Myosin Isoforms in Normal and Dystrophic Chickens†

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Received October 22, 1986; Revised Manuscript Received February 26, 1987

ABSTRACT: The myosin isoform content in the affected fibers of chickens with inherited muscular dystrophy has been investigated with a new high-performance liquid chromatographic procedure for separation of the tryptic fragments of myosin subfragment 1 (S-1). The results indicate that dystrophic muscle contains substantial amounts of normal adult myosin, together with various myosin species present in normal 5-day posthatch chickens. Confirmation was obtained by comparative peptide mapping of the S-1 tryptic fragments and by N-terminal sequencing of 20-kDa species. Together with data on other contractile proteins and certain metabolic enzymes [Obinata, T., Takano-Ohmura, H., & Matsuda, R. (1980) *FEBS Lett. 120*, 195–198; Mikasa, T., Takeda, S., Shimizu, T., & Kitaura, T. (1981) *J. Biochem. (Tokyo) 89*, 1951–1962; Feit, H., & Domke, R. (1982) *Cell Motil. 2*, 309–315; Cosmos, E. (1966) *Dev. Biol. 13*, 163–181; Cosmos, E., & Butler, J. (1967) in *Exploratory Concepts in Muscular Dystrophy and Related Disorders* (Milhorat, A. R., Ed.) pp 197–204, Excerpta Medica, Amsterdam], the results are consistent with the hypothesis that there is a general defect in muscle maturation in avian dystrophy.

Avian muscular dystrophy was first reported by Asmundsen and Julian in 1956. The autosomally inherited condition is characterized by progressive degeneration of fast white muscle fibers and is accompanied by infiltration of fat and connective tissue (Ashmore & Doerr, 1971; Somes, 1981; Cosmos et al., 1979; Barnard et al., 1982). Although the disease limits physical activities of the birds such as flying and righting from a supine position, it is not usually fatal.

Despite much research, the genetic defect has not been identified. Various abnormalities have been detected, however [e.g., Cosmos (1966), Cosmos and Butler (1967), and Wilson et al. (1979)], among them disorders of contractile proteins. In normal chickens, tropomyosin, troponin T, and the alkali light chains of myosin undergo isozymic changes during development (Roy et al., 1979; Matsuda et al., 1981; Dow & Stracher, 1971; Hoh, 1979), the extent of the switch varying with the different proteins. In dystrophic birds, these transitions do not proceed to their usual extent (Obinata et al., 1980; Mikasa et al., 1981; Feit & Domke, 1982).

Several years ago we identified small differences in SDS[1] gel peptide maps of the myosin heavy chain from dystrophic muscle (Rushbrook & Stracher, 1979; Rushbrook et al., 1981). The abnormality was present in the four strains of affected chickens investigated and absent in controls and appeared to be related to the disease. The observation was specific for the affected fast white muscle fibers, no differences being observed in the heavy chains from slow tonic fibers.

When subfragment 1 (S-1) and rod moieties of the heavy chain were generated and subjected to peptide mapping, differences were apparent in both moieties. Since the characterized posttranslational modifications of the myosin heavy chain are located in S-1 (Trayer et al., 1968; Kuehl & Adelstein, 1970), the peptide-mapping differences found in the rod were indicative of sequence differences. Both posttranslational and sequence differences were possibilities for the differences in S-1. Comparison of the heavy chain from adult dystrophic myosin with that from 12-day embryonic muscle ruled out an isoform from this stage as a major source of the differences.

Subsequently, using similar procedures, Bandman presented evidence that the abnormality was due to the persistance of the posthatch myosin isoform (Bandman, 1984). In a further study, immunological cross-reactivity/SDS gel peptide-mapping results (Bandman, 1985) suggested that about 60% of the myosin consisted of this form. Of the remainder, a small amount was identified immunologically as the normal adult species, the rest being unidentified.

Most recently, in apparent conflict with these results, Huszar's laboratory concluded from peptide-mapping and sequencing experiments that the post hatch form only of myosin is present (Huszar et al., 1985a,b).

In this study we apply to the problem a newly developed reverse-phase HPLC procedure for the separation of the tryptic fragments of S-1. This is the first physical–chemical procedure to separate the developmental isoforms of avian fast white muscle fibers. The results indicate that approximately 50% of the dystrophic myosin comprises that present in normal adult muscle, the remainder consisting of various species present in control 5-day posthatch chickens.

Materials and Methods

Chemicals. Chemicals used for routine analyses were of reagent grade or better. Chemicals used for HPLC were described previously (Rushbrook, 1985).

