Journal of Muscle Research and Cell Motility **12**, 242–246 (1991)

Changes in sarcoplasmic metabolite concentrations and pH associated with the catch contraction and relaxation of the anterior byssus retractor muscle of *Mytilus edulis* measured by phosphorus-31 nuclear magnetic resonance

NAOKATA ISHII[1]*, FUMIYUKI MITSUMORI[2] and KEIICHI TAKAHASHI[3]

[1] *Department of Sports Sciences, College of Arts and Sciences, University of Tokyo, Tokyo 153, Japan*
[2] *National Institute for Environmental Studies, Tsukuba, Ibaraki 305, Japan*
[3] *Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113, Japan*

Received 1 August 1990; accepted 1 November 1990

Summary

The sarcoplasmic concentrations of phosphorus metabolites and pH (pH_{in}) were measured in the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* by ^{31}P nuclear magnetic resonance spectroscopy. During an active contraction induced by 10^{-3} M acetylcholine, the concentration of arginine phosphate ($[Arg\text{-}P]_{in}$) decreased from the resting value of 7.47 ± 0.26 (mean $\pm$ SE, $n = 8$) to 6.67 ± 0.29 ($n = 6$) μmol g^{-1}, and that of inorganic phosphate (P_i) consistently increased from 0.84 ± 0.06 ($n = 7$) to 1.61 ± 0.12 ($n = 5$) μmol g^{-1}. In the 'catch' state following the active contraction, these concentrations were close to their resting levels, indicating that the catch is an inactive state. 5-hydroxytryptamine caused a rapid relaxation of the catch, which was associated with a slight decrease in $[Arg\text{-}P]_{in}$ and an increase in pH_{in} by ca 0.2 units. The sarcoplasmic concentration of ATP (mean, 1.6 μmol g^{-1}) did not change throughout the contraction-relaxation cycle.

Introduction

Some molluscan smooth muscles ('catch' muscles) exhibit the 'catch' contraction in which tension is maintained for a prolonged period of time (for reviews see Twarog, 1976; Rüegg, 1986). The catch state is thought to be an inactive state, since in this state the tension redevelopment after a quick release is practically absent (Jewell, 1959; Lowy & Millman, 1963), and the O_2 consumption is much lower in the catch than during the active tension development (Baguet & Gillis, 1968). Recently, the intracellular Ca^{2+} concentration in the catch state has been shown to be close to its resting level in intact smooth muscle cells isolated from the catch muscle (Ishii *et al.*, 1989b).

Several biochemical studies have shown that proteins, such as paramyosin (Achazi, 1979; Cooley *et al.*, 1979), a myosin heavy chain (Castellani & Cohen, 1987), and the myosin light chain (Sohma *et al.*, 1985, 1988) can be phosphorylated in a cyclic AMP (cAMP)-dependent manner. Since both cAMP and a catalytic subunit of cAMP-dependent protein kinase can cause relaxation of the catch-like state in skinned fibre preparations of the catch muscle (Marchand-Dumont & Baguet, 1975; Cornelius, 1982; Pfitzer & Rüegg, 1982), phosphorylation of these proteins may be involved in relaxation of the catch state.

On the other hand, the kinetics of actomyosin ATPase (for reviews see Trentham *et al.*, 1976; Goldman, 1987) indicates that either the decrease in the substrate (ATP) or the accumulation of one of the products (ADP) impedes detachment of actomyosin crossbridges. This suggests that a decrease in ATP concentration within the muscle cells can cause a catch-like state.

In order to see whether the catch is indeed an inactive state, and whether the catch state is attained without a decrease in ATP concentration, we have measured the sarcoplasmic concentrations of phosphorus metabolites in intact catch muscle, using ^{31}P nuclear magnetic resonance (NMR). We have also investigated changes in the sarcoplasmic pH, which might play a part in the regulation of the catch as suggested by the pH-dependency of the relaxation rate in skinned fibre preparations of the catch muscle (Rüegg, 1971; Ishii, 1987).

