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Paramyosin and the catch mechanism

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We propose here the formation by molluscan and notochord muscles in the catch state of three-dimensional, entangled network structures composed of bent and sometimes entwined paramyosin thick filaments including myosin intermediate filaments and disordered actin thin filaments; in the relaxed state the three forms lie in parallel. The intact forms of bivalve (*Andonta pacifica*, Heude) muscle paramyosin are those of 120 and 95 kDa (β-paramyosin). The 102 kDa form (α-paramyosin) is the proteinase cleavage product of 120 kDa paramyosin. Paramyosin could be phosphorylated in vitro by cyclic AMP-dependent protein kinase. The amino acid phosphorylated was at the serine residue. Paramyosin from muscles treated with acetylcholine (catch state) was phosphorylated to a greater extent than that of untreated muscles (normal state) and even more so in the case of serotonin-treated muscles (relaxed state). Actin markedly inhibited the phosphorylation of paramyosin in vitro.

1. Introduction

When molluscan smooth muscles are stimulated with a pulse of alternating current, they respond with a brief twitch similar to that produced by striated muscles. However, on stimulation by direct current or acetylcholine, they display prolonged tonic contraction or 'catch', as if they are locked in the contracted state from which they relax very slowly. Tension is maintained for long periods with a very low energy expenditure. ATP utilization, heat production and oxygen consumption are considerably lower in the catch state than in isometric tetanus. Unlike actively contracting muscles, tension is maintained during the catch state without participation of the 'active state', i.e., the muscle barely shortens after an isotonic 'quick release' produced by sudden unloading. The membrane potential remains at the resting level and there are no action potentials.

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To explain the phenomena exhibited by molluscan smooth muscles in the catch state, Lowy et al. [1] proposed that the contractile linkages are formed between thick and thin filaments during the contraction preceding the catch. After the cessation of active contraction these linkages are either locked in the attached state or decay very slowly. The very slow rate of breaking of linkages is responsible for the slow rate of detachment of the cross-bridges, however, it does not suggest a specific role for paramyosin in the catch mechanism. In addition, the theory cannot explain the energy expenditure. The total extra energy consumed over the period of slow relaxation is an order of magnitude greater than that expected from breakage of the known number of contractile cross-bridges only once. Different hypotheses involving paramyosin were proposed by Rüegg [2] and Johnson et al. [3]. The tension developed by the actomyosin system is maintained either by the formation of cross-links between the actomyosin system, between paramyosin molecules of adjacent fibrils, to the extent of forming branching