Chicken Strains. Line 412 (normal) and 413 (dystrophic) chickens of the ages specified were obtained from the University of California at Davis, and Rhode Island Red birds were obtained from the University of Connecticut at Storrs. Rhode Island Red birds are closely related to the New Hampshire strain that gave rise to lines 412 and 413 (R. G. Somes, Jr., personal communication). Five-day posthatch Rhode Island Red birds were the source of the posthatch myosin of Figure 3 and Table I, since line 412 birds could not be obtained in

† This work was supported by a grant from the National Institutes of Health (NS 18438).
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[1] Abbreviations: S-1, myosin subfragment 1; SDS, sodium dodecyl sulfate; TLCK, N^{α}-*p*-tosyllysine chloromethyl ketone; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; DTE, dithioerythritol; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; TFA, trifluoroacetic acid; HPLC, high-performance liquid chromatography; kDa, kilodalton(s); PTH, phenylthiohydantoin; LC1, myosin light chain 1; LC3, myosin light chain 3.

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the winter months due to inclement temperatures. No significant differences were apparent between posthatch Rhode Island Red and posthatch line 412 S-1 tryptic digests (J. I. Rushbrook and C. Weiss, unpublished observations, and Figures 1 and 3).

Protein Determinations. Protein concentrations were determined by the procedure of Lowry et al. (1951) using bovine serum albumin as a standard.

Myosin Preparation. Myosin from age-matched adult chickens (7.5–29 months) was prepared from the pectoralis major muscle, omitting the red strip (Gauthier & Lowey, 1977) and employing an extraction buffer:tissue ratio of 3:1 and a 35–50% ammonium sulfate cut (Rushbrook et al. 1981). Myosin from posthatch (5-day) chickens was prepared similarly, but the red strip was not removed and protease inhibitors (leupeptin, PMSF, and TLCK) were present in the early stages of the procedure. Adult preparations were from single birds, posthatch from as many as 100. No consistent differences were apparent with age in tryptic digests prepared from the adult birds.

SDS Gel Electrophoresis. SDS gel electrophoresis was carried out as described previously (Rushbrook & Stracher, 1979).

Preparation of Myosin Subfragment 1 (S-1) and Alkylated Tryptic Fragments of S-1. Chymotryptic S-1 was prepared by the procedure of Weeds and Taylor (1975) as modified for chicken myosin by Holt and Lowey (1977). Myosin (10 mg/mL) in 0.12 M NaCl, 1 mM EDTA, 1 mM DTE, and 0.02 M sodium phosphate, pH 7.0, was digested for 5 min at 25 °C with TLCK-treated chymotrypsin (0.025 mg/mL). The reaction was terminated with freshly prepared PMSF (0.01 M in ethanol) added to 0.1 mM. Rod and undigested myosins were precipitated by dialysis against 0.03 M NaCl, 0.5 mM DTE, 0.5 mM EGTA, and 0.01 M sodium phosphate, pH 7.0, and the S-1 preparation was concentrated and purified by fractionation between 43% and 58% saturated $(NH_4)_2SO_4$.

Tryptic fragments of S-1 were prepared according to the procedure of Sutoh (1982). Purified S-1 was solubilized in 2 M KCl, 1 mM EGTA, 0.5 mM DTE, and 0.2 M potassium phosphate, pH 6.8, and dialyzed into 0.03 M KCl, 0.1 mM DTE, and 0.02 M Tris-HCl, pH 8.0. The protein concentration was adjusted to 1 mg/mL, and digestion was carried out for 30 min at 25 °C with TPCK-treated trypsin. The reaction was terminated with TLCK (added to 0.23 mM), and the S-1 tryptic fragments were dialyzed against 0.5% acetic acid and 0.1 mM DTE prior to lyophilization. Alkylation was carried out by a modification of the procedure of Crestfield et al. (1963). The lyophilized S-1 tryptic digests were solubilized at 1 mg/mL in 6 M guanidine hydrochloride, 6 mM EDTA, and 0.36 M Tris-HCl, pH 8.6. DTE (1.19 M) in the same solution was added to a concentration of 0.108 M, and the solution was flushed with nitrogen and incubated for 1 h at room temperature. Freshly prepared iodoacetic acid (1.44 M) in 1.0 N sodium hydroxide was added in the dark to a concentration of 0.101 M, and the solution was incubated for 1 h in the dark. Mercaptoethanol was added to a concentration of 0.51 M, and the solution was incubated for 15 min at room temperature and then chilled on ice for 15 min. The alkylated preparations were dialyzed in the dark against several changes of 0.5% HOAc, 0.1 mM DTE, and stored at 4 °C prior to reverse-phase HPLC analysis. The samples (protein concentration approximately 0.8 mg/mL) were applied directly to the HPLC column without further treatment or additions.

Reverse-Phase HPLC Analysis of Alkylated Tryptic Fragments of S-1. The equipment and column were those described previously (Rushbrook, 1985). Solvent A consisted of 0.1% TFA in water, and solvent B consisted of 0.1% TFA in acetonitrile. A linear gradient of 30–60% B in 45 min at a flow rate of 1 mL/min was used. The column was conditioned overnight prior to separations with a solvent consisting of 50% 0.2% TFA with triethanolamine or triethylamine added to give pH 2.0 and 50% acetonitrile.