A part of this study has been reported elsewhere in a preliminary form (Ishii *et al.*, 1989a).

* To whom correspondence should be addressed.

0142-4319/91 $03.00 + .12

Materials and methods

Preparation

Specimens of *Mytilus edulis* were collected near the Misaki Marine Biological Station, University of Tokyo. Since Schanck and colleagues (1986) have reported the variation of metabolite concentrations and metabolic activities in the ABRM between breeding and non-breeding season of the mussel, all of the present measurements were made in the non-breeding season (from May through October). From 16–20 ABRMs (total weight, ca 800 μg) were excised from 8–10 individuals, and clamped at both ends firmly around a glass tubing (outer diameter, 5 mm; length, 60 mm) with their long axes parallel to that of the glass tubing. Since they often showed a catch contraction after the dissection, we stimulated them with an alternating current to release from them the catch state (Winton, 1937) and adjusted their lengths approximately to the *in situ* lengths. The glass tubing with the muscle preparations was connected to a Teflon inlet tubing for perfusion and inserted into a 10 mm NMR sample tube, which was then placed in the magnet. The preparations were continuously perfused (rate, 8 ml min^{-1}) with artificial sea water (ASW) consisting of 434 mM NaCl, 9.8 mM KCl, 52 mM $MgCl_2$, 10 mM $CaCl_2$ and 5 mM Hepes (pH 7.5) and bubbled with 95% O_2 and 5% CO_2. To induce a catch contraction, they were perfused with 10^{-3} M acetylcholine (ACh) in ASW for 6–10 min and subsequently washed with ASW for 10–15 min. Finally, complete relaxation was induced by perfusion of 10^{-4} M 5-hydroxytryptamine (5-HT) in ASW (Fig. 1).

NMR spectroscopy

^{31}P NMR measurements were performed at 161.8 MHz using a JEOL GX-400 spectrometer without proton noise decoupling. A series of spectra was obtained by accumulating 300–600 transients with a pulse recycling time of 1.0 s and a flip angle of 30°. A fully relaxed spectrum was also measured with a pulse recycling time of 30 s at resting stage. Chemical shift values were referred to the resonance of external methylene diphosphonic acid (MDP) solution sealed in a coaxial capillary. The amounts of the phosphorus metabolites were measured from the peak areas of their resonances relative to that of the MDP resonance. Peak area obtained from spectra with a pulse recycling time of 1.0 s were extrapolated to the condition of full relaxation by using a spectrum with a pulse recycling time of 30 s, assuming that no considerable change occurred in T_1 of metabolites throughout the contraction–relaxation cycle. The values of relative peak area were calibrated for the concentrations by using a solution containing 7.5 mM P_1. A β-phosphate resonance was used to determine the concentration of ATP. Sarcoplasmic pH (pH_{in}) was determined from the difference between the chemical shift of the P_1 resonance and that of the arginine phosphate (Arg-P) resonance by using the pH calibration curve drawn from measurements made in solutions containing 5 mM P_1, 5.5 mM Arg-P and 150 mM KCl. The sample temperature was maintained at 15° C.

Measurement of tension

Isometric tension was measured separately under the same condition as that for the NMR spectroscopy except that the measurement was made outside the spectrometer. Six out of 16–20 muscle preparations in the NMR sample tube were connected to a strain gauge (Kyowa, KSPH-4-2000-E4) the position of which was controlled by a DC motor. To test whether the catch state was attained, a quick release (rate, ca 0.5 length s^{-1}; amplitude, 0.04 length) was applied to the muscles by driving the DC motor with a damped (time constant, 4 ms) rectangular DC pulse. The output of the force transducer was stored in a microcomputer (NEC, PC-8801), and the relaxation rate of contraction was directly calculated with semi-logarithmic linear regressions.

