Histochemistry (1986) 86:19–23

Histochemistry

Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers

R.S. Staron and D. Pette*
Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz, Federal Republic of Germany

Accepted July 12, 1986

Summary. Combined histochemical and biochemical analyses were performed on single fibers of rabbit soleus muscle. Histochemically, four fiber types (I, IC, IIC, IIA) were defined. Of these, types I and IIA were separate, histochemically homogeneous groups. A heterogeneous C fiber population exhibited a continuum of staining intensities between types I and IIA. Microelectrophoretic analyses of specific, histochemically defined fibers revealed that type I fibers contained exclusively HCI, whereas type IIA fibers contained only HCIIa. The C fibers were characterized by the coexistence of both heavy chains in varying ratios, type IC with a predominance of HCI and type IIC with a predominance of HCIIa. A direct correlation existed between the myosin heavy chain composition and the histochemical mATPase staining and was especially evident in the C fiber population with its variable HCI/HCIIa ratio. This correlation did not apply to the myosin light chain complement.

Introduction

Histochemical staining for myofibrillar actomyosin ATPase (mATPase) distinguishes several fiber types in mammalian skeletal muscle (Guth and Samaha 1969; Brooke and Kaiser 1970). Immunohistochemical studies suggest a correlation exists between the mATPase staining and the type of myosin present (Gröschel-Stewart et al. 1973; Arndt and Pepe 1975; Gauthier and Lowey 1977, 1979; Lutz et al. 1979; Billeter et al. 1980; Pierobon-Bormioli et al. 1981). In support of this, Billeter et al. (1981) found, in addition to the set of slow myosin light chains (LC), various combinations of fast LCs in human type I fibers. Because these fibers were histochemically uniform, they concluded that the histochemical mATPase is determined by the myosin heavy chain (HC). In addition, they observed that histochemically discernible type IIA and IIB fibers contain identical sets of fast LCs but differ in their HCs. To investigate this proposed correlation, a combined histochemical and biochemical approach on single fibers was used in the present study. After histochemical typing of fibers in thin serial cross-sections, defined single fiber segments were dissected from thick serial freeze-dried cross-sections and analysed microelectrophoretically for their myofibrillar protein composition. Using this method, a relationship has been established between the myosin HC composition and the histochemical mATPase staining intensity.

* To whom offprint requests should be sent

Materials and methods

Muscles. Soleus muscles ($n=6$) were dissected from adult male White New Zealand rabbits. The muscles were cut into longitudinal strips of approximately 3 mm thickness and frozen in a slightly stretched position in melting isopentane (−159° C).

Histochemical analyses. Pieces 8 mm long were mounted on a chuck and thin (12 μm) cross-sections were cut at −25° C using a microtome in a cryostat. Sections were stained for mATPase using a modification (Staron et al. 1983) of the procedure of Brooke and Kaiser (1970). Preincubation pH values were 4.3, 4.5 and 9.6. The stained sections were either photographed using a polaroid attachment or direct tracings were made (×120) using a camera lucida. Subsequently, 50 fibres per muscle were identified, typed (Fig. 1) and numbered. Serial thick (200–300 μm) cross-sections were cut from the same block (Fig. 2) and carefully placed in precooled aluminum holders and freeze-dried at −38° C.

Biochemical analyses. Identified fibers from the thick freeze-dried cross-sections were microdissected under a stereomicroscope in a temperature (20° C) and humidity (30%–40%) controlled room. Segments from the same fiber were collected from successive cross-sections and stored in a micro-sample holder (Teutsch 1986) under vacuum at −38° C.

For microelectrophoresis, segments of a given fiber were transferred (under the stereomicroscope) from the sample holder (after being brought to room temperature) into a glass capillary (Fig. 3). Care was taken to insure that all pieces were at the bottom of the capillary. Preextraction of "soluble" proteins was performed using 15 μl of a medium (pH 7.0) containing 80 m*M* KCl, 1 m*M* Tes, 0.1 m*M* dithiotreitol, 0.1 m*M* phenylmethanesulfonyl fluoride.

