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BIOCHEMICAL COMPARISON OF FAST- AND SLOW-CONTRACTING SQUID MUSCLE

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Summary

The myofilament protein compositions of muscle fibres from the transverse muscle mass of the tentacles and the transverse muscle mass of the arms of the loliginid squid *Sepioteuthis lessoniana* were compared. These two muscle masses are distinct types, differing in their ultrastructural and behavioural properties. The transverse muscle of the tentacles consists of specialized muscle fibres that exhibit cross-striation and unusually short sarcomeres and thick filaments. The transverse muscle of the arms consists of obliquely striated muscle fibres that are typical of cephalopod skeletal muscle in general. The specialization of the tentacle muscle results in a high shortening speed and reflects its role in creating rapid elongation of the tentacles during prey capture. Comparison of samples of myofilament preparations of the two muscle fibre types using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) and peptide mapping of myosin heavy chains from the two muscle fibre types, however, showed little evidence of differences in contractile protein isoforms. Thus, specialization for high shortening speed appears to have occurred primarily through changes in the dimensions and arrangement of the myofilament lattice, rather than through changes in biochemistry. The thick filament core protein paramyosin was tentatively identified in the squid muscle fibres. This protein was less abundant in the short thick filament cross-striated tentacle muscle cells than in the obliquely striated arm cells.

Introduction

The biochemical basis of muscle specialization has been studied in a number of animals, most extensively in vertebrates. Molecular heterogeneity has been observed in comparisons among different muscle fibre types (e.g. Bandman, 1985; Bárány, 1967; Close, 1972; Edgerton, 1978; Reiser *et al.* 1985; Schachat *et al.* 1987; Schiaffino *et al.* 1988) as well as within single muscle fibre types classified by

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classical histochemical methods (Moore and Schachat, 1985; Moore *et al.* 1987; Schachat *et al.* 1985; Sweeney *et al.* 1988). These differences in biochemical composition are thought to allow fine tuning of the physiological performance of a muscle fibre.

The present study compares the myofilament protein composition of muscle fibres from the transverse muscle mass of the tentacles with that of the transverse muscle mass of the arms of the squid *Sepioteuthis lessoniana*. This comparison is of particular interest because it is likely that the transverse muscle of the tentacles is evolutionarily derived from the transverse muscle of the arms. Previous work on a number of squid species (Kier, 1982, 1985) revealed striking specialization of the transverse muscle of the tentacles when compared with the transverse muscle of the arms. This specialization is observed in behavioural, biomechanical and ultrastructural characteristics. In contrast to these observed specializations, the biochemical analysis described here suggests that relatively little biochemical specialization has occurred in the tentacle musculature. The results of this study contrast with most previous studies of biochemical specialization in vertebrate muscle systems and thus offer new insight into the ways in which muscle may evolve.

Materials and methods

Experimental animals

Specimens of the loliginid squid *Sepioteuthis lessoniana* Lesson were obtained from the Marine Biomedical Institute of the University of Texas Medical Branch at Galveston, USA. The animals were killed by overanaesthesia and the head and buccal mass were frozen quickly in liquid nitrogen and stored at −80 °C. Muscle tissue from four animals was used in this study. The wet mass of the animals ranged from 625 to 875 g.

Myofilament extraction

Samples of the transverse muscle mass from the arms and tentacles were chipped from the frozen buccal mass and ground to a fine powder in a mortar and pestle that had been cooled in liquid nitrogen. The following steps were performed at 4 °C. The powder was suspended in a 10-fold volume of extraction buffer (100 mmol l^{-1} NaCl, 10 mmol l^{-1} Tris–HCl, 10 mmol l^{-1} Tris base, 1.0 mmol l^{-1} EGTA, 1.0 mmol l^{-1} $MgCl_2$, 7 mmol l^{-1} β-mercaptoethanol, 0.1 mmol l^{-1} phenylmethylsulphonyl fluoride, 5 μg ml^{-1} each of Antipain, Chymostatin, Pepstatin and Leupeptin, pH 8.1) and mixed by hand. The sample was centrifuged briefly in an Eppendorf microfuge. The supernatant was discarded and the pellet was resuspended in the extraction buffer and centrifuged as before. The pellet was then resuspended and incubated in extraction buffer with 0.5 % Triton-X 100 for 10 min. The sample was centrifuged as above, the supernatant was discarded and the pellet was resuspended in extraction buffer without Triton-X. This last step was repeated twice.

Preparation of paramyosin

A sample from the myofilament extraction outlined above was used in a paramyosin purification procedure adapted from Waterston *et al.* (1974) and Shriefer and Waterston (1989). All steps were performed at 4 °C. The sample was centrifuged at 10 000 ***g*** for 10 min. The pellet was solublized for 20 min in 10 volumes of solubilization buffer (6 mol l^{-1} guanidine, 0.01 mol l^{-1} Tris–HCl, pH 7.5, 2 mmol l^{-1} EDTA, 1 mmol l^{-1} dithiothreitol (DTT), 1 mmol l^{-1} phenylmethylsulphonyl fluoride (PMSF), 1.0 mg ml^{-1} N^{α}-*p*-tosyl-L-arginine (TAME), 80 μg ml^{-1} Pepstatin and 80 μg ml^{-1} Leupeptin) and then passed through a 23 gauge needle. The sample was centrifuged as before, the supernatant was saved and was precipitated by 3 volumes of 95 % ethanol, 2 mmol l^{-1} DTT for 2 h. The precipitate was collected by centrifugation at 10 000 ***g*** for 10 min, resuspended in 0.6 mol l^{-1} NaCl, 10 mmol l^{-1} sodium phosphate (pH 6.0), 2 mmol l^{-1} DTT and dialyzed overnight against the same buffer. The sample was centrifuged as before and the supernatant was dialyzed against 0.1 mol l^{-1} NaCl, 10 mmol l^{-1} sodium phosphate (pH 6.0) for 3–4 h. The paramyosin-containing precipitate was then collected by centrifugation at 16 000 ***g*** for 10 min. The precipitate was resuspended in 0.6 mol l^{-1} NaCl, 10 mmol l^{-1} sodium phosphate (pH 6.0) and 2 mmol l^{-1} DTT. A partial purification of paramyosin was also performed as part of the procedures (outlined below) for purification of actomyosin.