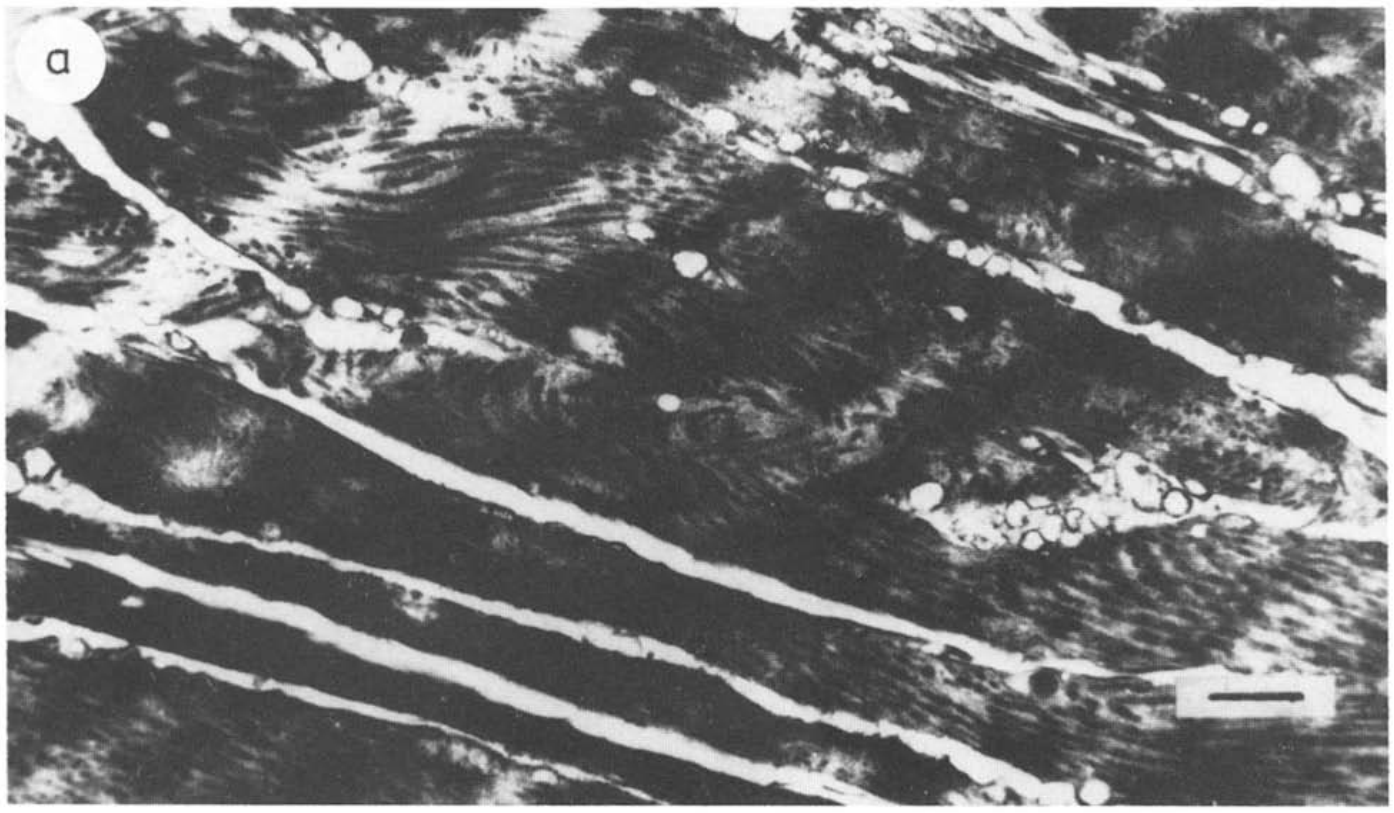

Fig. 1. *Amphioxus* notochord paramyosin. (a) *Amphioxus* notochord paramyosin, (b) *Amphioxus*.

filaments, generating frictional forces or shearing forces between adjacent thick filaments, or between paramyosin and myosin of the same filament as a result of a phase transition [4] of the paramyosin core during the catch state, hindering the detachment of actomyosin bridges. The in vitro experimental evidence available for the latter interaction is, however, contradictory [4,5].

Szent-Györgyi et al. [4] also postulated that the filament diameter may be the critical determinant of whether a cooperative transition can take place and underlined the distinction between a cooperative transition and the distribution between muscles in the catch and noncatch states. The thick filaments of catch muscles were assumed to have checker-board structures [6,7].

In the present paper, we propose, on the basis of electron-microscopic evidence obtained on bivalve (*Andonta pacifica*, Heude) adductor and foot muscles, mollusc, i.e., abalone (*Haliotis gigantea*) foot muscles and *Amphioxus (Branchiostoma belcheri tsingtasensis* Tchang et Koo) notochord muscles (fig. 1) the formation in the catch state of three-dimensional, entangled network structures composed of bent and sometimes entwined paramyosin thick filaments including myosin intermediate filaments and disordered actin thin filaments. In the relaxed state the three forms lie in parallel. The Bear-Selby [8] network structure undergoes no change in form. Details of the above-mentioned structures are discussed below.

Fig. 2. Molluscan smooth muscle in the catch state. Three-dimensional anastomosed networks are formed between disordered thin filaments and bent thick filaments. Note how a single thin filament can become attached to different thick filaments.

Fig. 3. Molluscan smooth muscle in the catch state. Entangled and entwined (arrow) thick filaments are shown.

3. Muscle structure in the relaxed state

In the relaxed state, both thick and thin filaments exhibit an ordered parallel arrangement. The thick filaments show the same cross-striations of 72.5 and 14.5 nm. No difference could be found in the periodic structures of the paramyosin core between the relaxed and catch states [9] (fig. 4).

