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Comparison of adult, embryonic, and dystrophic myosin heavy chains from chicken muscle by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and peptide mapping*

(fast white fiber type/slow red fiber type/embryonic presumptive fast white fiber type)

JULIE IVORY RUSHBROOK AND ALFRED STRACHER†

Department of Biochemistry, State University of New York-Downstate Medical Center, Brooklyn, New York 11203

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ABSTRACT **Chicken myosin heavy chains from adult fast white muscle fibers (both normal and dystrophic), adult slow red fibers, and embryonic presumptive fast white fibers were compared by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and by peptide mapping. The heavy chain of slow red myosin migrated electrophoretically more slowly than the heavy chains of the other myosins and differed markedly from them in its peptide maps. The heavy chain of dystrophic fast white myosin was similar to its normal counterpart by peptide mapping but showed slight differences. The peptide map of the heavy chain of embryonic presumptive fast white myosin had the general features of that of the heavy chain of fast white, not slow red, fibers but contained definite differences from the former. The results are consistent with the existence of a separate gene for the heavy chain of embryonic presumptive fast white myosin.**

Subunits of myosin isozymes of two of the three major fiber types of adult chicken skeletal muscle have been fairly well defined. Sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis has revealed three low molecular weights components (light chains) with different amino acid sequences in the myosin of fast white fibers (1–3); two components in the same size range, but differing from those in fast white fibers, are found in the myosin of slow red fibers (1). The fiber types also appear to differ in their myosin heavy chains. By immunochemical criteria, distinct differences are apparent (4, 5). In another species, the cat, comparisons of the products of tryptic digestion and chemical cleavage of the two myosins indicate sequence differences in the heavy chains (6). Myosin of the third major fiber type of chicken skeletal muscle, fast red, has not been characterized.

The nature of the isozymes of chicken embryonic skeletal muscle myosin is unclear. The light chain complement of myosin isolated from presumptive fast white fibers electrophoretically resembles that of the adult tissue but frequently contains none or diminished amounts of the smallest light chain, light chain-3 (7–10). In myosin isolated from presumptive slow red muscle, the light chain patterns of both presumptive fast white fibers and adult slow red fibers are present, with a shift in the relative amounts of protein in each toward the adult slow red pattern as development proceeds (9). Immunochemical examination of heavy chain components isolated from the two tissue types shows that the heavy chain of presumptive fast white fibers resembles that of the adult tissue, whereas presumptive slow red myosin contains two types of heavy chains, one similar to that of adult fast white fibers and the other similar to that of adult slow red fibers (9). These results may be interpreted to indicate that the earliest myosin synthesized in embryonic muscle resembles that of adult fast white fibers, differing only in the amount of light chain-3. However, studies done directly on the tissue, using antibodies apparently specific for the heavy chains of adult fast white, slow red, and cardiac muscle fibers, show the presence of antigens of all three heavy chains in embryonic presumptive fast white fibers (11). Similar work in the rat, using antibodies to heavy chains of both adult fast white and slow red myosins, indicates the presence of these heavy chains or similar species in embryonic presumptive slow red fibers (12).

The following study was directed at further definition of the myosin isozymes of adult and embryonic skeletal muscle of the chicken. The procedures used were direct electrophoresis in $NaDodSO_4$ slab gels and one-dimensional peptide mapping by the procedure of Cleveland *et al.* (13). Because of interest in the possibility of a genetic defect in myosin in muscular dystrophy, myosin of the affected fast white fibers of adult dystrophic chickens was also included.

MATERIALS AND METHODS

Embryonic myosin was prepared from White Leghorn chicken eggs obtained from Shamrock Farms (NJ). Normal adult myosins were prepared from chickens raised in our facilities from such eggs. Dystrophic myosin was prepared from dystrophic chickens from the Department of Animal Genetics, University of Connecticut.

