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PRESSURE ADAPTATION OF Na^+/K^+-ATPase IN GILLS OF MARINE TELEOSTS

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Summary

The effects of pressure and temperature on an integral membrane protein, Na^+/K^+-adenosine triphosphatase (Na^+/K^+-ATPase), were studied in fish gill membrane preparations from shallow- and deep-living marine teleosts. The inhibition by pressure of maximal velocity of the enzyme is nonlinear, increasing at higher pressures. Na^+/K^+-ATPases from deep-sea fish were less inhibited by pressure than those of shallow-living species. Habitat temperature also affected the pressure response of the enzyme. As a function of physiological pressure and temperature, the order of increasing pressure-sensitivity was cold, deep-sea < warm, deep-sea (hydrothermal vents) < polar = shallow and mid-depth, cold < shallow, warm. Activation volumes in all species were conserved at 30–60 ml mol^{-1} at physiological pressures, which may reflect a similar membrane physical state at the actual pressure the animal experiences. Arrhenius plots [ln(Na^+/K^+-ATPase activity) *vs* 1/T] were steeper for warm-water and shallow-living species than for deep-sea species. The depth at which adaptation was first observed was about 2000 m (≈ 200 atm; 1 atm = 101·3 kPa). The data are consistent with a model of increased membrane fluidity resulting in reduced pressure-sensitivity of Na^+/K^+-ATPase from deep-sea species.

Introduction

Pressure provides a unique selective feature in the largest portion (by volume) of the biosphere, the deep sea. Deep-sea organisms not only tolerate pressures lethal to most surface-living life, but some even require high pressures (bacteria: Yayanos & Dietz, 1983; Yayanos *et al.* 1981; crustaceans: Macdonald & Gilchrist, 1978, 1980; Yayanos, 1981; fish: Macdonald *et al.* 1987; W. W. Wakefield, personal communication). The biochemical basis of pressure tolerance has been investigated in only a very few systems. Most studies have focused on soluble enzymes and structural proteins. In all systems examined, the homologous proteins of deep-sea organisms have been found to be significantly less sensitive to pressure than those of shallow-living species (Siebenaller, 1987; Siebenaller & Somero, 1989; Somero *et al.* 1983). The threshold pressure at which perturbation is adequate to

Key words: deep sea, homeoviscous adaptation, Na^+/K^+-ATPase, pressure.

favour selection for pressure-adapted variants of these proteins differs, showing that different biochemical systems differ substantially in their sensitivities to pressure (Siebenaller & Somero, 1989).

Membranes and their associated proteins should be especially sensitive to pressure and, therefore, apt to show adaptation in deep-living species. Membrane proteins contact two very different environments: the non-polar, hydrophobic interior of the membrane and the polar, aqueous medium on either side of the bilayer. The complexity of these systems allows many sites for pressure to perturb protein function, but also provides additional evolutionary opportunities for adaptation.

Two pressure-adaptive strategies can be postulated for membrane proteins. The first is evolution of a pressure-adapted protein. This has been demonstrated in soluble proteins such as dehydrogenases (Siebenaller, 1984; Siebenaller & Somero, 1978) and structural proteins such as actin (Swezey & Somero, 1985), but membrane proteins have not been examined in this regard. Second, modification of the membrane lipid composition could modulate protein function to offset the effects of pressure. At high pressures or low temperatures, membrane lipids become more viscous (less fluid), which may inhibit function of membrane proteins. According to homeoviscous theory, this inhibition can be offset by increasing the proportion of short-chain or unsaturated fatty acids in the membrane phospholipids (Macdonald & Cossins, 1985). Changes in phospholipid head groups or amount of other lipids such as cholesterol may also play a role. In the limiting case, no changes in the primary structure of a membrane protein would be necessary, given the proper lipid environment. Homeoviscous adaptation to temperature is well-documented (Cossins, 1983), and recent work has also demonstrated a homeoviscous response to pressure in fish (Avrova, 1984; Cossins & Macdonald, 1984, 1986) and bacteria (DeLong & Yayanos, 1985, 1986).

Na^+/K^+-ATPase (EC3.6.1.3) is a particularly attractive protein for pressure studies for several reasons. It is a heterodimeric integral membrane protein with an absolute requirement for phospholipids. Its primary role in osmoregulation of marine fishes (Philpott, 1980) makes it a likely candidate for adaptation, and teleost gills are an excellent, readily accessible source of the enzyme. Finally, Na^+/K^+-ATPase is one of the very few membrane enzymes for which any effects of pressure have been described (Chong *et al.* 1985; Moon, 1975; Pfeiler, 1978; de Smedt *et al.* 1979).

The primary questions of interest in this study were as follows. (1) Is Na^+/K^+-ATPase pressure-adapted? (2) What properties of the enzyme (V_m, K_m) are important in adaptation? (3) How much pressure is required to select for adaptation? (4) Is adaptation of Na^+/K^+-ATPase due to changes in the protein, changes in the lipid environment, or both? To attempt to answer these questions, we investigated the effects of pressure and temperature on gill membrane preparations from fishes occurring from surface waters to depths greater than 4 km. We examined deep-sea species from typical cold (2–3 °C) environments and warm (3–20 °C) hydrothermal vent sites. Our results show distinct adaptation t

pressure and support a role for homeoviscous adaptation in the adaptation process.

Materials and methods

Specimen collection

Species used in this study are listed in Table 1. Deep-sea, mid-depth and shallow-water temperate species were collected by otter trawl in Monterey Bay, or off the coasts of Oregon or New Jersey. The hydrothermal vent zoarcid *Thermarces cerberus* and an undescribed bythitid (genus *Bythites*) were captured using the submersible DSRV *Alvin* at the 13°N and Galapagos vent sites, respectively. Gills were excised from freshly caught fish, wrapped in aluminium foil and frozen on dry ice or in liquid nitrogen. Whole, frozen specimens of antarctic fish were provided by Dr William Stockton. Warm-water species were purchased in Hawaiian fish markets by Dr Margaret McFall-Ngai and frozen whole on dry ice. Gills were removed from thawed specimens and processed as described below.

