Contractile properties and protein isoforms of single fibres from the chicken pectoralis red strip muscle

Peter J. Reiser*, Marion L. Greaser† and Richard L. Moss

Department of Physiology, School of Medicine and †Muscle Biology Laboratory, University of Wisconsin, Madison, WI 53706, USA

1. The contractile properties of single muscle fibres of the red strip region of adult chicken pectoralis major (PM) muscle, some of which are known to express an embryonic isoform of myosin heavy chain (MHC), were determined and compared with the properties of the fast white fibres of the PM and the slow tonic fibres of the anterior latissimus dorsi (ALD) muscle.

2. The red strip fibres could be classified into two groups, fast and slow. The mean velocity of unloaded shortening (V_{max}) in fast red strip fibres was approximately half the V_{max} of fast white fibres. V_{max} of slow red strip fibres was less than 20% of the value for fast red strip fibres and was not different from V_{max} of ALD fibres.

3. The tension-generating ability, i.e. the maximal isometric tension/fibre cross-sectional area (P_0/CSA), was the same in fast red strip fibres and fast white fibres. P_0/CSA was approximately 30% lower in slow red strip fibres compared with fast red strip fibres but was 70% greater in slow red strip fibres compared with ALD fibres.

4. The tension–pCa relation of fast red strip fibres was shifted to lower pCa values, indicating a lower calcium sensitivity compared with fast white fibres, and this difference was associated with a difference in troponin T isoform composition. The tension–pCa relation of slow red strip fibres was not different from that in ALD fibres.

5. The difference in V_{max} between fast red strip fibres and fast white fibres was associated with different MHC compositions of these fibres.

6. The myofibrillar protein isoform composition of slow red strip fibres was identical to that of the slow tonic fibres of ALD muscle and these two groups of fibres had very similar contractile properties.

Deep within the pectoralis major (PM) muscle of adult chickens is a small distinct band of muscle fibres, most commonly referred to as the 'red strip', due to its marked colouration in contrast to the pale (or 'white') fibres that comprise the bulk of the PM (Gauthier & Lowey, 1977). This set of fibres constitutes less than 1% of the mass of the adult PM muscle (Matsuda, Bandman & Strohman, 1983).

Red strip fibres differ from the other fibres of the PM with respect to several characteristics. Based on histochemical myosin ATPase staining intensities, two types of fibres comprise the red strip (Grove, Cerny, Perriard, Eppenberger & Thornell, 1989). Type 1 fibres express the same slow-type myosin heavy chains (MHCs) present in the slow tonic fibres of the anterior latissimus dorsi (ALD) muscle (Williams & Dhoot, 1992). Others have reported that the fast red strip fibres express an embryonic isoform of MHC (Crow & Stockdale, 1986; Shear, Bandman & Rosser, 1988). The significance of the persistent expression of an embryonic MHC isoform in adult skeletal muscle is unclear. However, these fibres provide an opportunity to physiologically assess the role of this isoform because of their relatively large size compared with embryonic muscle fibres. Differences also exist between red strip fibres and fast white fibres with respect to troponin T (TnT) isoform and tropomyosin expressions (Matsuda *et al.* 1983; Dhoot, 1988). The ultrastructural characteristics of red strip fibres also differ markedly from those of the fast white fibres (Edman, Lexell, Sjostrom & Squire, 1988).

* To whom correspondence should be addressed at: Oral Biology, College of Dentistry, The Ohio State University, Columbus, OH 43210-1241, USA.

Thus, based on biochemical and ultrastructural studies, the red strip appears to be a highly specialized set of muscle fibres. This band of fibres spans a relatively short distance from the anterior edge of the sternum to the proximal end of the humerus and is, therefore, in a position to keep the wing closely apposed to the body. It is probably capable of serving this function for prolonged periods, given the presence of slow fibres. This function could have a role as a thermoregulatory mechanism to retain body heat. Rosser & George (1986) discuss the exclusive presence of slow tonic fibres in the deep portion of the pectoralis in a soaring bird, which also requires wing stabilization although, in this case, in an extended position. However, the physiological properties of the red strip fibres in any avian species have not yet been reported. The objective of the present study was to measure the contractile properties of chicken red strip fibres and to compare these properties with those of fast white PM fibres and slow tonic fibres that comprise the ALD. The results indicate that fast red strip fibres have a slower shortening velocity and lower calcium sensitivity of tension generation than the fast white fibres. Compared with the adult fast MHC isoform in fast white fibres, the MHC in fast red strip fibres has a different electrophoretic mobility and has been identified by others as being an embryonic MHC isoform. This is, therefore, the first report of measurements of the maximal shortening velocity (V_{max}) in single muscle fibres that are known to contain chicken embryonic MHC. The contractile properties of slow red strip fibres are virtually identical to those of ALD slow tonic fibres except for a difference in the tension-generating ability.

