Purification of Myosin Heavy Chain Isoforms by Electroendosmotic Preparative Gel Electrophoresis: Characterization of Embryonic Slow Myosin Heavy Chain

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Abstract

Myosin is a hexapolypeptide constituted by four light and two heavy chains the isoforms of which segregate differently in specific stages of animal development and in different fiber types in adulthood. In mammals the Myosin Heavy Chains (MHC) are polypeptides with a molecular mass of about 200 KDa which isoforms can be identified by SDS PAGE and/or immunochemistry. A method for the purification of myosin heavy chain isoforms using a SDS Electrendosmotic Preparative Gel Electrophoresis (SDS EPGE) is described. Semplicity and reproducibility of this approach permits purification of single isoforms from complex mixture with a sufficient high recovery to perform immunochemical or biochemical studies. The possibility to apply useful tool as SDS removal and protein concentration by KDS precipitation before further analysis on the sample is described. Application of the methodology to the study of slow type embryonic MHC by enzymatic as well as chemical peptide mapping and amino acid composition is described.

Key words: EPGE, KDS-precipitation, MHC isoforms, peptide mapping, amino acid analysis, muscle.

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Several Myosin Heavy Chain (MHC) with distinctive fiber types specific and developmental stage-specific distribution have been identified in skeletal muscle [19]. In mammals an embryonic MHC isoform is initially expressed, later this isoform declines being replaced by a neonatal isoform which is then replaced by the adult isoforms. Type 1 (slow-twitch) and type 2 (fast-twitch) fibers are known to contain different MHCs, which are responsible for the different myosin ATPase activity and speed of shortening of the fiber types. Three subpopolation of type 2 skeletal muscle fibers, referred to as type 2A, 2B and 2X/d, have been described in rat skeletal muscles using anti-MHC mAbs [18] and accordingly three type of fast MHC isoforms have been identified by electrophoretic and immunoblotting analyses [1, 5, 11]. Type 2A, 2X/d and 2B MHCs, as well as the 1/slow (type 1) MHC present in slow skeletal muscle fibers and ventricular myocardium, are coded by distinct genes. In addition to α-cardiac MHC, the presence of different genes encoding for multiple slow MHC is referred by some studies. Two reports describe two α-cardiac MHC in humans. Others using monoclonal antibody characterization demonstrate a difference in epitopes distribution between three stage-specific slow MHC during late embryonic life [10].

The need of MHC isolation in structural/immunological studies and to perform amino acid sequence analysis is clearly understood, and continually call for improved and cheap methods. Polyacrilamide gel electrophoresis is commonly used for its high resolution power, but the low capacity of most system, the variable recovery of proteins from gel matrix and the low resolution of some preparative gel electrophoresis limit the practical application as a preparative method. A preparative gel electrophoresis system based on elution of proteins plates by electroendosmosis, which is the backward flow occurring between the electrodes during electrophoresis, has been proposed. This paper describe an efficient purification of MHC from adult and embryonic muscles. The use of SDS removal and protein concentration by KDS precipitation before further analysis is described.

Materials and Methods

Myosin

Myosin was extracted essentially according to Carraro et al. [6] with the following specifications. All procedures were performed at 2-4°C. Muscle tissue was homogenized with a Polytron apparatus (PT 10 0D) in 50 mM KCl containing 10 mM EGTA, 30 mM Pepstatin, 12 mM phenylmethylsulfonylfluoride (PMSF) and 1mM benzamidine (Sol A) to reduce effects of endogenous proteases. After centrifugation at 650 x g for 10 minutes, the pellet was resuspended and centrifuged in Sol A three or more times until the supernatant became clear. Myosin was then solubilized for 20 minutes in 0.3 M KCl, 0.15 M potassium phosphate pH 6.5, 10 mM Mg acetate, containing 10 mM EGTA, 30 mM pepstatin, 12 mM PMSF, 1mM benzamidine (Sol B) to which 5 mM ATP was added before using. We used approximately 25 ml of Sol B for 1 g of original fresh muscle tissue. Samples were centrifuged at 150.000 x g for 4 hours. The final supernatant was dialyzed for 4-12 hours in 1 mM Tris-HCl pH 7.2, 10 mM KCl, 0.1 mM dithiothreitol and 5 mM EGTA. Myosin was collected by centrifugation at low speed, dissolved in 0.5 M NaCl, 50% v:v glycerol and stored at -20°C.