Preparation of actomyosin

A sample resulting from the myofilament preparation above was used to prepare an actomyosin extract, incorporating a procedure adapted from Harris and Epstein (1977). The myofilament preparation was centrifuged at 5000 ***g*** for 10 min. The pellet was suspended in 1 volume of a high-salt buffer [20 mmol l^{-1} Tris–HCl, 0.6 mol l^{-1} KCl, 1 mmol l^{-1} DTT, 1 mmol l^{-1} PMSF (pH 8.0)] and centrifuged at 20 000 ***g*** for 15 min. The supernatant (S-2) was saved and this step was repeated. The supernatants (S-2) were combined and were centrifuged at 100 000 ***g*** for 3 h. This supernatant (S-3) is enriched in paramyosin and was therefore saved for a paramyosin analysis. The pellet (P-3) was suspended gently in 0.5 volume 20 mmol l^{-1} Tris–HCl, 0.6 mol l^{-1} KCl, 5 mmol l^{-1} ATP, 5 mmol l^{-1} $MgCl_2$, 1 mmol l^{-1} DTT, 1 mmol l^{-1} PMSF (pH 8.0) and centrifuged at 100 000 ***g*** for 15 min. This step was repeated and the supernatants (S-4) from both steps were saved. One volume of glycerol was added to the combined supernatants and the sample was stored at −20 °C.

Preparation of myosin

To identify the myosin light chains, 0.3 ml of arm and tentacle myofilaments, prepared as described above, were pelleted by centrifugation in an Eppendorf microfuge for 30 s. The myofilaments were then extracted with 0.5 ml of 0.6 mol l^{-1} NaCl, 10 mmol l^{-1} Tris, 7 mmol l^{-1} β-mercaptoethanol, 2 mmol l^{-1} $MgCl_2$ and 1 mmol l^{-1} ATP, pH 7.5. Following a 20 min spin in an Eppendorf microfuge,

0.25 ml of the supernatant was diluted with an equal volume of 0.8 mol l^{-1} KI in the same buffer to depolymerize actin. The resulting supernatant was applied to a 10 ml Sephacryl S-300 (Pharmacia) column. 0.2 ml fractions were collected and each was analyzed by electrophoresis on a 10 % SDS–polyacrylamide gel to identify the fractions containing purified myosin and the myosin light chains.

Gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) was performed as described by Laemmli (1970) with modifications described by Schachat *et al.* (1985). 10.5 % polyacrylamide gels were used for comparisons of the myofilament homogenates and 8 % polyacrylamide gels were used for the paramyosin analyses. 4.75 % polyacrylamide gels and 5.0 % Neville gels (Neville, 1971; Neville and Glossman, 1971) were used to compare the myosin heavy chains from the two muscle fibre types. The gels were stained with either Coomassie Brilliant Blue or with silver as in Schachat *et al.* (1985).

Peptide mapping

A comparison of the myosin heavy chains of the tentacle and arm muscles was made using a modification of the cyanogen bromide peptide mapping technique of Pepinsky (1983) and a V8 protease peptide mapping technique (Hames, 1981).

Cyanogen bromide

Myofilament extracts from the arm and tentacle were electrophoresed on a 4.75 % SDS–polyacrylamide gel. The protein bands were visualized with 1.0 mol l^{-1} KCl and the bands containing the myosin heavy chains were excised from the gel. The gel slices were washed twice for 10 min in water and then incubated in 20 volumes of 100 mg ml^{-1} cyanogen bromide, 53 % formic acid, 30 mmol l^{-1} β-mercaptoethanol for 30 min at room temperature. The cyanogen bromide was diluted from freshly prepared 700 mg ml^{-1} stock in formic acid. The gel slices were washed twice in water and then in 0.25 mol l^{-1} Tris (pH 6.8) for 3 min each. The gel slices were incubated in SDS sample buffer (2 % SDS, 50 mmol l^{-1} Tris, pH 6.8, 20 % glycerol) for 5 min at 37 °C and the polypeptides from these gel slices were electrophoresed into 15 % SDS polyacrylamide gels by loading the gel slices directly into the stacking gel wells. The gels were stained with silver as described by Schachat *et al.* (1985).