An abstract of the results of this study was presented previously (Reiser, Graham, Greaser & Moss, 1988).

METHODS

Samples

The methods, experimental solutions and statistical analysis used in this study were identical to those described in detail in an earlier report (Reiser, Greaser & Moss, 1992). All the samples were obtained from the PM, ALD and posterior latissimus dorsi (PLD, fast-twitch) muscles of adult White Leghorn chickens which were killed by rapid cervical dislocation. Samples were removed from the superficial layer (less than 5 mm deep) of the PM as a source of fast white fibres. Care was taken when removing the red strip to prevent inclusion of any white fibres in this tissue. The results of this study (in particular, non-overlapping ranges of V_{max}) suggest that fast white fibres were never included when the red strips were isolated. Thin bundles of fibres were dissected from the PM and ALD samples, tied to glass capillary tubes and stored in glycerinating solution for up to 22 days to render the sarcolemma hyperpermeable. Single fibres were isolated from a glycerinated bundle on the day of an experiment and mounted in the experimental chamber. Slow and fast fibres in the red strip could be distinguished from one another during their isolation because the slow fibres had a more grainy appearance when transilluminated and viewed through a stereomicroscope. The temperature-controlled chamber was mounted on the stage of a Zeiss WL microscope equipped with a Polaroid film camera. The chamber included a spring-mounted stainless-steel plate insert with three wells containing activating or relaxing solutions. The bottom of each well was sealed with a glass coverslip through which the mounted fibre was transilluminated for viewing and photographing. One end of the fibre was attached to an isometric tension transducer (model 403, Cambridge Technology Inc., Watertown, MA, USA) and the other end was connected to the arm of a servo-controlled DC torque motor that was used to introduce slack in the fibre at desired times. The motor and transducer were mounted on three-way positioners that could be moved to set the sarcomere length, which was measured on photographs. Fibre width was determined from the same photographs and fibre depth was measured as the vertical displacement of the microscope stage while focussing on the top and bottom surfaces of the fibre. Fibre cross-sectional area (CSA) was calculated using the width and depth measurements and assuming an elliptical circumference. The diameter of a skinned muscle fibre is approximately 20% greater than its diameter prior to skinning (Godt & Maughan, 1981). Thus, the maximal isometric tension/fibre cross-sectional area (P_0/CSA) values of intact fibres would be about 44% greater than in skinned fibres when referenced to the intact diameter. The P_0/CSA values in this report were not adjusted for fibre swelling. Resting sarcomere length was set between 2·25 and 2·42 μm in fibre segments in which tension–pCa measurements were made and between 2·44 and 2·64 μm in segments in which V_{max} was determined.

Mechanical measurements

All of the measurements in this study were made at 15 °C. V_{max} was determined by the slack-test method (Edman, 1979; Reiser, Moss, Guilian & Greaser, 1985). Briefly, the fibre was transferred to the maximally-activating solution and tension was allowed to develop. Slack (ΔL) was rapidly introduced into the fibre by movement (complete within 2 ms) of the torque motor arm when the developed tension reached a plateau. The total range of ΔL imposed was between 6 and 23% of fibre length and the mean ΔL range on a given fibre was 8 ± 2% (means ± S.D.) of fibre length. The time interval (Δt) between the instant at which slack was introduced and when tension started to redevelop was measured directly from the screen of a digital storage oscilloscope. The magnitude of ΔL was varied with each activation and the corresponding Δt was recorded. V_{max} was determined as the slope of the linear regression of ΔL against Δt. The calcium sensitivity of tension production in a single fibre was determined by measuring the peak active tension (P) generated in a series of activating solutions in which the pCa ($-\log[Ca^{2+}]$) ranged from 4·5 to 7·4, and normalizing P with respect to the peak active tension in the maximally activating solution (P_0; pCa = 4·0). The resting peak tension in each fibre was subtracted from the total peak tension in each activating solution to obtain the tension actively generated.

Electrophoresis and immunoblotting

MHC isoforms were electrophoretically separated using a protocol identical to that in Reiser *et al.* (1988). Five fast and four slow red strip fibres were randomly chosen for MHC analysis. The identity

of troponin T (TnT) isoforms on SDS-PAGE gels was determined using the same monoclonal antibody and immunoblotting procedure as reported in Reiser *et al.* (1992), and was based on several assumptions. Firstly, isoforms from PLD and PM muscle were assumed to be essentially all fast, and ALD muscle isoforms were assumed to be slow. Secondly, the troponin extracts were assumed to primarily contain the same isoforms as the fibres, and the red strip only contained mixtures of the fast and slow isoforms found in the other muscles studied. We thus identified fast and slow TnT in red strip fibres and fibre bundles based on these assumptions. Briefly, troponin-containing fractions from PM, PLD and ALD were prepared for isoform identification as described previously (Reiser *et al.* 1992), up to the step before hydroxylapatite chromatography, and electrophoresed in a Hoefer Mighty Small gel unit. Proteins were transferred to nitrocellulose in a Hoefer TE transfer unit. The blot was reacted with a monoclonal anti-TnT antibody (Lim, Tu & Lemansky, 1984), washed, reacted with a horseradish peroxidase-labelled anti-mouse antibody and incubated with the 4-chloro-1-naphthol substrate for colour development. Tropomyosin isoforms were identified on gels, based on known molecular weights and stoichiometries.