Muscle in the catch state which had been homogenized in a medium containing acetylcholine showed the presence of two types of myofilaments, viz., thick and thin. The underlying periodic structure of the paramyosin core is discernible, and both the 14.5 nm pitch periodicity and the Bear-Selby net are evident. Muscle in the relaxed state which had been homogenized in a medium without acetylcholine also showed thick and thin filaments, the thick filament also exhibiting both cross-striated and net structures (fig. 5).

4. Paramyosin of *Amphioxus* notochord, bivalve adductor muscle and abalone foot muscle

4.1. Amphioxus notochord

Amphioxus notochord paramyosin [10,11] was crystallized in a buffer of isoelectric point pH 5.9, giving rise to spindle-shaped paracrystals with cross-striations of 14.5 nm. The subunit molecular mass is 105 kDa.

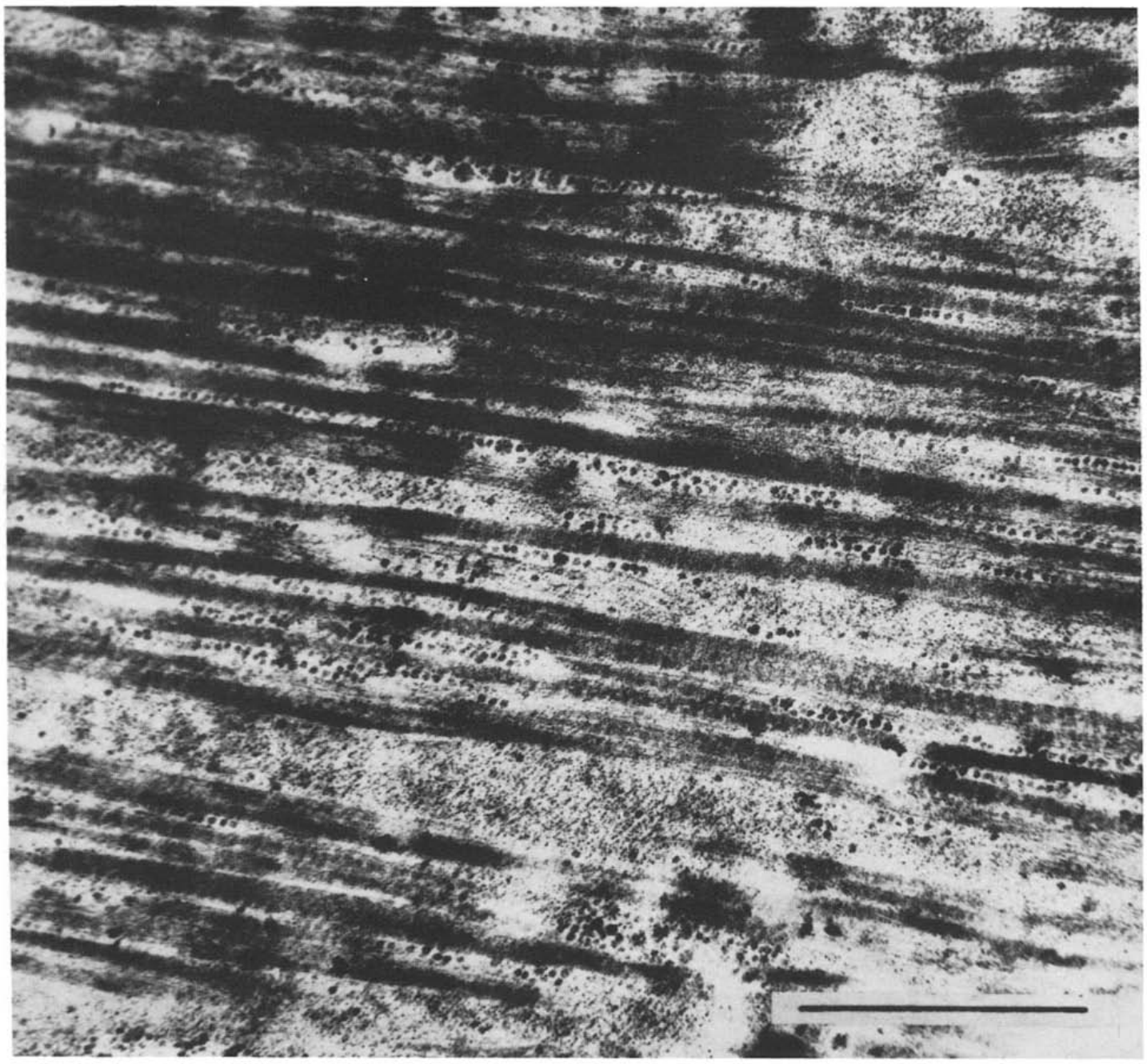

Fig. 4. Molluscan smooth muscle in the relaxed state. Two-dimensional order. Note the 14.5 and 72.5 nm periodicities.