V8 protease

Myofilament extracts from the arm and tentacle were electrophoresed on a 7 % SDS–polyacrylamide gel. The gel was stained with 0.3 mol l^{-1} copper chloride, which precipitates Tris/SDS complexes in the gel, resulting in an opaque blue/green background on which proteins appear as clear bands (Lee *et al.* 1987). The bands containing the myosin heavy chains were excised from the gel. The gel slices were washed three times for 15 min in 0.25 mol l^{-1} Tris, pH 9.0, 0.25 mol l^{-1} EDTA, followed by a 15 min rinse in water. Sample wells of an 11 % SDS–polyac-

rylamide gel were filled with 0.125 mol l^{-1} Tris–HCl (pH 6.8), 0.1 % SDS and each gel slice was positioned horizontally on the bottom of the well. Each gel slice was then overlayered with 0.125 mol l^{-1} Tris–HCl (pH 6.8), 0.1 % SDS, 20 % glycerol. A buffer containing 0.125 mol l^{-1} Tris–HCl (pH 6.8), 0.1 % SDS, 10 % glycerol, Bromophenol Blue and V8 protease (75 and 150 ng per gel slice) was added to the well. Electrophoresis was started and continued until the running front was two-thirds of the distance down the stacking gel. The power was then turned off for 30 min. Finally, electrophoresis was completed and the gel was stained with Coomassie Brilliant Blue as described above.

Electron microscopy

Cross-sectional slabs of the arms and tentacles, 2–3 mm thick, were obtained from animals killed by overanaesthesia. The slabs were placed in 3.0 % glutaraldehyde, 0.065 % phosphate buffer, 0.5 % tannic acid and 6.0 % sucrose and fixed for 10 h at 4 °C. Portions of the slabs adjacent to the fixation surface were then cut into smaller blocks, approximately 1 mm×1 mm×4 mm and washed overnight in buffer. The blocks were rinsed for 20 min in chilled 0.065 % phosphate buffer and postfixed for 40 min in a 1:1 mixture of 2 % potassium ferrocyanide in 0.13 mol l^{-1} cacodylate buffer and 2 % osmium tetroxide. The blocks were dehydrated in ethanol and embedded in Epon. Thin sections were stained with saturated aqueous uranyl acetate and with lead citrate (Reynolds, 1963) and examined with a Zeiss EM 10CA electron microscope. For additional details concerning the electron microscopical methods used, see Kier (1985).

Results

Fig. 1 shows a 10.5 % SDS–PAGE of the myofilament extracts from *Sepioteuthis lessoniana* tentacle and arm muscle and, for comparison, myofilament extracts from erector spinae and soleus muscles of a New Zealand White rabbit. Comparison of the cross-striated tentacle muscle with the obliquely striated arm muscle lanes reveals few significant differences in myofilament protein composition. The similarity between the two squid muscle fibre types is made even more striking when compared to the molecular heterogeneity revealed by these techniques applied to the rabbit muscle fibre types shown in Fig. 1. This control demonstrates that the procedure is capable of resolving the remarkable variety of differences in protein composition that have been reported previously for well-studied mammalian muscle fibre types. The only difference in protein composition between the arm and tentacle transverse muscle revealed with this technique is the absence of two weak bands that run immediately in front of actin in the tentacle muscle. These proteins have not yet been identified.

Tentative identifications of several of the *Sepioteuthis lessoniana* muscle proteins are provided in Fig. 1. The myosin light chains and heavy chains are identified on the gel. No differences in myosin light chains are observed, based on the purifications of myosin from the myofibrils described above. In addition to the