Results

When the bundles of ABRM were perfused with 10^{-3} M ACh in ASW and subsequently washed with ASW, they responded with a typical catch contraction (Fig. 1). The peak tension was 2.0 ± 0.5 kg cm^{-2} (mean $\pm$ SE, $n = 4$). During the slow relaxation, no significant tension redevelopment was observed after a quick release by 4% muscle length, indicating that the muscle was in the catch state. The perfusion of 10^{-4} M 5-HT in ASW caused a rapid relaxation. The rates of relaxation before and after the perfusion of 5-HT were 1.2 ± 0.1 and $23.0 \pm 3.0 \times 10^{-3}$ s^{-1} (mean $\pm$ SE, $n = 4$), respectively.

The NMR measurements were carried out at stages of resting, contraction in ACh ASW, catch, relaxation in 5-HT ASW (Fig. 1a–e), and recovery after washing the muscles for more than 1 h. Representative spectra taken in each state are shown in Fig. 2.

Immediately after the dissection, we observed a large P_1 signal (concentration, 2–3 μmol g^{-1}) which was presumably caused by excitation during the dissection procedure (Schanck *et al.*, 1986). Following the perfusion with ASW, the P_i signal gradually decreased and reached an equilibrium (average concentration, 0.8 μmol g^{-1}) within 1 h. The shape of the whole spectrum for the resting state was thereafter kept unchanged for more than 10 h. In some spectra with relatively low signal-to-noise ratio, the small P_i signal often hindered the estimation $[P_i]_{in}$ and pH_{in} (see below).

The metabolite concentrations for four different states – resting, contraction in ACh ASW, catch, and relaxation in 5-HT ASW (Fig. 1a–e) – measured from spectra taken in six experiments are shown in Fig. 3. In the resting muscle, the average intracellular concentrations of Arg-P ($[Arg\text{-}P]_{in}$), P_1 ($[P_i]_{in}$) and ATP ($[ATP]_{in}$) were 7.47 ± 0.26 (mean $\pm$ SE, $n = 8$), 0.84 ± 0.06 ($n = 7$) and 1.55 ± 0.09 ($n = 8$) μmol g^{-1}, respectively. During the active contraction, $[Arg\text{-}P]_{in}$ decreased to 6.67 ± 0.29 ($n = 6$) μmol g^{-1} with a corresponding increase in $[P_i]_{in}$ to 1.61 ± 0.12 ($n = 5$) μmol g^{-1}. These changes were statistically significant ($p < 0.05$, Student's t-test), when the concentrations were normalized to their resting values in each experiment. The smaller number of observation for the value of $[P_i]_{in}$ is due to the small P_i signal relative to the noise level in some spectra.

During the catch state, $[Arg\text{-}P]_{in}$ and $[P_i]_{in}$ were

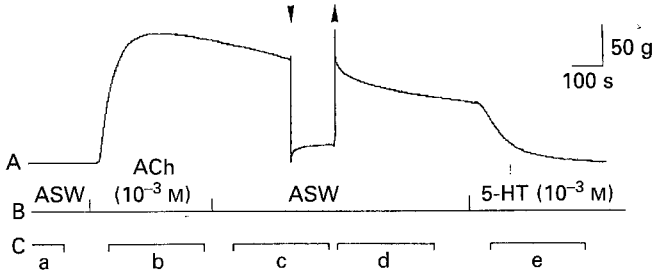

Fig. 1. A typical tension response (A) of the preparation induced by the perfusion procedure shown in B. Tension was measured for six out of 16 muscles mounted in a NMR tube. A quick release and a following quick stretch by 4% muscle length (arrowheads) were applied during the slow relaxation process. The absence of significant tension redevelopment after the quick release indicates that the muscles were in the catch state. NMR measurement (C) was made separately following the same perfusion procedure as that for the tension measurement. Spectra during the resting state (a), contraction in ACh ASW (b), catch (c, d), relaxation in 5-HT ASW (e), and recovery after wash-out 5-HT of (not shown), were obtained by accumulating 300 transients for each state with a pulse recycling time of 1.0 s.