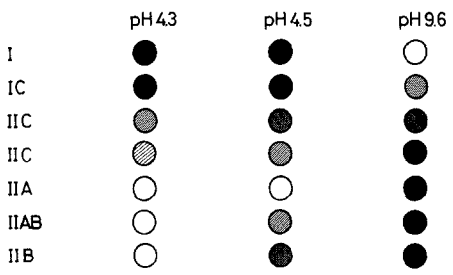

Fig. 1. Schematic illustration of histochemical fiber typing based upon mATPase staining intensity following preincubation at various pH values. As indicated, the C fiber population spans a range between types I and IIA

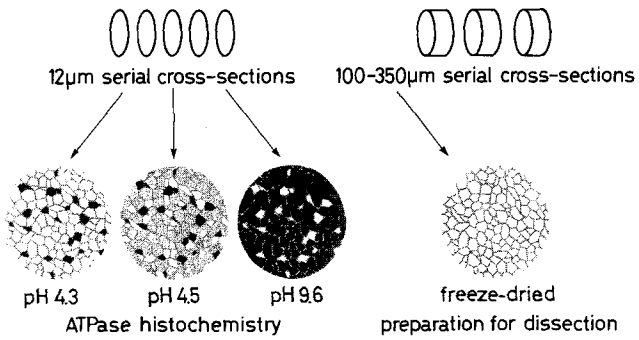

Fig. 2. Diagrammatic illustration of combined histochemical and biochemical analyses on defined single fiber fragments

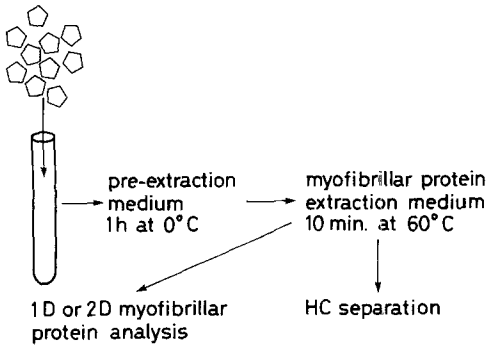

Fig. 3. Illustration of the process for single fiber fragment loading and subsequent extraction for myosin light and heavy chain analyses.

The capillaries were sonicated for 60 min in an ice-bath. Thereafter, the supernatant was withdrawn using a polyethylene capillary. Extraction of myofibrillar proteins was accomplished following 10 min at 60° C using 10 µl of a medium containing 10% (w/v) glycerol, 5% (v/v) mercaptoethanol and 2.3% (w/v) sodium dodecylsulfate in 0.0625 *M Tris*-HCl buffer (pH 6.8).

Microelectrophoresis for myosin LC analysis was performed according to Laemmli (1970) on 0.75 mm thick slab gels (1 cm high stacking gel, 4 cm high separating gel) at 80 V for 160–170 min. The amount of protein applied (approximately 1 µg) was estimated from the dry weight of the samples as determined on a quartz-fiber balance (Lowry and Passonneau 1972). Macroelectrophoretic separation of myosin HCs was accomplished either with or without preextraction of soluble proteins using a 4% stacking and 5% separating gel (Rushbrook and Stracher 1979; Carraro and Catani 1983). The electrophoresis was run at 120 V for 19 h. The amount of protein applied was in the range of 0.3–0.5 µg (dry weight). Micro- and macrogels were silver-stained according to the method of Oakley et al. (1980).

Results

Combined histochemical and biochemical analyses demonstrated a correlation between the mATPase staining intensities (pH 4.3, 4.5, 9.6) and the myosin heavy chain (HC) content (Fig. 4). The majority of the rabbit soleus fibers consisted of type I. These fibers were histochemically uniform (Fig. 4) and contained exclusively the slow-myosin HCI (Fig. 5A, fiber 12).

Some regions of the rabbit soleus muscle contained a number of fibers classified as either IIA, IIC or IC (Fig. 4). The IIA fibers displayed a histochemical pattern opposite to that observed for the type I fibers (Fig. 1). These fast fibers contained exclusively the fast-myosin HCIIa