Statistical analysis

Student's t test was used to test for statistical significance of differences between mean values following analyses of variance. $P < 0{\cdot}05$ was accepted as significant. All of the stated differences are statistically significant. Results are given as the means ± S.D. unless indicated otherwise. The calcium sensitivities of the different types of fibres were determined by performing Hill linear transformations (equation: $y = \log P/[P_0 - P]$) of the tension–pCa data. Only data within the 10–90% P_0 range were transformed. The pCa_{50} values (i.e. the pCa at which tension corresponding to 50% P_0 was generated) were calculated from the transformed data. The threshold pCa values for tension production were estimated by inspection of the tension–pCa curves.

RESULTS

The distribution of V_{max} values obtained from all the fast white fibres, and fast and slow fibres of the red strip examined in this study are shown in Fig. 1. Besides being morphologically distinguishable (see Methods) and having different anatomic locations within the pectoralis muscle, the fast white (i.e. the superficial PM) and fast and slow fibres of the red strip formed three groups with non-overlapping ranges in V_{max}: the slow red strip fibres ($n = 19$) had a mean V_{max} of 0·38 ± 0·12 muscle lengths (ML) s^{-1}, while the fast red strip ($n = 21$) and fast white fibres ($n = 24$) had mean V_{max} values of 2·43 ± 0·45 and 4·20 ± 0·64 ML s^{-1}, respectively (all differences were statistically significant). Although the fibres in the red strip have been referred to as 'fast' and 'slow' in the literature for more than 10 years, the mean V_{max} of the fast red strip fibres was approximately one-half that of the fast white fibres. These fibres thus form three functionally distinct groups. For consistency with studies from other laboratories, we retain the 'fast' and 'slow' nomenclature for the red strip fibres in this report. There was no difference in the peak isometric tension generated during maximal calcium activation per unit of fibre cross-sectional area (i.e. P_0/CSA) between the fast white and the fast red strip fibres (192 ± 39 and 205 ± 51 kN m^{-2}, respectively), but P_0/CSA was significantly lower ($P < 0{\cdot}005$) in the slow red strip fibres (124 ± 29 kN m^{-2}). The fast red strip fibres are smaller (1720 ± 630 μm^2; $P < 0{\cdot}005$) than the fast white and slow red strip fibres (2480 ± 590 and 2530 ± 670 μm^2, respectively).

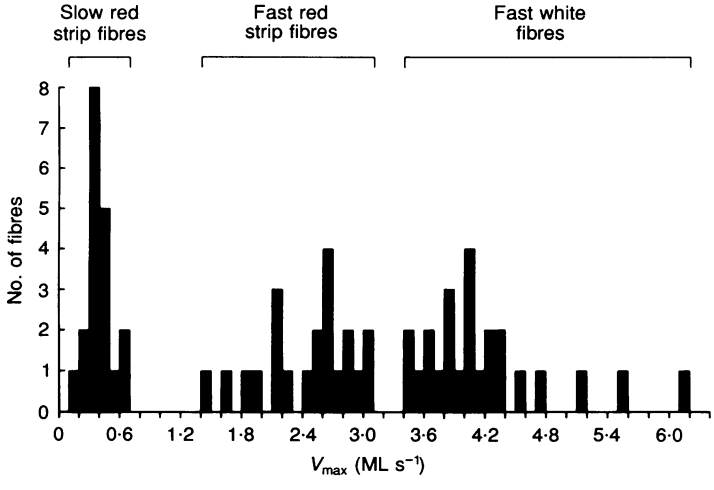

Figure 1

Distribution of the maximal velocities of shortening (V_{max}) in muscle lengths per second (ML s^{-1}), measured on slow and fast fibres from the red strip and on fibres from the white PM (pectoralis major) superficial layer.

Table 1. Summary of measurements on subsets of single fibres from the white pectoralis major and red strip muscles in which tension–pCa relations were determined

	CSA (μm^2)	P_0 (μN)	P_0/CSA (kN m^{-2})	V_{max} (ML s^{-1})	V_{max} range (ML s^{-1})
White	2270 ± 380	372 ± 69	165 ± 21	4·66 ± 0·78	3·78–6·10
Red strip, slow	2330 ± 450	292 ± 45	126 ± 10	0·45 ± 0·13	0·31–0·63
Red strip, fast	2190 ± 200	379 ± 35	174 ± 16	2·59 ± 0·31	2·13–2·90

Values are expressed as the means ± S.D. of measurements on 7 slow and 7 fast fibres from the red strip and 15 fibres from the fast white pectoralis (except for V_{max} of the fast white fibres where $n = 9$). Abbreviations used are explained in the legend of Table 3.