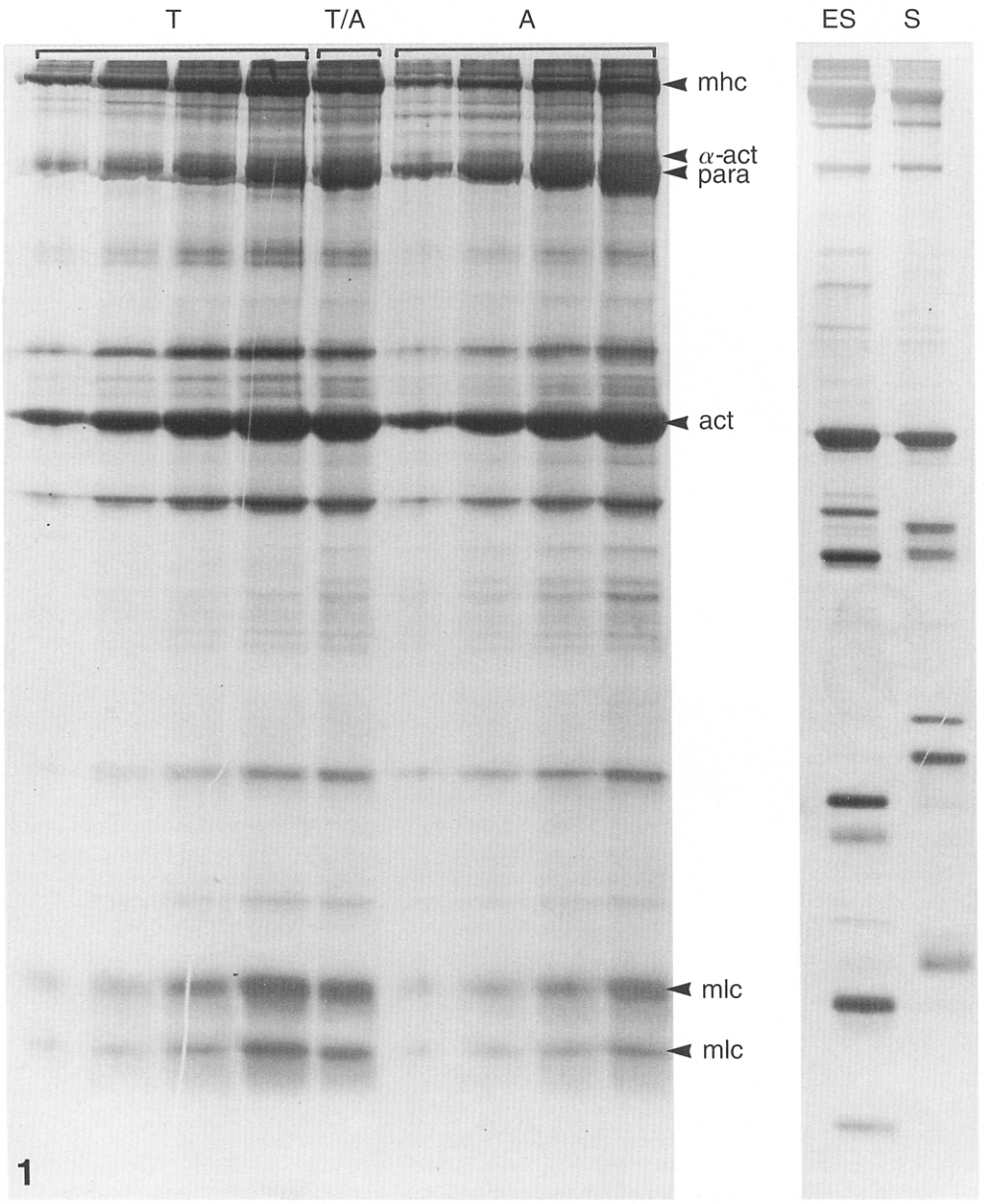


Fig. 1. Photograph of a silver-stained SDS–polyacrylamide gel (10.5 % acrylamide) of a range of loadings (increasing from left to right) of myofilament extracts of the transverse muscle cells of the tentacle (T) and arm (A) of *Sepioteuthis lessoniana*. The lane labelled T/A was loaded with 50 % tentacle and 50 % arm extract. Note that the protein composition of the arm and tentacle transverse muscles is remarkably similar. For comparison, identically prepared myofilament extracts of an erector spinae muscle (ES) (a fast muscle) and a soleus muscle (S) (a slow muscle) from a New Zealand White rabbit were run in adjacent lanes. Several of the bands are identified, including α-actinin (α-act), actin (act), the myosin heavy chains (mhc), the myosin light chains (mlc) and paramyosin (para).

comparisons shown here, no heterogeneity was observed in comparisons of the myosin heavy chains from each muscle type using 4.75% SDS–PAGE or 5% Neville gels. Tentative identification of actin and α-actinin is also provided in Fig. 1. The identity of the α-actinin band is based on an estimate of molecular weight and on its reactivity with a polyclonal antibody (generously supplied by Dr K. Burridge, University of N. Carolina). Slight staining of the myosin heavy chain band and actin band with the antibody was also observed, but since these proteins are much more abundant than α-actinin, the staining of the myosin heavy chain and actin normalized to protein amount was extremely weak.

In addition to the electrophoretic techniques outlined above, a more detailed analysis of the myosin heavy chains was performed using cyanogen bromide and V8 protease peptide mapping. The myosin heavy chains from arm and tentacle muscle were digested in cyanogen bromide and the polypeptide products produced by the digestion were compared by SDS–PAGE. Cyanogen bromide splits polypeptide chains specifically on the carboxyl side of methionine residues. Any differences in position or number of methionine residues between the two proteins under comparison will result in a different number and/or molecular weight of polypeptide digestion products. A 15% SDS–polyacrylamide gel of the products of such a digestion of the myosin heavy chains from arm and tentacle transverse muscle is shown in Fig. 2. There is no difference in the pattern of cyanogen bromide fragments or even in their relative intensities.

The results of the V8 protease peptide mapping technique are shown in Fig. 3. V8 protease cleaves primarily on the carboxyl side of glutamic acid residues; it also cleaves aspartic acid residues when they are present in excess of the substrate. As in the case of the cyanogen bromide peptide mapping technique, the size, number and relative intensities of the V8 protease fragments are the same in both muscles. These techniques have been used previously to reveal differences in avian myosin isoforms as well as myosin isoforms in mammalian and nematode muscles. The results of both the cyanogen bromide and V8 protease peptide mapping, together with the low-percentage acrylamide SDS–PAGE and Neville gel analysis, suggest that the myosins are quite similar if not identical. Thus, the differences in physiological performance between the two muscle fibre types are unlikely to be accounted for by differences in myosin isoform.

Paramyosin was purified from squid muscle and was used to identify the protein on the gels of arm and tentacle myofilament extracts. Comparison of the amount of paramyosin in the tentacle with that in the arm muscle using SDS–PAGE shows that less paramyosin is present in the cross-striated tentacle muscle. This difference is seen clearly in the 8.0% SDS–polyacrylamide gel shown in Fig. 4, which includes extracts from the cross-striated tentacle and obliquely striated arm muscle in addition to purified paramyosin from the arm muscle. The paramyosin content, referenced to that of the myosin heavy chains, is reduced in the cross-striated tentacle muscle. The paramyosin/myosin heavy chain ratio, as determined by densitometry, was 0.22 (S.D.=0.03) in the cross-striated tentacle muscle and 0.57 (S.D.=0.03) in the obliquely striated arm muscle.