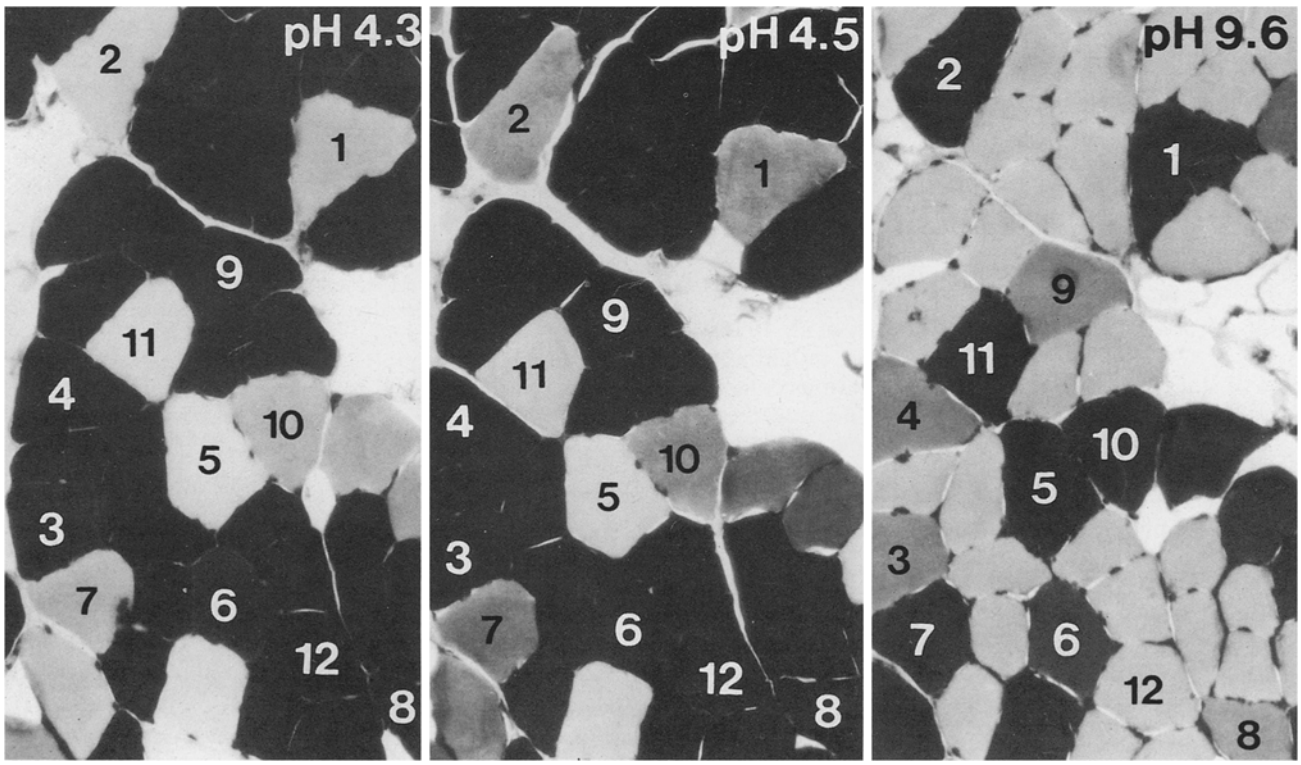

Fig. 4. Histochemically defined fiber types in rabbit soleus muscle. Serial cross-sections were stained for mATPase after preincubation at pH values 4.3, 4.5, 9.6. ×160

Table 1. Myosin light and heavy chain combinations in specific histochemically defined fibers of rabbit soleus muscle. The fiber numbers refer to those used in Figs. 4 and 5. Differences in relative contents are indicated by ++ or +. (+) indicates that, depending on the muscle, the respective component may be present or not (see Staron and Pette 1986a)

Fiber no.	1	2	3	4	5	6	7	8	9	10	11	12
Histochemical type	IIC	IIC	IC	IC	IIA	IIC	IIC	IC	IC	IIC	IIA	I
LC1s	++	++	++	++	++	++	++	++	++	++	++	++
LC2s	+	+	++	++	(+)	++	+	++	++	+	(+)	++
LC1f	++	++	+	+	++	++	++	+	+	++	++	
LC2f	++	++	+	+	++	++	++	+	+	++	++	
LC3f	++	++			++	+	++			++	++	
HC I	+	+	++	++		++	+	++	++	+		++
HC IIa	++	++	+	+	++	++	++	+	+	++	++	