SDS Electroendosmotic preparative gel electrophoresis (SDS EPGE)

Aliquots of stored myosin were precipitated by 10-fold dilution in ice cold distilled water and centrifugation at 650 x g for 10 minutes. The pellet was resuspended in 10% v:v glycerol, 2.3% SDS, 5% 2-mercaptoethanol, 62.5 mM Tris-HCl pH 6.8 (Sol C). For preparative separation of MHC we used the following conditions: EPGE was performed as described in Curioni et al. [8] using the EPGE system produced by Elettrofor S.a.S. of Dr M. Ruggero, Rovigo, Italy. The cylindrical gel, 2 cm in diameter and 6.7 cm in height, was made of 11 ml of 7% polyacrylamide separating gel and 10 ml of 3.8% polyacrylamide stacking gel. One mg of myosin was loaded over the gel in a volume up to 2 ml of Sol C containing 100 ml of bromophenol blue to dye the electrophoretic front. A constant current of 10 mA was applied. After 8-10 hours, when the electrophoretic front has reached the bottom end of the gel, the effluent was collected in fractions lasting 12 minutes (about 2 ml). MHC eluted from the gel during the following eight hours. Due to their low extinction coefficient, MHC were identified by analytical SDS PAGE. Fractions containing the eluted MHC were pooled and the MHC recovered by KDS-protein precipitation.

High resolution SDS PAGE of MHC

Analytical SDS PAGE of MHC was performed on 7% polyacrylamide slabs 0.75 mm x 130 mm x 130 mm [4]. Slabs were prepared according to Laemmli [12], but 37.5% v:v glycerol was present in the separating and stacking gels [9]. Electrophoretic buffer was: 50 mM Tris-HCl, 384 mM glycine pH 8.3 and 0.2% SDS. The stacking gel was prepared by mixing 0.8 ml of 29.2% acrylamide 0.8% bisacrylamide in 50% glycerol, 1.5 ml 0.5M Tris-HCl pH 6.8 0.4% SDS, 3.7 ml 50% glycerol (37.5% glycerol, 4% Acrilamide-bis (36.5:1), 125 mM Tris-HCl (pH 6.8), 0.1% SDS final concentration) 40 ml 10% ammonium persulfate, 10 ml tetramethylenediamine. The separating gel was prepared by mixing 3.75 ml of 29.2% acrylamide 0.8% bisacrylamide in 50% glycerol, 3.75 ml 1.5M Tris-HCl pH 8.8 0.4% SDS, 7.5 ml 50% glycerol (37.5% glycerol, 7% Acrilamide-bis (36.5:1), 375 mM Tris-HCl (pH 8.8), 0.1% SDS final concentration) 75 ml 10% ammonium persulfate, 20 ml tetramethylenediamine. Separation of MHC was achieved using a constant current mode at 4 mA per slab, corresponding to the voltage of about 40 Volts. Buffer was changed twice during the run restarting the electrophoresis with the above current value. Usually gel electrophoresis started at late morning and the buffer was changed at late afternoon and next day early morning. After 24 hours of electrophoresis the voltage rose to 150-170 Volts and the run was stopped. When bands contained more than 0.2 mg of protein the slabs were stained with 0.1% Coomassie blue in 5% acetic acid, 40% methanol and destained with 40% methanol 7% acetic acid. If bands contained less than 0.1 mg of protein the slabs were stained with the silver method [13].

KDS-Protein Precipitation.