As required (see below), peaks were collected manually for further analysis by peptide mapping or sequencing. During these preparative runs when as much as 0.5 mg of protein was applied, the 50-kDa fragments tended to stick to the column. To avoid contamination between samples, only one kind of sample was applied to the column during a series of runs and the column was washed with 6 M guanidine hydrochloride prior to switching to a new sample.

Further Digestion of Alkylated 20-kDa Fragments. As specified in the text, 20-kDa fragments were purified by HPLC as described above and lyophilized. The fragments were digested at 0.125 mg/mL with thermolysin in 0.05 M ammonium bicarbonate buffer, pH 7.8, and 1.0 mM calcium chloride, for 24 h at 37 °C. The thermolysin concentration was 0.017 mg/mL. The reaction was terminated by the addition of HOAc to 2.5%. Samples were stored at −20 °C prior to chromatography.

Reverse-Phase HPLC Peptide Mapping of Thermolytic Peptides of Alkylated 20-kDa Fragments. The equipment and column used were those described above. Solvent A was 0.25% phosphoric acid with triethanolamine added to give pH 2.0, and solvent B was acetonitrile. Peptides were chromatographed with a linear gradient from 1% to 29% B in 28 min at a flow rate of 1 mL/min.

Amino Acid Sequence Determinations. Edman degradations and PTH-amino acid analyses were carried out on an Applied Biosystems Model 470A gas-phase protein sequencer connected on line to a microbore HPLC PTH-amino acid analyzer (Model 120A) from the same company (Applied Biosystems, Foster City, CA). The 20-kDa fragments specified in the text were purified by HPLC as described above and the sample volumes reduced by lyophilization prior to application to the filter.

Results

Reverse-phase HPLC of the alkylated tryptic fragments of S-1 from normal myosin produced five major peaks (Figure 1b). SDS gel electrophoresis (Figure 2A) revealed that the order of elution of components was 20 kDa (peak 1), 23 kDa (peak 2), 18 kDa (peak 3), myosin light chain 3 (LC3) (peak 4), and 50 kDa (peak 5). The 20-kDa region (peak 1) was consistently found to contain three components, in contrast to the other peaks, which are fairly symmetrical. This heterogeneity may be correlated with the diffuse nature of the 20-kDa band on SDS gel electrophoresis (Figure 2 and Figure 4).

Comparative reverse-phase HPLC peptide mapping of the 18-kDa fragment and of LC3 using thermolysin revealed marked similarities (J. I. Rushbrook, C. Weiss, and A. G. Wadewitz, unpublished results). This confirmed previous results indicating that the 18-kDa species is derived from myosin light chain 1 (LC1) by tryptic cleavage (Mornet et al., 1979). LC1 is identical with LC3 in 141 of its 191 residues (Nabeshima et al., 1984; Henry et al., 1982).

The reverse-phase HPLC profile of the S-1 tryptic fragments from 5-day posthatch chickens is shown in Figure 1c. The profile resembles in a general fashion that of the adult chromatogram, and SDS gel analysis of the peaks reveals a similar elution order for the 20-, 23-, and 50-kDa species

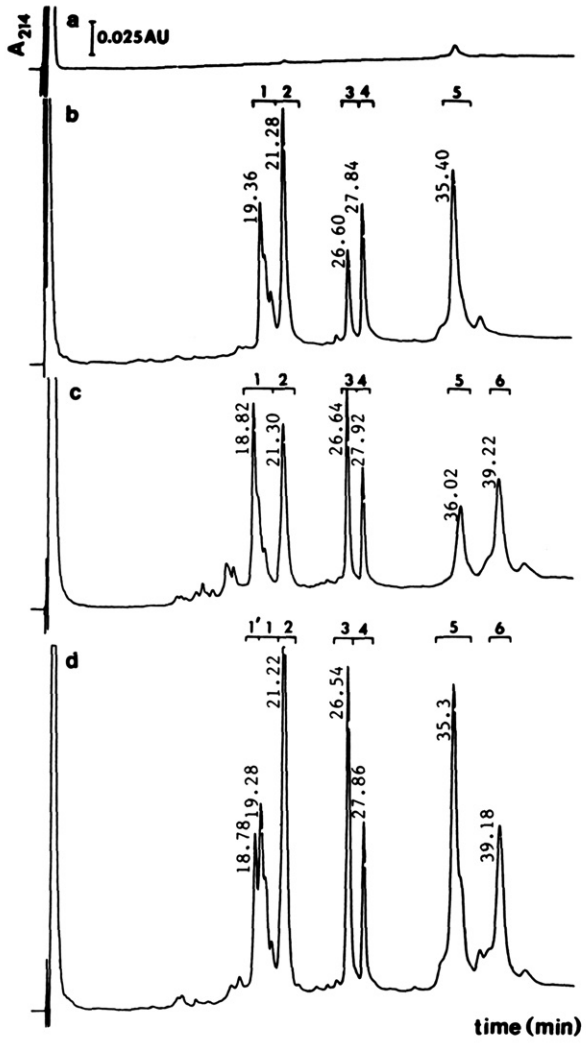

FIGURE 1: Reverse-phase HPLC analysis of alkylated tryptic fragments of S-1 from normal adult, normal posthatch, and dystrophic adult myosins: (a) base line, (b) normal adult (line 412), (c) normal 5-day posthatch (line 412), and (d) dystrophic adult (line 413). For elution conditions, see Materials and Methods. Approximately 200 μg of protein was applied in each run.