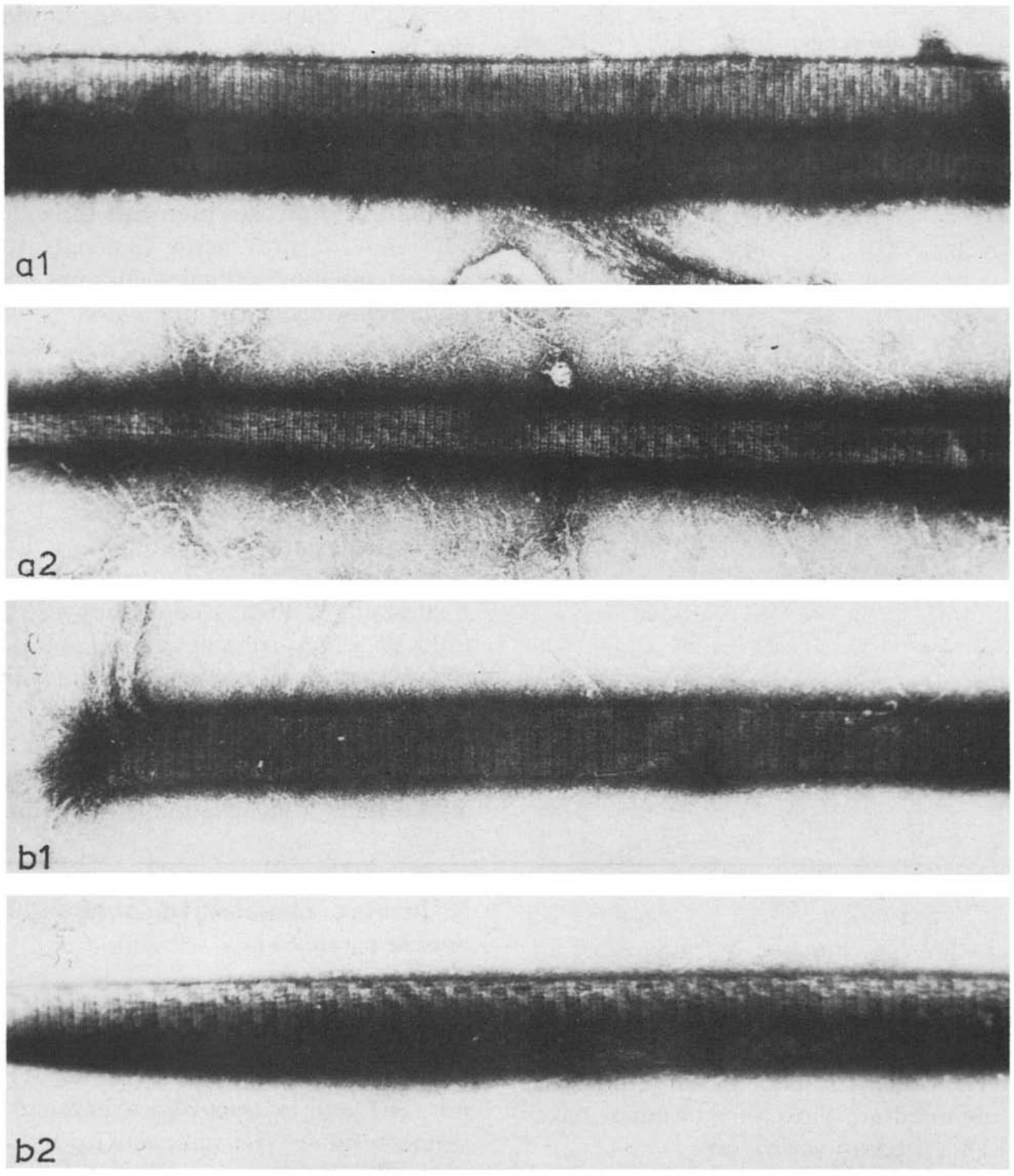

Fig. 5. Periodic structure of the paramyosin core. (a) Filament isolated from molluscan smooth muscle in the catch state in medium containing acetylcholine. 1, cross-striated structure; 2, Bear-Selby network. (b) Filament isolated from molluscan muscle in the relaxed state in medium without acetycholine. 1, cross-striated structure; 2, Bear-Selby network.