The procedure of KDS-protein precipitation was performed as described [7, 14] with the following modifications. Briefly: add to the eluted fractions NaOH 1N to reach pH 12, at room temperature add KCl to a final concentration of 180 mM, centrifuge at 650g for 15 min at 2-4°C and separate the supernatant with a Pasteur . Add ice-cold trichloroacetic acid (TCA) to lower the pH to less than 2 (usually to a final concentration of 10% TCA). Keep the acidified solution on ice for 10 min and then add 2 M KCl to a final concentration of 180 mM. Centrifuge in a conical glass test tube at 650g for 15 min at 2-4°C. Remove the supernatant. KDS-protein pellets were resuspended with 10% glycerol, 62.5 mM Tris-HCl, pH 6.8 (Sol D) to a final volume of 200-300 ml. Protein concentration was determined according to Sandri et al.[16], that is, by SDS PAGE and densitometry using MHC standards subjected to electrophoresis in the same slab. The KDS-precipitation resulted in a 100 fold increase in protein concentration of the EPGE fractions, i.e., to about 1 mg/ml.

Quantitative Densitometry of SDS PAGE electrophoretograms

SDS PAGE slabs were scanned in a GS 300 Transmittance-Reflectance Scanning Densitometer (Hoefer

Scientific Instruments) connected to a Macintosh SE (Apple Computer). Data were processed using the GS-370 Data System for the Hoefer GS 300 Scanning Densitometer, Macintosh version. The slabs were scanned both along the electrophoretic migration and along the axis perpendicular to the direction of the electrophoretic migration. Densitometric values of MHC standards were linear between 0.25-10 mg of protein.

Peptide mapping by steady-state enzymatic proteolysis

Enzymatic peptide mapping of EPGE-purified MHC1 was performed using as proteolytic agents S-V8 protease or a-Chymotrypsin. Aliquots of 5 mg of MHC (3-5 ml of KDS concentrated MHC) were digested at 37°C adding 80 ml of 100 mM NH4HCO3 pH 7.8. Proteolysis was performed at different substrate/protease ratios, i.e. the enzyme to protein ratios varied from 1/20 to 1/1 w/w. After 15 minutes at 37°C, proteolysis was stopped by adding 25 ml of 2.3% w/v SDS and 5% v/v 2-mercaptoethanol 10% glycerol 62.5 mM Tris-HCl (pH 6.8) and boiling for 2 minutes. The digests were subjected to SDS-PAGE on 8% polyacrylamide slabs (0.75 mm x 130 mm x 130mm). Slabs were prepared according to Talmadge et al. [23] but the electrophoretic run was performed using constant current mode at 5 mA for 16-18 hours at 18-20°C. Slabs were stained with the silver technique [12].

Peptide mapping by chemical proteolysis

Peptide mapping of MHC1 was performed using hydroxylamine with the following modification [17]: 1 mg aliquots of MHC 1 were dissolved in 100 ml of Sol. C and after addition of 200 ml of the reaction buffer, incubated at 40°C; the chemical reaction was stopped after 4 hours by dialysis against water for 1 hour then against a solution of 0.05% SDS, 96 mM glycine 12.5 mM Tris-HCl for 1 hour. After KDS protein precipitation, the digest was subjected to electrophoresis as described for enzymatic peptide mapping.

Amino acid analysis

The second pellets after KDS protein precipitation were washed twice with 5 ml of 2 M KCL, pH 1 (acidified with TCA). After each washing samples were centrifuged at 650g for 10 min at 2-4°C. The final pellets were dissolved in 1 ml of 6 N HCl and hydrolyzed at 110°C for 24 h in N2 atmosphere. The HCl was removed by evaporation in a speed-Vac concentrator and the residue containing amino acids was reconstituted in 300 ml of loading buffer (Buffer-S, Beckman). A 50 ml aliquot was loaded on a System 6300 Bekman amino acid analyzer equipped with a Na high performance ion exchange column. The amino acid were separated by using a buffer elution program (Buffer Na-E, -F, -D) corresponding to the expanded hydrolyzate method I, as indicated by the manufacturer, and revealed by reaction with ninhydrin. Quantitation was carried out by an external standard method using a standard calibration mixture (Pierce) containing 5 nM of each amino acid (25 nM for PRO and HYP).