7.61 ± 0.32 ($n = 7$) and 1.24 ± 0.23 ($n = 5$) μmol g^{-1}, respectively, and these values were not significantly different from their original resting values, despite the maintenance of tension.

During the relaxation induced by 5-HT, however, $[\text{Arg-P}]_{in}$ significantly ($p < 0.05$) decreased to 6.99 ± 0.46 ($n = 6$) μmol g^{-1} without a corresponding increase in $[P_i]_{in}$(1.24 ± 0.23 μmol g^{-1}, $n = 5$). This lowered $[\text{Arg-P}]_{in}$ was completely recovered to the resting value after washing the muscles with normal ASW for 1 h (Fig. 2). $[\text{ATP}]_{in}$ was kept constant at ca 1.6 μmol g^{-1} throughout the contraction-relaxation cycle.

The pH_{in} decreased from 7.10 ± 0.01 ($n = 6$) to 6.99 ± 0.06 ($n = 4$) during an active contraction, stayed at 6.98 ± 0.03 ($n = 4$) during the subsequent catch state, and rose to 7.16 ± 0.02 ($n = 5$) when rapid relaxation was induced by 5-HT. All of these changes in pH_{in} were statistically significant ($p < 0.05$). However, the P_i resonance showed broad peak width relative to those of Arg-P and ATP, and in some cases, was split into several small peaks, suggesting an inhomogenous population of cells with respect to pH_{in}, or a heterogeneous circumstance of P_i within the cells.

Discussion

Quantitative ^{31}P NMR studies on resting catch muscles have been made in anaerobic condition by Ellington (1983) and Schanck and colleagues (1986). Their results are practically consistent with ours except the higher $[\text{Arg-P}]_{in}$ (12.9 μmol g^{-1}) reported by Schanck and colleagues. The present NMR measurements are also consistent with both the measurement of O_2 consumption of the ABRM (Baguet & Gillis, 1968) and the biochemical analysis of the frozen ABRM (Nauss & Davies, 1966; Gies, 1988) which have shown a low metabolic activity during the catch state. In combination with the observations that both the intracellular free calcium concentration (Ishii *et al.*, 1989b) and the force-generating activity (Jewell, 1959; Lowy & Millman, 1963) are already close to their resting level during the catch state, the present results suggest that the ATPase activity, attributable mainly to the actomyosin crossbridges, is much lower in the catch than in the active contraction. Importantly, this catch state is attained at normal (resting) concentrations of ATP and P_i, and therefore would not be caused by the formation of rigor-like crossbridges.

The decrease in $[\text{Arg-P}]_{in}$ by ca 0.5 μmol g^{-1} during the relaxation of the catch might be associated with either cAMP-dependent activation of Ca^{2+} extrusion from sarcoplasm (Ishii *et al.* 1989c) or phosphorylation of myofilament proteins. Recent biochemical studies have suggested that cAMP-dependent phosphorylation of either paramyosin (Achazi, 1979; Cooley *et al.*, 1979), a myosin light chain (Sohma *et al.*, 1985, 1988), or the myosin heavy chain (Castellani & Cohen, 1987) is involved in the relaxation of the catch state. The amount of these proteins within the muscle cell can be estimated at 0.1–0.3 μmol g^{-1} (unpublished observation), and they would have several sites for phosphorylation (Cooley *et al.*, 1979), so that the phosphorylation process might consume ca 0.5 μmol ATP g^{-1}. The observation that no significant increase in $[P_i]_{in}$ occurred on application of 5-HT appears to favour the latter possibility.