Membrane preparation

Thawed gill filaments were trimmed from the gill arches and homogenized in 5–10 vol. of EIS buffer (1 mmol l^{-1} EDTA, 50 mmol l^{-1} imidazole, 250 mmol l^{-1} sucrose, 5 mmol l^{-1} 2-mercaptoethanol, pH 7·5). Homogenates were centrifuged for 15 min at 2000 ***g***. The supernatant was carefully removed and spun for 90 min at 19 000 ***g***. The pellet was resuspended in approximately 1 ml of EIS buffer per gram of original tissue. Samples were stored in Eppendorf microfuge tubes at −80°C until needed.

Enzyme assays

A coupled pyruvate kinase/lactate dehydrogenase (PK/LDH) assay was used to determine ATPase activity. Decrease in absorbance at 340 nm was followed spectrophotometrically using a high-pressure optical cell (Mustafa *et al.* 1971). At pressures above 1 atm (101·3 kPa), the path length for the light beam increases by 12 % as a result of pressure exerted on the quartz–sapphire windows. Measured high-pressure activities were therefore corrected by 12 %. The assay medium contained 100 mmol l^{-1} NaCl, 20 mmol l^{-1} KCl, 5 mmol l^{-1} $MgCl_2$, 3 mmol l^{-1} disodium adenosine-5′-triphosphate (ATP), 0·5 mmol l^{-1} phosphoenolpyruvate (PEP) (tricyclohexylammonium salt), 0·05 mmol l^{-1} fructose-1,6-bisphosphate (FBP) (tetracyclohexylammonium salt), 0·2 mmol l^{-1} β-nicotinamide adenine dinucleotide, reduced (NADH), 30 mmol l^{-1} Tris buffer (pH 7·5 at 10°C) and excess coupling enzymes. Tris buffer was used because of its very low pressure-dependence (Neumann *et al.* 1973). All reagents were the best grade available. EDTA, FBP, NADH and ouabain were from Sigma, and ATP, PEP and PK/LDH were purchased from Boehringer-Mannheim. (Pyruvate kinase from Sigma retained less than 0·5 % of its activity at 10°C in the presence of 3 mmol l^{-1} ATP.)

Thawed membrane preparations were divided into two portions. One fraction was diluted with at least 1 vol. of EIS buffer containing 10 mmol l^{-1} ouabain (a specific Na^+/K^+-ATPase inhibitor) and incubated for 30–60 min at room temperature. The other fraction was treated identically, except that no ouabain was present. No loss of total ATPase activity occurred in up to 2 h under these conditions. Reactions were started by addition of membrane preparations. After a nonlinear phase lasting a few minutes, the reaction was linear for at least 30 min, enough time to assay ATPase at three pressures. All assays were run in triplicate or better. Na^+/K^+-ATPase activity was calculated as the difference between total ATPase activity (no ouabain present) and ouabain-insensitive Mg^{2+}-ATPase, and was stable during the course of the experiment. Membranes prepared in this manner form fragments rather than sealed vesicles. We did not investigate coupling of ATP hydrolysis to cation transport.

Na^+/K^+-ATPase was at least half of the total activity at 1 atm, and a smaller fraction at higher pressures. Activities at high pressures were expressed as fractions of the 1 atm activity. Intraspecific variability in pressure effects was low, so error bars have been omitted from the figures for clarity. Standard deviations for data from *Anoplopoma fimbria* are shown in Fig. 5. This species showed the greatest intraspecific variation. Na^+/K^+-ATPase activities were usually 2–10 μmol ATP hydrolysed mg protein^{-1} h^{-1} at 1 atm. On a mass-specific basis, activities were 10–30 μmol ATP h^{-1} g gill filament^{-1}, approximately half of the activity measured in crude gill homogenates. Deep-sea fishes generally had lower Na^+/K^+-ATPase activities than shallow-living species.

For assays at temperatures other than 10°C, the pH was adjusted to follow the temperature-dependence of intracellular pH ($\Delta pH/\Delta T \approx -0{\cdot}017\,°C^{-1}$) (Somero, 1981). By combining data from pressure effects at different temperatures and temperature effects at 1 atm, Arrhenius plots [ln(Na^+/K^+-ATPase activity) *vs* 1/T] could be generated for a range of pressures.

Curve fitting

ln(Na^+/K^+-ATPase activity) *vs* pressure data for each species were fitted to quadratic equations by nonlinear regression. The slope of such a curve equals $-\Delta V‡/RT$ (Morild, 1981), where $\Delta V‡$ is the apparent activation volume, R is the gas constant and T is the absolute temperature. Apparent activation volumes were calculated from the derivatives of the fitted equations. Intraspecific variability was low (see Fig. 5), so means from several individuals are shown in the figures. Although Arrhenius plots appeared to be slightly curved, there were not enough measurements over a wide enough range of temperatures to distinguish the data statistically from straight lines.

Results

The effect of pressure on ln(Na^+/K^+-ATPase activity) at 10°C is illustrated in Fig. 1 for each species listed in Table 1. Two aspects of these data are of particula

Table 1. *Species used in the study*

Species	Depth range (m)	Habitat temperature (°C)
Deep-sea cold[a]		
Coryphaenoides armatus	1885–4815	2–4
C. leptolepis	2288–4639	2–4
Deep-sea, warm (hydrothermal vents)[b]		
Thermarces cerberus	2600	4–10
Bythites sp.[c]	2500	4–10
Shallow and mid-depth, cold[d]		
Coryphaenoides acrolepis	700–1820	4–8
Antimora microlepis	360–3000	4–8
Sebastolobus altivelis	330–1500	4–10
S. alascanus	25–1500	4–10
Anoplopoma fimbria	0–1500	4–10
Porichthys notatus	0–300	6–15
Polar (antarctic)[e]		
Gymnodraco acuticeps		−1·8
Rhigophila dearborni		−1·8
Trematomus bernacchii		−1·8
T. centronotus		−1·8
T. loenbergi		−1·8
Shallow, warm[f]		
Sphyraena barracuda	0–20	24
S. helleri	0–20	24
Lutjanus kasmira	0–?	24
Mulloidichthyes auriflamma	6–30	24

[a] Depth ranges from Siebenaller *et al.* (1982). Capture depth was 3025 m.

[b] Depths are depth of vent where fish was captured.

[c] Undescribed species.