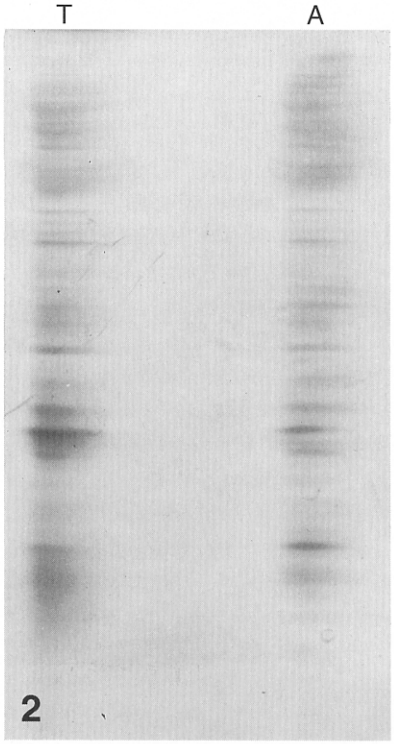


Fig. 2. Photograph of a silver-stained SDS–polyacrylamide gel (15 % acrylamide) of products of cyanogen bromide digestion of the myosin heavy chains from tentacle (T) and arm (A) transverse muscle of *Sepioteuthis lessoniana*. Note that an identical assemblage of polypeptides is produced from the two muscle cell types.

Discussion

Morphology and function of arm and tentacle musculature

Most squid species capture fast-swimming, alert prey by rapidly extending a pair of tentacles. This tentacle strike in *Loligo pealei* occurs in only 15–30 ms and involves an elongation by the tentacles of 70–100 % of their resting length. During the strike, suckers on the terminal portion of the tentacles attach to the prey and the tentacles then shorten, bringing the prey within reach of the remaining eight appendages, termed arms. The arms are capable of complex bending and manipulative movements and are responsible for subduing and orienting the prey.

Biomechanical analysis suggests that the transverse muscle mass of the tentacles is responsible for generating the force that rapidly extends the tentacles during prey capture. Contraction of the transverse muscle mass decreases tentacle diameter and thus increases the length of the tentacles (Kier, 1982; Kier and Smith, 1985; Smith and Kier, 1989). An estimate of shortening speed based on high-speed ciné films and a biomechanical model of the tentacles supports the

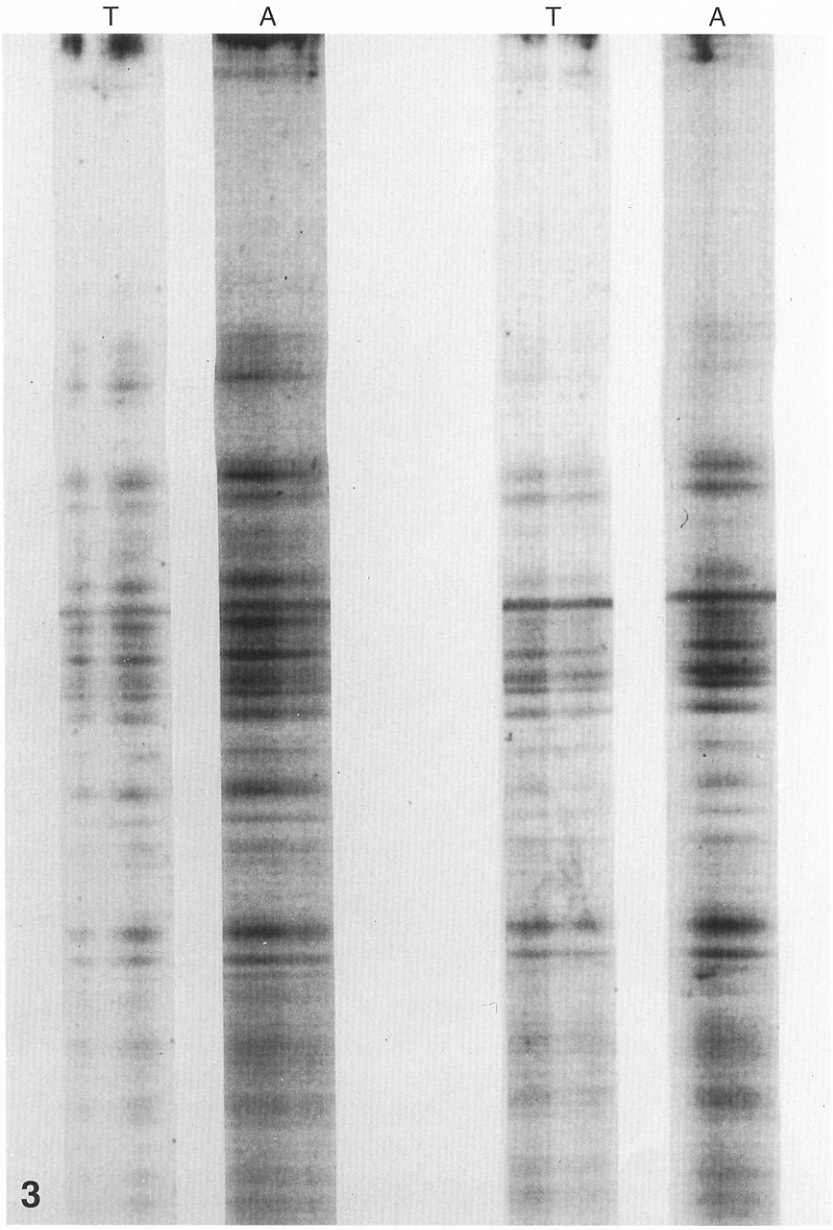


Fig. 3. Photograph of a Coomassie Brilliant Blue stained SDS–polyacrylamide gel (12.5 % acrylamide) of products of V8 protease digestion of the myosin heavy chains from tentacle (T) and arm (A) transverse muscle of *Sepioteuthis lessoniana*. The lanes on the left used a lower concentration of V8 protease than those on the right. Note that an identical assemblage of polypeptides is produced from the two muscle cell types.

