STRUCTURAL DIFFERENCES BETWEEN THE HEAVY CHAINS OF MYOSIN SUBFRAGMENT-1 FROM BOVINE, PORCINE AND HUMAN HEARTS

J. J. LEGER, C. KLOTZ, F. CAVAILLE and F. MAROTTE
INSERM U 127, Hopital Lariboisière, 41 Bd de la Chapelle, 75010 Paris, France

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1. Introduction

The detailed structural analysis of myosins from different muscles has led to the classification of myosin structures into four main groups corresponding to fast, slow or cardiac, smooth and embryonic muscles [1–3]. The myosin activities and the physiological performances of these four muscle types are very different and it is clear that these differences in structure and activity are related. Similar variations are observed between the activities of myosin from different hearts but few studies compare the structures of different cardiac myosins.

The majority of studies on myosin structure concern the light chains. The heavy chains with mol. wt 200 000 represent >80% of the whole molecule and the sites of myosin activity are located on their N-terminal portion. Two peptides, of mol. wt 9000, isolated, respectively, from the heavy chains of rabbit skeletal muscle myosin and of bovine cardiac myosin, have been sequenced and compared [4,5]. Other information on the structure of myosin heavy chains has been obtained after chemical cleavage at specific residues [2,6–8], after limited enzymatic hydrolysis [2,3], or using an immunological approach [9]. The above studies concern the whole myosin or intact heavy chains isolated from different muscles or from the same muscle in different pathological states.

Here an unfragmented portion of the myosin heavy chain corresponding to subfragment-1 and therefore to the active site of myosin is isolated from bovine, porcine and human hearts. These 90 000 mol. wt polypeptides are cleaved either chemically or enzymatically in the presence of SDS. The related peptides are analysed on polyacrylamide gels according to their molecular weight or to their charge. It is shown that the simultaneous use of both chemical and enzymatic cleavage of the same polypeptide chain provides complementary information on the differences and similarities of the cleaved structure. The one-dimensional analysis of the peptides obtained after cleavage of the three cardiac subfragment-1 heavy chains is sufficient to assess the existence of structural differences between these three homologous proteins.

2. Materials and methods

Myosin was isolated from the left ventricles of bovine, porcine and human hearts and from rabbit skeletal muscle as in [11]. The chymotryptic digestion of myosin was carried out using insoluble myosin [12]. The heavy chains of subfragment-1 were isolated by chromatography on Biogel A 15m (2 X 150 cm) in the presence of SDS at pH 7.0. The chromatographied heavy chains, if not immediately used, were concentrated by ammonium sulfate and stored at −20°C. The preparations were tested by polyacrylamide gel electrophoresis [13].

The heavy chain samples were dialysed against water, then either concentrated and treated with cyanogen bromide or dialysed against a buffer containing 0.4 M boric acid, 0.08 M Tris, 5 mM EDTA, 2% SDS, 0.2% 2-mercaptoethanol (pH 7.0) before proteolytic treatment. In this last case, each sample was adjusted to ~1.3 mg/ml, corresponding to A_{280} = 1 unit [12]. The samples were then treated at 100°C

for 2 min and proteolysed as described [10] at 37°C, for 2 h, with either chymothypsin (0.5 mg/ml) or papain (0.005 mg/ml). After chemical or enzymatic cleavage, the samples were desalted on Sephadex G-25 then dissolved either in SDS buffer [14] or in urea–Ampholine buffer [15] at 5 mg/ml.

The peptides were analysed on polyacrylamide gel by isoelectrofocusing and by electrophoresis in the presence of urea–SDS gels. The procedure of isoelectrofocusing in [15] was used with the following range of ampholines: 0.2 ml (pH 3–10); 0.2 ml (pH 4–6); 0.2 ml (pH 5–7); in 10 ml gel mixture. The procedure used for urea–SDS polyacrylamide gels was derived from that of [14]. An exponential gradient of both acrylamide and urea was obtained using a two-chamber gradient mixer [15]. The front chamber which has a constant volume contained 5 M urea and 12% acrylamide solution; the other chamber contained 7.8 M urea and an acrylamide solution variable between 18% and 24%. With convenient volumes of these two solutions, the gradient of urea in the separating gel (1.5 mm × 12 cm) lies between 5 and 7 M; the gradient of acrylamide lies between 12% and 18% for analysis of cyanogen bromide peptides and between 12% and 16% for analysis of other peptides. The stacking gel (1.5 mm × 3 cm) contained 5 M urea. All the acrylamide–urea solutions contained 1 mM EDTA and 5% glycerol. The buffers of the cathodic and anodic reservoirs were continuously exchanged during the running time (~20 h).