4.2. Bivalve adductor muscle

This form of paramyosin [12] was obtained from the crude extract isolated using phosphate buffer (pH 7.5) by immediate analysis on SDS-polyacrylamide gel electrophoresis. No band corresponding to α-paramyosin of 102 kDa was detected. In addition to β-paramyosin of 95 kDa, a band at 120 kDa was visible. On incubation at 37°C for various periods of time, the amount of 120 kDa protein present gradually decreased and that of 102 kDa α-paramyosin steadily increased as shown by SDS-polyacrylamide gel electrophoresis (fig. 6). The 120 kDa protein and the α- and β-paramyosin forms were precipitable using antiserum against the peptide fragments com-

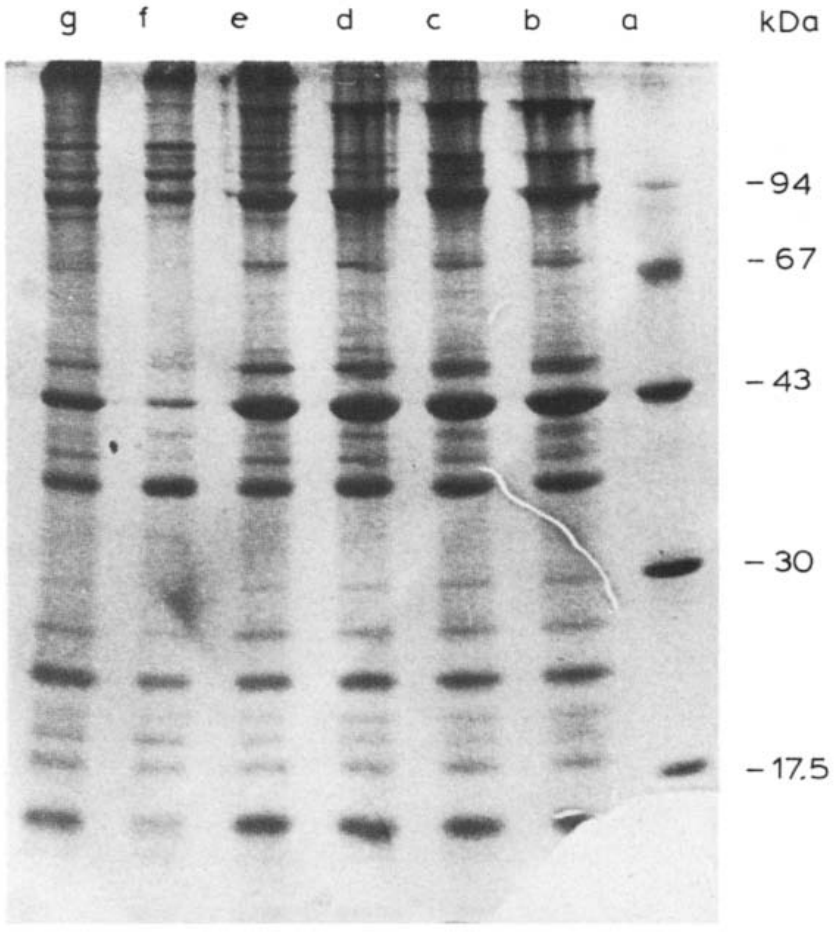

Fig. 6. Bivalve adductor muscle paramyosin, analyzed immediately by means of SDS-polyacrylamide gel electrophoresis. (a) Standard marker proteins (Sigma; in kDa); (b) crude extracts. Crude extracts incubated at 37° C for (c) 1 h, (d) 2 h, (e) 4 h, (f) 8 h, (g) 24 h.