[d] Depth ranges from Miller & Lea (1972). Capture depths indicated in Fig. 7. *P. notatus* were trawled at 85 m.

[e] We could not find data on depth ranges for antarctic species. These fish were collected in McMurdo Sound (W. Stockton, personal communication), which has a maximum depth of 600 m.

[f] Depth ranges for *Sphyraena* sp. are estimated assuming same depth range as *Sphyraena argentea* (Miller & Lea, 1972). *L. kasmira* belongs to a shallow-water family of fish (Gosline & Brock, 1960). Depth range for *M. auriflamma* from Gosline & Brock (1960).

importance. The first is the nonlinearity of the curves, indicating an increase in activation volume with pressure. Second, Na^+/K^+-ATPases of species living under similar temperature and pressure regimes have very similar pressure responses, but significant intergroup differences exist, with the enzymes from deep-sea species being the least pressure-sensitive.

The nonlinearity of the pressure response could have several causes. Denaturation or dissociation of subunits is a possibility, but membrane preparations

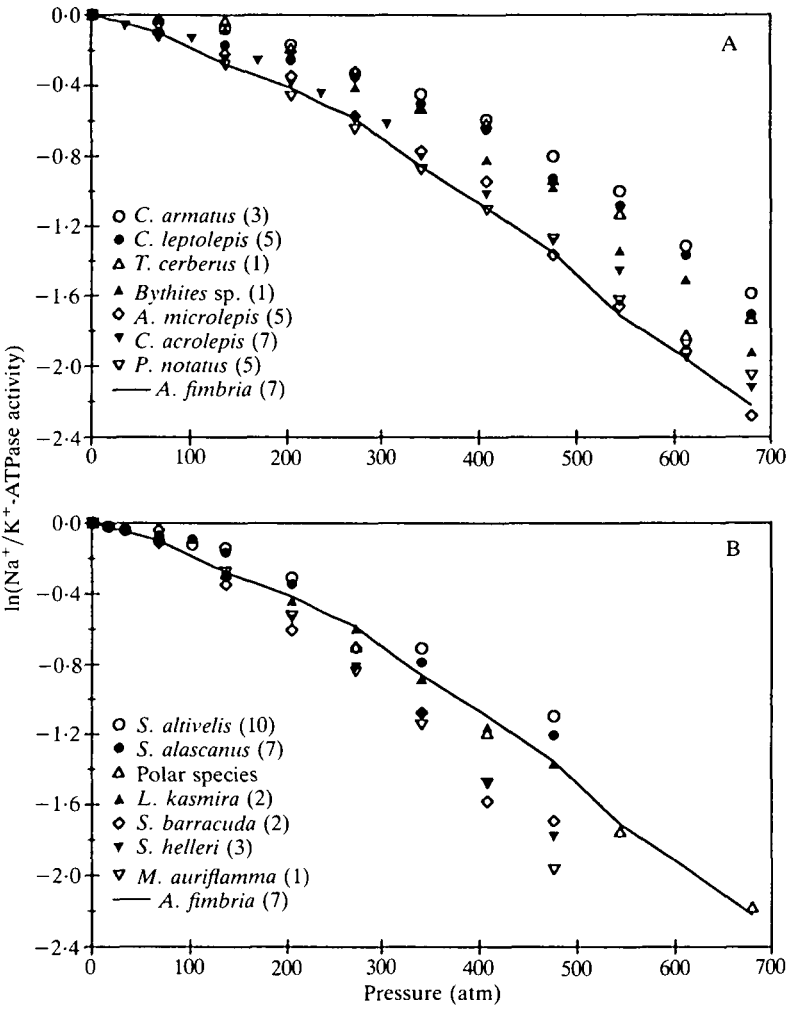

Fig. 1. Effect of pressure on maximal velocity of Na^+/K^+-ATPase at 10°C. Membrane preparations were assayed as described under Materials and methods. The lines indicate data for the sablefish, *Anoplopoma fimbria*, and are included in both panels to facilitate comparison. *N* values for each species are in parentheses. (A) Cold-living species inhabiting depths of 0 to greater than 4000 m, and hydrothermal vent species. (B) Shallow-living species living at −1·8 to 24°C. Antarctic species had barely measurable ATPase levels, so data from one individual each from five species were averaged. Complete scientific names are given in Table 1.

incubated for 1 h at >680 atm at 10–25°C showed no loss of Na^+/K^+-ATPase activity following pressure release (data not shown). Alternatively, the curves could reflect a decrease in affinity for ATP or for cations at high pressures. Even if V_m were unaffected by pressure, a rise in K_m with increasing pressure would result in pressure inhibition as the enzyme became progressively less saturated with substrate at higher pressures. Estimated $K_{0.5}$ (half-saturating concentration)

values of ATP (data not shown) for Na^+/K^+-ATPases from *Sebastolobus alascanus* and *Porichthys notatus* are less than 0·1 mmol l^{-1} at pressures up to 476 atm. Thus, in the presence of 3 mmol l^{-1} ATP, slight affinity changes should not affect the observed activities. Also, reducing the ATP concentration to 1 mmol l^{-1} had no measurable effect on Na^+/K^+-ATPase from several species, even at 680 atm. Varying Na^+ and K^+ concentrations also had no effect for fish from shallow and deep-sea habitats, at pressures up to 408 atm (Fig. 2), well beyond the pressure range of the shallow-living species. Thus, we conclude that the nonlinearity of the ln(Na^+/K^+-ATPase activity) *vs* pressure data is due to effects on V_m rather than affinity for substrate(s).

The effects of pressure on Na^+/K^+-ATPase activities of cold-living species from a range of depths, as well as two species of hydrothermal vent fish, are shown in Fig. 1A. Na^+/K^+-ATPases from the two deepest cold-living species and the hydrothermal vent fishes were the least inhibited by pressure. The shallower species all generally inhabit depths of less than 2000 m (equivalent to 200 atm), suggesting that pressures greater than 200 atm are required for pressure adaptation. A comparison of the effect of pressure on Na^+/K^+-ATPases from several shallow-living species, spanning an environmental temperature range of over 25 °C, showed that the enzymes from warm-water fishes were much more pressure-sensitive than those of temperate and polar species (Fig. 1B).

