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FIBRE TYPES IN LEECH BODY WALL MUSCLE

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Summary

The fibre type composition of obliquely striated muscle of adult *Hirudo medicinalis* was investigated by enzyme histochemistry, by immunohistochemistry and by SDS–PAGE. The oxidative capacity of the fibres, assessed by succinate dehydrogenase activity, was similar in all three layers of body wall muscle (longitudinal, oblique and circular) and in dorsoventral muscles. Histochemical localisation of Mg^{2+}-activated actomyosin ATPase activity gave stronger staining in the longitudinal muscle than in other layers. As muscle shortening speed is directly related to this form of ATPase activity, this suggests that the longitudinal layer fibres are faster contracting than the circular, oblique or dorsoventral muscles. Results with polyclonal antibodies specific for vertebrate myosins were consistent with the ATPase results, i.e. fibres with the lowest actomyosin ATPase activity reacted preferentially with an antibody for a slower myosin. Thus, anti-T2, selective for vertebrate tonic fibre myosin, bound preferentially to fibres in oblique, circular and dorsoventral muscles, whereas anti-S, selective for vertebrate slow twitch fibre myosin (faster than vertebrate tonic fibre myosin), bound preferentially to the bulk of longitudinal layer fibres. Whereas most of the longitudinal layer stained uniformly with the anti-S antibody, some fibres in the outermost bundles were negative for the anti-S antibody and were, therefore, different from the main mass of longitudinal fibres. SDS–PAGE analysis of contractile protein preparations from body wall muscle also revealed a difference in the composition of the oblique, circular and dorsoventral muscles compared to the longitudinal layer, supporting the conclusion that leech body wall muscle contains two fibre types.

Introduction

It is well known that most skeletal muscles in vertebrates contain a mixture of

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muscle fibres of several differentiated types, and that the different types of fibre are contacted by motoneurones with different and characteristic properties, each motoneurone innervating only fibres of one particular type (Burke, 1981). Whether the same is true for muscles of the hydroskeleton in soft-bodied animals such as the leech is not known. Studies of muscle fibre types in invertebrates are rare: most of them are of crustacean or chelicerate muscles (e.g. Ogonowski and Lang, 1979; Levine *et al.* 1989) and demonstrate the existence of fast and slow types (often with different metabolic capacities as well) comparable in some respects to fast and slow types in vertebrates. Two muscle fibre types have also been found in *Drosophila* (Raghavan, 1981) and in the earthworm (D'Haese and Carlhoff, 1987). In both vertebrates and invertebrates, the contractile protein myosin consists of two kinds of subunit: heavy chains (relative molecular mass approximately 200×10^3) which form the force-generating ATPase sites, and associated light chains (two or more forms with relative molecular masses of about 20×10^3). Analytical techniques used to study vertebrate contractile proteins can also be applied successfully to invertebrate muscle (e.g. Costello and Govind, 1984), and invertebrate myosin light chains have received some attention because, in some species, calcium regulation of contraction is mediated by one of the light chains (Kendrick-Jones *et al.* 1976). By contrast, there is very little information available on invertebrate myosin heavy chains, yet studies of vertebrate myosin have shown that the heavy chain of myosin exists in multiple isoforms that are usually characteristic of particular muscle types (fast, slow, etc.) and determine their histochemical ATPase activity as well as some important functional properties (Bandman, 1985; Mascarello *et al.* 1986; Schiaffino *et al.* 1988; Sweeney *et al.* 1988).

Body wall muscle in *Hirudo medicinalis* has characteristics that place it as intermediate in type between the classic smooth and striated muscles of vertebrates. Although originally classified as 'helical smooth' on the basis of X-ray diffraction (Hanson and Lowy, 1960), later fine structural studies showing highly ordered arrays of obliquely staggered myofilaments have led to its separate designation as 'obliquely striated' muscle (Rosenbluth, 1972). We were interested in whether this obliquely striated muscle, like striated muscle in vertebrates, contains a mixture of differentiated fibre types with different contractile proteins and different metabolic machinery producing energy for contraction.

To investigate fibre type composition of adult leech muscle we have used enzyme histochemistry for fibre typing of metabolic enzymes and localisation of ATPase activity, immunohistochemistry for myosin, and gel electrophoresis. Some of this work has appeared in preliminary form (Blackshaw and Rowlerson, 1989).

Materials and methods

Histochemistry and immunohistochemistry

Frozen 10 μm sections of body wall were stained for succinic dehydrogenase and

alpha-glycerophosphate dehydrogenase (αGPDH). To look for differences in muscle contractile proteins we used methods developed mainly for use in mammalian muscle. For histochemical localisation of magnesium-activated actomyosin ATPase we used the method of Mabuchi and Sreter (1980), which includes magnesium as well as calcium in the ATP-containing incubation medium. The polyclonal antibodies tested against leech muscle are described in Tables 1 and 2. Antibodies were visualised by indirect fluorescence or by indirect immunoperoxidase staining of the sections.