Chemicals. $NaDodSO_4$, acrylamide, *N,N'*-methylenebisacrylamide, *N,N,N',N'*-tetramethylethylenediamine, and ammonium persulfate were obtained from Bio-Rad Laboratories. Other chemicals were reagent grade or better. *Staphylococcus aureus* V8 protease was obtained from Miles and stored at 1 mg/ml in water at −60°C. Papain was obtained from Sigma and stored at 1 mg/ml in 0.1 mM EDTA/0.01 M Na acetate, pH 4.5, at −60°C.

Preparation of Myosins. Myosins were prepared by classical procedures (14) at 4°C. The homogenized tissue was extracted briefly in 0.6 M KCl/5 mM $MgCl_2$/1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid/1 mM Na pyrophosphate/0.5 mM dithiothreitol/10 mM K phosphate, pH 6.8. After centrifugation at 27,000 × *g* for 10 min the extracted myosin was precipitated by dilution to 0.046 M KCl. The precipitate was redissolved in 2 M KCl/0.5 mM dithiothreitol/0.2 M K phosphate, pH 6.8, and the volume was adjusted with water to give 0.5 M KCl. Concentrated ATP and $MgCl_2$ solutions (pH 6.8) were added to give final concentrations of 1 mM and the solution was centrifuged at 160,000 × *g* for 2 hr to remove F-actin and undissociated actomyosin. A 35–50% saturated ammonium sulfate fractionation step was occasionally added but did not noticeably improve the purity.

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

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† To whom reprint requests should be addressed.

The myosin was then usually dialyzed against 0.5 M KCl/0.5 mM dithiothreitol/1 mM EDTA/0.01 M K phosphate, pH 6.8, and stored at −20°C after addition of glycerol to 50% (vol/vol). Proteins were also stored at −60°C in $NaDodSO_4$ sample buffer (see below).

Protein was determined by the procedure of Lowry *et al.* (15) with bovine serum albumin as a standard.

$NaDodSO_4$ Electrophoresis. $NaDodSO_4$ electrophoresis was carried out in the slab gel mode (16) (1.5-mm-thick gels) according to King and Laemmli (17) with minor modifications (18). Samples were prepared for electrophoresis by the addition of 10% (wt/vol) $NaDodSO_4$ to achieve 2% $NaDodSO_4$ and mercaptoethanol to 5% (vol/vol), incubation for 3 min at 100°C, and dialysis against electrophoresis sample buffer. The mercaptoethanol addition and heating procedure were repeated prior to application to the gel.

One-Dimensional Peptide Mapping. One-dimensional peptide maps of myosin heavy chains were obtained by the procedure of Cleveland *et al.* (13) by using heavy chain bands cut out of 5% $NaDodSO_4$ slab gel electrophoretograms of whole myosin preparations. To obtain these bands, proteins were applied to 5% slab gels at 40 μg per well and the bands were visualized by staining for 10 min in the stain of Cleveland *et al.* and rinsing briefly in water. Mercaptoethanol was added (final concentration, 1 mM) to the solution used to incubate the gel slices. The digestion and second-stage electrophoresis were carried out in a slab gel containing a 15% polyacrylamide separating gel.

The proteases used were *S. aureus* V8 protease (2 μg per well) and papain (2 ng per well). Papain was activated by incubation at room temperature for 1.5–2 hr in buffer that was 60 mM in mercaptoethanol.

The digestion patterns of the myosin heavy chains were surprisingly reproducible. The molecular weight ranges of the patterns were insensitive to the amount of heavy chain applied, to at least 25% of the amount used in the maps shown; only the intensity of the bands varied. Variation in the amount of *S. aureus* V8 protease similarly varied only the intensity of the bands, more myosin remaining undigested as the amount of protease was decreased. Variation in the amount of papain by factors of 10, however, shifted the molecular weight range of the digestion products, which is the more usual finding (13). Papain was especially effective in digesting the denatured myosin heavy chain. Small variations observed on repeated mapping of a given heavy chain appeared to involve slight shifts in the size range of digestion products. These seemed to arise from differences in digestion times due to variation in the length of the stacking gel. Differences cited in maps of heavy chains of different fiber types are among those that appeared consistently in digestion patterns of different electrophoretic runs for which the digestion times appeared to be comparable.