Table 2. Summary of tension–pCa relations of fibres from the white pectoralis major and red strip muscles

	n_1	n_2	pCa_{50}	pCa_{th}
White	1·49	4·30	6·15	6·8
Red strip, slow	1·71	3·30	5·90	7·0
Red strip, fast	2·18	4·70	5·95	6·4

The values in this table are single determinations obtained from analyses of the curves shown in Fig. 2. n_1, Hill coefficient for tension greater than one-half maximal tension (i.e. P_{50}); n_2, Hill coefficient for tension less than P_{50}; pCa_{50}, pCa at which P_{50} is generated; pCa_{th}, threshold pCa for tension development.

To determine if the three groups of fibres are also functionally diverse with respect to the calcium sensitivity of tension development, tension–pCa relations were determined on a randomly selected subset of the fibres represented in Fig. 1. V_{max} and tension–pCa relations were determined on separate adjacent segments of each of nine fibres from the fast white portion of the pectoralis and seven slow and seven fast fibres from the red strip. Additional tension–pCa data alone were obtained from six other fibres from the fast white pectoralis so that seven measurements were performed at each pCa for each of the three fibre types. The tension–pCa curves obtained are shown in Fig. 2 and the results of measurements of CSA, P_0/CSA and V_{max} on these fibres are summarized in

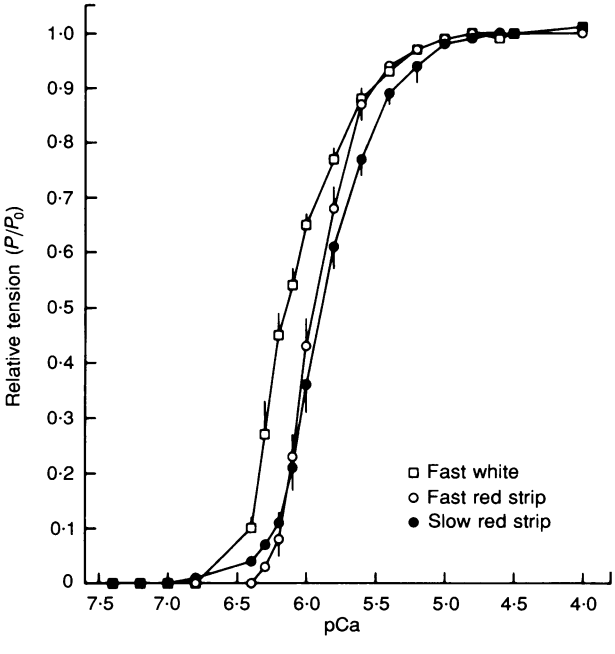

Figure 2. Relative tension *vs.* pCa for fast white pectoralis fibres and fast and slow fibres from the red strip

Each point represents the mean ± S.E.M. of measurements on 7 fibres. In some cases the size of the standard error is less than the symbol size. A summary of the Hill transformation of the data is presented in Table 2.

Table 3. Summary of measurements on and tension–pCa relations in slow fibres from the red strip and the anterior latissimus dorsi (ALD) muscles

	CSA ($\mu m)^2$	P_0 (μN)	P_0/CSA (kN m^{-2})	n_1	n_2	pCa_{50}	pCa_{th}
ALD	2750 ± 590	203 ± 43	75 ± 15	2·53	3·15	5·95	7·2
Red strip, slow	1610 ± 280	198 ± 26	125 ± 17	1·83	2·90	5·95	7·2

CSA, fibre cross-sectional area; P_0, absolute tension generated during maximal calcium activation; P_0/CSA, tension generated per unit fibre CSA during maximal calcium activation. Other abbreviations are explained in the legend of Table 2. Values for CSA, P_0 and P_0/CSA are expressed as the means ± S.D. ($n = 7$).

Table 1. The curve representing the fast white fibres is shifted, relative to the curves for the other fibres, to higher pCa values, indicating that the fast white fibres are most sensitive to calcium with respect to tension development. The tension–pCa data for all three fibre types were linearized by performing the Hill transformation (a method used to make quantitative comparisons between different tension–pCa relations, as described in Moss, Swinford & Greaser, 1983) and the results are summarized in Table 2. The fast red strip fibres had tension–pCa relations that were steeper and had higher thresholds for activation than the slow red strip fibres.