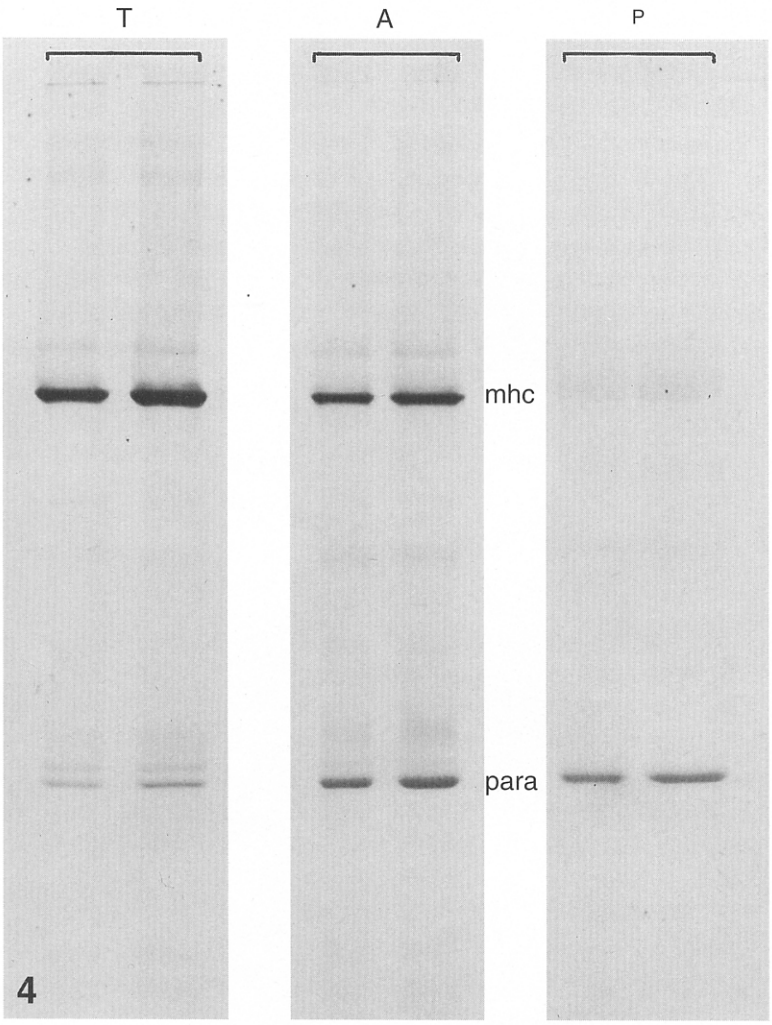


Fig. 4. Photograph of a silver-stained SDS–polyacrylamide gel (8.0% acrylamide) of myofilament extracts of the transverse muscle cells of the tentacle (T) and arm (A) of *Sepioteuthis lessoniana*. Paramyosin was purified from the transverse muscle of the arm of *Sepioteuthis lessoniana* and loaded in the lanes labelled P. Note that the arm muscle contains significantly more paramyosin than the tentacle muscle. The myosin heavy chain (mhc) and paramyosin (para) bands are labelled.

hypothesis that the transverse tentacle musculature is capable of high shortening speeds (see Kier, 1985).

The biomechanical analysis suggests that the transverse muscle mass of the arms serves a different function from that of the tentacles. In the arms, the transverse muscle mass provides the support that is required to create bending movements in a structure lacking hardened skeletal elements. [For discussions of the mechanics of bending in muscular structures such as the arms, see Kier (1982, 1987, 1988,

1989) and Kier and Smith (1985)]. Thus, behavioural and biomechanical analyses predict dramatically different functional roles for the transverse muscle mass of the tentacles and the transverse muscle mass of the arms.

An analysis of the ultrastructure of the muscle cells from the two muscle masses of squid revealed striking differences that correspond to these functional differences (Kier, 1985). Muscle cells from the transverse muscle mass of the tentacles are cross-striated. They differ from the obliquely striated cells that are present in the transverse muscle mass of the arms and that characterize all other cephalopod 'skeletal' musculature (Fig. 5). The ultrastructure of the cross-striated cells is indicative of high shortening speed. In particular, the thick filament and sarcomere length were found to be unusually short: 0.5–0.9 μm and 0.9–1.6 μm, respectively. For comparison, the thick filament length of the obliquely striated cells was 2.8 μm or greater. It should be emphasized that these ultrastructural differences have been observed in all squid species examined (Family Ommastrephidae: *Illex illecebrosus*; Family Loliginidae: *Loligo pealei, Loligo opalescens, Loliguncula brevis* and *Sepioteuthis sepioidea*).