The ^{31}P-NMR studies of ABRM by Zange and colleagues (1990a, b) with special reference to the pH_{in} have shown the increase in pH_{in} by ca 0.2 units during the relaxation induced by 5-HT, and suggested the activation of Na^+/H^+ exchange by cAMP. Their results are consistent with ours for this 5-HT-induced alkalization, though they have shown no change in pH_{in} during the active contraction and the catch, compared with that in the resting state. The present decrease in pH_{in} during the catch was associated with the recovery of $[\text{Arg-P}]_{in}$ and $[P_i]_{in}$ (Fig. 3), and would not therefore be caused by a fatiguing effect of prolonged activation by ACh. Thus the main reason for the above discrepancy may be the difference in the condition of measurement. In the present study, more than 16 muscle bundles were placed in a NMR sample tube to reduce the extracellular space and

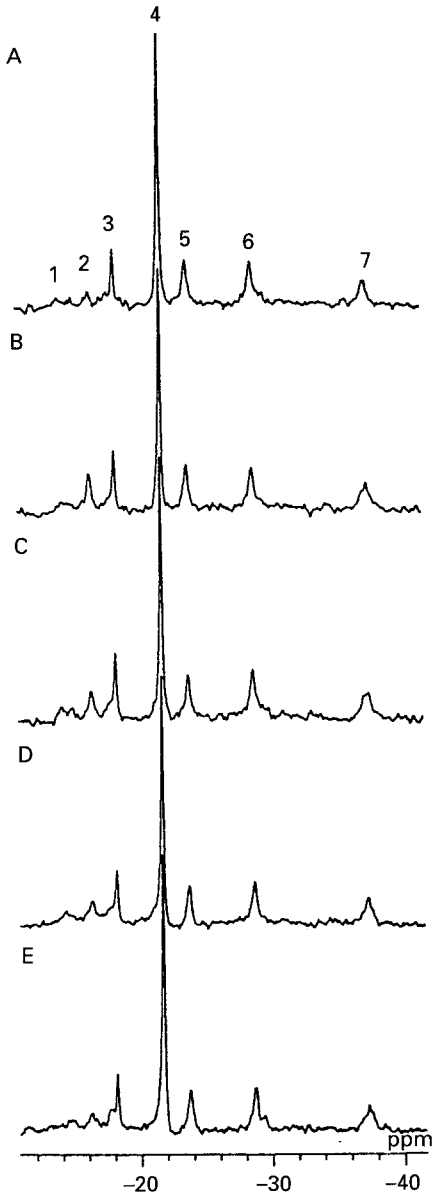

Fig. 2. ^{31}P NMR spectra taken during resting state (A), contraction in ACh ASW (B), catch (C), relaxation in 5-HT ASW (D), and recovery after wash-out 5-HT of (E). Chemical shift values are in ppm from external MDP. Spectra are all averages of two spectra which were obtained by accumulating 300 transients. Signal identifications: 1, sugar phosphates; 2, P_i 3, unidentified; 4, arginine phosphate; 5, γ-phosphate of ATP; 6, α-phosphate of ATP; 7, β-phosphate of ATP.