RESULTS

The heavy chains of myosin from adult normal and dystrophic pectoralis major muscle [essentially pure fast white fibers (19)], adult anterior latissimus dorsi muscle [primarily slow red fibers (5)], and 16-day-embryo pectoralis muscle (presumptive fast white fibers) were compared by direct electrophoresis in Laemmli $NaDodSO_4$ slab gels and by partial protease digestion followed by electrophoresis of the products by the procedure developed by Cleveland *et al.* (13).

Analysis by direct electrophoresis in 5% polyacrylamide gels showed that the heavy chains from adult normal and dystrophic fast white fibers and embryonic presumptive fast white fibers had similar migration rates (Fig. 1*A*). However, the predominant heavy chain of the anterior latissimus dorsi (i.e., that of the slow red fibers) migrated more slowly than the heavy chains

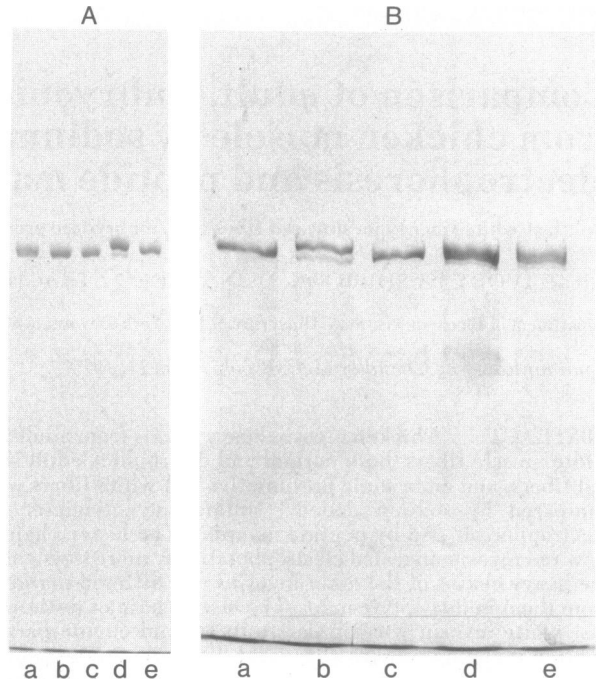

FIG. 1. Electrophoresis of myosin in $NaDodSO_4$/5% acrylamide slab gels to investigate the heavy chain component in myosin from the various fiber types. (*A*) Lanes: a, fast white; b, dystrophic fast white; c, 16-day-embryo presumptive fast white; d, slow red; e, fast white. (*B*) Lanes: a and c, fast white; b, slow red; d and e, mixture of equal amounts of myosin from fast white and slow red fibers. All lanes contained 2 μg of total protein except lane d in *B* had 4 μg. The slow red myosin preparation in *B* is different from that used in *A*.

from the other tissues under these conditions. This difference remained upon coelectrophoresis of myosin preparations from fast white fibers and slow red fibers, if the amount of protein applied was low (Fig. 1*B*, lane e). Use of a low percentage of acrylamide gel (5%) was necessary to show the difference. On electrophoresis in a 7% gel, the difference was small and would ordinarily have been overlooked. A small amount of protein migrating just in front of the fast white heavy chains is present in the electrophoretogram of the anterior latissimus dorsi preparation and may constitute the heavy chain complement from the small number of fast white fibers in this muscle. No difference was apparent in heavy chains from 16-day-embryo pectoralis major or mixed pectoralis major and minor muscle (not shown).

Mapping of the heavy chains of adult fast white and slow red fibers produced digestion patterns with large differences (Figs. 2 and 3, lanes a and d). The pattern from dystrophic fast white fibers was essentially identical to that of normal tissue (Figs. 2 and 3, lanes b and a). One small region of difference that appeared consistently is bracketed (Fig. 3).