Based on the results of measurements in a previous study (Reiser *et al.* 1988) on forty-two fibres from adult ALD muscle (which is composed almost exclusively of slow tonic fibres), the mean V_{max} of the slow red strip fibres was not different from the mean V_{max} of adult ALD fibres (0·37 ± 0·14 ML s^{-1}, Reiser *et al.* 1988). However, P_0/CSA of the slow red strip fibres was approximately 70% greater ($P < 0·005$) than that of ALD fibres. Therefore, even though the mean V_{max} is not different between slow fibres in these two muscles, the fibres are not equivalent in their maximum tension-generating abilities. The slow fibres from the ALD and the red strip muscles do not differ from each other with respect to the calcium sensitivity of tension development (Fig. 3), as determined on a separate set of fibres from these two muscles isolated from the same adult chicken. The results from these two groups of fibres are summarized in Table 3. Thus, although the slow fibres in the red strip and the ALD have very similar physiological properties, they are not identical.

To determine if the diversity in physiological properties between the fast white and fast and slow red strip fibres is related to differences in myofibrillar protein composition,

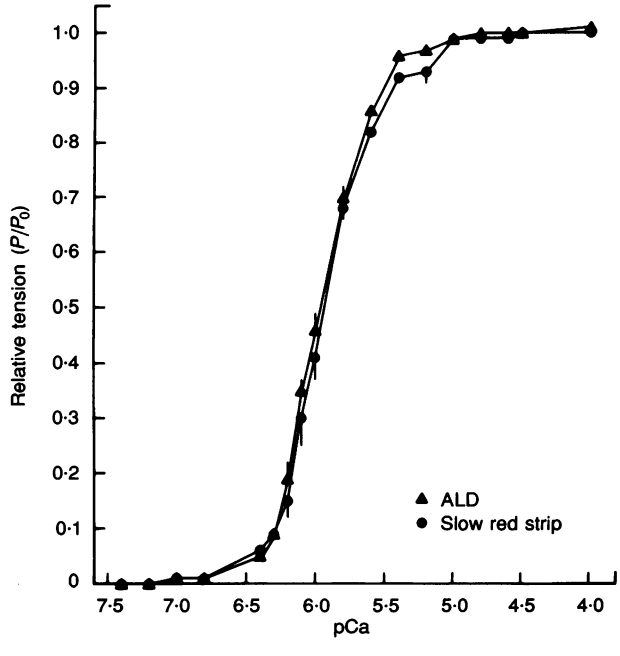

Figure 3. Relative tension *vs.* pCa for slow fibres from the red strip and from the anterior latissimus dorsi (ALD) muscle

Each point represents the mean ± S.E.M. of measurements on 7 fibres. A summary of the Hill transformation of the data is presented in Table 3.

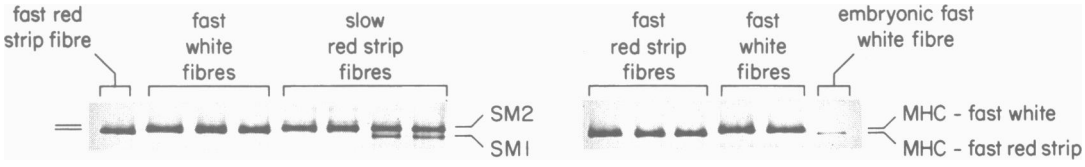

Figure 4. The myosin heavy chain region of an SDS gel

Each lane represents a different single fibre. Note the distribution of four myosin heavy chains (MHCs) among the different fibres: two (SM1 and SM2) are present in slow muscle, while the MHC band of the fast red strip fibres, which others have identified as an embryonic MHC (see Discussion), migrates slightly ahead of the MHC of the white portion of the PM. An embryonic fast white fibre was loaded on the extreme right-hand lane as a marker of the embryonic MHC. The slow red strip fibres contain slow type MHC SM1 and/or SM2.

several of the fibres on which the combined measurements of V_{max} and tension–pCa relations were made were analysed with SDS-polyacrylamide gels. Each gel lane shown in Fig. 4 represents a single fibre. The type of MHC is different between each of the three groups of fibres. The slow red strip fibres have the same two slow-type MHC isoforms found in the ALD muscle (i.e. SM1 and SM2), based on their co-migration with the two ALD MHC isoforms (not shown). The slow fibres were arranged on the gel in order of increasing V_{max}, from left to right. As V_{max} increased among these fibres, so did the proportion of the SM1 isoforms. All of these fibres had exclusively slow-type myosin light chains LC1 and LC2 (not shown). The type of MHC in fast red strip fibres differed (i.e. had a different electrophoretic mobility) from that of the fast white pectoralis fibres. These two groups of fast fibres had exclusively fast-type LCs but the relative proportions of LC1 and LC3 were not quantified in this study, although they were in another recent study (Reiser, 1994; see Discussion). We cannot assign the identity of the MHC in fast red strip fibres. However, its migration, relative to the MHC of the adult fast white fibres, is the same as that of fast white pectoralis fibres at late embryonic ages. The results from other laboratories have identified the MHC of fast red strip fibres as being an embryonic fast MHC, based on the use of immunohistochemical staining (Crow & Stockdale, 1986; Shear *et al.* 1988; see Discussion).