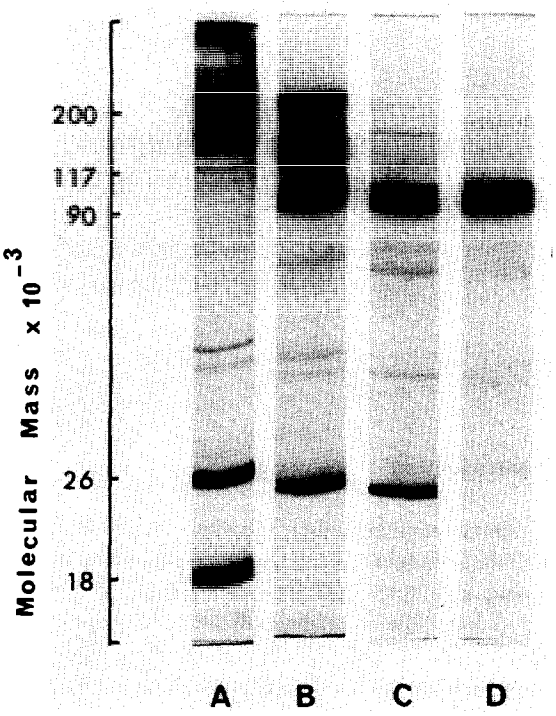


Fig.1. SDS–polyacrylamide gel electrophoresis [13]: (A) bovine cardiac myosin; (B) the same after 10 min digestion in 0.12 M NaCl, 1 mM EDTA with chymotrypsin at 0.1 mg/ml 25°C; (C) subfragment-1 soluble at low ionic strength; (D) heavy chain of subfragment-1, after Biogel A 15 m column in dissociating medium.

3. Results

The heavy chain of myosin subfragment-1 migrates as an unique band of mol. wt 90 000 after filtration of the dissociated subfragment-1 on agarose beads (fig.1). This purification step permits removal of the light chain and of some minor proteolytic products. The protease chymotrypsin was chosen in order to obtain an unfragmented portion of the whole myosin heavy chain. The monodispersity of this polypeptide is assessed by the fact that only one band is seen on SDS or SDS–urea gels. Isoelectricfocusing is of no use for this problem as the protein does not enter the gel in our experiments.

The heavy chains of myosin subfragment-1 from porcine, bovine and human hearts, cleaved either by cyanogen bromide or by two different proteases in the presence of SDS, give rise to different peptide maps as observed on polyacrylamide gels (fig.2,3). The peptide maps of the heavy chain of myosin subfragment-1 from rabbit skeletal muscle are also presented. Each map is a set of many bands corresponding each to one or more peptides; these bands are characterized by their molecular weight on SDS–urea gels, by their charge on isoelectricfocusing gels and by their capacity of fixing Coomassie blue. The observed bands are thus distributed in major and minor bands according to their intensity of staining.

It can be observed, in both gel systems, that the number of major bands produced after the three cleavage procedures is higher for cardiac than for the skeletal muscle samples. On SDS–gels, the distribution of the major bands according to molecular weight is