The results from all species from a given pressure–temperature regime have been combined to facilitate comparisons among the major environmental classes examined in this study (Fig. 3). The order of increasing pressure-sensitivity was deep-sea < shallow, cold < shallow, warm. The two hydrothermal vent species appeared to be slightly more pressure-sensitive than the non-vent deep-sea fishes. The actual temperatures experienced by the hydrothermal vent fishes are unknown, and may vary considerably. The vent bythitid was captured in 6 °C water (J. O'Brien, personal communication), but waters as warm as 20–25 °C may be accessible to this species and the zoarcid, *Thermarces cerberus*. Thus, increased pressure-sensitivity of Na^+/K^+-ATPases in vent fishes relative to other deep-sea species may be related to higher physiological temperatures, as in shallow-living species.

Quadratic equations fitted the ln(Na^+/K^+-ATPase activity) *vs* pressure data very well (Fig. 4A). The apparent activation volumes shown in Fig. 4B were calculated from the fitted quadratic equations for several species. At physiologically relevant pressures, Na^+/K^+-ATPases from all species had activation volumes of 30–60 ml mol^{-1}.

Na^+/K^+-ATPases from several species were assayed over a range of temperatures and pressures. In general, the enzyme was less inhibited by pressure at higher temperatures, as shown by the data for the sablefish (*Anoplopoma fimbria*) enzyme (Fig. 5, data for other species not shown). By combining these data with Na^+/K^+-ATPase activities as a function of temperature at 1 atm, the effects of temperature at higher pressures could be calculated.

Plots of ln(Na^+/K^+-ATPase activity) *vs* 1/T (Arrhenius plots) were generated

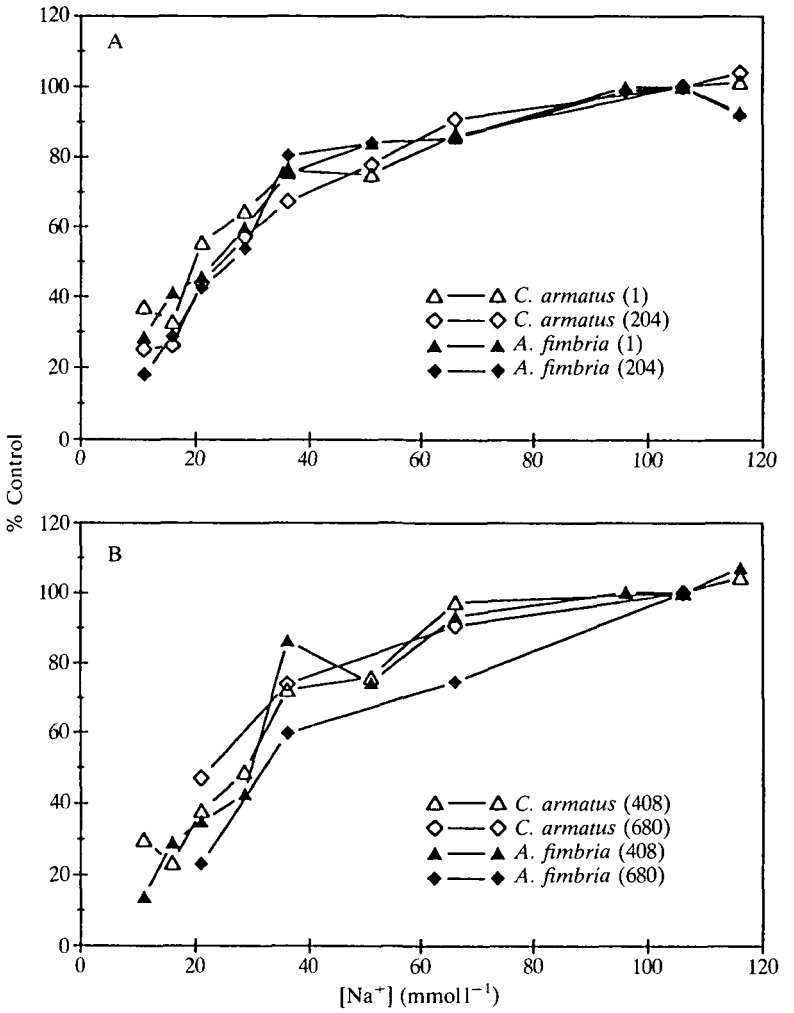

Fig. 2. Effect of cation composition and pressure on Na^+/K^+-ATPase activity in *Anoplopoma fimbria* and *Coryphaenoides armatus*. ATPase was assayed at 10°C as described in Materials and methods, except that NaCl and KCl levels were covaried to maintain a constant total cation concentration of 126 mmol l^{-1}. Enzyme activities are presented as a percentage of the control (100 mmol l^{-1} NaCl, 20 mmol l^{-1} KCl, 3 mmol l^{-1} Na_2ATP) activities. Assay pressures are given in parentheses in atmospheres.

for several pressures. The slope of an Arrhenius plot is equal to $-E_a/\boldsymbol{R}$, where E_a is the apparent activation energy and $\boldsymbol{R}$ is the gas constant. A higher apparent activation energy corresponds to a more temperature-sensitive enzyme. Arrhenius relationships at 1 atm were generated and calculated apparent activation energies at higher pressures are shown in Fig. 6B. The apparent activation energy for Na^+/K^+-ATPase increased at higher measurement pressures. The deeper-living grenadier (*Coryphaenoides armatus*) had the least temperature-sensitive enzyme;

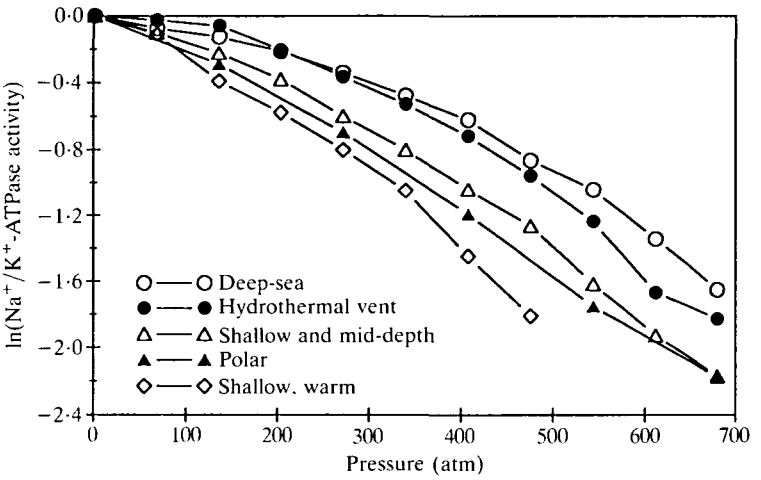

Fig. 3. Environmental comparison of pressure-sensitivity of Na^+/K^+-ATPase at 10°C. Data were averaged for each group of species from a given pressure–temperature habitat (Table 1).

that of the warm-living barracuda (*Sphyraena barracuda*) was the most temperature-sensitive.