Results

The typical SDS PAGE analysis of 1 mg proteins eluted during a preparative electrophoresis of a crude homogenate of an adult rat EDL muscle is shown in figure 1. Lane 1 shows the fraction containing pure actin; after numerous fractions in which desmin, a-actinin, albumin are contained, MHC emerge from the gel after 10-15 h in a pure form; lane 18 shows the partially purified MHC2B, while in successive lanes starts the elution of MHC2X. Purification of MHC isoforms from prepurified myosins is shown in figure 2. High resolution SDS-PAGE of fractions from SDS EPGE of adult skeletal muscle myosin demonstrate that the different MHC can be collected in pure fractions; note isolation of MHC2A in lane 7. MHC2X and MHC2B are eluted by SDS EPGE of EDL myosin, while MHC2A and MHC1 are prepared from soleus myosin. The a-cardiac MHC from ventricular myosin has also been purified (result not shown). Identity of the MHC bands in SDS-PAGE was confirmed by Western blot analysis in a previous study [15]. The slow type myosin heavy chain (MHC 1) runs well ahead of the fast/embryonic MHC. MHC1 represents over 90% of total MHC in soleus myosin, while a band with identical mobility is present in neonatal and embryonic myosins preparations at less than 10% concentrations of total MHC protein. Figure 3 shows that the EPGE fractions contain different proportion of the MHC isolated from myosin of 18 days embryo. Note in lane 2 the purified MHC1, while in the successive fractions the MHCemb appears in increasing proportion, lanes 3-5, and then isolated (lanes 11-12).

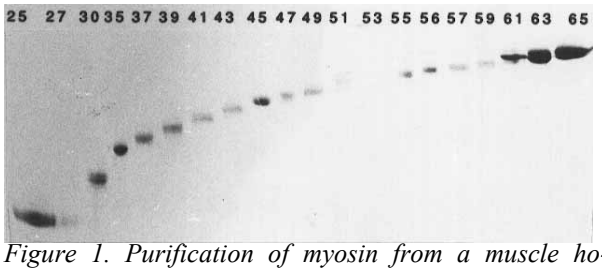

Figure 1. Purification of myosin from a muscle homogenate. Aliquots of fractions from preparative electrophoresis were analyzed on 7% SDS-PAGE and protein was detected by Coomassie blue staining. Lanes 18-20 show the pure myosin heavy chain fractions ,in fraction 61 starts the elution of MHC2B. Lane 13 represents fraction 53 which contains no protein.

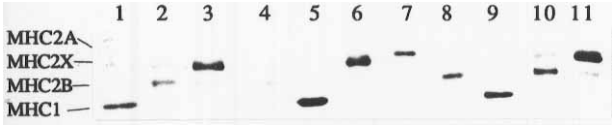

Figure 2. Purification of adult MHC by EPGE. Fractions containing the pure MHC isoforms were subjected to high resolution glycerol/SDS-PAGE (7% polyacrylamide) and proteins were detected by Silver staining. Lane 1-3, 50ng myosin marker extracted from adult rat soleus, EDL and tongue respectively; lane 4, MHC2B; lane 5, MHC1; lane 6 MHC2X; lane 7 MHC2A; lane8 MHC2B. Lane 9-11, 100ng myosin marker extracted from