Evolution of the transverse tentacle muscle

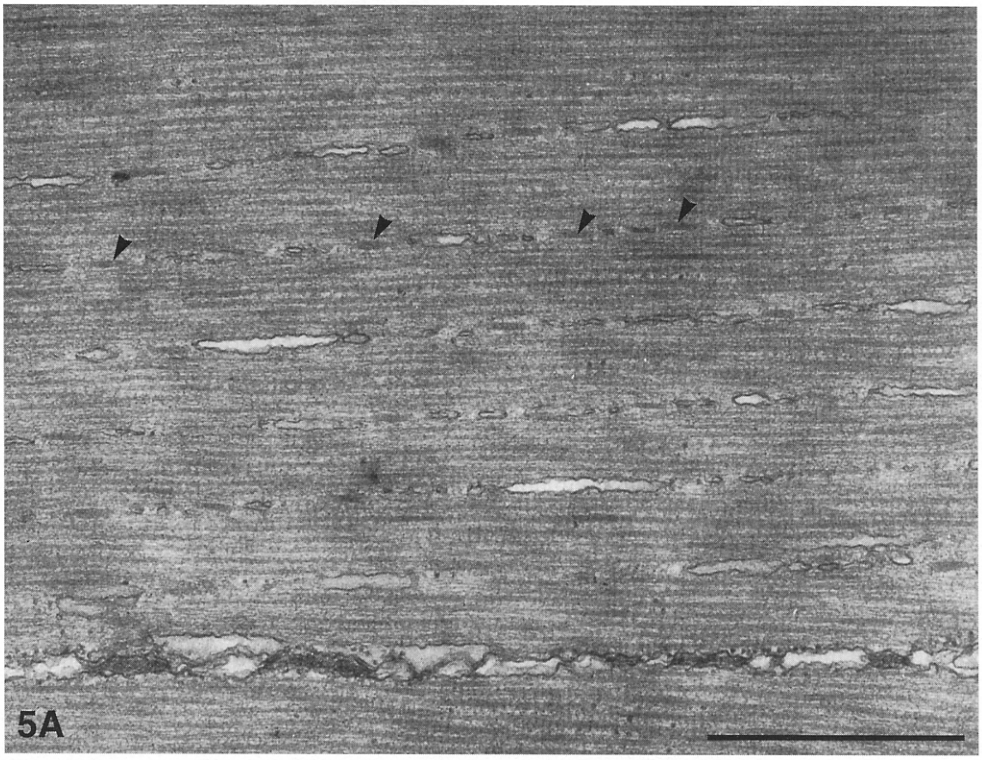
Comparative morphological, neuroanatomical and developmental considerations (von Boletzky, 1987, 1992; Donovan, 1977; Naef, 1921/1923) suggest that the tentacles of squid and cuttlefish have evolved through modification of the fourth pair of arms. The transverse muscle of the arms and the transverse muscle of the tentacles are thus homologous. If the tentacles are modified arms, it is most parsimonius to hypothesize that the cross-striated cells evolved from the obliquely striated cells. Indeed, aspects of the ultrastructure, e.g. the structure of the Z disc, the details of the arrangement of the sarcoplasmic reticulum and lack of an M line, suggest that the cross-striated muscle evolved by a reorganization of the obliquely striated cells (see Kier, 1985, 1991), supporting Prosser's (1982) contention that cross-striation has evolved many times in parallel.

Biochemical comparison of arm and tentacle muscles

Paramyosin content

The results presented here provide a tentative identification of the thick filament core protein paramyosin and suggest that the paramyosin content of the cross-striated tentacle muscle is reduced when compared to the obliquely striated muscle cells. A reduction in paramyosin in the cross-striated muscle cells is also suggested by ultrastructural observations. An electron-lucent core was observed in electron micrographs of transverse sections of the thick filaments of the cross-striated cells (Kier, 1985). The electron-lucent core was more apparent in regions towards the ends of the thick filaments than at their centre. An electron-lucent core was not observed in the thick filaments of the obliquely striated cells.

The reduction in paramyosin content is in agreement with previous studies that have shown a correlation between thick filament length and paramyosin content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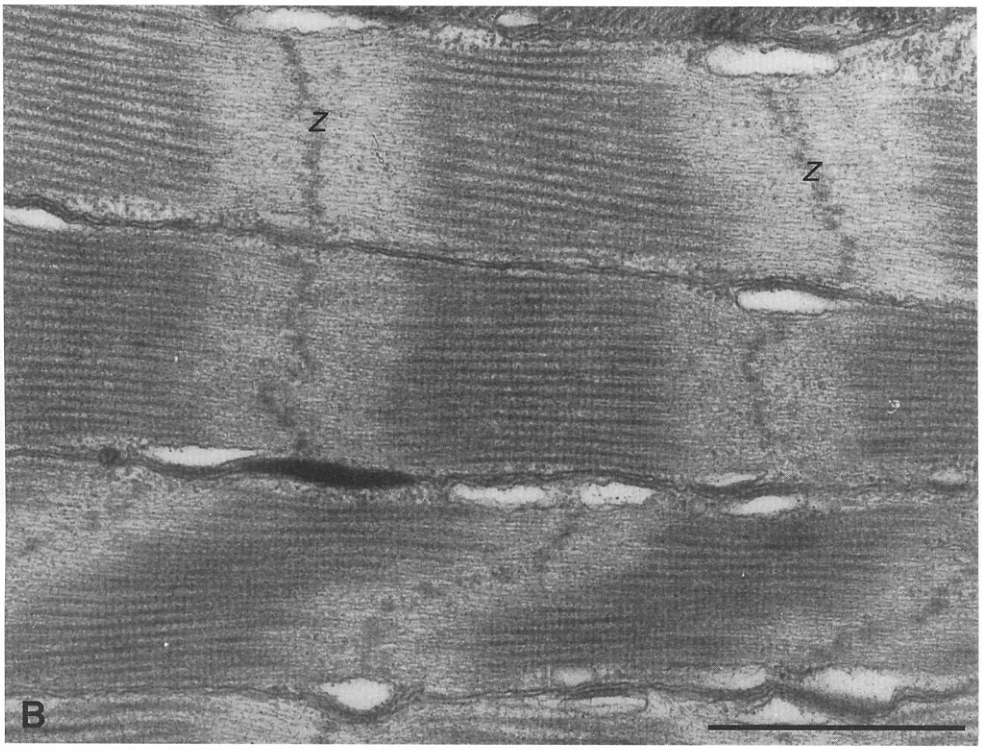
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Fig. 5. (A) Electron micrograph of a longitudinal section of obliquely striated muscle cells of the transverse muscle of the arm of the squid *Illex illecebrosus*. The ultrastructure of the transverse muscle of the arm of *Sepioteuthis lessoniana* is the same (see Kier, 1985). The long axis of the muscle fibre is oriented horizontally on the page. The arrowheads indicate a series of dense bodies and shows that their alignment is oriented obliquely to the long axis of the muscle fibre. (B) Electron micrograph of a longitudinal section of cross-striated muscle fibres of the transverse muscle of the tentacle of *Loligo pealei*. The ultrastructure of the transverse muscle of the tentacle of *Sepioteuthis lessoniana* is the same. The long axis of the muscle fibre is oriented horizontally on the page and two Z discs (*z*) are labelled. Scale bars, 1 μm.