As a fish moves vertically in the water column, it may be able to modify its membrane lipid composition to counteract the effects of varying pressures. Such changes may be reflected in differing pressure-sensitivities of membrane proteins in individuals of a species captured at different depths. We made intraspecific comparisons of five species (Fig. 7, also *Sebastolobus alascanus* and *Coryphaenoides acrolepis*). No intraspecific differences in pressure response were observed.

Discussion

This study provides evidence for pressure adaptation of an integral membrane protein, Na^+/K^+-ATPase, in marine teleosts. Deep-sea and cold-water species have less pressure-sensitive Na^+/K^+-ATPases than shallow- and warm-water species. Habitat pressures in excess of 200 atm are required to select for a pressure-adapted enzyme. Circumstantial evidence implicates a role for the membrane lipid environment in adaptation to pressure of Na^+/K^+-ATPase.

The high apparent activation volume (30–60 ml mol^{-1}) of Na^+/K^+-ATPase relative to many other enzymes (see table II in Morild, 1981) leads us to expect adaptation of V_m. Deep-sea fish maintain plasma Na^+ and K^+ concentrations similar to those of shallow species (Blaxter *et al.* 1971; Shelton *et al.* 1985). Although they are primarily benthic fish, grenadiers (*Coryphaenoides* spp.) may make large vertical excursions and certainly undergo ontogenetic vertical migrations of thousands of metres. For example, *C. leptolepis* has been caught 2000 m off the bottom in over 4000 m of water (Stein, 1985). If *C. leptolepis* Na^+/K^+-

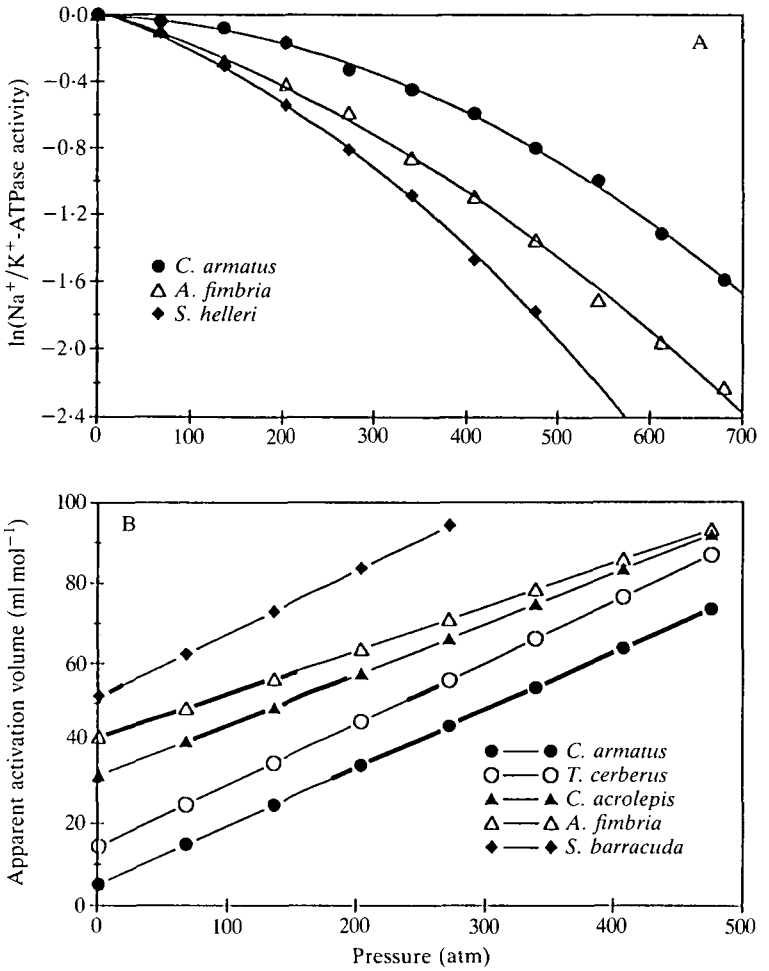

Fig. 4. (A) Quadratic equations fitted to ln(Na^+/K^+-ATPase activity) *vs* pressure data at 10°C for three species. (B) Apparent activation volumes of Na^+/K^+-ATPase at 10°C. Data from Fig. 1 were fitted to quadratic equations by nonlinear regression. The slope of such a curve (calculated by taking the derivative) is equal to $-\Delta V‡/RT$. Bold lines indicate physiological pressure ranges.

ATPase were as pressure-sensitive as that of barracudas, a 75% decrease in Na^+/K^+-ATPase activity would occur over this depth range, rather than the 33% variation we observed. This could cause severe problems in ion regulation, since passive ion fluxes differ in pressure-sensitivity from Na^+/K^+-ATPase, and may even increase in high pressures (Macdonald, 1984). *C. armatus* may also undergo large vertical excursions (Haedrich & Henderson, 1974; Pearcy & Ambler, 1974).