Fractions containing the purified MHC1 from several EPGE are pooled, usually in a final volume of 10-20 ml, and the MHC are concentrated by the KDS-protein precipitation method. The pellets of concentrated MHC are resuspended in 200-300 ml of sol D at a concentration of about 1 mg/ml, or are treated with 6 N HCl for amino acid analysis. Actual concentration of the purified MHC is determined by SDS PAGE and densitometry. Figure 4A shows peptide pattern of MHC1 from soleus and from embryo using the S-V8 protease at protein/protease ratio of 1:1, 1:5, 1:10 and 1:20 w/w. At high ratio all the MHC is digested. Lowering the S-V8 concentration, the pattern become more complex and shifts at high MW. The peptide analyses are performed in condition of high MW peptide production since smaller peptides produced by digestion of the three MHC isoforms at low protein/protease ratio are very similar, if not identical. When 5 mg of MHC1 from embryonic myosin are digested with S-V8 protease, at any protein/protease ratio, the peptide pattern is peculiarly different from that of soleus MHC1 and a-cardiac MHC. Differences in the pattern of cleavage using S-V8 between MHC1 from embryonic myosin and that from soleus is confirmed by codigestion experiments while MHC1 and a-cardiac MHC codigestion give almost identical patterns (data not shown). Figure 4B shows the enzymatic cleavage of the MHC by a-chymotrypsin at enzyme/protein ratio of 1:1, 1:5, 1:10, 1:20. The peptide patterns of MHC1 from soleus and from embryo are very similar except for two peptides (compare lane 2 with lane 10). Codigestion of soleus and embryonic MHC1 confirms the presence of the two peptides (arrows in figure 4 B). On the contrary the codigestion of soleus MHC1 and a-cardiac MHC shows a peptide pattern identical to that of the individual digestions (results not shown).

The patterns of the chemical digestion of the three MHC with hydroxylamine are presented in Figure 5. Hydroxylamine recognizes the Asparagine-Glycine bonds which are present in position 65-66 , 696-697 and 1284-1285 in the adult MHC1. When we treated 1 mg of MHC1 according to Sandri el al. [17] four peptides of

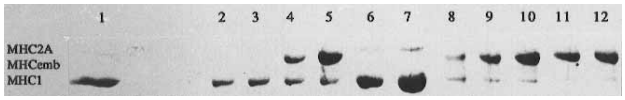

Figure 3. Purification of embryo MHC by EPGE. Aliquots of purified MHC were subjected to high resolution glycerol/SDS-PAGE (7% polyacrylamide). Coomassie blue stain. Lane 1, 2 mg myosin marker extracted from adult soleus; lane 2-5, successive EPGE fractions of embryonic MHC after 7 hours; lane 6-7, 2-3 mg myosin marker purified from adult soleus; lane 8-12, successive EPGE fractions of embryonic MHC after 8 hours. Note the purified slow and fast forms of embryonic proteins at the beginning and at the end of the electroendosmotic elution of MHC.

70-65-60-6 kDa, are produced from all MHC1; the higher Mw peptides were identically produced. Ahead of the MHC1 band few additional bands are present; they are not products of the chemical digestion since they were present in the protein preparations. These polypeptides are products of "spontaneous" degradation of MHC during freezing, thawing and storage.

Amino acid analysis on 5-10 mg of adult MHC1, a-cardiac MHC and MHC1 from embryonic muscle after SDS/glycine/Tris removal and protein concentration are performed. The chromatographic analysis give amino acid concentration, since adult MHC1 sequence is known the concentration of single amino acid is calculated. In table 1 the ratio between amino acid concentration calculated from sequence and amino acid measured is shown.

Discussion

Electroendosmotic Preparative Gel Electrophoresis

High resolution SDS PAGE is the only approach for separation of the myosin heavy chain family [15]. Electroendosmotic Preparative Gel Electrophoresis permits to purify the individual isoforms and to process them for structural studies. SDS EPGE takes advantage of the discriminating power of SDS-PAGE combined with the elution of MHC bands by the backward electroendosmotic flow occurring between the electrodes of EPGE. Purification of MHC depends on their absolute and relative amount in complex mixtures, on polyacrylamide concentration and on buffer ionic strength which affects MHC separation and flow rate. The volume of fractions also influences protein purification and concentration, as described in Curioni et al. [8]. The SDS PAGE permits to purify significant amounts of individual isoforms of MHC. Recovery of MHC 1 is excellent (near 100%) when prepared from the soleus and cardiac myosins. The MHC1 band present in the embryonic myosin is purified with more difficulty due to its relative low content (less than 10%). In several EPGE runs of embryonic