Analysis of leech muscle myosin by SDS–PAGE

In a recent study of *Limulus polyphemus* L. muscle, two isoforms of myosin could be resolved by electrophoresis on pyrophosphate gels (Levine *et al.* 1989). However, as this method can reflect differences in either heavy or light chains of myosin, it does not provide an unambiguous demonstration of heavy chain differences. We used instead the method of Carraro and Catani (1983), in which different isoforms of the myosin heavy chain alone are separated by their differing mobility on electrophoresis in the presence of SDS in 5 % polyacrylamide gels.

Strips of body wall muscle were dissected from the leech. Two kinds of sample were removed: (1) 'body wall' samples, which contained longitudinal, circular and oblique muscle together with dorsoventral muscle and (2) 'longitudinal' samples, which were strips dissected from the muscle that constitutes the bulk of the body wall, the longitudinal layer. These samples were taken in the ventral midline from the deepest layer of muscle adjacent to the ventral nerve cord. Muscle samples were homogenised in an ice-cold low-salt buffer (0.1 mol l^{-1} KCl, 5 mmol l^{-1} EDTA, 0.01 % 2-mercaptoethanol, pH 7.5). The homogenate was centrifuged briefly and the supernatant discarded. The precipitate was resuspended in low-salt buffer, and the cycle of precipitation and resuspension was repeated three more times. This sort of procedure is often used to isolate well-washed myofibrils from vertebrate skeletal muscle and produced a sample of comparable composition (in terms of its contractile protein content) from the leech muscle, as shown in Fig. 3A. This contractile protein sample was suspended in gel sample buffer (0.075 mol l^{-1} Tris–HCl, pH 6.8, 2 % SDS, 10 % glycerol, 5 % 2-mercaptoethanol, 0.001 % Bromophenol Blue) and heated to 100 °C for 3 min to dissociate all protein subunits in preparation for their separation by gel electrophoresis. Conditions for electrophoresis were essentially as described by Laemmli (1970), and the separated protein bands were then visualised by silver staining. The overall composition of the leech contractile protein sample was compared with that of vertebrate myofibrillar samples on a two-stage separating gel permitting resolution of proteins covering a wide range of relative molecular masses (see Fig. 3A; sample loads 1–5 μg per lane). The myosin heavy chain composition of the contractile protein sample was analysed on 5 % separating gels, with electrophoresis conditions and staining as before, but with much lower sample loads of about 100 ng. It has been shown that, despite their very similar primary structures, different isoforms of the heavy chain of myosin show small differences in

migration rate in such gels (Carraro and Catani, 1983). Again the contractile protein sample was compared with reference samples of vertebrate fast and slow myosins (see Fig. 3B).

Results

The tubular body wall of the leech contains three distinct muscle layers of differing thickness; an outer thin layer of circular muscle, one or two fibres thick, lying immediately beneath the skin; a thick inner layer of longitudinal muscle, and an orthogonal grid of oblique muscle fibres sandwiched between the other two layers. The longitudinal muscle forms the bulk of the body wall and consists of many rows of fibres arranged in bundles (Fig. 1). These three layers, together with the dorsoventral muscles that span the body cavity, are largely responsible for the posture of the leech and its locomotory activities of swimming, crawling and walking.

Histochemistry and immunohistochemistry

Succinic dehydrogenase, a mitochondrial enzyme, is an indicator of oxidative metabolism and in vertebrate muscle is strongly correlated with fatigue resistance. In the leech muscle it was found only in the central core of the fibres, where the mitochondria are located. Staining was uniform across circular and oblique layers, but in the longitudinal layer there appeared to be a gradual transition in the density of the stain, with the densest staining located in bundles at the outer edge