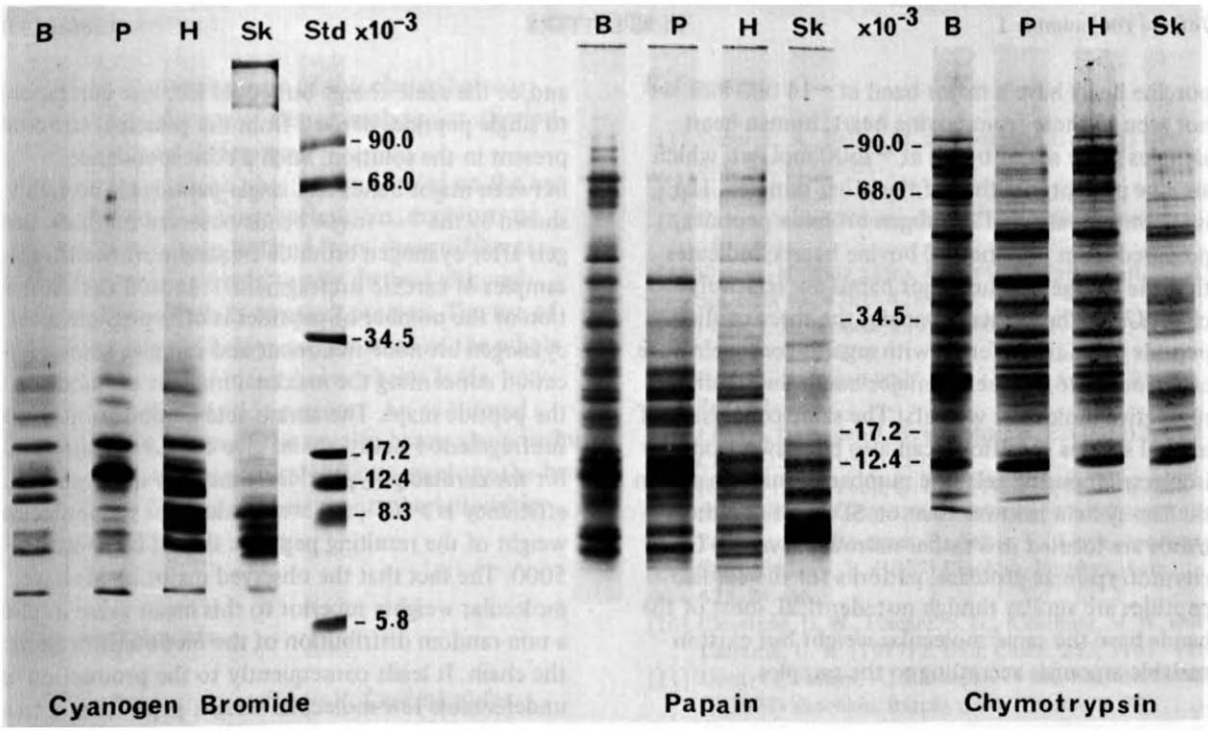
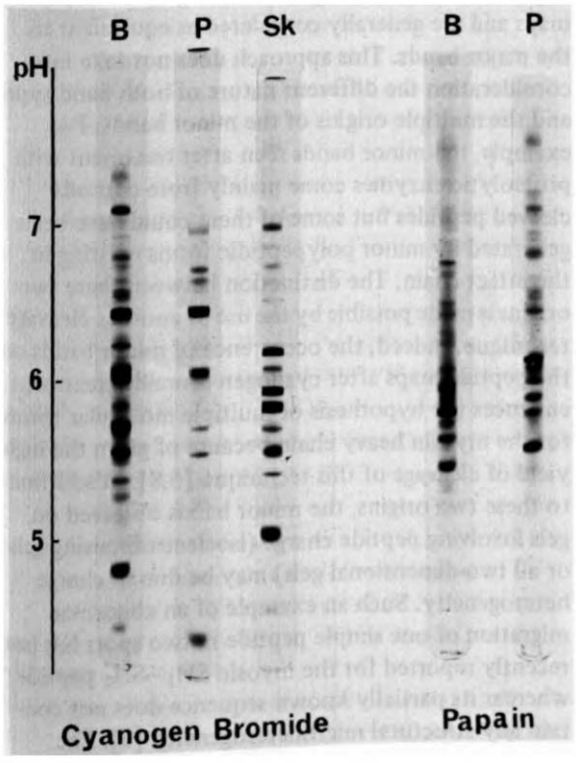


Fig.2. SDS–urea gels of peptides from the heavy chains of myosin subfragment-1 from bovine (B), porcine (P), human (H) hearts and from rabbit skeletal muscle (SK) obtained by digestion with cyanogen bromide, papain or chymotrypsin (see section 2). 50 μg sample well of each Std., different standards of molecular weight.



generally wider for cardiac than for the skeletal muscle samples. On isoelectricfocusing gels, the distribution of the major bands according to the charge varies with the muscle type. The skeletal peptides, obtained after cyanogen bromide treatment, are slightly more acidic than the equivalent cardiac peptides, as observed in fig.3. These observations confirm therefore the structural specificity of the myosin heavy chain according to muscle type.