The peptide maps obtained with the heavy chain of embryonic presumptive fast white fibers had the general features of those of the heavy chain of adult fast white fibers in each case (Figs. 2 and 3, lanes c and a). There was no indication of the presence of a species similar to the heavy chain of slow red fibers (lane d). Although the resemblance to the maps of the fast white heavy chain was strong, with each protease comparison of the embryonic heavy chain map with the adult fast white heavy chain map reveals distinct differences (see arrowheads, lane c). Additional bands are present in the patterns of the embryonic heavy chain, and some bands present in the pattern of the adult heavy chain are missing. The reproducibility of these differences is illustrated in Fig. 4. These results indicate the presence of a major new species in the embryonic heavy chain. It should

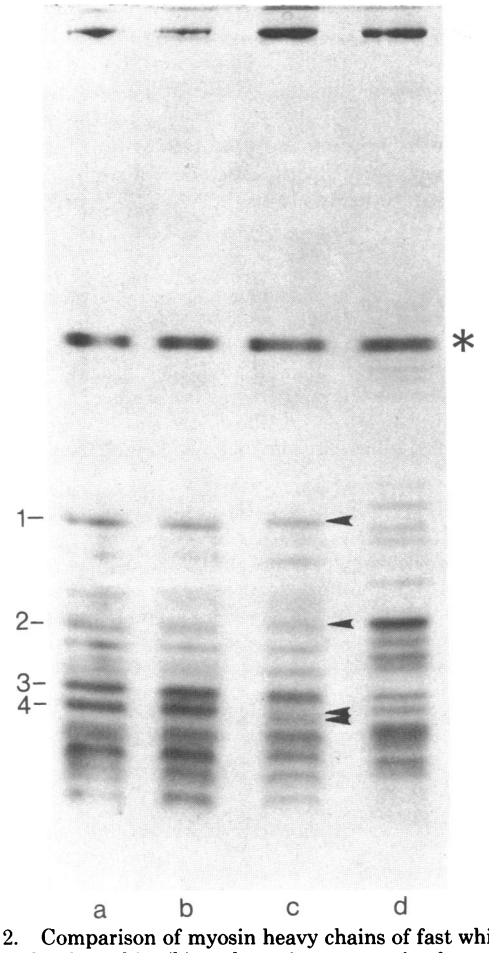

FIG. 2. Comparison of myosin heavy chains of fast white (lane a), dystrophic fast white (b), embryonic presumptive fast white (c), and slow red (d) fibers by the peptide mapping procedure of Cleveland *et al.* (13) using *S. aureus* V8 protease (2 μg per lane). Heavy chain from 40 μg of myosin was used in each lane. The asterisk locates the *S. aureus* V8 protease band. Arrowheads indicate certain differences in the pattern of lane c compared with lane a. The numbers locate prominent bands in the map of the fast white fiber heavy chain to facilitate comparison with the map of Fig. 4*A*.