(Figure 2B). A number of differences are notable, however. Most obviously, 50-kDa species are present in not one but two peaks, peaks 5 and 6. Peak 6 in the posthatch chromatogram clearly elutes after the 50-kDa species in the adult profile. Cochromatography of adult and posthatch preparations indicates that the posthatch peak 5 also elutes significantly later than its adult counterpart, appearing as a shoulder to the adult peak (Figure 3d).

SDS gel electrophoresis shows that the posthatch peak 5 contains two species (Figure 2B, track f). One migrates with the main 50-kDa species in the starting material (track a), and the other, the more intense, migrates slightly faster. Peak 6 contains one major component which migrates with the main band in the starting material (Figure 2B, tracks g and a). Peak 5 becomes insignificant in amount by 12 days posthatch (Rushbrook et al., 1987).

The second major difference between the posthatch and adult profiles resides in the 20-kDa species. The 20-kDa region in the posthatch profile (Figure 1c, peak 1), though a composite triplet peak like the adult species (Figure 1b, peak 1), elutes earlier than the adult triplet. That this difference is significant is confirmed when the adult and posthatch samples are cochromatographed (Figure 3d). Differences are also apparent in the 20-kDa species on SDS gel electrophoresis. The diffuse posthatch 20-kDa band consistently migrated more slowly on SDS gel electrophoresis than the normal adult 20-kDa band (Figure 4, compare lanes a and c).

Differences were also present between the posthatch and adult profiles in peak 2, the 23-kDa fragment. At the posthatch stage the main peak height is lower and there is a prominent early eluting shoulder (Figure 3c). The shoulder is marginally resolved, however, and this paper will focus on the more clearly defined points of differences.

The reverse-phase HPLC profile of S-1 tryptic fragments from adult dystrophic myosin is shown in Figure 1d and SDS gel analyses of the peaks in Figure 2C. The HPLC profile differs significantly when compared with that of the normal adult (Figure 1b). The dystrophic 20-kDa region contains an additional prominent early eluting peak (peak 1'). On SDS gel electrophoresis this shoulder can be seen to consist of a