Dehydrogenases show pressure adaptation of both V_m and K_m. We did not investigate in detail the effects of pressure on affinity of Na^+/K^+-ATPase for ATP or cations. Na^+/K^+-ATPase is believed to be regulated by intracellular Na^+ level

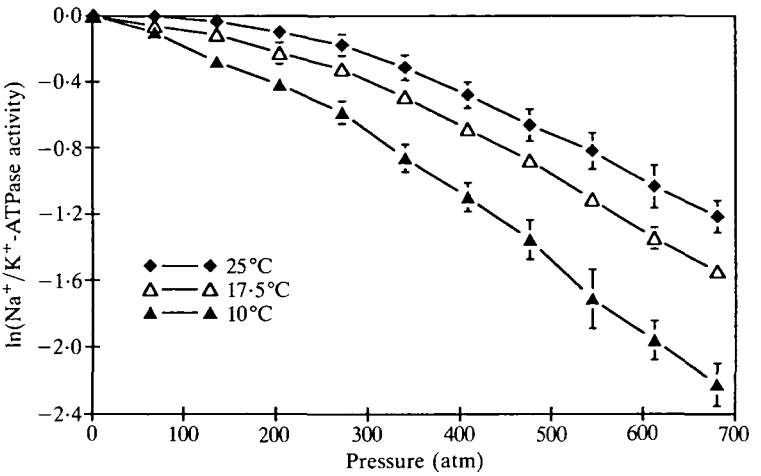

Fig. 5. Activity–pressure relationships at three temperatures of Na^+/K^+-ATPase from *Anoplopoma fimbria*. The pH of the Tris buffer was adjusted to follow the temperature response of intracellular pH (Somero, 1981). Error bars indicate standard deviations of measurements from seven individuals at each pressure. Na^+/K^+-ATPases from other species showed less variation than that of *A. fimbria*.

(Yamamoto *et al.* 1979). A comparison of the cation sensitivities of Na^+/K^+-ATPases from a deep-sea and a shallow-living species is shown in Fig. 2. The cation responses of both enzymes are identical at pressures up to 408 atm. Only at pressures well above the physiological range does the affinity for Na^+ decrease in Na^+/K^+-ATPase from *A. fimbria*. Intracellular Na^+ concentrations are generally in the range 30–70 mmol l^{-1} (Shelton *et al.* 1985). Estimated $K_{0.5}$ values for ATP at all pressures (data not shown) were less than 0·1 mmol l^{-1}, well below the typical physiological concentration of several mmol l^{-1}.

Two previous studies of teleost Na^+/K^+-ATPase (Moon, 1975; Pfeiler, 1978) found activation by pressure of Na^+/K^+-ATPases from deep-sea fish, in the physiological pressure range. The discrepancy between our data and Moon's may be due to differences in membrane preparations. Moon used NaI-treated membranes. He also had difficulty obtaining stable enzyme activity from *Antimora microlepis* (formerly *A. rostrata*) gills. Moon's data for the coho salmon are consistent with our results in shallow-living, cold-water species.

We prepared our membranes in a similar manner to Pfeiler (1978), and our ATPase assay technique was almost identical to his. However, Pfeiler (personal communication) did not correct for expansion of the cuvette due to pressure (see Materials and methods). When this is taken into account, his data (at 18°C) and ours for the sablefish *Anoplopoma fimbria* at 17·5°C (Fig. 5) are in complete agreement. Na^+/K^+-ATPases from mammals (Chong *et al.* 1985; Grisham & Barnett, 1973; de Smedt *et al.* 1979) and several ectothermic vertebrates (Pequeaux & Gilles, 1978) are consistently inhibited by pressure.

The close correlation between Na^+/K^+-ATPase activity and membrane fluidity,

as measured by a variety of techniques (Chong *et al.* 1985; Harris, 1985; Sinensky *et al.* 1979), argues strongly that the physical state of the membrane is of critical importance in establishing enzyme activity. Na^+/K^+-ATPase alternates between two distinct conformations during the catalytic cycle, and the conformational

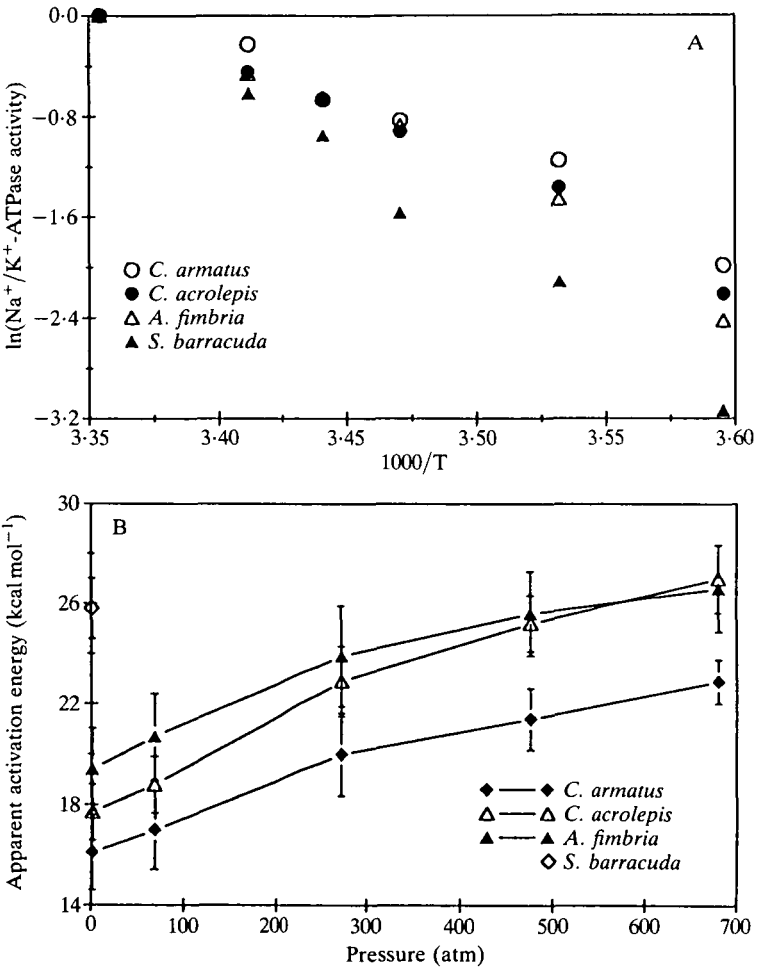

Fig. 6. (A) Arrhenius plots at 1 atm of Na^+/K^+-ATPase for fish from different habitats. (B) Effect of pressure on apparent activation energy. Data from Fig. 6A and effects of pressure at six temperatures between 5 and 25°C (e.g. Fig. 5) were combined to generate Arrhenius plots at high pressures. The slope of such a plot (calculated by linear regression) is equal to $-E_a/R$. Error bars indicate standard errors of estimated E_a values.