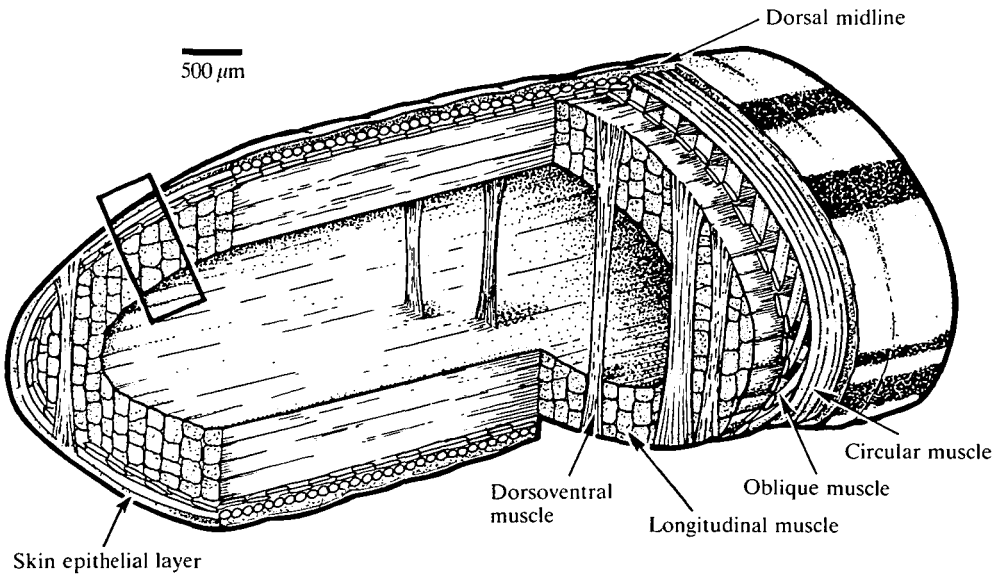

Fig. 1. Diagram showing the arrangement of the main muscles in the body wall of *Hirudo medicinalis*. Transverse sections through the body wall shown in Fig. 2 correspond to the area outlined in the rectangle.

of the layer, adjacent to the oblique muscle. αGDPH activity, an indicator of glycolytic pathways and associated with fast twitch fibres in vertebrate muscle, was not seen in any of the muscle layers, though it was detected in the skin epithelial layer on the outer edge of the body wall sections.

The methods used to look for differences in muscle contractile proteins were developed mainly for use in mammalian muscle. The method of Mabuchi and Sreter (1980) for histochemical localisation of magnesium-activated actomyosin ATPase gave stronger staining in the longitudinal muscle than in the other layers (Fig. 2A). As muscle shortening speed is directly related to this form of ATPase activity (Barany, 1967) this result suggests that the longitudinal layer fibres, which are used in swimming, are faster contracting than fibres in other layers, which are thought to have a largely postural function. The dorsoventral muscles, for example, which produce flattening of the body, are known to be tonically active throughout swimming (Ort *et al.* 1974).

Several polyclonal antibodies specific for vertebrate fast and slow myosins were tested against the leech. Only two of these anti-myosin antibodies gave selective staining. Their characteristics are summarised in Table 1. Anti-T2, selective for vertebrate tonic fibre myosin, also reacted against leech muscle, where it bound preferentially to fibres in oblique, circular and dorsoventral muscles (Fig. 2B). Anti-S, selective for vertebrate slow twitch fibre myosin in mammals and other vertebrates, reacted weakly with the superficial circular and oblique layers but strongly with the bulk of the longitudinal layer fibres (Fig. 2C). In vertebrates, slow twitch fibres are faster than tonic fibres. Assuming the binding specificity of anti-T2 and anti-S has the same significance in leech as in vertebrate muscle, our results are consistent with the ATPase results, i.e. the fibres reacting preferentially with the antibody selective for tonic myosin have the lower ATPase activity.

Whereas most of the longitudinal layer stained uniformly with the anti-S antibody, i.e. were anti-S positive, fibre bundles on the outer edge of the longitudinal layer adjacent to the oblique muscle contained a mixture of fibres – some of these were like the majority of the longitudinal layer fibres, but others were negative for the anti-S antibody and, therefore, different from the main mass of longitudinal fibres (Fig. 2D).

The vertebrate-raised polyclonal antibodies which were tested against the leech and did not give selective staining are listed in Table 2.

Analysis of leech muscle myosin by SDS–PAGE

The contractile protein sample derived from leech body wall was similar in composition to myofibrillar preparations from mammalian fast and slow muscles as shown by SDS–PAGE (Fig. 3A). The major components were myosin heavy chain and actin; minor components with a wide range of relative molecular masses were also present, and these included two low molecular mass bands tentatively identified as the myosin light chains (Fig. 3A). SDS–PAGE on 5% gels revealed two clearly resolved myosin heavy chain components in the leech body wall sample (which contains muscle from all three layers and dorsoventral fibres). This is