The three cardiac subfragment-1 heavy chains, treated with cyanogen bromide, generate some well-defined major bands, whose number and the widths of distribution in molecular weight and charge are relatively close; however the values of the apparent molecular weights of the major bands are characteristic for each animal species. For example, samples from

Fig.3. Isoelectricfocusing gels of peptides from the heavy chains of myosin subfragment-1 from bovine (B) and porcine (P) hearts and from rabbit skeletal muscle (SK), obtained by digestion with cyanogen bromide or with papain (see section 2). 25 μg sample/tube.

porcine heart have a major band at ~14 000 mol. wt not seen in these from bovine heart; human heart samples show major bands at ~7500 mol. wt, which are not present in either of the other samples. The isoelectricfocusing of cyanogen bromide peptides, prepared from porcine and bovine hearts, indicates that the charges of the major bands are relatively close. Using the protease papain, the three cardiac peptide maps are different with regards to the cleavage efficiency, the number of major bands and their respective molecular weights. The same conclusion of animal species specificity can also be drawn from the isoelectricfocusing gels; the number of major bands in the last system is lower than on SDS gels and these bands are located in a rather narrow pH range. Using chymotrypsin as protease, patterns for the cardiac peptides are similar though not identical; most of the bands have the same molecular weight but exist in variable amounts according to the samples.

4. Discussion

The cleavage technique employed thus provides different information according to the number and the dimension of the peptides produced. Chymotryptic treatment induces a rather mild cleavage and the resulting peptides have a structure relatively close to that of the unfragmented polypeptide. The similarities between the three relatively close structures are thus observed. In contrast, cyanogen bromide breaks down the initial structure into many peptides of low molecular weight thus revealing some structural differences. The concentration of papain used here leads to an intermediate extent of cleavage.

The one-dimensional peptide maps of myosin heavy chains published present a large proportion of high molecular weight products some of which do not enter the gel [2,7]. They are not seen on the subfragment-1 peptide maps and are consequently due to the rod portion of the myosin heavy chains. Some of the observed large fractions could also be explained by the known properties of aggregabilities of this rod portion.

Major bands or major spots, as defined here, are clearly observed on all published one- or two-dimensional peptides maps. These bands could be generated by several peptides having the same molecular weight and/or the same charge but could likewise correspond to single peptides derived from the principal structure present in the solution. Such a correspondance between major bands and single peptides is probably shown by the 7–9 major bands observed on SDS–urea gels after cyanogen bromide treatment of the three samples of cardiac subfragment-1. Indeed the estimation of the number of peptides is only possible after cyanogen bromide treatment and can give some indication concerning the maximal number of bands on the peptide maps. The amino acid composition of the subfragment-1 heavy chains give 17–19 methionines for the cardiac polypeptides. Since the cleavage efficiency is >90%, the mean values for the molecular weight of the resulting peptides should be about 5000. The fact that the observed major bands have molecular weights superior to this mean value implies a non-random distribution of the methionines along the chain. It leads consequently to the production of undetectable low-molecular weight peptides and to a restricted number of detectable peptides. The 7–9 major bands seen in the cardiac samples probably correspond to such single peptides since in addition, the sum of their app. mol. wt is ~90 000.

Minor bands are also present in all published peptide maps and are generally considered as equivalent to the major bands. This approach does not take into consideration the different nature of both band types and the multiple origins of the minor bands. For example, the minor bands seen after treatment with proteolytic enzymes come mainly from partially cleaved peptides but some of them could have been generated by minor polypeptidic forms existing in the intact chain. The distinction between these two origins is made possible by the use of another cleavage technique. Indeed, the occurrence of minor bands on the peptide maps after cyanogen bromide treatment enhances the hypothesis of multiple molecular forms for the myosin heavy chain because of given the high yield of cleavage of this technique [6,8]. In addition to these two origins, the minor bands observed on gels involving peptide charge (isoelectrofocusing gels or all two-dimensional gels) may be due to charge heterogeneity. Such an example of an abnormal migration of one simple peptide in two spots has been recently reported for the myosin SH_1–SH_2 peptide whereas its partially known sequence does not contain any structural microheterogeneity [7]. The

possibly artefactual nature of this charge heterogeneity limits the use of charge analysis for discriminating structural differences.

In summary, these observations, based on the use of two different cleavage techniques, demonstrate that the heavy chain isolated from three different cardiac myosins are structurally distinct although homologous in their N-terminal portion. The use of half of the myosin heavy chain instead of the whole myosin molecule or intact heavy chains leads, however, to complex peptide patterns. An additional initial fragmentation of the myosin heavy chain is thus required to locate structural differences along the heavy chain and to determine their eventual relationship with differences in activity.

Acknowledgements

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