Fig. 7. Intraspecific comparison of effect of pressure on Na^+/K^+-ATPase from individuals captured at different depths. (A) *Anoplopoma fimbria*. (B) *Sebastolobus altivelis*. (C) *Antimora microlepis*. Na^+/K^+-ATPases from *S. alascanus* (1000–1400 m) and *Coryphaenoides acrolepis* (1200–1400 m) also showed no intraspecific differences related to capture depth. ATPase was assayed at 10°C. *N* values are in parentheses.

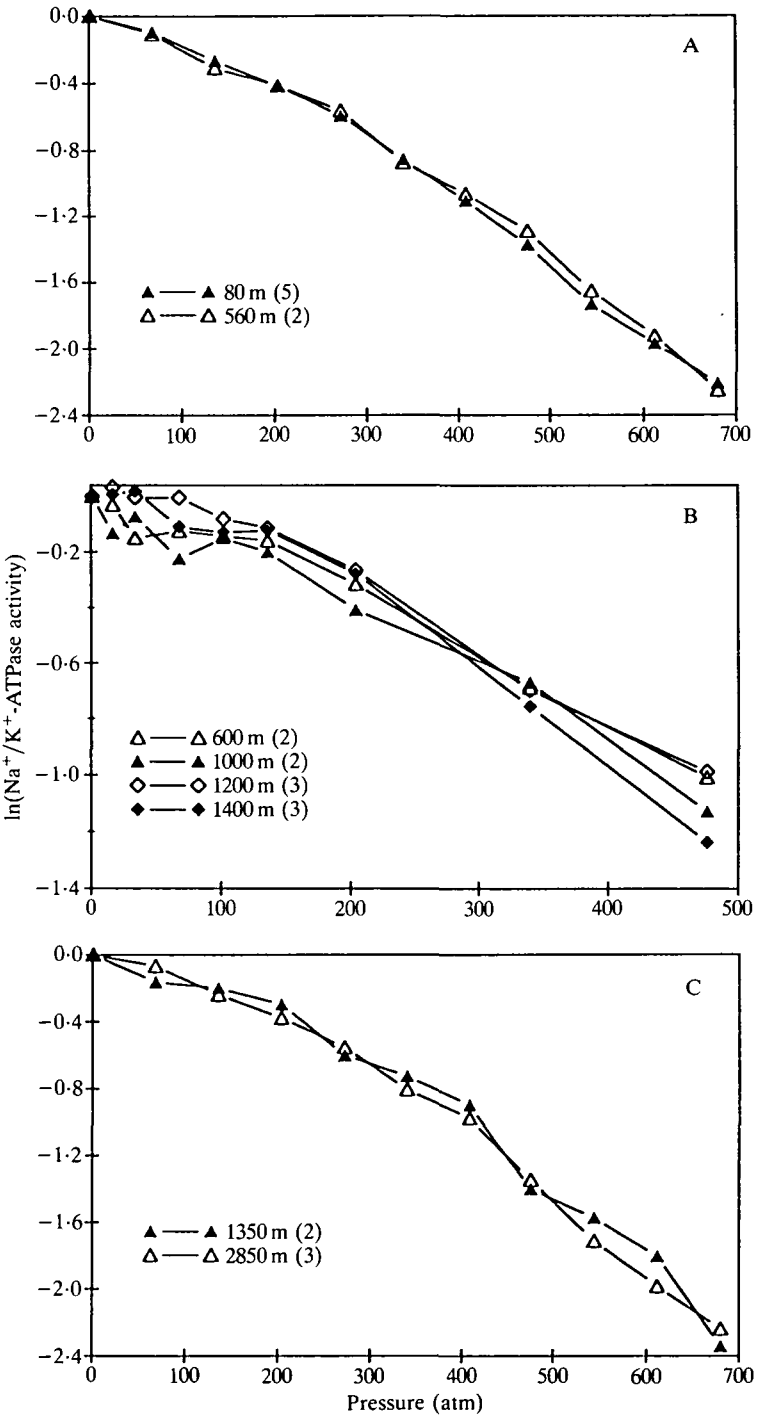
A
80 m (5)
560 m (2)
B
600 m (2)
1000 m (2)
1200 m (3)
1400 m (3)
C
1350 m (2)
2850 m (3)
ln(Na+/K+-ATPase activity)
Pressure (atm)

Fig. 7

changes are probably rate-limiting for enzyme activity (Jørgensen, 1982). Rearrangements of the peripheral membrane lipids are likely at this time. Lipid bilayers are more compressible than proteins by an order of magnitude (Macdonald, 1984), so volume changes associated with lipids are probably important.

A hypothetical model for lipid rearrangements during the rate-limiting step for Na^+/K^+-ATPase is shown in Fig. 8. The main assumption of this model is that the transition state for the reaction is similar at all temperatures and pressures; in particular, the packing of the peripheral lipids around the enzyme molecule is the same. In the ground state, the peripheral lipids will be more subject to the ordering and compacting effects of high pressure or low temperature than in the transition state. The lipids are disordered to a similar extent in the transition state, so the difference in volume between the ground and transition states (i.e. the activation volume) will be greater at increased pressures (Fig. 4). Higher temperatures will offset the ordering effects of pressure, returning the lipids to the state shown on the left side of Fig. 8, so Na^+/K^+-ATPase activity should be less pressure-sensitive at higher temperatures (Fig. 5). Homeoviscous adaptation will also offset the ordering effects of pressure.

When the apparent activation volumes of Na^+/K^+-ATPases from fishes from different depths are compared (Fig. 4B), they fall in the range of 30–60 ml mol^{-1} at physiological pressures for all species. Homeoviscous theory predicts that fish from deep-sea or cold-water habitats will have a greater proportion of short-chain and unsaturated fatty acyl chains in their phospholipids, tending to counteract environmental conditions by disordering the membrane. Deep-sea fish at high pressure will have membranes resembling those on the left side of Fig. 8. Conservation of apparent activation volume may thus reflect a similar membrane physical state at the actual pressure the animal experiences.

