The Pi liberated from ATP was determined by the method of Bonting, Simon & Hawkins (1961).

Sucrose gradient analysis of the asolectin-reconstituted preparation was performed as follows: 100–200 μl aliquots of the reconstituted preparation were layered onto a linear gradient (5 ml) of 0–20 (w/v) % sucrose, containing 20 mM Tris-maleate (pH 7.0) and 0.12 M KCl. The tubes were centrifuged at 150,000 × *g* in a Hitachi P55ST2 rotor for 16 h at 4°C. The gradient tubes were fractionated 270 μl each from the top of the tubes. Total phospholipids of the DOC-washed preparations and the asolectin-reconstituted preparations were extracted by the method of Folch, Lees & Stanley (1957). The amounts of phosphorus in the extracted phospholipids and the asolectin were determined by the method of Bartlett (1959). The protein concentration was determined by the biuret method (Gornall, Bardawill & David, 1949) and the method of Lowry et al. (1951) with the use of bovine serum albumin as the standard.

Results

A temperature of more than 20°C is fatal to the scallop, which lives in the cold (≤20°C) sea. The activity of the scallop ATPase in a range of 0–20°C is, therefore, mainly discussed here by referring to that of the rabbit ATPase in that temperature range.

The DOC-washed Scallop and Rabbit ATPases

Scallop and rabbit ATPase preparations, which were purified by washing scallop and rabbit SR with DOC, were employed to obtain the solubilized preparations with detergent ($C_{12}E_8$) and to reconstitute the membranous ATPase preparations with asolectin. These DOC-washed ATPase preparations were, therefore, used as the preparation having native membrane lipids. The DOC-washed scallop (Fig. 1*A*) and rabbit (Fig. 1*B*) ATPase preparations exhibited their peak activities of 1,158 and 5,282 nmol Pi/mg of protein/min, respectively, at 30 and 50°C. At 0–20°C, the scallop ATPase activity was 7–60% (81–692 nmol Pi/mg of protein/min) of the peak activity, while the rabbit activity was 0–7% (0–328 nmol Pi/mg of protein/min) of the activity. The temperatures at which the scallop and rabbit activities were irreversibly lost were 42 and 55°C, respectively. The temperature-dependent profiles of the DOC-washed scallop and rabbit preparations are almost the same as those (Abe et al., 1992) of their native SR preparations, respectively. This suggests that DOC-washing of scallop and rabbit SR does not affect the temperature-dependent profiles of their ATPase activities.