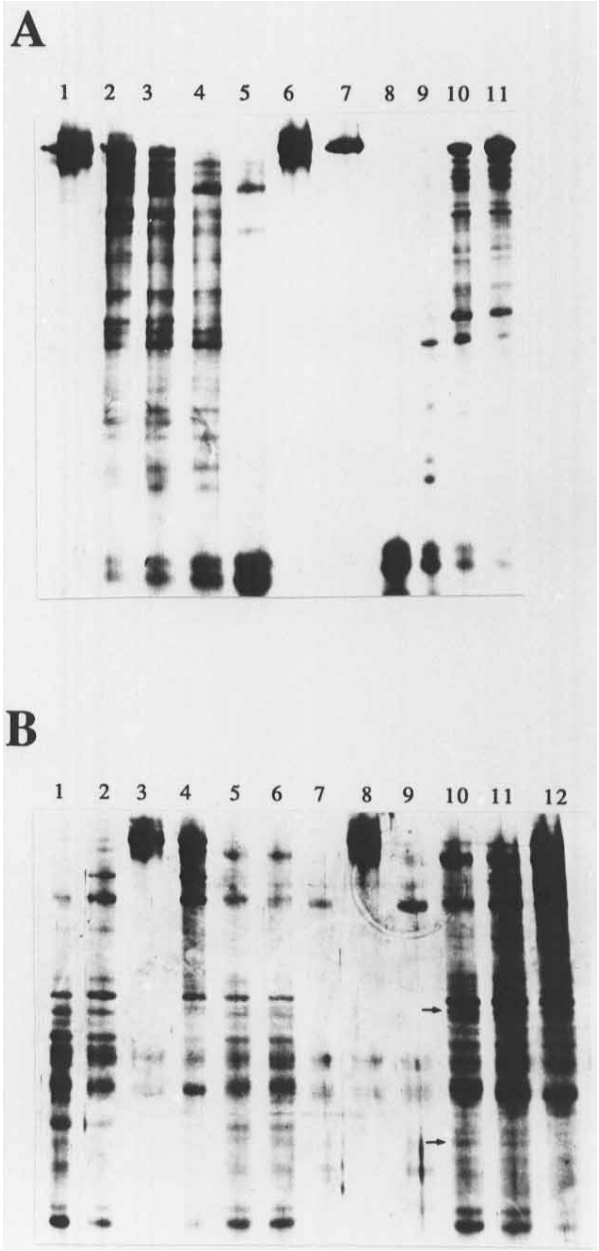

Figure 4. (A) S-V8 Steady-State Peptide Mapping of MHC 1. The purified MHC 1 preparations were digested with S-V8 protease for 15 min at 37°C, then subjected to SDS 8% PAGE and silver-stained. A, lanes 1 and 6, MHC 1 purified from embryonic muscle before S-V8 addition. Lanes 2-5 S-V8 digest of MHC 1emb at enzyme/protein ratio of 1/20,1/10,1/5 and 1/1, respectively. Lane 7, MHC1 purified from soleus muscle before S-V8 addition. Lanes 8-11 S-V8 digest of soleus muscle MHC 1 at enzyme/protein ratios of 1/1,1/5,1/10 and 1/20 respectively. Note that the peptide patterns of embryo MHC1 and soleus muscle MHC1 differ regardless of the protease/protein ratios employed. (B) a-Chymotryptic Steady-State Peptide Mapping of MHC 1. The purified MHC 1 were digested with a-chymotrypsin for 15 min at 37°C and then, electrophoresed on a SDS 8% PAGE and silver stained. Lanes 1-2 the a-chymotrypic digest of soleus MHC1 at enzyme/protein ratio of 1/10,1/20 respectively. Lane 3, MHC1 purified from soleus. Lanes 4-7 a-chymotrypic codigests of soleus MHC 1 and MHC 1 from embryo at enzyme/protein ratios of 1/20,1/10,1/5,1/1 respectively. Lane 8, embryo MHC 1 purified before a-chymotrypsin addition. Lanes 9-12 a-chymotrypic digests of embryo MHC 1 at enzyme/protein ratios of 1/1,1/5,1/10,1/20 respectively. Note that the peptides which distinguish chymotryptic pattern of embryo MHC 1 from adult MHC 1 are present also in the codigestion analyses.