Several protein fractions were isolated (see Methods) and were used to identify troponin T and tropomyosin bands on gels by first determining their electrophoretic migration patterns (Fig. 5). The fast white and fast red strip fibres

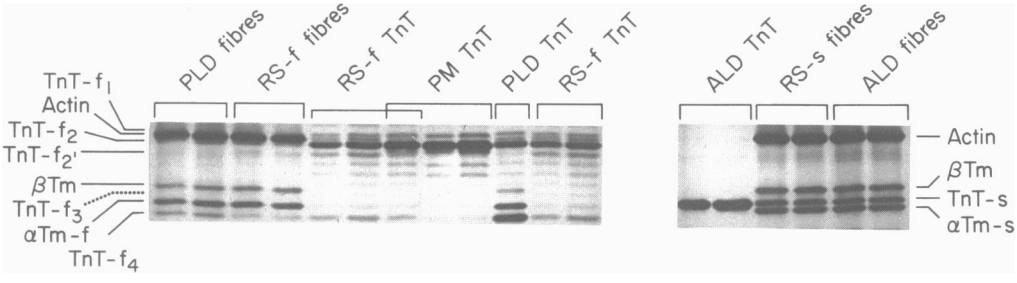

Figure 5

Silver-stained SDS-polyacrylamide gel containing samples of either single muscle fibres or troponin T (TnT) fractions (see Methods) from the fast-twitch posterior latissimus dorsi (PLD) and PM muscles, slow anterior latissimus dorsi (ALD) muscle, and fast and slow (RS-f and RS-s, respectively) fibres of the red strip. Note that the extraction procedure preferentially removed the fast isoforms of TnT from the red strip sample (hence labelled 'RS-f'). Fast and slow fibres of the red strip contain different isoforms of α-tropomyosin (αTm) and TnT, as observed when comparing the RS-f fibre and RS-s fibre lanes. The TnT fractions from RS-f and PM were co-electrophoresed in the seventh lane, as indicated by the overlapping brackets. The migration of the proteins in these fractions assisted in the identification of TnT isoforms on the gel shown in Fig. 6. The identification of TnT isoforms was verified with immunoblots, as in Reiser *et al.* (1992). The Tm and TnT compositions of the RS-f and PLD fibres are similar, consisting primarily of TnT-f_2 and TnT-f_4 (note that the PLD fraction also contained some αTm and βTm).

contained only the fast type isoforms of troponin (Tn) and tropomyosin (Tm) subunits (Fig. 6). Troponin C (TnC) and troponin I (TnI) of these two groups of fibres co-migrated, suggesting that they are the same isoforms. TnT, on the other hand, was different between the fast white and fast red strip fibres. None of these fibres contained the slow-type TnT that was present in the slow red strip fibres, so all the TnT isoforms in the fast fibres were considered to be fast type. The fast white fibres contained one form of TnT that we refer to as TnT-f_2. TnT-$f_{1'}$ and TnT-f_3 were observed in neonatal chicken fast twitch fibres in a previous study (Reiser *et al.* 1992) but were not observed in either fast red strip or fast white fibres in the present study. The fast red strip fibres also contained TnT-f_2, but in much lower amounts relative to that in fast white fibres, as well as other isoforms of fast-type TnT that we refer to as TnT-$f_{2'}$ and TnT-f_4. These differences in TnT composition between the two groups of fast fibres could also be seen in the gel shown in Fig. 6. The fast red strip fibres contained βTm, as well as the fast-type isoform of αTm, whereas the fast white fibres contained fast-type αTm exclusively. The slow red strip fibres contained βTm, the slow-type isoform of αTm and only slow-type isoforms (one each) of TnC, TnI and TnT.