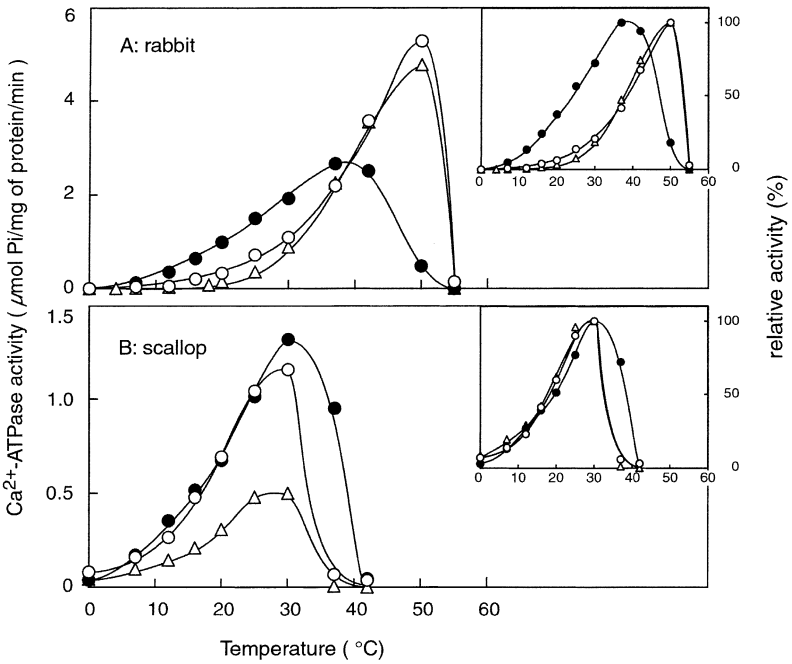

Fig. 1. Temperature dependence of the Ca^{2+}-ATPase activity of DOC-washed, detergent-solubilized and asolectin-reconstituted preparations of rabbit and scallop ATPases. (*A*) ATPase activities of rabbit DOC-washed (○), detergent-solubilized (●) and asolectin-reconstituted (Δ) preparations. (*B*) ATPase activities of scallop DOC-washed (○), detergent-solubilized (●) and asolectin-reconstituted (Δ) preparations. Insets in *A* and *B* are the temperature-dependent profiles of the rabbit and scallop ATPase preparations, which are normalized by setting each peak level of the activity at 100%, respectively.

The above-described peak activity is a function of the fraction of undenatured ATPase molecules present and of some collision-type factor in the Arrhenius equation. At a temperature lower than the peak temperatures of 30 and 50°C, respectively, for the scallop and rabbit ATPases, the denaturation of these ATPase proteins and/or the disruption of the lipid bilayers of their DOC-washed and asolectin-reconstitued membrane preparations and/or the oxidation of the membrane lipids of these preparations, etc., seem to begin. Namely, these processes are thought to decrease the slope of the activity vs temperature plots below the peak temperature, followed by a steep fall of the activity above the peak temperature.

The Detergent-solubilized and the Asolectin-reconstituted Rabbit ATPases

The solubilization of the DOC-washed preparation of the rabbit ATPase with the detergent lowered the temperature at which the activity was at a peak level from 50 to 37°C, and also lowered the peak level of the activity by about half (from 5,282 to 2,660 nmol Pi/mg of protein/min) (Fig. 1*A*). At 7–20°C, however, the activity (37–328 nmol Pi/mg of protein/min) was increased 3–7 times by the solubilization, though no activity at 0°C was maintained. At more than 37°C, the activity was decreased, and it was irreversibly lost at 55°C. The solubilization seems to shift the temperature-dependent profile of the rabbit ATPase to the lower temperature side. The detergent has been shown to change the oligomeric state (Vanderkooi et al., 1977; Pick & Karlish, 1980; Nakamura & Tajima, 1997) of the rabbit ATPase molecules in the SR membrane to a monomeric state (Møller, le Maire & Andersen, 1988). However, it has also been shown that the monomeric and oligomeric molecules in the membrane, which are reconstituted with an excess amount of phospholipids, have almost the same ATPase activity (Starling, East & Lee, 1995) and that the effects of the detergent on the ATPase are quite similar to those of phospholipids (Dean, 1982). Such a shift of the temperature-dependent profile, therefore, seems to be induced by the detergent surrounding the ATPase molecules rather than by the monomerization of the molecules with the detergent. It is thought that compared with the native lipids of the rabbit SR membrane, the detergent provides a lipid environment suitable for the rabbit ATPase to support its activity at low but not at higher temperatures. On the other hand, the reconstitution of the DOC-washed rabbit preparation with asolectin did not affect the temperature (50°C) at which the activity was at a peak level. The peak activity (4,770 nmol Pi/mg of protein/min) of the reconstituted ATPase preparation was near that (5,280 nmol Pi/mg of protein/min) of the DOC-washed preparation. At 7–20°C, however, the activity (8–153 nmol Pi/mg of protein/min) was at a level 1/5 to 1/2 of that (37–328 nmol Pi/mg of protein/min) of the washed preparation. As in the case of the washed preparation, no activity at 0°C was observed. At 30–50°C, the activity (888–4,770 nmol Pi/mg of protein/min) of the reconstituted preparation was close to that (1,095–5,282 nmol Pi/mg of protein/min) of the washed preparation. The homogeneity of the reconstituted preparation was examined as to the ratio of the membrane lipid to the ATPase protein (Fig. 2*A*); the preparation was sedimented onto a continuous sucrose gradient of 0–20%. The fraction pattern of