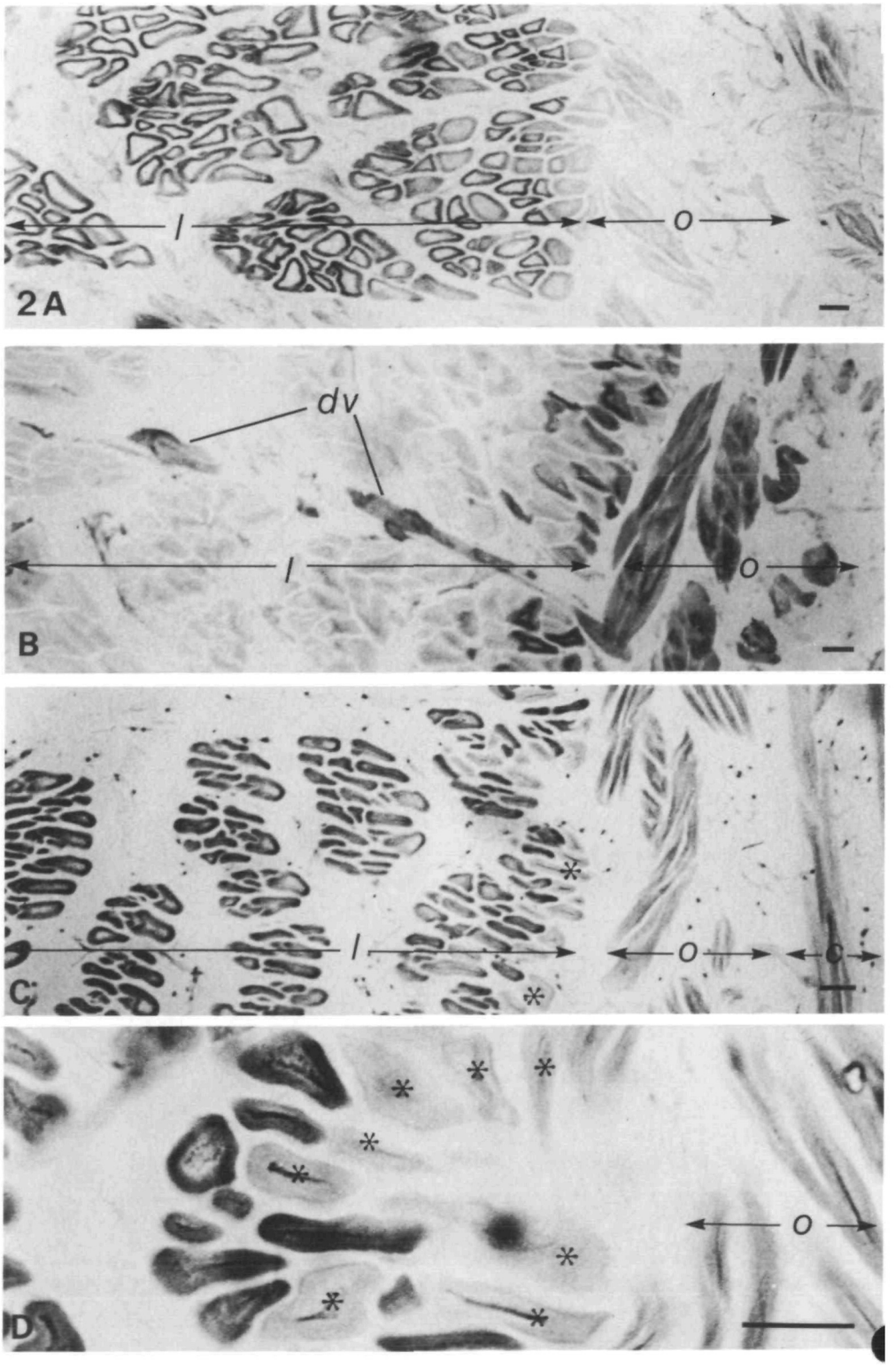
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Fig. 2. (A–C) Transverse sections of body wall showing part of the longitudinal muscle layer (*l*) and the overlying oblique layer (*o*). A few longitudinal layer fibres located just below the oblique layer are shown in D at higher magnification. (A) Actomyosin ATPase. Staining is stronger in the longitudinal layer fibres than in the oblique layer fibres. As expected, the central core of the fibres does not stain: this contains organelles such as mitochondria, but no myofilaments. (B) Anti-T2, an antibody selective for tonic fibre myosin, binds preferentially to fibres of the oblique and circular layers and to dorsoventral muscles (*dv*). The dark streak in the core of some fibres is due to peroxidase-like staining of the granular material located here, and is unrelated to the antibody. (C) Anti-S, an antibody selective for slow twitch fibre myosin, binds preferentially to the majority of the longitudinal layer fibres. A few fibres in the peripheral bundles of the longitudinal layer (e.g. at the points marked *) react weakly, like the fibres of the oblique, circular (*c*) and dorsoventral layers (not shown). (D) Anti-S, showing the heterogeneity of fibres in a small bundle at the periphery of the longitudinal layer (* indicates fibres negative for the anti-S antibody). Scale bars, 10 μm.

shown in Fig. 3B with mammalian fast and slow myosin heavy chains for comparison. The separation of the two bands in the leech contractile protein sample is as good as between the mammalian fast and slow isoforms of the myosin heavy chain; presumably the two leech bands are also due to different isoforms. In the muscle sample dissected from the deepest part of the longitudinal layer, only one heavy chain band was present, the more abundant component.