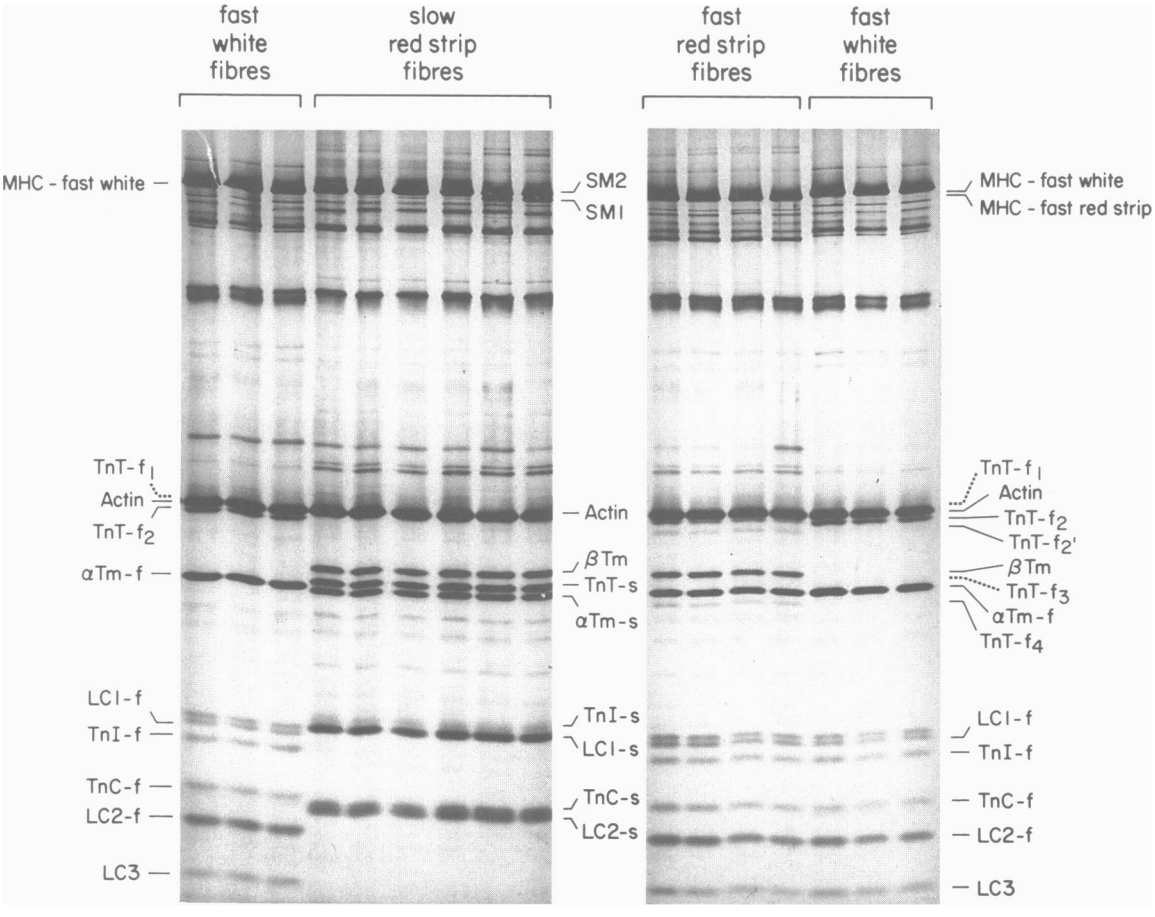

Figure 6. Silver-stained SDS-polyacrylamide gel with each lane representing a different single muscle fibre on which physiological measurements were made

Fibres from the white portion of the PM and slow and fast fibres from the red strip are represented. The slow fibres of the red strip contain exclusively slow isoforms of troponin-T (TnT) and troponin-C (TnC) and myosin light chains 1 and 2 (LC1 and LC2). The fast fibres of the red strip and the fibres of the white portion of the pectoralis contain fast isoforms (with identical mobilities on the gel) of myosin light chains 1, 2 and 3 (LC3) and of troponin-I (TnI) and TnC; differences exist in the TnT composition between these two groups of fast fibres. The fast fibres of the red strip contain troponin-T-$f_{2'}$ (TnT-$f_{2'}$) and troponin-T-f_4 (TnT-f_4), as well as troponin-T-f_2 (TnT-f_2), while the latter isoform is present to the virtual exclusion of the other isoforms in the white portion of the pectoralis. The migration positions of troponin-T-f_1 (TnT-f_1) and troponin-T-f_3 (TnT-f_3) are indicated with dotted lines because they are present in only trace amounts in adult fibres but represent a greater fraction of the TnT in neonatal fast-twitch fibres as previously reported (Reiser *et al.* 1992).

DISCUSSION

The main result of this study is that there is extensive functional diversity among single fibres of the chicken pectoralis muscle, especially between the fibres that comprise the fast white portion of the muscle and the fibres within the red strip. Furthermore, this functional diversity is related to heterogeneity in myofibrillar proteins at the single fibre level. Specifically, when comparing fast white fibres to fast red strip fibres, variations in the maximal velocity of shortening are related to the myosin heavy chain composition, and differences in the calcium sensitivity of tension development are related to troponin T isoforms and possibly to Tm subunit composition, as discussed below. The functional significance of the red strip within the chicken PM is unclear but, given its fibre type composition (see below) and anatomic location, it may have a role in holding the wing in a forward position and/or in stabilizing the shoulder joint for long periods (Rosser & George, 1986).