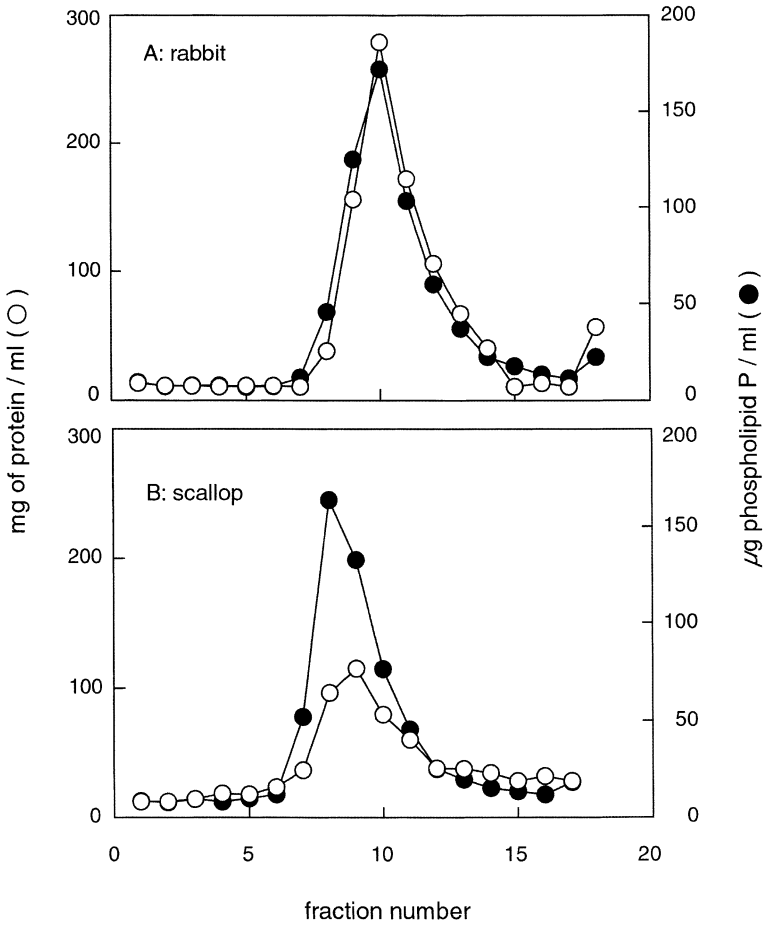

Fig. 2. Concentrations of ATPase protein and phospholipid phosphorus in sucrose gradient fractions of reconstituted rabbit and scallop preparations. ○, concentration of ATPase protein (mg of protein/ml); ●, concentration of phospholipid phosphorus (μg of P/ml).

protein concentration in the gradient showed a single peak and was consistent with that of the lipid concentration. This suggests that the preparation is composed of a single population of reconstituted membrane vesicles. The phospholipid phosphorus content of the total vesicles is 667 μg P/mg of protein. The lipids (23.0 μg P/mg of protein) of the DOC-washed preparation seem to be diluted by about 1/29 with the addition of asolectin (32.3 μg P/mg). It is, therefore, thought that asolectin does not serve as a lipid environment suitable for the catalytic activity of the rabbit ATPase at 0–20°C, while at a temperature of more than 30°C, it facilitates such activity in the same way as native lipids of the SR membrane.