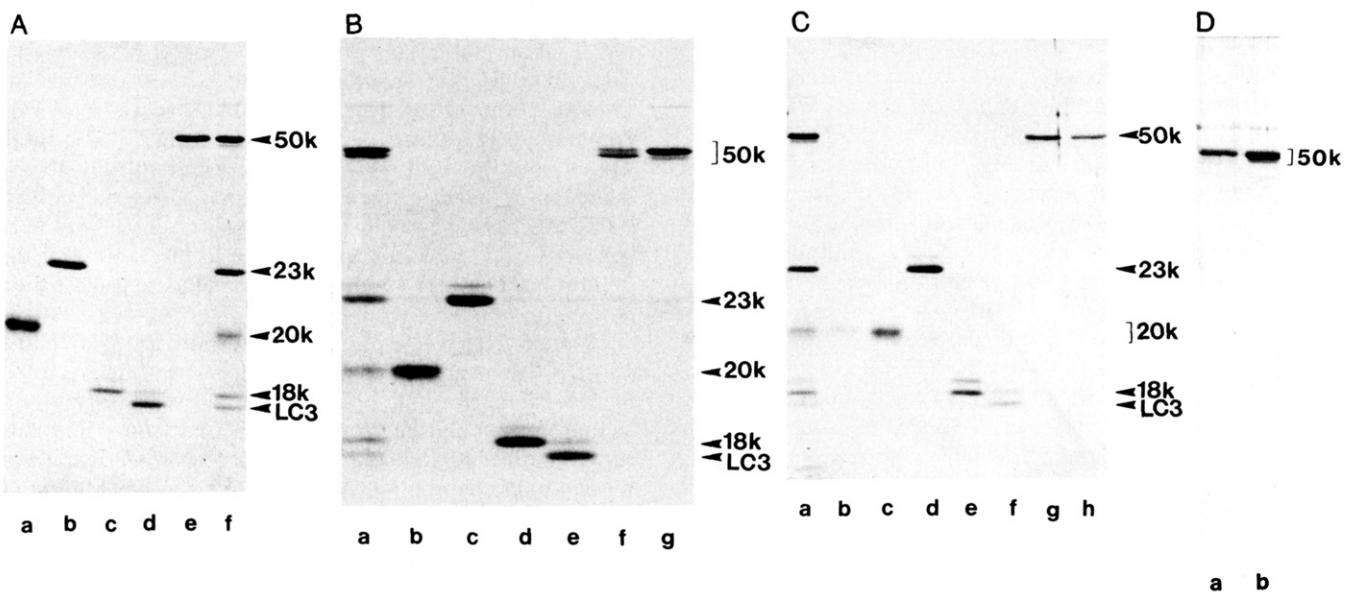

FIGURE 2: SDS gel analysis of the peaks in the separations of Figure 1. (A) Peaks from Figure 1b: (a) peak 1, (b) peak 2, (c) peak 3, (d) peak 4, (e) peak 5, and (f) starting material. (B) Peaks from Figure 1c: (a) starting material, (b) peak 1, (c) peak 2, (d) peak 3, (e) peak 4, (f) peak 5, and (g) peak 6. (C) Peaks from Figure 1d: (a) starting material, (b) peak 1' (c) peak 1, (d) peak 2, (e) peak 3, (f) peak 4, (g) peak 5, and (h) peak 6. (D) Peak 5 (Figure 1d) without shoulder (a) and with shoulder (b). An amount of 2–5 μg was applied per lane.

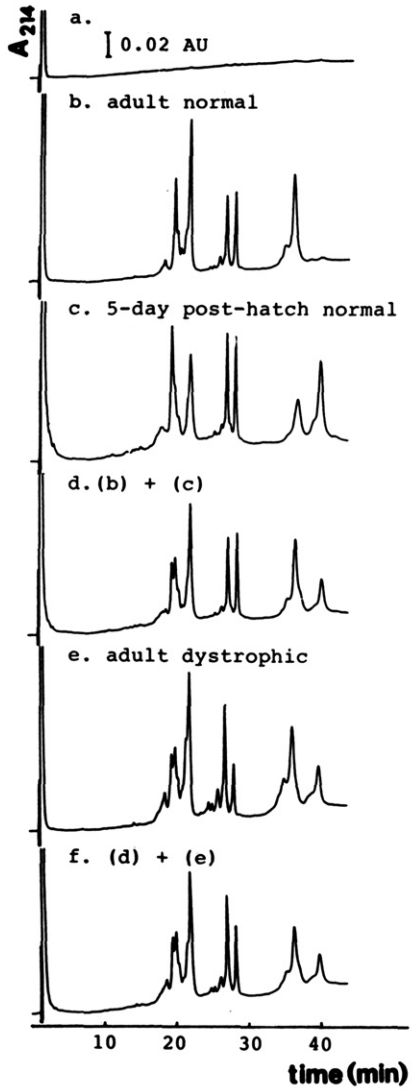

FIGURE 3: Cochromatography of normal adult, normal posthatch (5-day), and dystrophic adult alkylated tryptic fragments of S-1: (a) base line, (b) normal adult (line 412), (c) normal 5-day posthatch (Rhode Island Red), (d) (b+c), (e) adult dystrophic (line 413), and (f) (d+e). Approximately 60 μg of total protein was applied per run. Column and elution system as in Figure 1.

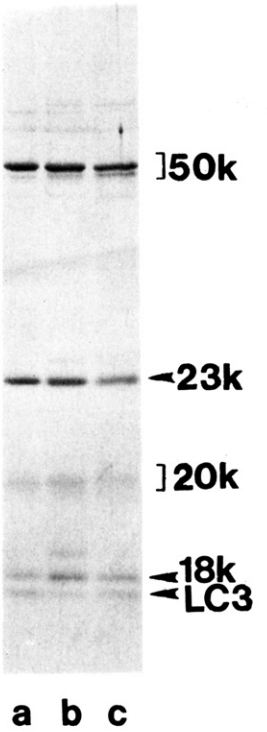

FIGURE 4: SDS gel comparison of alkylated tryptic digests of S-1 from (a) normal adult (line 412), (b) dystrophic adult (line 413), and (c) normal 5-day posthatch (line 412) myosin. Five micrograms of protein was applied per lane.

20-kDa component migrating slightly more slowly than the material in peak 1 (Figure 2C, compare tracks b and c). In addition, the dystrophic HPLC profile contains two 50-kDa peaks, peak 5 and peak 6. Peak 5 has an HPLC elution time similar to that of peak 5 of the adult normal profile but contains a prominent late eluting shoulder. Peak 6 has no counterpart in the normal adult digest.

SDS gel electrophoresis shows that peak 5 (shoulder not collected) contains a band migrating with the major 50-kDa species in the unfractionated S-1 tryptic digest (Figure 2C, compare lanes a and g). When the entire peak including the shoulder is analyzed (Figure 2D), an additional component is seen moving slightly faster than the main 50-kDa species. Peak 6 contains a species with SDS gel electrophoretic mobility identical with that of the major 50-kDa species in the unfractionated digest (Figure 2C, compare lanes a and h).