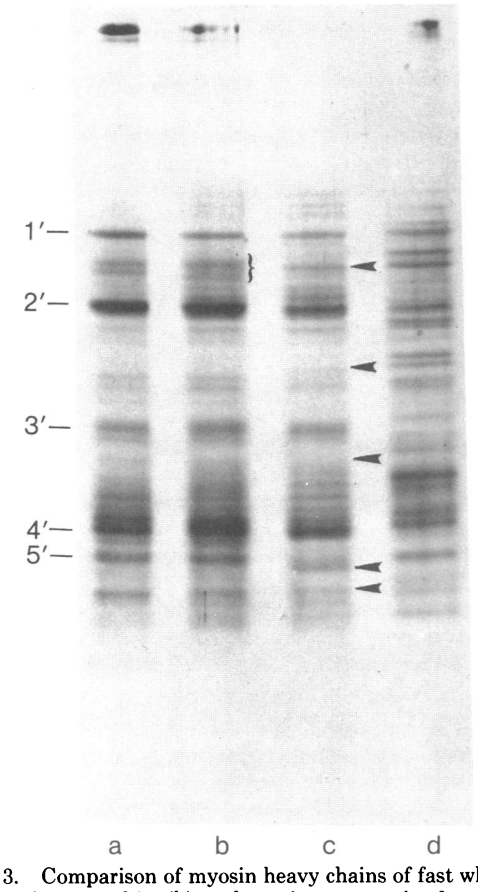

FIG. 3. Comparison of myosin heavy chains of fast white (lane a), dystrophic fast white (b), embryonic presumptive fast white (c), and slow red (d) fibers by the peptide mapping procedure of Cleveland *et al.* (13) using papain (2 ng per well). Other conditions were as in Fig. 2. The amount of papain applied was too low to produce a band. Arrowheads and brackets indicate certain differences in lanes b and c compared with lane a. The numbers locate prominent bands in the map of the fast white fiber heavy chain to facilitate comparison with the map in Fig. 4*B*.

be noted that control digestions using a mixture of adult fast white and slow red heavy chains produced additive patterns that were different from those obtained with the embryonic heavy chain (unpublished data).

DISCUSSION

Comparison of the myosin heavy chains of fast white and slow red fibers by peptide mapping according to the procedure of Cleveland *et al.* (13) reveals striking differences in the two species, which are not unexpected in view of their quite distinct antigenicities (4, 5) and the sequence differences found by peptide mapping in the heavy chains of the two fiber types of the cat (6). The separation of the heavy chains of fast white and slow red fibers by direct electrophoresis in the Laemmli Na-DodSO$_4$ electrophoretic system, however, was an unexpected finding because there have been no previous reports of such a separation. In the study of other proteins, the Laemmli system has been noted for its high degree of resolution (20–22). The probable basis for the electrophoretic separation is indicated by the sequence differences in the heavy chains apparent in the peptide maps, although slight differences in size could also be responsible.

Previously, myosins of adult dystrophic fast white fibers and embryonic presumptive fast white fibers have been shown to be similar in possessing diminished amounts of the electrophoretically fastest moving light chain (7–10, 23). Similarities in the myosins of the two fiber types have also been implied by the proposal that muscular dystrophy may involve a failure of embryonic muscle cells to undergo cell death so as to allow development of adult muscle cells (24). The results of the present study, however, indicate that this is not the case. The peptide map of the dystrophic heavy chain closely resembles that of the normal and does not show the differences typical of the embryonic species. Thus, the study fails to show any further resemblance between embryonic and dystrophic myosins. The small difference that is detected consistently in the papain digestion products of the dystrophic compared with the normal heavy chain has not yet been explained.

Peptide mapping of the myosin heavy chain of embryonic presumptive fast white fibers produced a pattern similar to that of adult fast white fibers, with no evidence for the presence of a component like that of adult slow red fibers. Thus, the results support the previous biochemical finding that the embryonic presumptive fast white heavy chain resembles the adult type (9), rather than the histochemical data indicating the presence of components similar to adult fast white, slow red, and cardiac heavy chains (11). The reason for the disparity between the biochemical and histochemical studies is not clear at this stage. The possibility existed that purification of the embryonic myosin for the biochemical study might have entailed preferential extraction of only one of perhaps several myosin species present. However, peptide maps of heavy chains obtained from whole