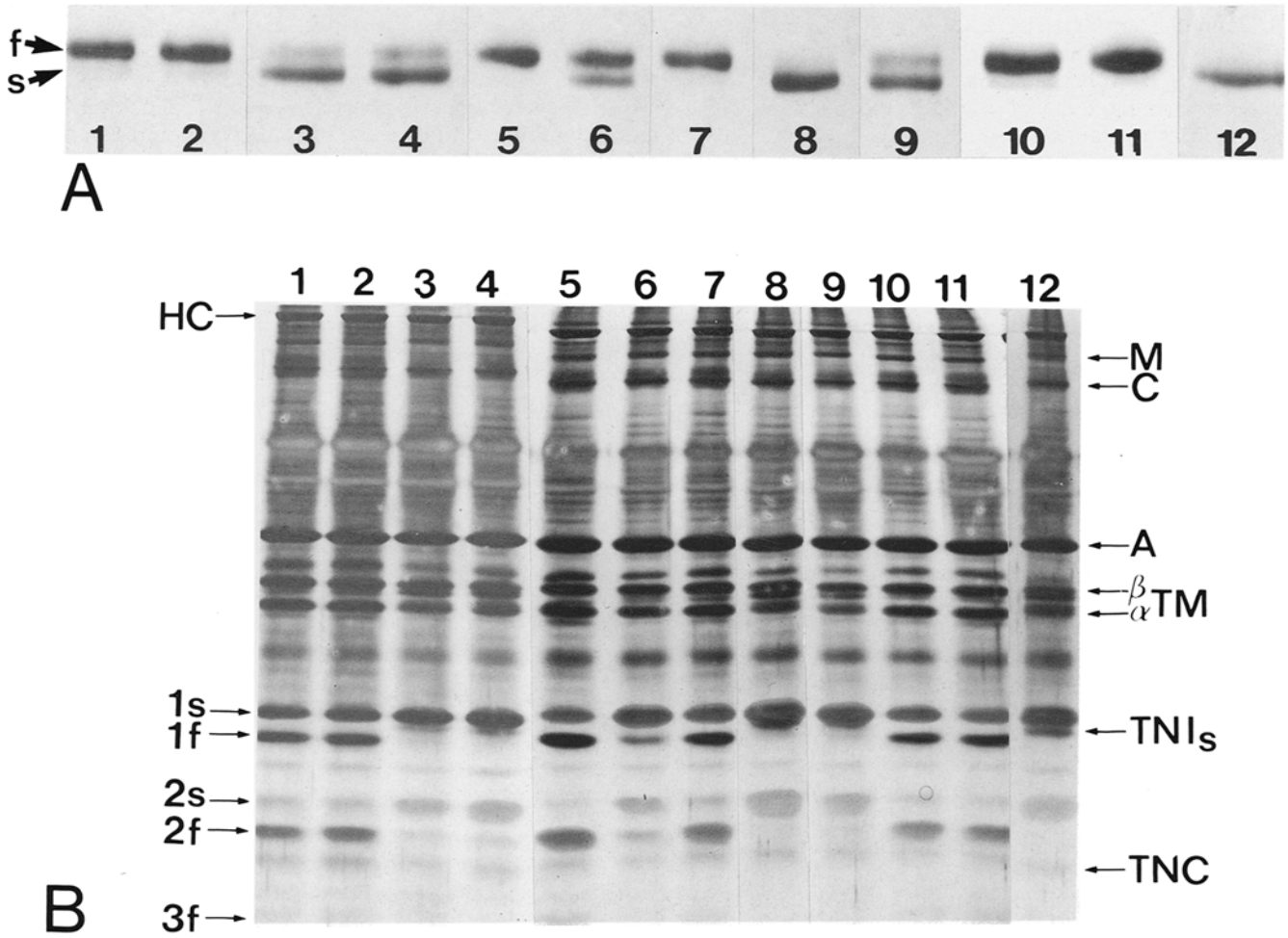

Fig. 5. Single fiber analyses for **A** myosin heavy chains and **B** myosin light chains of specific, histochemically defined rabbit soleus fibers. The fiber numbers correspond to those same fibers shown in Fig. 4 and Table 1. Abbreviations: A = actin; C = C-protein; HC = myosin heavy chain; TM = α and β tropomyosin subunits; TNC = troponin C; TNI_s = slow troponin I; f = fast (IIa) heavy chain; s = slow heavy chain; 1f, 2f, 3f = fast light chains LC1f, LC2f, LC3f; 1s, 2s = slow light chains LC1s, LC2s

(Fig. 5A, fibers 5, 11). The identity of the HCIIa was based on previous work demonstrating differences in the electrophoretic mobilities of the two fast-myosin heavy chains HCIIa and HCIIb (Staron and Pette 1986b).