The above results suggest strongly that the dystrophic digest contains a mixture of the normal adult and 5-day posthatch digests. The peak 1′ and peak 1 dystrophic 20-kDa species correlate with species in posthatch and adult digests, respectively. The peak 5 50-kDa species of the dystrophic digest, excluding the shoulder, has a counterpart in the normal adult peak 5 50-kDa species, while the shoulder to the dystrophic peak 5, and peak 6, may be correlated with peak 5 and peak 6, respectively, in the posthatch digest. The conclusion is supported by both HPLC and SDS gel data. It is particularly reinforced by comparison of the dystrophic chromatographic profile with that of cochromatographed normal adult and posthatch myosins (Figure 3, compare parts d and e) and by cochromatography of the dystrophic profile with the mixed adult and posthatch digests (Figure 3e,f).

The relationship between the adult normal, posthatch, and adult dystrophic myosins was explored further by comparison of the thermolysin digests of their tryptic fragments using HPLC. The peaks chosen for digestion are identified as in Figure 1. The 20-kDa maps from the three sources are shown in Figure 5. The adult normal and dystrophic peak 1 digests were very similar (Figure 5, parts b and c, respectively), differing only in the presence of several additional peaks in the dystrophic digest (arrowheads). These additional peaks are increased in size in Figure 5d, the map of peak 1′, the shoulder to the dystrophic peak 1, and are thus derived from the shoulder material. Comparison of the dystrophic peak 1′ and normal posthatch peak 1 digests (Figure 5, parts d and e, respectively) shows that the peaks are prominent in the posthatch digest. Certain peaks indicated by asterisks diminish in amount throughout the series concomitantly with these changes.

The 20-kDa peptide maps thus support the view that the dystrophic myosin contains a mixture of the normal adult myosin isoform and the isoform(s) present in 5-day posthatch myosin. Similar conclusions could be drawn from maps of the other fragments (not shown).

Further confirmation was sought from comparative N-terminal amino acid sequencing of 20-kDa fragments present in the reverse-phase chromatograms of the various alkylated tryptic digests of S-1. The peaks chosen were the first peak of the adult 20-kDa triplet, the first peak of the 5-day posthatch 20-kDa triplet, and the peaks proposed to correspond

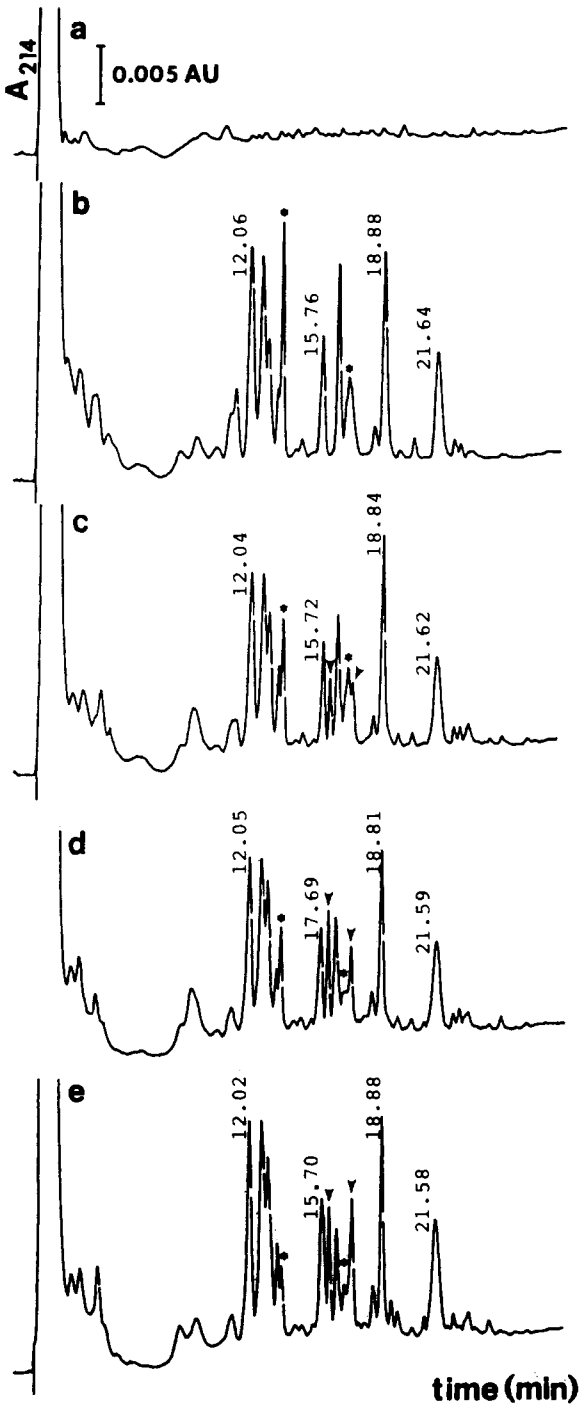

FIGURE 5: Comparison of thermolysin digests of the 20-kDa fragments from normal and dystrophic adult and normal posthatch myosins by reverse-phase HPLC: (a) base line; (b) peak 1, normal adult (line 412); (c) peak 1, dystrophic adult (line 413); (d) peak 1′, dystrophic adult (line 413); (e) peak 1, normal posthatch (line 412). (For peak identification, see labeling in Figure 1 and text). Peaks present in the dystrophic peak 1 digest (c) and not in the adult digest (b) are indicated with arrowheads. These peaks are intensified in the dystrophic peak 1′ digest (d) and are prominent in the 5-day posthatch digest (e). Complementing these changes are peaks that diminish in amount throughout the series (asterisks, b–e). For elution conditions see Materials and Methods. Approximately 8 μg of material was applied in each chromatogram.