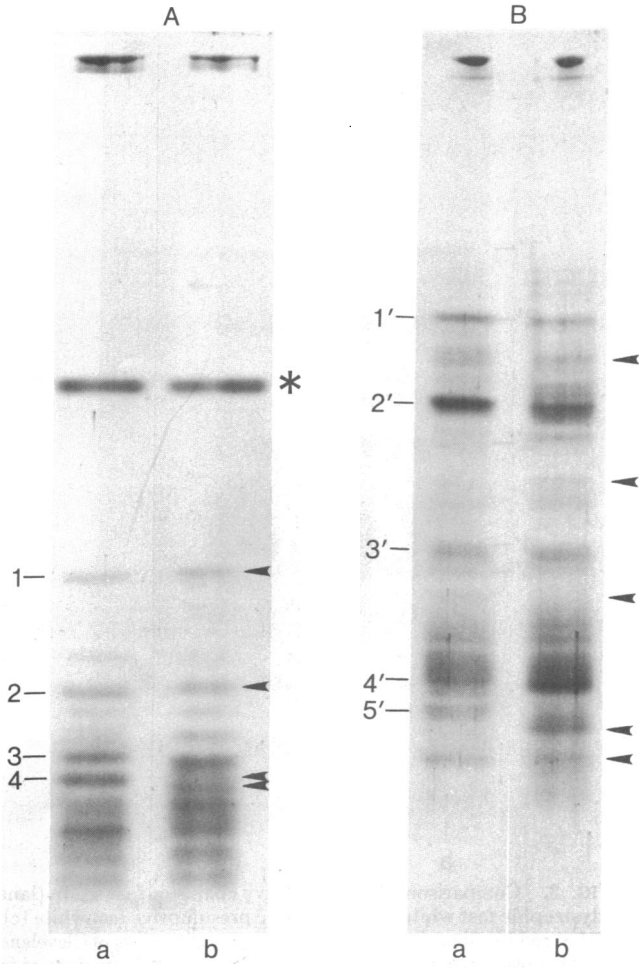

FIG. 4. Peptide maps of fast white and 16-day-embryo presumptive fast white myosin heavy chains to demonstrate the reproducibility of the patterns shown in Figs. 2 and 3. (*A*) Digestion with *S. aureus* V8 protease as in Fig. 2; lane a, myosin heavy chain from adult fast white fibers; lane b, myosin heavy chain from 16-day-embryo presumptive fast white fibers. (*B*) Digestion with papain as in Fig. 3; lane a, myosin heavy chain from fast white fibers; lane b, myosin heavy chain from 16-day-embryo presumptive fast white fibers. Arrowheads indicate certain differences in patterns between lanes b and a. The asterisk locates the *S. aureus* V8 protease band. The numbers are as in Figs. 2 and 3.

embryonic muscle taken up in $NaDodSO_4$ electrophoresis sample buffer resembled those of the heavy chains of the purified embryonic myosin and did not support this explanation (unpublished data). Approximately 40 μg of myosin heavy chain was used to produce the peptide maps in the present study. When approximately 10 μg of protein was applied, the digestion patterns were barely discernible. Thus, up to 25% of the heavy chain protein could have differed from the bulk of the protein—i.e., been of the slow red type—and would have contributed little to the digestion patterns. However, one would have expected this proportion of slow red type heavy chain to have been detected with the immunodiffusion technique used in the previous biochemical study (9).

The peptide map study presented here showed strong similarities between the myosin heavy chains of embryonic presumptive fast white myosin and of the adult tissue but it also revealed definite differences. Certain of these differences—namely, the absence of bands present in the pattern of adult species—indicate the presence of a major new myosin heavy chain species in the embryonic tissue. Unless this species is merely the result of the probable absence of one *N*-methylhistidine per heavy chain in the embryonic myosin (25), which seems unlikely, the result indicates amino acid sequence differences between the embryonic and adult heavy chains, implying the presence of a distinct gene for the myosin heavy chain of embryonic presumptive fast white fibers. Sequence determinations indicate the existence of such a gene for the embryonic presumptive fast white heavy chain of the rabbit (26).

Note Added in Proof. While this manuscript was in press, two relevant papers appeared. In one (27), evidence was presented from peptide mapping for a unique gene for the embryonic fast-twitch myosin heavy chain from rabbit. The other (28) indicates that previous immunohistochemical studies showing the presence of adult fast and slow fiber myosin heavy chains in embryonic muscle may have been due to the presence of impurities in the myosin used to raise the antibodies.

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