monly obtained from purified paramyosin. α-Paramyosin was cleaved with endopeptidase during isolation. γ-Paramyosin, like α-paramyosin, is the proteinase cleavage product of 120 kDa paramyosin. The intact forms of paramyosin that are actually obtained are those of molecular mass 120 and 95 kDa (β-paramyosin), i.e.,

$$\left.\begin{matrix}\text{120 kDa paramyosin}\\ \text{95 kDa paramyosin}\end{matrix}\right\} \xrightarrow{\text{trypsin}} \left\{\begin{matrix}\text{102 kDa } \alpha\text{-paramyosin}\\ \text{93 kDa } \gamma\text{-paramyosin}\end{matrix}\right.$$

Bivalve paramyosin paracrystals showed cross-striations of 14.5 nm at pH 6.5 and 36 nm at pH 4.5. The homologous antigen formed a spur, which is very closely related and shows only minor differences.

4.3. Abalone foot muscle

Abalone foot muscle paramyosin [13,14] comprises three different filaments: thick filaments varying in diameter from 30 to 100 nm and showing cross-striations of 14.5 nm; i.e., paramyosin filaments. Intermediate filaments (diameter 12 nm) arranged in an orderly manner around thick filaments in the relaxed state. In some areas, cross-bridges between paramyosin filaments and intermediate and myosin filaments were clearly visible. The thin (5 nm) actin filaments were demonstrated via longitudinal sectioning. No difference in cross-bridge structure exists between the relaxed and catch states. The most striking feature of the catch state was the disorderly arrrangement of the filaments. A continuous process of breakage and formation of cross-bridges between paramyosin, myosin and actin filaments might be involved in the tonic contractions.

Abalone paramyosin comprises three subunits: α-subunit, 102 kDa, isofocussing pH 6.50–6.00; β-subunit, 99 kDa, isofocussing pH 5.45; α-subunit, 95 kDa, isofocussing pH 4.80. The β- and γ-paramyosins of abalone are larger than those of bivalve adductor muscle. Abalone α-, β- and γ-paramyosins crystallize over a wide range of pH (pH 6.5, 5.45 and 4.5, respectively) in paracrystals, all showing cross-striations of 14.5 nm.

5. In vitro phosphorylation of bivalve adductor muscle paramyosin

Paramyosin was phosphorylated in vitro [15] using cyclic AMP-dependent protein kinase (concentration, 0.4 μg/μl) and [γ-^{32}P]ATP at 30 μCi/ml. The amino acid phosphorylated was at the serine residue. The radioactivity incorporated (in cpm ^{32}P incorporated) was as follows: catch state – acetylcholine (27 μg/μl)-treated, 71 200; usual state – untreated, 43 300; relaxed state – serotonin (1 mg/30 ml)-treated, 35 000.

Actin markedly inhibited the in vitro phosphorylation of paramyosin. This may be due to conformational changes which expose more serine residues: ^{32}P incorporated (in cpm) – paramyosin, 43 288; paramyosin-M, 39 290; paramyosin-A, 2655; paramyosin-M-A, 2475.

The catch state was in the phosphorylated form, the relaxed state being in the dephosphorylated form.

6. Isolation of mRNA from bivalve adductor muscle

Isolation of mRNA from bivalve adductor muscle [16] was carried out by means of oligo(dT)-cellulose chromatography. Agarose electrophoretograms produce three clearly distinguishable bands, with values ranging from 16 to 34 S. Using a rabbit reticulocyte lysate cell-free system, we were able to direct protein biosynthesis. The translation products obtained on employing SDS-polyacrylamide gel electrophoresis revealed 13 bands at 200, 120, 95, 90, 85, 60, 58, 53, 38, 26, 18 and 15 kDa. The 200, 26, 18 and 15 kDa bands are most likely to be due to the heavy chain and the light chains of myosin. The 120 kDa band represents the intact subunit of paramyosin and that of 95 kDa corresponds to the β-subunit. The 38 kDa protein is probably tropomyosin; we are currently investigating the 53 kDa protein. The results described above provide further evidence in support of our proposal that the 102 kDa α-subunit and the 93 kDa γ-subunit of paramyosin are proteinase fragments of the 120 kDa subunits of paramyosin.

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