to these in the dystrophic profile, i.e., the second peak of the dystrophic 20-kDa quadruplet and peak 1′, the first peak of the dystrophic quadruplet. Although in some instances minor sequences were also present, as might be expected from the closeness of the peaks and the proposed overlapping triplets in the dystrophic chromatogram, there was no difficulty in identifying the sequence of the major component in any instance.

The fragments of interest were found to differ in sequence at residue 22 (Table I). The first peak of the normal adult 20-kDa triplet contained Ala at this position, and this residue was also found at position 22 in peak 1′ of the dystrophic chromatogram. The results are consistent with dystrophic myosin containing the isozymes of normal adult and 5-day posthatch myosins.

Discussion

The reverse-phase procedure described in this study separates the 20-, 23-, and 50-kDa tryptic fragments of S-1, significantly upgrading conventional chromatographic procedures in elution time and resolution (Tong & Elzinga, 1983; Muhlrad & Morales, 1984; Mornet et al., 1980). Single major peaks are obtained for the 23- and 50-kDa components of normal adult myosin while the 20-kDa species consistently chromatographs as a composite peak with three components (Figure 1b). A similar triplet is found for the 20-kDa species in the posthatch myosin digest (Figure 1c), suggesting that the heterogeneity is a general characteristic of this fragment.

The presence of the triplet may be correlated with the diffuse band found for the 20-kDa fragment on SDS gel electrophoresis (Figures 2 and 4). In work reported in detail elsewhere, we show that the three peaks correspond to bands of increasing mobility on SDS gel electrophoresis (Rushbrook et al., 1987). Amino acid sequence analysis shows that the protein fragments in each peak differ at their N-termini due to differential cleavage by trypsin at the lysine triplet located at residues 638–640 of the myosin heavy chain (numbering according to Strehler et al., 1986), the earliest eluting peak containing two lysines, and the latter two peaks, one and none, respectively. Whether these differences alone account for the different gel mobility and chromatographic properties of the fragments remains to be determined.

The study of myosin in avian muscle development has been hindered by the fact that, unlike their mammalian counterparts (Fitzsimmons & Hoh, 1981; Whalen et al., 1981), avian developmental isoforms do not separate by nondenaturing gel electrophoresis (Hoh, 1979; Lowey et al., 1983; Bandman, 1984). The resolution of the reverse-phase HPLC procedure described in this paper is sufficient to distinguish between the S-1 tryptic digests of myosin isoforms present at the adult and early posthatch stages of development. The 5-day posthatch myosin possesses a 20-kDa peak and two 50-kDa peaks, peaks 5 and 6, which differ clearly from their adult counterparts in elution time.

The presence of two 50-kDa peaks in the 5-day posthatch myosin is notable. The first eluting peak, peak 5, diminishes in amount by 12 days posthatch while the second, peak 6, is the dominant species between 12 and 20 days (Rushbrook et al., 1987), identifying it as belonging to the posthatch isoform as defined by the studies of Bandman et al. (1982) and Lowey et al. (1983). The first 50-kDa peak would then represent the remnants of a late embryonic or perhaps new perihatching isoform. Alternatively, the several 50-kDa species at 5 days posthatch may differ in methylated amino acid content. Myosin from the perinatal chicken and rat has been shown to contain methylated arginine, a modification not present in adult myosin (Reporter & Corbin, 1971; Helm et al., 1977) and, in the perinatal rat, the amount of trimethylated lysine rises dramatically before decreasing to adult levels (Helm et al., 1977). Whether such modifications alone would be sufficient to account for the large retention time differences observed remains to be determined. Interestingly, the first 50-

Table I: Comparison of N-Terminal Sequences of 20-kDa Fragments from Normal Adult, Dystrophic Adult, and 5-Day Posthatch Myosins

	1 10 20 23
normal adult (line 412) (first peak of 20-kDa triplet)	Lys-Lys-Gly-Ser-Ser-Phe-Gln-Thr-Val-Ser-Ala-Leu-Phe-Arg-Glu-Asn-Leu-Asn-Lys-Leu-Met-Ala-Asn-
dystrophic adult (line 413) (second peak of 20-kDa quadruplet)	Lys-Lys-Gly-Ser-Ser-Phe-Gln-Thr-Val-Ser-Ala-Leu-Phe-Arg-Glu-Asn-Leu-Asn-Lys-Leu-Met-Ala-Asn-
dystrophic adult (line 413) (first peak of 20-kDa quadruplet, peak 1′)	Lys-Lys-Gly-Ser-Ser-Phe-Gln-Thr-Val-Ser-Ala-Leu-Phe-Arg-Glu-Asn-Leu-Asn-Lys-Leu-Met-Thr-Asn-
normal 5-day posthatch (Rhode Island Red) (first peak of 20-kDa triplet)	Lys-Lys-Gly-Ser-Ser-Phe-Gln-Thr-Val-Ser-Ala-Leu-Phe-Arg-Glu-Asn-Leu-Asn-Lys-Leu-Met-Thr-Asn-

kDa peak has been consistently larger in 5-day New Hampshire than White Leghorn birds (Rushbrook et al., 1987), presumably a reflection of the different genetic backgrounds with respect to muscle development of the two strains (Randall & Wilson, 1980).