myosin only a few fractions contain pure MHC 1, the large majority being contaminated by the most abundant MHCemb, indeed recovery decreases around 25%. In any case MHC1 from embryonic muscle are easily concentrated from fractions of several electroendosmotic runs by the KDS-protein precipitation method [14]. Recovery of fast isoforms is good for MHC2B (around 40%) and MHC2X (around 30%) when prepared from EDL myosin. Purification of MHC2A from soleus myosin needs an approach similar to that employed for purification of embryonic MHC1 due to its low content in soleus muscle (less than 10%) that is their proportion is increased by a first EPGE run, and they are purify to homogeneity with a second EPGE.

Isolated isoforms may be processed by methods as hydroxylamine reaction and amino acid analysis which are deeply influenced by glycine, Tris and SDS. The overall procedures were tested using MHC1 from cardiac and adult soleus or embryonic skeletal muscle. The latter comigrate also in SDS PAGE and question is open if the three proteins derived from a unique gene. With the modifications described in the methods we are able to remove SDS and solutes by the KDS method. Removal of the interfering solutes permits to obtain the amino acid composition of MHC1 expected from their known gene sequence, table 1. Glycine was always slightly overestimated due to the high amount present in the elution buffer. More exhaustive washing with 2M KCl pH1 eliminates the influence of undesired solutes but reduce protein recovery. Washing with absolute ethanol and ether even more reduces protein recovery and glycine overestimation. Overall errors due to MHC purification, recovery method and chromatographic separation of amino acid were assessed using these MHC which sequence is known at gene level. The expected to measured ratios are for all the amino acid near to unit with low SEM, in the case of MHC1 and a-cardiac MHC. We conclude that the method is useful not only for separa-

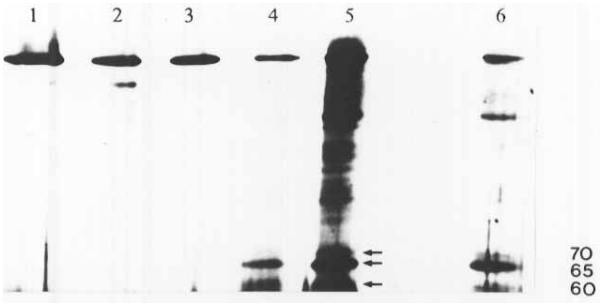

Figure 5. Hydroxylamine Peptide patterns of MHC 1. The purified MHC were digested with hydroxylamine for 4 hours at 40°C , KDS precipitated, analyzed on a SDS 8% PAGE and silver stained. Lane 1, purified a-cardiac MHC before digestion 1 mg; lane 2, embryo MHC 1 before digestion 1 mg; lane 3, soleus MHC 1 before digestion 1 mg; lanes 4-5-6 digests of a-cardiac MHC, embryo MHC 1 and soleus MHC 1 respectively 1 mg each. Peptide patterns are identical suggesting that the embryo MHC 1 and adult MHC 1 have identical primary sequences at the Asparagine-Glycine bonds at positions 65, 696 and 1284.

tion of MHC in amounts useful for their use as immunogens, but also to determine their amino acid composition.

Slow type myosin heavy chain family

Reports based on immunochemical analysis suggest that at least tree different MHC 1 appear during development in rat and human [10]. In a previous study we confirm that an additional mAb (BA-D5), which recognizes a-cardiac MHC and MHC 1, poorly reacts with the MHC 1 band present in late embryonic/neonatal myosins [2]. The MHC 1 of embryonic muscle is not the a-cardiac MHC, since we previously reported that this band is also negative after reaction with an anti-a-cardiac MHC monoclonal.