The reduced pressure-sensitivity of Na^+/K^+-ATPase at high temperatures suggests that the habitat-related differences seen in Fig. 3 should be reduced when the enzyme is assayed at physiological temperatures, as is indicated by Fig. 9. In particular, Na^+/K^+-ATPases from the shallow, warm-water barracuda and the deep-sea, cold-water grenadier (*C. armatus*) show almost identical pressure responses at their respective environmental temperatures. If the hydrothermal vent bythitid does enter warm water, it, too, has a similarly pressure-sensitive Na^+/K^+-ATPase.

Cossins (1983) uses the term 'efficacy' to describe the extent to which homeoviscous adaptation compensates for the effects of the physical environment on membranes. If apparent activation volume reflects the physical state of the membrane (Fig. 8), then the efficacy of homeoviscous pressure adaptation is nearly 100% (Fig. 4). Note that the apparent activation volume of Na^+/K^+-ATPase from the barracuda is in the conserved range of 30–60 ml mol^{-1} at 10°C, but is lower ($\approx$25 ml mol^{-1} calculated from data in Fig. 9) at the physiologically relevant temperature of 25°C. This accords with Cossin's (1983) suggestion that homeoviscous adaptation to temperature is usually incomplete.

Arrhenius plots [ln(Na^+/K^+-ATPase activity) *vs* 1/T] were steeper in warm

than in cold-water fish and in shallow-living than in deep-living species. These data, too, can be explained by homeoviscous adaptation. A protein in a more viscous lipid environment (e.g. a warm-adapted membrane) will find it more difficult to undergo conformational changes owing to the greater difficulty of peripheral lipid rearrangement. This should be reflected in a higher activation energy for catalysis in warm-living species. Enzymes from deep-sea species, with

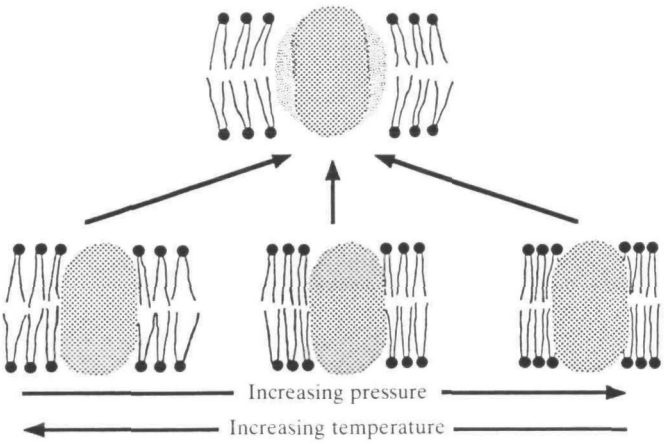

Fig. 8. A hypothetical model portraying the effects of pressure and temperature on the organization of the lipid bilayer surrounding a Na^+/K^+-ATPase molecule, with concomitant effects on the activation volume of the reaction. Increasing pressure compresses the bilayer, leading to a larger change in volume during the rate-limiting step of the Na^+/K^+-ATPase reaction. The ground-state enzyme is shown with large stippling; the change in enzyme conformation which occurs during formation of the activated complex, which is assumed to be the same at all pressures, is indicated by light stippling.

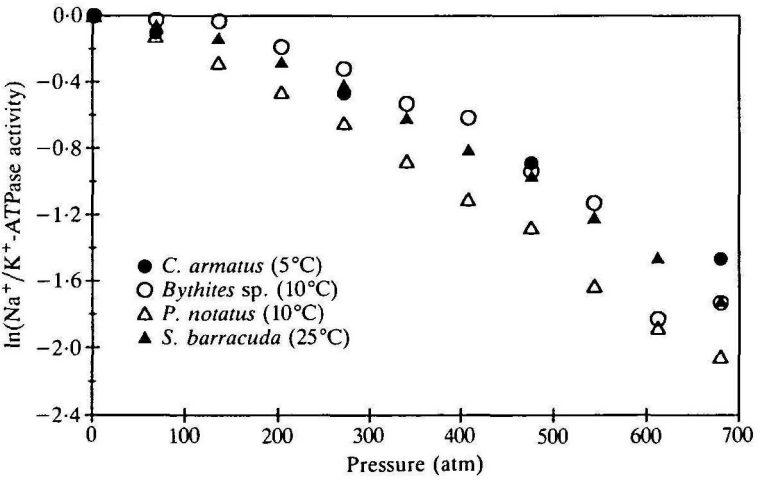

Fig. 9. Effect of pressure on Na^+/K^+-ATPase assayed at each species' habitat temperature.

the most fluid membranes, should have lower apparent activation energies, and high pressures should increase the activation energy as a result of increased membrane viscosity. Again, our data are consistent with both these predictions (Fig. 6B). Note also that, in the cold-living species, apparent activation energies are similar at habitat pressures. Analogous to conservation of activation volumes (Fig. 4B), similar activation energies may reflect similar membrane physical states under physiological conditions.

A final prediction of homeoviscous adaptation is that a species inhabiting a wide depth range could change its membrane lipid composition as a function of environmental pressure. This has been observed for bacteria (DeLong & Yayanos, 1985, 1986), but the effects of varying lipid composition on membrane enzyme function have not been investigated. We observed no intraspecific differences in pressure-sensitivity of Na^+/K^+-ATPase as a function of capture depth (Fig. 7), but only one of our species (*Antimora microlepis*) spanned the threshold range of pressures (>200 atm). This is further evidence that pressures greater than 200 atm are required to select for a pressure-adapted Na^+/K^+-ATPase.

The magnitude of the threshold adaptation pressure may have implications concerning the corresponding threshold temperature, i.e. the minimal change in body temperature required to elicit homeoviscous adaptation. The increase in viscosity of the lipid bilayer caused by 1000 atm of hydrostatic pressure corresponds to that caused by a decrease in temperature of 15–20°C (Macdonald, 1984). Therefore, the threshold adaptation pressure of 200 atm is equivalent to a temperature decrease of 3–4°C. We predict that the temperature threshold for homeoviscous adaptation in membrane systems will be in this range. A soluble enzyme, lactate dehydrogenase, does show adaptive variation related to differences in body temperature of this magnitude (Graves & Somero, 1982), but we are not aware of any study which has addressed the question of a threshold for homeoviscous adaptation to temperature.

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