The Detergent-solubilized and the Asolectin-reconstituted Scallop ATPases

The peak ATPase activities of the detergent-solubilized and the asolectin-reconstituted preparations of the scallop ATPase were observed at 30°C, similar to the case of the DOC-washed preparation (Fig. 1*B*). The peak level of the activity (1,320 nmol Pi/mg of protein/min) of the solubilized preparation was almost the same as that (1,158 nmol Pi/mg of protein/min) of the washed preparation. The solubilized preparation was active at 0°C with an activity of 38 nmol Pi/mg of protein/min, though this level of activity was low compared with that (81 nmol Pi/mg of protein/min) of the washed preparation. The reconstituted preparation, however, exhibited about half (34–61%; 42–500 nmol Pi/mg of protein/min) of the activity (81–1,158 nmol Pi/mg of protein/min) of the washed preparation over a temperature range of 0–30°C. This is different from the observations (Fig. 1*A*) that the activity of the reconstituted rabbit preparation is comparable to that of the washed preparation at more than 30°C. It is, therefore, probable that the low activity of the reconstituted scallop preparation is due to the scallop ATPase itself rather than to the asolectin that was used for the reconstitution. In the presence of high calcium of $\geq$1 mM and glycerol (20% v/v), the solubilized scallop ATPase has been shown to be stable (Kalabokis & Hardwicke, 1988; Kalabokis, Santoro & Hardwicke, 1993 and *see* "Assays"). Based on the present and earlier observations, however, compared with solubilized rabbit ATPase, solubilized scallop ATPase is thought to be less stable: (i) In the absence of glycerol and the presence of a lower level of calcium of 0.1 mM, the ATPase activity of the scallop ATPase, which was solubilized by the detergent with a ratio of

Table 1. Relative ATPase activities of DOC-washed, detergent-solubilized and asolectin-reconstituted preparations of rabbit and scallop ATPases in a temperature range of 0–20°C

Temperature (°C)	0	4	7	12	16	20
Rabbit						
DOC-washed	0 (0)	–	0.70 (0.037)	0.96 (0.051)	3.85 (0.203)	6.21 (0.328)
Detergent-solubilized	0 (0)	–	4.70 (0.126)	13.3 (0.355)	24.2 (0.643)	37.2 (0.990)
Asolectin-reconstituted	0 (0)	0.05 (0.002)	0.17 (0.008)	0.60 (0.029)	1.61 (0.077)	3.21 (0.153)
Scallop						
DOC-washed	7.00 (0.081)	–	13.8 (0.160)	22.9 (0.226)	41.3 (0.478)	59.8 (0.692)
Detergent-solubilized	2.88 (0.038)	–	13.0 (0.171)	26.8 (0.354)	39.2 (0.517)	51.1 (0.675)
Asolectin-reconstituted	8.40 (0.042)	–	19.4 (0.097)	29.0 (0.145)	42.0 (0.210)	61.6 (0.308)

The activities of each preparation were normalized by setting its peak activity at 100%, as shown in the insets of Fig. 1*A* and 1*B*. The numbers in parentheses are the observed values (μmol Pi/mg of protein/min) of the activities.

detergent to ATPase protein of 2.5:1, decreased to a level of about 10% of that before the solubilization within 10 min after the solubilization (*data not shown*). The rabbit ATPase, which was solubilized with the same ratio of detergent:protein, has been previously shown to be stable (Møller, le Maire & Andersen, 1988). (ii) In the presence of calcium (0.1 mM) and glycerol (20% v/v), the ATPase activity of the scallop ATPase, which was solubilized by the detergent with a much higher ratio (50:1) of detergent to ATPase protein, decreased to a level of about 30% of that before the solubilization within 4 min (*data not shown*). As for the rabbit ATPase which was solubilized with the same high ratio of detergent:protein, the ATPase activity has been found to be fully maintained (Møller et al., 1980). Therefore, it is likely that the above-mentioned low activities of the reconstituted scallop ATPase result from inactivation of the ATPase molecules during the reconstitution process (about 4 h) of the solubilized ATPase molecules into the asolectin membrane, due to the unstability of the solubilized scallop molecules. Moreover, the observed values of the Ca^{2+}-ATPase activities of the asolectin-reconstituted scallop preparations were found to significantly fluctuate from preparation to preparation and to be irrelevant to the Arrhenius analysis with statistical confidence. The whole temperature-dependence of the scallop ATPase activity in the experimental temperature range of 0–42°C fluctuated only slightly and constantly exhibited a profile with a temperature of 30°C at which the activity reached its peak. It is also likely that the fluctuation in the observed values of the activities is mainly derived from that in the content of the undenatured ATPase molecules in the reconstituted preparations, which is caused by the unstability of the solubilized scallop ATPase. Sucrose gradient analysis of the reconstituted scallop preparation (Fig. 2*B*) showed that the fraction patterns of protein and lipid concentrations in the gradient were single and almost the same. The preparation seems to be composed of a single population of reconstituted membrane vesicles, similar to the case of the reconstituted rabbit preparation. Phospholipid phosphorus content of the total vesicles is 919 μg P/mg of protein (Fig. 2*B*). The lipids (25.1 μg P/mg of protein) of the scallop DOC-washed preparation, therefore, are thought to be diluted about 1/37 by the addition of asolectin.