To obtain reliable results in enzymatic peptide mapping a stringent control on digestion conditions is essential, in particular when denatured polypeptides are used as substrates, which the case of MHC, since SDS PAGE is the only method which allows separation and preparation of individual isoforms. Using the KDS-precipitations few microliters of resuspended MHC samples are used in the digestion mixture, minimizing inhomogeneities among samples that could influence enzymatic activity and its specificity. To obtain good results KDS content and pH must be carefully controlled. In any case we performed codigestion experiments to be sure that differences in the polypeptide patterns among MHC are due to their primary sequence, not to amounts of interfering compounds. The difference in protein amount shown in gel slabs is mainly due to a differential evaporation of the buffer during the post digestion boiling step. As to the differences between adult and embryo MHC1 we conclude that: 1) Asparagine-Glycine bonds are conserved since Hydroxylamine digestion produces identical peptide patterns; 2) the two polypeptides differ in Glutamic and Aspartic acid content or in amino acid composition near the cleavege site since S-V8 protease produces different patterns. 3) Differences in hydrophobic groups are minimal since the pattern produced by a-Chymotrypsin differs only in two peptides. These conclusions are in keeping with the amino acid analysis which shows that amino acid composition differs mainly in serine, treonine, arginine and much less in hydrophobic groups. This could explain the different peptide pattern with S-V8 protease. Indeed its rate is affected by amino acid composition near Aspartic and Glutamic residues, that is the reaction is faster when serine or other small amino acids are present, slower when large hydrophobic residues occupy the sites near the cleavege point . After short incubation time and at low protein/protease ratio the different cleavege rate is shown by a different peptide pattern. Instead increasing protease concentration all the sites (more than 300) are attached resulting in an identical pattern of very small peptide. Structural informations are obtained by comparing the peptides of MW higher than 100 kDas. The S-V8 maps show difference in two major peptides which are in adult MHC1 of 170 and 130 KDas, while in MHC 1emb they are of 150 and 110 kDas. These peptides may only derive from removal of the N and/or C-terminal portions of MHC suggesting heterogeneity of different

Table 1. Reproducibility of amino acid composition after MHC elution expressed as ratio between the calculation of single amino acid concentration expected from sequence and the measurement observed from chromatography. Values are means ± SEM of values from four different MHC purification. Each purification were subjected to a duplicate hydrolysis and duplicate analyses for amino acid determination were performed.

Amino acid	Adult a-cardiac MHC 1	Embryo MHC1	Ratio
K	1.03 ± 0.07	1.00 ± 0.03	1.06
H	0.87 ± 0.03	0.97 ± 0.03	0.90
R	1.01 ± 0.03	1.02 ± 0.05	1.30
D	1.10 ± 0.10	1.10 ± 0.05	1.03
E	0.86 ± 0.03	0.87 ± 0.02	0.85
T	0.90 ± 0.02	0.84 ± 0.02	0.74
S	0.92 ± 0.03	0.97 ± 0.05	0.69
G	0.70 ± 0.10	0.70 ± 0.11	0.71
A	0.93 ± 0.03	0.98 ± 0.02	1.00
V	1.05 ± 0.05	1.05 ± 0.05	0.91
I	1.10 ± 0.05	1.10 ± 0.05	1.06
L	1.03 ± 0.03	1.04 ± 0.03	1.05
Y	1.06 ± 0.03	1.10 ± 0.08	1.10
F	1.07 ± 0.05	1.04 ± 0.02	0.99

MHC1 in these terminal regions.

In summery a reliable method to purify individual MHC from prepurified myosin or total muscle extracts using electroendosmotic preparative gel electrophoresis is here validated and after selective KDS precipitation of SDS-solubilized polypeptides its application as preparative method for protein analyses which are highly affected by interfering solutes is demonstrated. We confirm by an independent approach the presence of an embryonic MHC1 which differs in amino acid composition from adult type MHC1, but remains to be determined if the slow MHC isoforms are products of different genes or of alternative splicing of a common gene.

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