Unfortunately, identification of the minor heavy chain isoform as that which binds anti-T could not be done by immunoblotting, as leech myosin heavy chain (both types) consistently fails to react with any of our antibodies to myosin on both Western and dot blots. We can only conclude that the antigenic similarity between leech and vertebrate slow and tonic myosins does not survive the denaturation process used to prepare samples for gel electrophoresis.

Discussion

The histochemistry and immunohistochemistry of obliquely striated muscle from leech body wall show two fibre 'types' with different ATPase activity and different myosin composition. The circular, oblique and dorsoventral muscles, and a small number of fibres in the longitudinal layer, are slower than the bulk of the longitudinal layer fibres, as judged by their lower ATPase activity. This conclusion is supported by their different immunoreactivity. For example, the slower (circular, oblique and dorsoventral) fibres react preferentially with an antibody that, in all vertebrate species examined, is specific for tonic fibres, which are the slowest of all. The results of the SDS–PAGE analysis of a contractile protein preparation obtained from leech body wall muscle support the conclusion from the histochemical experiments that leech body wall muscle consists of two fibre types. On SDS gels two distinct myosin heavy chain bands were found in the samples of body wall muscle that included all three layers together with dorsoventral fibres,

Table 1. *Antibody specificity*

Antibody	Raised against	Specificity of reaction: Immunoblotting and/or GEDELISA against contractile proteins	Specificity of reaction: Reaction with fibres of known type in vertebrate muscle	Reference
Anti-S	Whole muscle from mammalian slow twitch muscle (cat soleus)	Strong reaction with slow myosin heavy chain Weak reaction with myosin light chains and one intermediate molecular mass protein	Strong reaction with all slow twitch fibres Cross reacts with avian tonic and reptile slow fibres Selective for type 4 fibres in *Xenopus*	Rowlerson *et al.* (1981) Mascarello *et al.* (1982) Rowlerson and Spurway (1988)
Anti-T2	Heavy chain only of myosin from tonic muscle (chick anterior latissimus dorsi)	Reacts with heavy chain of tonic myosin only No reaction with light chains or other contractile proteins	Selective for tonic fibres in mammals and reptiles Selective for type 5 (true tonic) fibres in *Xenopus*	A. M. Rowlerson (unpublished observations)

Table 2. *Polyclonal antibodies tested (by immunoblotting or immunohistochemistry, or, usually, both) that did not give selective staining of leech muscle*

Antibody	Known binding properties	Reference
Anti-fast	Preference for type IIB over IIA in some mammals General anti-fast in other mammals Specific for type 3 in Amphibia	Anti-IIB; Snow *et al.* (1982) Anti-FM1; Rowlerson and Spurway (1988)
Anti-fast	Preference for type IIB in some mammals General anti-fast in others For types 2 and 3 in Amphibia	 Anti-FM2; Rowlerson and Spurway (1988)
Anti-fast	Preference for type IIA in most mammals Pink muscle myosin in some fish General anti-fast in some other vertebrates	Snow *et al.* (1982) Scapolo *et al.* (1988) A. M. Rowlerson (unpublished observations)
Anti-slow	Specific for slow-twitch fibre myosin (e.g. type I in mammals and type 4 in Amphibia) Some cross reaction with 'tonic' myosin in mammals, but not in most other vertebrates	 A. M. Rowlerson (unpublished observations)
Anti-embryonic/neonatal	Specific for anti-embryonic/neonatal myosins in mammalian muscle, but little reaction in other vertebrates tested so far	A. M. Rowlerson (unpublished observations)
117 and 118	React with most fast and, to a lesser extent, slow myosins in mammals No reaction with either mammalian IIM or mammalian and chick tonic myosin Not yet tested on other species	 A. M. Rowlerson (unpublished observations)
Anti-IIM	Specific for IIM myosin in mammals Specific for IIM myosin in reptiles	Rowlerson *et al.* (1981) A. M. Rowlerson (unpublished observations)