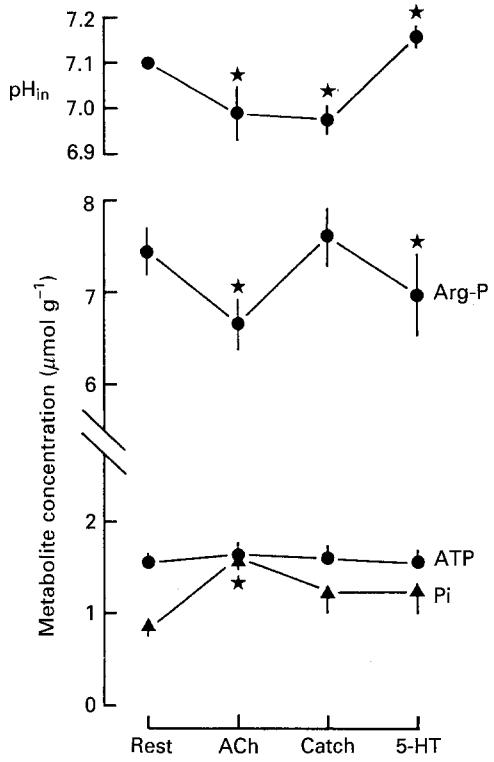

Fig. 3. Changes in pH_{in} (A) and metabolite concentrations (B) during the contraction-relaxation cycle. Each point represents the mean value obtained from six experiments. Bars indicate standard errors. Values denoted by asterisks showed significant differences ($p < 0.05$, Student's t-test) from those in the resting state. Abbreviations used for the stages; Rest, resting state; ACh, contraction in ACh ASW; Catch, catch state; 5-HT, relaxation in 5-HT ASW.

obtain a high signal-to-noise ratio. Such a circumstance may lower the efficiency of acid-base exchange coupled with active transport mechanisms and slow the recovery of pH_{in}. In addition, recent measurement of pH_{in} with a fluorescent pH indicator BCECF in isolated ABRM cells (Ashley *et al.*, 1989) have shown no change in pH_{in} upon successive application of carbachol, KCl and 5-HT. Therefore, the regulation of pH_{in} under various extracellular conditions should be studied further. However, since several studies with skinned fibre preparations of ABRM (Rüegg, 1971; Ishii, 1987) have shown that the increase in pH of the bathing medium dramatically accelerates the relaxation, the alkalization of the sarcoplasm induced by 5-HT may play, at least, a part in the relaxation of the catch state in the whole muscle.

References

ACHAZI, R. K. (1979) Phosphorylation of molluscan paramyosin. *Pflügers Arch.* **379**, 197–201.

ASHLEY, C. C., ISHII, N. & SIMPSON, A. W. M. (1989) Intracellular pH (pH_i) is not altered by agents which promote contraction and relaxation in smooth muscle cells isolated from the ABRM of *Mytilus edulis*. *J. Physiol. (Lond.)* **415**, 102P.

BAGUET, F. & GILLIS, J. M. (1968) Energy cost of tonic contraction in a lamellibranch catch muscle. *J. Physiol. (Lond.)* **198**, 127–43.

CASTELLANI, L. & COHEN, C. (1987) Myosin rod phosphorylation and the catch state of molluscan muscles. *Science* **235**, 335–7.

COOLEY, L. B., JOHNSON, W. H. & KRAUSE, S. (1979) Phosphorylation of paramyosin and its possible role in the catch mechanism. *J. biol. Chem.* **256**, 3178–81.

CORNELIUS, F. (1982) Tonic contraction and the control of relaxation in a chemically skinned molluscan smooth muscle. *J. Gen. Physiol.* **79**, 821–34.

ELLINGTON, W. R. (1983) The extent of intracellular acidification during anoxia in the catch muscle of two bivalve molluscs. *J. Exp. Zool.* **227**, 313–7.

GIES, A. (1988) Changes of metabolite contents and of energy charge induced by contraction, catch and relaxation in smooth molluscan muscle fibres. An analysis using reversed-phase ion-pair high-performance liquid chromatography. *Comp. Biochem. Physiol.* **91B**, 483–7.

GOLDMAN, Y. E. (1987) Kinetics of the actomyosin ATPase in muscle fibers. *Ann. Rev. Physiol.* **49**, 637–54.

ISHII, N. (1987) Mechanical properties of saponin-treated smooth muscle cells isolated from a molluscan smooth muscle. *J. Musc. Res. Cell Motil.* **8**, 281.

ISHII, N., MITSUMORI, F., TAKAHASHI, K., SIMPSON, A. W. M. & ASHLEY, C. C. (1989a) Intracellular metabolite and free calcium concentrations during the 'catch' contraction and relaxation in a molluscan smooth muscle. In *Muscle Energetics* (edited by PAUL, R. J., ELZINGA, G. & YAMADA, K.) pp. 463–4. New York: Alan R. Liss.