Application of the reverse-phase HPLC procedure to the S-1 tryptic fragments of dystrophic myosin indicates the presence of isoforms from normal adult and 5-day posthatch chickens. This conclusion is reinforced by cochromatography of the dystrophic profile with that of a mixture of normal adult and posthatch fragments, by SDS gel analysis of the fragments, and by further peptide mapping and N-terminal sequencing of 20-kDa fragments. A ratio of developmental to adult isoforms in the dystrophic myosin of approximately 1:1 may be obtained by comparing the areas of peak 1′ and the peak that follows it, allowing for a contribution of the second peak of the posthatch triplet to the main adult peak.

The finding of appreciable amounts of posthatch myosin isoform in the pectoralis major muscle of mature dystrophic chickens concurs with the conclusions of the laboratories of Bandman (1984, 1985) and Huszar (Huszar et al., 1985a,b). However, our results differ in the determination that approximately 50% of the protein consists of the normal adult isoform.

Bandman (1985) concluded from cross-reactivity and peptide-mapping analyses that approximately 60% of dystrophic myosin consisted of the posthatch isoform. An antibody specific to the normal adult isoform cross-reacted only slightly, and he concluded that the remainder was composed mostly of as yet unidentified isoform(s).

The reason for the differences with this work are unclear. The two might be reconciled if the adult-specific antibody used by Bandman were generated against a methylated amino acid sequence and if this sequence were undermethylated in the dystrophic muscle. Strain differences in the two studies are a possible but unlikely source of the discrepancy since peptide mapping of heavy chains from four strains of dystrophic birds showed remarkable similarities and all maps contained bands present also in normal birds [Rushbrook et al., 1981; see also Bandman (1984)].

Huszar and co-workers (Huszar et al., 1985a,b) concluded from peptide-mapping and sequence studies that dystrophic myosin consisted of the posthatch myosin isoform; i.e., other isoforms were not present. Scrutiny of the peptide maps suggests that they are in general compatible with the presence of a mixture of normal adult and posthatch isoforms. These investigators, however, found only a single sequence containing 3-methylhistidine in dystrophic myosin. The sequence differed from that of normal chickens in two positions out of 31, Glu at position 11 in the normal being changed to Gln, and Met at position 13 being changed to Ile. The reason for the discrepancy between this study and the present work is unclear.

The presence of the isoforms of 5-day posthatch myosin in avian dystrophic muscle resembles in some ways the situation in Duchenne dystrophy (Fitzsimmons & Hoh, 1981). Here isoforms characteristic of fetal and neonatal myosin, usually absent from 1 month after birth, were routinely detected. In Duchenne dystrophy, however, the amount of these isoforms was quite variable from patient to patient and did not exceed 12% of the total myosin. A possible explanation for their origin might thus be the presence of regenerating fibers. In avian dystrophy the proportion of developmental isoforms was consistently high (preparations from seven birds).

The results are consistent with the hypothesis that myosin in avian dystrophy belongs in the group of contractile proteins comprising tropomyosin, troponin C, and the LC1/LC3 pair which fail to complete their usual developmental isoform transitions (Obinata et al., 1980; Mikasa et al., 1981; Feit & Domke, 1982). The maturation problems seems not to be limited to contractile proteins since a similar phenomenon occurs with enzymes of energy metabolism (Cosmos, 1966; Cosmos & Butler, 1967). In dystrophic fast white muscle fibers the normal development change from an oxidative to a glycolytic metbolism is incomplete. Dystrophic fibers possess a heterogeneous response to histochemical stains for succinate dehydrogenase and phosphorylase, a finding present in normal birds only in the transitional stage after hatching.

The genetic defect underlying these observations remains unknown, as does the particular event leading to the deterioration and destruction of the muscle fiber. Persistence of the posthatch myosin isoform per se would appear not to be responsible, since an essentially benign condition involving the presence of human neonatal myosin has been described (Fitzsimmons & Hoh, 1982). While the disease is manifested as a muscle disorder, origins outside the muscle should not be ruled out; for example, thymectomy of normal chickens has been found to arrest the developmental metabolic changes in normal chickens in a way simulating that found in avian dystrophy (Cosmos et al., 1977).

ACKNOWLEDGMENTS

We acknowledge the careful technical assistance of Patti Yao and thank Ellen Factor Levy for photographic reproductions.

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