The C fiber population was histochemically heterogeneous. Some fibers stained more like IIA fibers (Fig. 4, fibers 1, 2, 7, 10) and were classified as IIC. Others stained more like type I fibers (Fig. 4, fibers 3, 4, 8, 9) and were typed as IC. Both, IC and IIC fibers formed groups which were by no means uniform. Thus, IC and IIC fibers represented a histochemical continuum between types I and IIA. Likewise, the myosin HC analysis reflected a spectrum. A basic characteristic found for this C fiber population was the coexistence of slow-myosin HCI and fast-myosin HCIIa (Staron and Pette 1986a, b). The IC fibers displayed a predominance of HCI (Fig. 5A, fibers 3, 4, 8, 9), whereas

HCIIa predominated in the IIC fibers (Fig. 5A, fibers 1, 2, 7, 10).

The HCI/HCIIa ratio was variable within both the IC and the IIC fiber populations. Thus, the continuum observed histochemically was further reflected by the myosin HC composition. In those cases where the HCI/HCIIa was approximately equal (Fig. 5A, fiber 6), assignment of fibers to either group was difficult (Fig. 4, fiber 6) and other criteria had to be used.

All type I fibers contained the slow-myosin light chain (LC) complement, LC1s and LC2s (Fig. 5B, fiber 12). Although differences were also found between LC1sa and LC1sb (Staron and Pette 1986a), no attempt was made to consider this point in the present study. Confirming previous results (Staron and Pette 1986a), all IIA fibers of rabbit soleus muscle contained, in addition to the fast-myosin LC complement LC1f/LC2f/LC3f, either the slow-myosin LC1s or both LC1s and LC2s (Fig. 5B, fibers 5, 11). Likewise, the IIC fibers displayed a coexistence of slow- and fast-myosin light chains (Fig. 5B, fibers 1, 2, 6, 7, 10). The IC fibers contained predominately the slow-myosin LC1s and LC2s and, in addition, small amounts of LC1f and LC2f (Fig. 5B, fibers 3, 4, 8, 9).

A closer inspection of the distribution of myosin light and heavy chains in the various fiber types revealed an apparent relationship between the HC composition and the ratio between fast- and slow-myosin LC2 (DTNB-light chain). Thus, LC2s predominated in types I and IC, whereas LC2f was prominent in the type IIA and IIC fibers.

Discussion

Using single fiber techniques, Reiser et al. (1985a, b) have recently established a correlation between myosin HC composition and maximum velocity of shortening for developing and adult rabbit muscles. Similarly, it has been previously suggested that the histochemical staining intensity of the myosin ATPase is determined by the myosin heavy chain composition (Gauthier and Lowey 1979; Billeter et al. 1981; Salviati et al. 1983). Using the combined histochemical and biochemical technique for single fiber analysis (Staron and Pette 1986a, b), this correlation has now been established.

The slow type I and the fast type IIA represent the extreme fiber types in soleus muscle. A population of C fibers with coexisting fast- and slow-myosins form a continuum between these two extremes with the IC between types I and IIC and the IIC between types IC and IIA. According to the histochemical staining pattern for mATPase, those fibers which stain similar to type I (IC) display a higher HCI/HCIIa ratio than those which stain similar to the type IIA (IIC). Since this ratio spans the entire range, it is not surprising that some fibers are between types IC and IIC (e.g. fiber 6, Figs. 4 and 5).

It has recently been shown that the myosin heavy chains of mammalian IIA and IIB fibers are different (Dalla Libera et al. 1980; Salviati et al. 1982; Billeter et al. 1981; Mabuchi et al. 1984). These two fast-myosin heavy chains were shown to be distinct in rabbit tibialis anterior muscle and to be specifically distributed within histochemically distinct fast fiber subtypes (Staron and Pette 1986b). The IIA fibers contained HCIIa, IIB fibers contained HCIIb and an intermediate population (IIAB) displayed coexistence of the two fast-myosin heavy chains in varying ratios. A correlation was, therefore, established between the myosin HC complement and the histochemical staining for mATPase in these fast fiber subgroups (Staron and Pette 1986b).

A summary of the myosin light and heavy chain distribution observed in the present study is given in Table 1. A comparison of the various fiber types demonstrates the lack of a correlation between the light chain composition and the histochemical mATPase appearance. Thus, fibers which are similar in their light chain complement but vary in their heavy chains, are histochemically classified as different types (e.g. fibers 10 and 11 in Table 1). However, there appears to exist a preferential combination between either light chain LC2s and the heavy chain HCI or LC2f and HCIIa. Therefore, slow- and fast-myosin heavy chains appear to be correlated in some way with the slow and fast DTNB light chains, respectively.