Previous results from this laboratory (Reiser *et al.* 1985; Reiser *et al.* 1988) have clearly demonstrated a functional relationship between V_{max} and the MHC composition of single muscle fibres. The relationship between V_{max} and LC composition in these studies was less firm and extended over a narrower range in V_{max} than that between V_{max} and MHC content. The same result is observed in the present study when comparing fast white and fast red strip fibres which have significantly different V_{max} values and different MHC isoforms. These two groups of fibres have exclusively fast-type LCs. Thus, it appears that the MHC composition is the primary determinant of V_{max} of fast fibres from different portions of the chicken PM. The fast red strip fibres contain embryonic MHC (Crow & Stockdale, 1986; Shear, Bandman & Rosser, 1988) and fast white fibres contain adult fast MHC. We therefore conclude that the embryonic MHC regulates muscle shortening at a significantly lower velocity than does adult fast MHC. This conclusion is particularly important because fibres of the embryonic PM (wherein the embryonic MHC is most prevalent) are extremely small, which makes it difficult to make reliable physiological measurements on single fibres at this developmental stage. Lowey, Waller & Trybus (1993*a*) showed, using an *in vitro* motility assay, that chicken embryonic myosin moves actin filaments more slowly than 3- and 12-day post-hatch myosin. They concluded that the difference in velocity was due primarily to the presence of the neonatal isoform of MHC in the post-hatch myosin. The agreement between this result and our present results is particularly interesting when considering the disparity in methodology. The *in vitro* motility assay measures the velocity of filaments that are untethered except for the binding that occurs during cross-bridge attachments, while the single fibre approach measures the velocity within the physical constraints of the myofilament lattice.

A nearly twofold range in V_{max} (~3·4–6·2 ML s^{-1}) was observed in the fast white fibres in the present study. This is very similar to the range observed (3·1–5·8 ML s^{-1}) in another study (Reiser, 1994) on a separate set of fast white fibres in which a very strong linear correlation was found to exist between the alkali light chain ratio (LC3/[LC1 + LC3]) and V_{max}. This result and those from studies on mammalian muscle fibres (e.g. Eddinger & Moss, 1987; Greaser, Moss & Reiser, 1988; Sweeney, Kushmerick, Mabuchi, Sreter & Gergely, 1988; Bottinelli, Betto, Schiaffino & Reggiani, 1994; Bottinelli, Canepari, Reggiani & Stienen, 1994) provide evidence that the alkali light chains probably have a modulatory role in the regulation of V_{max}. Additional evidence for a role of the alkali light chains in modulating V_{max} has been derived from studies employing an *in vitro* motility assay (Lowey, Waller & Trybus, 1993*a*,*b*) and using the chicken pectoralis muscle as a source of myosin. The results from all of these studies show that higher V_{max} values are associated with greater amounts of LC3. It is, therefore, likely that the large range in V_{max} among the fast white fibres is due to variations in the LC3 content.

The calcium sensitivities of tension development are also different between the fast white fibres and the fast red strip fibres and both are different from those of slow red strip fibres. Both troponin T and tropomyosin compositions differ between the two groups of fast fibres. In an earlier study (Reiser *et al.* 1992), we observed that a few fast white fibres contained a significant amount of βTm, as well as αTm, while the majority contained exclusively αTm. All of these fibres contained almost exclusively TnT-f_2 and all had virtually identical calcium sensitivities, independent of whether they contained only αTm or αTm in combination with βTm (the $\alpha:\beta$ ratio in the mixed fibres in the earlier study was approximately 4 : 1). From this observation, we concluded that the presence of approximately 20% βTm in fast white fibres does not modulate their calcium sensitivity. We cannot conclude from the present set of results whether the TnT isoforms, the presence of βTm, both of these, or some other factor is responsible for the fast red strip fibres having a different calcium sensitivity from that of the fast white fibres. However, a role of TnT isoforms in modulating calcium sensitivity is consistent with the conclusion from our previous study (Reiser *et al.* 1992).

The mean P_0/CSA of adult ALD fibres was shown to be lower than that in adult PM fast white fibres in an earlier study (Reiser *et al.* 1992). The results of the present study show that P_0/CSA differs between two sets of fibres (ALD and slow red strip) that express the same MHC isoforms.

The basis for this difference is not known but could be due to a possible difference in myofibril density between ALD and slow red strip fibres.

The present results show strong similarities in the contractile properties of slow red strip fibres and ALD fibres and should at least partially resolve whether the former are considered tonic or slow twitch fibres. Several studies have reported evidence for the presence of an avian slow-twitch fibre type while others suggest that all avian slow fibres are tonic (Pierobon Bormioli, Sartore, Vitadello & Schiaffino, 1980; Shafiq, Shimizu & Fischman, 1984; Rosser & George, 1986; Rosser, George & Frombach, 1987; Hikida, 1987). None of these studies involved measurements of the contractile properties of avian slow fibres. Our results demonstrate that V_{max} and the calcium sensitivity of tension development in slow fibres from the red strip and from the ALD are identical to each other and, therefore, suggest that slow red strip fibres should be considered to be tonic to the same extent as ALD fibres. However, our measurements on skinned fibres do not directly relate to the twitch *versus* tonic characteristics of intact cells.

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