(Levine *et al.* 1976). Although paramyosin has been found in all molluscan muscles studied and in muscles from a variety of invertebrates, its function remains somewhat unclear (Chantler, 1983; Cohen *et al.* 1971; Elfvin *et al.* 1976; Levine *et al.* 1976; Szent-György *et al.* 1971; Waterston *et al.* 1974; Winkelman, 1976). There is evidence that paramyosin is necessary for determination of the length of the longer thick filaments of many invertebrates (Ikemoto and Kawaguti, 1967; Levine *et al.* 1976; Mackenzie and Epstein, 1980) and the reduced paramyosin content of the cross-striated cells may be related to length determination of these short thick filaments.

Biochemical composition

Although the arm and tentacle muscles contained different amounts of paramyosin, the striking difference in physiology and ultrastructure between these two muscle types was not reflected in differences in protein composition. The techniques used in this study are the same as those used to reveal biochemical heterogeneity in a variety of other muscle fibre types (e.g. Briggs *et al.* 1987; Schiaffino *et al.* 1988). The similarity in protein composition between the two squid muscle fibre types provides an important contrast to the situation observed in vertebrate skeletal muscle fibre types. Comparisons of the biochemical composition between vertebrate skeletal muscle fibre types show consistent biochemical heterogeneity. Indeed, variation has been found in the biochemistry of single muscle fibre types classified by classical histochemical methods (e.g. Sweeney *et al.* 1988; Moore and Schachat, 1985). Differences in the myosin light chains, myosin heavy chains, troponins and tropomyosins have been noted and, in some cases, these differences have been correlated with differences in physiological performance. Differences in shortening speed between various vertebrate muscle fibre types seem to be primarily a function of differences in the myosin isoforms. Variation in the myosin isoforms is reflected in variation in myosin ATPase activity and hence rate of cross-bridge cycling (Bandman, 1985; Bárány, 1967; Close, 1972; Reiser *et al.* 1985; Schiaffino *et al.* 1988; Sweeney *et al.* 1988). In spite of the observed biochemical heterogeneity, the sarcomere length and thick filament length remain relatively constant between different vertebrate skeletal muscle fibre types (Eisenberg, 1983; Hoyle, 1983).

In contrast, the specialization of the squid tentacle fibres for fast contraction

involved a different mechanism. In the cross-striated squid fibres, a rearrangement and redimensioning of the myofilaments provided for an increase in shortening speed. The most important aspects of this rearrangement, in terms of increased shortening velocity, are the thick filament and sarcomere lengths. In general, there is an inverse correlation between the unloaded shortening velocity of a muscle fibre and its thick filament length (Josephson, 1975; Millman, 1967). This is because muscles with short thick filaments and short sarcomeres have more sarcomeres in series per unit length and the shortening velocities of elements in series are additive (Huxley and Simmons, 1972; Josephson, 1975). Thus, by decreasing the sarcomere length, the shortening velocity of a muscle cell can be increased, even if other aspects of the cell remain unchanged. The relationship between sarcomere length and shortening speed has also been exploited in the evolution of muscle fibre types in other invertebrates, such as brachiopods (Reed and Cloney, 1977), cercaria larvae of trematodes (Reger, 1976; Sundaraman and Nadakal, 1979) and especially in crustaceans (Hoyle, 1967, 1983).

In summary, as Hoyle (1983, p. 268) noted, 'the quite small range of sarcomere lengths that exists in chordates is not known to be associated with significant speed differences and has certainly not been exploited by vertebrates as a major variable'. Instead, it appears that vertebrates utilize biochemical specialization for the fine tuning of muscle fibre performance. In this study, we present a case in which the opposite situation obtains; major ultrastructural specialization has occurred but the biochemical composition has remained relatively constant. This case contributes to the growing appreciation of the diverse means by which muscle specialization can evolve.

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