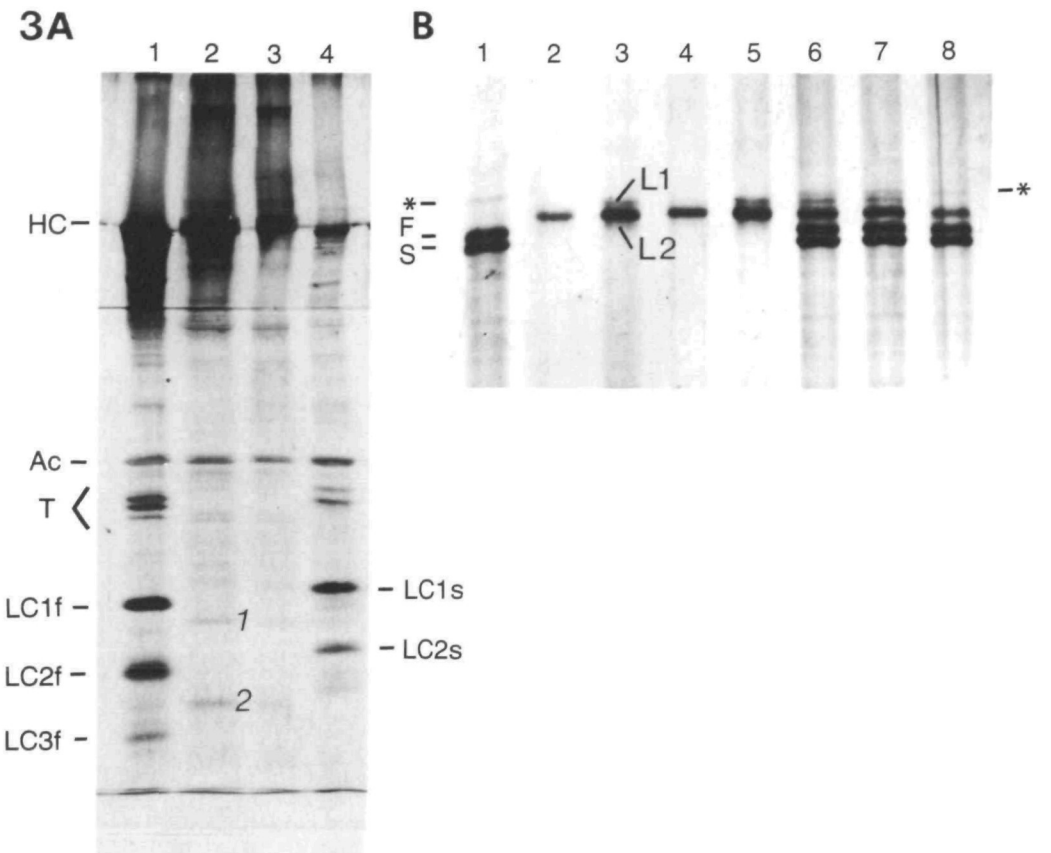

Fig. 3. SDS–PAGE of leech contractile protein samples compared with equivalent mammalian samples. (A) The overall composition of the samples as revealed on a two-stage (8% and 15% acrylamide) separating gel. Samples were: lane 1, guinea pig sartorius (fast) muscle myofibrils; lanes 2 and 3, leech body wall muscle (double sample load in lane 2); lane 4, rat soleus (slow) muscle myofibrils. Major protein bands are indicated: HC, myosin heavy chain; Ac, actin; T, tropomyosin and troponin-T; LC1f, LC2f, LC3f, fast myosin light chains; LC1s, LC2s, slow myosin light chains; *1,2*, presumed light chains of leech myosin. (B) The portion of a 5% acrylamide gel containing the heavy chains of myosin. Samples in the eight lanes were: lane 1, rat extensor digitorum longus (fast) and soleus (slow) muscle myofibrils; lanes 2 and 4, leech longitudinal muscle; lanes 3 and 5, leech body wall (longitudinal+circular+oblique muscle); lanes 6 and 7, co-migration of leech body wall muscle with rat extensor digitorum and soleus myofibrils; lane 8, co-migration of leech longitudinal muscle with rat extensor digitorum and soleus myofibrils. The myosin heavy chain bands are labelled: F, mammalian fast; S, mammalian slow; L1, L2, leech isoforms; * non-myosin high molecular mass component.

but the weaker of these two bands was not present in samples of longitudinal muscle only. This indicates that the oblique, circular and dorsoventral muscles contain a myosin heavy chain isoform which is distinct from the isoform in the

longitudinal muscle, and most probably accounts for the different ATPase activity and immunoreactivity shown by these muscle layers.

The proposal that different myosin heavy chains are present in functionally specialised invertebrate muscles is supported by studies of obliquely striated muscle in the nematode *Caenorhabditis elegans* (for a review see Waterston, 1988). In *C. elegans*, four electrophoretically distinct heavy chain isoforms are present; two of these are constituents of body wall, vulval and anal sphincter muscles, the other two are found exclusively in the pharynx. It may be that additional myosin heavy chain isoforms are present in other muscles in the leech, such as heart or jaw muscles, which were not included in this study. The *C. elegans* studies, however, show that more than one isoform may be present in a single fibre. For example, both myoA and myoB are expressed in all 95 body wall muscle cells, but are confined to distinct regions of the thick filament. Their location suggests that the different isoforms may be specialised for different assembly functions, since myosin is assembled in a different way in the different regions. Our gel electrophoresis results on adult leech muscle showed only a single myosin heavy chain band present in deep longitudinal fibres; this does not exclude the possibility that it may have contained two or more unresolved isoforms, and we did not attempt to investigate this further.