In summary, the present investigation establishes a direct correlation between the histochemical reactivity for mATPase and the myosin heavy chain composition of a given fiber. This relationship is derived from single fiber analyses on rabbit soleus (Staron and Pette 1986a and present study) and tibialis anterior (Staron and Pette 1986b) muscles but may also apply to other mammalian fibers as well.

Acknowledgements. This study was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 156. R.S. gratefully acknowledges his support by an A. von Humboldt stipend. The authors thank Ms. B. Gohlsch for excellent technical assistance.

References

Arndt T, Pepe FA (1975) Antigenic specificity of red and white muscle myosin. J Histochem Cytochem 23:159–168

Billeter R, Weber H, Lutz H, Howald H, Eppenberger HM, Jenny E (1980) Myosin types in human skeletal muscle fibers. Histochemistry 65:249–259

Billeter R, Heizmann CW, Howald H, Jenny E (1981) Analysis of myosin light and heavy chain types in single human skeletal muscle fibers. Eur J Biochem 116:389–395

Brooke MH, Kaiser KK (1970) Three "myosin adenosine triphosphatase" systems: the nature of their pH lability and sulfhydryl dependence. J Histochem Cytochem 18:670–672

Carraro U, Catani C (1983) A sensitive SDS-PAGE method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. Biochem Biophys Res Commun 116:793–802

Dalla Libera L, Sartore S, Pierobon-Bormioli S, Schiaffino S (1980) Fast-white and fast-red isomyosins in guinea pig muscles. Biochem Biophys Res Commun 96:1662–1670

Gauthier GF, Lowey S (1977) Polymorphism of myosin among skeletal muscle fiber types. J Cell Biol 74:760–779

Gauthier GF, Lowey S (1979) Distribution of myosin isoenzymes among skeletal muscle fiber types. J Cell Biol 81:10–25

Gröschel-Stewart U, Meschede K, Lehr I (1973) Histochemical and immunochemical studies on mammalian striated muscle fibres. Histochemie 33:79–85

Guth L, Samaha FJ (1969) Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. Exp Neurol 25:138–152

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lowry OH, Passonneau JV (1972) A flexible system of enzymatic analysis. Academic Press, New York London

Lutz H, Weber R, Billeter R, Jenny E (1979) Fast and slow myosin within single skeletal fibres of adult rabbits. Nature 281:142–144

Mabuchi K, Pinter K, Mabuchi MS, Sréter F, Gergely J (1984)

Characterization of rabbit masseter muscle fibers. Muscle Nerve 7:431–438

Oakley BR, Kirsch DR, Morris NR (1980) A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. Anal Biochem 105:361–363

Pierobon-Bormioli S, Sartore S, Dalla Libera L, Vitadello M, Schiaffino S (1981) "Fast" isomyosins and fiber types in mammalian skeletal muscle. J Histochem Cytochem 29:1179–1188

Reiser PJ, Moss RL, Giulian GG, Greaser ML (1985a) Shortening velocity in single fibers from adult rabbit soleus muscles is correlated with myosin heavy chain composition. J Biol Chem 260:9077–9080

Reiser PJ, Moss RL, Giulian GG (1985b) Shortening velocity and myosin heavy chains of developing rabbit muscle fibers. J Biol Chem 260:14403–14405

Rushbrook JI, Stracher A (1979) Comparison of adult, embryonic, and dystrophic myosin heavy chains from chicken muscle by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and peptide mapping. Proc Natl Acad Sci USA 76:4331–4334

Salviati G, Betto R, Danieli Betto D (1982) Polymorphism of myofibrillar proteins of rabbit skeletal-muscle fibres. Biochem J 207:261–272

Salviati G, Betto R, Danieli Betto D, Zeviani M (1983) Myofibrillar-protein isoforms and sarcoplasmic-reticulum Ca^{2+}-transport activity of single human muscle fibres. Biochem J 224:215–225

Staron RS, Pette D (1986a) The multiplicity of myosin light and heavy chain combinations in histochemically typed single fibres. II. Rabbit tibialis anterior muscle. Biochem J (in press)

Staron RS, Pette D (1986b) The multiplicity of myosin light and heavy chain combinations in histochemically types single fibres. I. Rabbit soleus muscle. Biochem J (in press)

Staron RS, Hikida RS, Hagerman FC (1983) Reevaluation of human muscle fast-twitch subtypes: evidence for a continuum. Histochemistry 78:33–39

Teutsch HF (1986) A new sample isolation procedure for microchemical analysis of functional liver cell heterogeneity. J Histochem Cytochem 34:263–267