ISHII, N., SIMPSON, A. W. M. & ASHLEY, C. C. (1989b) Free calcium at rest during "catch" in single smooth muscle cells. *Science* **243**, 1367–8.

ISHII, N., SIMPSON, A. W. M. & ASHLEY, C. C. (1989c) Effects of 5-hydroxytryptamine (serotonin) and forskolin on intracellular free calcium in isolated and fura-2 loaded smooth-muscle cells from the anterior byssus retractor (catch) muscle of *Mytilus edulis*. *Pflügers Arch.* **414**, 162–70.

JEWELL, B. R. (1959) The nature of the phasic and tonic responses of the anterior byssal retractor muscle of *Mytilus*. *J. Physiol. (Lond.)* **149**, 154–77.

LOWY, J. B. M. & MILLMAN, B. M. (1963) The contractile mechanism of the anterior byssus retractor muscle (ABRM) of *Mytilus edulis*. *Phil. Trans. R. Soc. (Lond.)* B **246**, 105–48.

MARCHAND-DUMONT, G. & BAGUET, F. (1975). The control mechanism of relaxation in molluscan catch muscle (ABRM). *Pflügers Arch.* **354**, 87–100.

NAUSS, K. & DAVIES, R. E. (1966) Changes in inorganic phosphate and arginine during the development, maintenance and loss of tension in the anterior byssus retractor muscle of *Mytilus edulis*. *Biochem. Z.* **345**, 173–87.

PFITZER, G. & RÜEGG, J. C. (1982) Molluscan catch muscle: Regulation and mechanics in living and skinned anterior byssus retractor muscle of *Mytilus edulis*. *J. Comp. Physiol.* **147**, 137–42.

RÜEGG, J. C. (1971) Smooth muscle tone. *Physiol. Rev.* **51**, 201–48.

RÜEGG, J. C. (1986) *Calcium in Muscle Activation*. Berlin: Springer.

SCHANCK, A., VERBAERT, B., VAN MEERSCH, M., BAGUET, F. & DEVROEDE J. (1986) ^{31}P-nuclear-magnetic resonance study of muscles from *Mytilus edulis*. *Eur. J. Biochem.* **156**, 625–9.

SOHMA, H., YAZAWA, M. & MORITA, F. (1985) Phosphorylation of regulatory light chain a (RLC-a) in smooth muscle myosin of scallop, *Papinopecten yessoensis*. *J. Biochem. (Tokyo)* **98**, 569–72.

SOHMA, H., INOUE, K. & MORITA, F. (1988) A cAMP-dependent regulatory protein for RLC-a myosin kinase catalyzing the phosphorylation of scallop smooth muscle myosin light chain. *J. Biochem. (Tokyo)* **103**, 431–5.

TRENTHAM, D. R., ECCLESTON, J. F. & BAGSHAW, C. R. (1976) Kinetic analysis of ATPase mechanisms. *Quart. Rev. Biophys.* **9**, 217–81.

TWAROG, B. M. (1976) Aspects of smooth muscle function in molluscan catch muscle. *Physiol. Rev.* **56**, 829–38.

WINTON, F. R. (1937) The changes in viscosity of an unstriated muscle (*Mytilus edulis*) during and after stimulation with alternating, interrupted and uninterrupted direct currents. *J. Physiol. (Lond.)* **88**, 492–511.

ZANGE, J., GRIESHABER, M. K. & JANS, A. W. (1990a) The regulation of intracellular pH estimated by ^{31}P-NMR spectroscopy in the anterior byssus retractor muscle of *Mytilus edulis* L. *J. Exp. Biol.* **150**, 95–109.

ZANGE, J., PÖRTNER, H. O., JANS, A. W. H. & GRIESHABER, M. K. (1990b) The intracellular pH of a molluscan smooth muscle during a contraction-catch-relaxation cycle estimated by the distribution of [^{14}C]DMO and by ^{31}P-NMR spectroscopy. *J. Exp. Biol.* **150**, 81–93.