Our finding that the outermost bundles of longitudinal muscle are different from the main mass of longitudinal muscle in containing a mixture of fibres is interesting. The morphological studies of Lanzavecchia and colleagues (Lanzavecchia, 1977; Lanzavecchia *et al.* 1977) in a different species of leech, *Glossiphonia complanata*, show two distinct regions of longitudinal muscle: a smaller outer zone where the fibres are arranged in 'rosettes' and a larger interior zone. One possibility is that different regions of the longitudinal muscle are functionally specialised, and may receive different innervation. In *Hirudo*, the motoneurones and modulatory neurones supplying the longitudinal layer that lie within the segmental ganglia have been identified and characterised in terms of their biochemistry and fields of innervation (Stuart, 1970; Ort *et al.* 1974; Kuffler, 1978; Cline, 1983); and physiological studies have shown that individual motoneurones elicit contractions with characteristic and different rise times and peak tensions (Mason *et al.* 1979; Mason and Kristan, 1982). It may be that in the leech the two kinds of fibre in the longitudinal muscle each have a specific and different combination of excitatory or inhibitory innervation.

An alternative possibility is that the heterogeneity in the leech longitudinal muscle is associated with the sensory rather than the motor innervation of the muscle. The longitudinal muscle is innervated by large peripheral neurones that respond to stretch and release of the muscle with hyperpolarising and depolarising potentials (Blackshaw and Thompson, 1988) and whose input influences the rhythmical contractions of the longitudinal muscle during swimming (Blackshaw and Kristan, 1990). *H. medicinalis* does not appear to have structurally distinct receptor muscles like those found in vertebrates or articulated invertebrates. Rather, the dendrites of the leech stretch receptors innervate specific fibres within

the longitudinal layer and these fibres are located within the outermost bundles, adjacent to the oblique muscle. The association of individual sensory or motor neurones with the slower or faster fibres in the longitudinal layer could be tested by combining intracellular staining of the neurones with immunohistochemistry.

References

BANDMAN, E. (1985). Myosin isoenzyme transitions in muscle development, maturation and disease. *Int. Rev. Cytol.* **97**, 97–131.

BARANY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. gen. Physiol.* **50**, 197–218.

BLACKSHAW, S. E. AND KRISTAN, W. B. JR (1990). Input from single stretch receptor neurones influences the centrally generated swim motor pattern in the leech. *J. Physiol.,Lond.* **425**, 93P.

BLACKSHAW, S. E. AND ROWLERSON, A. M. (1989). ATPase activity and antibodies to vertebrate slow myosins distinguish fibre types in leech body wall muscle. *J. Physiol., Lond.* **418**, 51P.

BLACKSHAW, S. E. AND THOMPSON, S. W. N. (1988). Hyperpolarising responses to stretch in neurones innervating body wall muscle in the leech. *J. Physiol., Lond.* **396**, 121–137.

BURKE, R. E. (1981). Motor units: anatomy, physiology and funcional organisation. In *Handbook of Physiology*, section 1, *The Nervous System*, vol. II, *Motor Control*, part 1 (ed. J. M. Brookhout and V. B. Mountcastle), pp. 345–422. Bethesda: American Physiological Society.

CARRARO, U. AND CATANI, C. (1983). A sensitive SDS–PAGE method for separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. *Biochem. biophys. Res. Commun.* **116**, 793–802.

CLINE, H. T. (1983). ^{3}H-GABA uptake selectively labels identifiable neurons in the leech central nervous system. *J. comp. Neurol.* **215**, 351–358.

COSTELLO, W. J. AND GOVIND, C. K. (1984). Contractile proteins of fast and slow fibres during differentiation of lobster claw muscle. *Devl Biol.* **104**, 434–440.

D'HAESE, J. AND CARLHOFF, D. (1987). Localisation and histochemical characterisation of myosin isoforms in earthworm body wall muscle. *J. comp. Physiol.* **157**, 171–179.

HANSON, J. AND LOWY, D. (1960). Structure and function of the contractile apparatus in the muscles of invertebrate animals. In *Structure and Function of Muscle*, vol. I, (ed. G. H. Bourne), pp. 265–335. New York: Academic Press.

KENDRICK-JONES, J., SZENTKIRALYI, E. M. AND SZENT-GYORGYI, A. G. (1976). Regulatory light chains in myosins. *J. molec. Biol.* **104**, 747–775.