Discussion

The temperature dependences of the scallop (Kalabokis & Hardwicke, 1988), rabbit (Dean & Suarez, 1981; Bigelow & Thomas, 1987) and lobster (Deamer, 1973; Madeira & Antunes-Madeira, 1975) Ca^{2+}-ATPases have been studied by subjecting data to Arrhenius analysis. Arrhenius analysis of the present data of the scallop ATPase is, therefore, important for comparison of the present data with those of previous studies. However, due to their fluctuation with each preparation, the observed values of the Ca^{2+}-ATPase activities of the asolectin-reconstituted preparations of the scallop ATPase were found to be irrelevant to Arrhenius analysis with statistical confidence. Thus, we attempted to find the difference in the temperature sensitivities of the rabbit and scallop ATPase proteins by normalizing the ATPase activities of each ATPase preparation (DOC-washed, $C_{12}E_8$-solubilized and asolectin-reconstituted preparations of the rabbit and scallop ATPases) to its peak activity (Fig. 1*A*, inset and Fig. 1*B*, inset). Such normalization is thought to be of no quantitative significance in the examination of the temperature sensitivities of these ATPase molecules, because the peak activity is not the maximum activity of the Ca^{2+}-ATPase molecules in each ATPase preparation, but rather the activity of the undenatured ATPase molecules that are present at the peak temperature, as described in the Results section. However, it is thought that the difference in the temperature sensitivities of these ATPases, which appears due to this normalization, can serve as basic information on their sensitivities. The relative activi-

ties of the rabbit and scallop ATPase preparations in a low temperature range of 0–20°C, which were normalized by setting their peak activities at 100%, are summarized in Table 1.

As for the rabbit ATPase (Fig. 1*A*, inset), the relative activity (0.7–7.0%) of the DOC-washed preparation at 7–20°C became higher (4.7–40%; 6–15 times) and lower (0.17–3.2%; 1/4–1/2), respectively, by the solubilization and the reconstitution; the solubilization lowered the peak temperature of the DOC-washed preparation from 50 to 37°C, while the reconstitution did not affect the peak temperature. This indicates that the detergent and the asolectin operate on the rabbit ATPase activity in opposite directions at a lower temperature, suggesting the importance of the effect of the lipid environment on the temperature sensitivity of the ATPase. Warren et al. (1974) earlier observed that the rabbit ATPase activity is highly sensitive to the structure of the lipid chain at low temperatures and suggested that the activity is inhibited below temperatures that correspond to a phase transition of the lipid compounding with the ATPase protein. The opposite effects of the detergent and the asolectin, observed here, are also thought to be the result of the difference in their transition temperatures.

As for the scallop ATPase, the entire temperature-dependent profiles of all of the washed, solubilized and reconstituted preparations at 0–42°C, which were normalized, were almost the same (Fig. 1*B*, inset). At 0–20°C, the relative activities of the solubilized and the reconstituted preparations were 3–50 and 9–60%, respectively, very close to that (7–60%) of the DOC-washed preparation. These results indicate that the detergent and the asolectin scarcely affect the temperature dependence of the scallop ATPase activity, suggesting no significant effect of the lipid environment on the temperature sensitivity of the scallop ATPase. As to the temperature sensitivity of the rabbit ATPase, a significant effect of the lipid environment was observed at low temperature, as shown in Fig. 1*A*. In contrast to the case of rabbit ATPase, it is, therefore, thought that the scallop ATPase is capable of operating independently at low temperature rather than with the help of the SR membrane lipids. The present study provides a new viewpoint of temperature sensitivity useful in comparing the structure of the scallop (Nagata et al., 1998) and rabbit (Brandl et al., 1986) ATPases, which differ by about 30% in their amino-acid sequences.

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