KUFFLER, D. P. (1978). Neuromuscular transmission in longitudinal muscle of the leech *Hirudo medicinalis*. *J. comp. Physiol.* **124**, 333–338.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

LANZAVECCHIA, G. (1977). Morphological modulations in helical muscles (Aschelminthes and Annelida). *Int. Rev. Cytol.* **51**, 133–186.

LANZAVECCHIA, G., DE EGUILEOR, M., VAILATI, G. AND VALVASSORI, R. (1977). Studies on the helical and paramyosinic muscles. *Boll. Zool.* **44**, 311–326.

LEVINE, R. J. C., DAVIDSHEISER, S., KELLY, A. M., KENSLER, R. W., LEFEROVICH, J. AND DAVIES, R. E. (1989). Fibre types in *Limulus* telson muscles: morphology and histochemistry. *J. Muscle Res. Cell Motility* **10**, 53–66.

MABUCHI, K. AND SRETER, F. A. (1980). Actomyosin ATPase II: fibretyping by histochemical ATPase reaction. *Muscle Nerve* **3**, 233–239.

MASCARELLO, F., CARPENE, E., VEGETTI, A., ROWLERSON, A. AND JENNY, E. (1982). The tensor tympani muscle of cat and dog contains IIM and slow tonic fibres: an unusual combination of fibre types. *J. Muscle Res. Cell Motility* **3**, 363–374.

MASCARELLO, F., SCAPOLO, P. A., VEGETTI, A. AND ROWLERSON, A. (1986). Functional adaptation of the fibre type composition of skeletal muscle in mammals. *Bas. appl. Histochem.* **30**, 279–283.

MASON, A. AND KRISTAN, W. B. (1982). Neuronal excitation, inhibition and modulation of leech longitudinal muscle. *J. comp. Physiol.* **146**, 527–536.

MASON, A., SUNDERLAND, A. J. AND LEAKE, L. D. (1979). Effects of leech Retzius cells on body wall muscles. *Comp. Biochem. Physiol.* C **63**, 359–361.

OGONOWSKI, M. M. AND LANG, F. (1979). Histochemical evidence for enzyme differences in crustacean fast and slow muscle. *J. exp. Zool.* **207**, 143–154.

ORT, C. A., KRISTAN, W. B. AND STENT, G. S. (1974). Neuronal control of swimming in the medicinal leech. *J. comp. Physiol.* **94**, 121–154.

RAGHAVEN, K. V. (1981). Evidence for myosin heterogeneity in *Drosophila melanogaster*. *Wilhelm Roux's Arch. devl Biol.* **190**, 297–300.

ROSENBLUTH, J. (1972). Obliquely striated muscle. In *Structure and Function of Muscle*, vol. 1 (ed. G. H. Bourne), pp. 389–419. New York: Academic Press.

ROWLERSON, A., POPE, B., MURRAY, J., WHALEN, R. B. AND WEEDS, A. G. (1981). A novel myosin present in cat jaw-closing muscles. *J. Muscle Res. Cell Motility* **2**, 415–438.

ROWLERSON, A. AND SPURWAY, N. C. (1988). Histochemical and immunohistochemical properties of skeletal muscle fibres from *Rana* and *Xenopus*. *Histochem. J.* **20**, 657–673.

SCAPOLO, P. A., VEGGETTI, A., MASCARELLO, F. AND ROMANELLO, M. G. (1988). Developmental transitions of myosin isoforms and organisation of the lateral muscle in the teleost *Dicentrarchus labrax* L. *Anat. Embryol.* **178**, 287–295.

SCHIAFFINO, S., ANSONI, S., GORZA, L., GUNDERSEN, K. AND LOMO, T. (1988). Myosin heavy chain isoforms and velocity of shortening of type II skeletal muscle fibres. *Acta physiol. Scand.* **134**, 575–576.

SNOW, D. H., BILLETER, R., MASCARELLO, F., CARPENE, E., ROWLERSON, A. AND JENNY, E. (1982). No classical type IIB fibres in dog skeletal muscle. *Histochemistry* **75**, 53–65.

STUART, A. E. (1970). Physiological and morphological properties of motoneurones in the central nervous system of the leech. *J. Physiol., Lond.* **209**, 627–646.

SWEENEY, H. L., KUSHMERICK, M. J., MABUCHI, K., SRETER, F. A. AND GERGELY, J. (1988). Myosin-alkali light chain and heavy chain variations correlate with altered shortening velocity of isolated skeletal muscle fibres. *J. biol. Chem.* **263**, 9034–9039.

WATERSTON, R. H. (1988). Muscle. In *The Nematode* C. elegans (ed. W. B. Wood), pp. 281–336. Cold Spring Harbor Laboratory Monograph.