STRUCTURAL DIFFERENCES IN THE HEAVY CHAINS OF RAT VENTRICULAR MYOSIN ISOENZYMES

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Received 10 November 1978

1. Introduction

Intact myosin from the rat ventricular myocardium can be electrophoretically separated into 3 distinct components V_1, V_2 and V_3, in the order of decreasing Ca^{2+}-activated ATPase activity and mobility [1]. The relative proportions of these forms of myosin is a function of age and the thyroid state of the animal. Week 3–4 rats show only V_1 while those rendered hypothyroid show predominantly V_3. Previous studies on these 3 forms of myosin using SDS–gel electrophoresis showed that the molecular sizes of the 2 types of light chains from each component are the same. The number of light chains per heavy chain is also identical for these components suggesting structural differences may exist in the heavy chains. We have now studied the amino acid composition of the heavy chains and their CNBr-peptides using SDS–gel electrophoresis and two-dimensional peptide mapping. The results suggest that the 3 forms of ventricular myosin are isoenzymes which differ in heavy chain composition.

2. Experimental

2.1. *Materials*

Ventricular myosin with all 3 components was obtained from adult Wistar rats. V_1 was obtained from week 3–4 rats or adult rats made hyperthyroid by daily intraperitoneal injection of 100 μg triiodothyronine for 3 weeks. V_3 was obtained from adult rats rendered hypothyroid by:

(i) A single injection of 0.5 mCi ^{131}I intraperitoneally;

(ii) Surgical thyroidectomy 4–6 weeks before experiment.

Before use, a sample from each heart was tested by pyrophosphate gel electrophoresis to determine the distribution of myosin components.

2.2. *Preparation of* S-*carboxymethylated myosin heavy chains*

Myosin heavy chains for peptide mapping were prepared by gel filtration after reduction in denaturing solvent and *S*-carboxymethylation of the crude myosin extract. About 1 g tissue was homogenized with 3 ml 40 mM NaCl and 3 mM sodium phosphate buffer (pH 7.0). The homogenate was spun at 5000 rev./min in a refrigerated centrifuge for 20 min. The pellet was resuspended and repelleted in the same solution to remove soluble proteins. The pellet was then suspended for 1 h in a solution containing 0.1 M $Na_4P_2O_7$, 5 mM EGTA, 5 mM dithiothreitol (pH 8.6) to extract myosin. The mixture was centrifuged at 108 000 × *g* for 3 h. To the supernatant was added recrystallized urea to 8 M and 2-mercaptoethanol to final conc. 0.1 M. The mixture was bubbled with nitrogen and maintained at 50°C in a water bath for 3 h to denature and reduce the myosin. The mixture was cooled to room temperature and solid iodoacetic acid was added to final conc. 0.2 M. The pH was maintained at 8.5 by titrating with triethylamine. Light was excluded from the reaction mixture by wrapping the tube containing it with aluminium foil. Alkylation was terminated within 30 min by the addition of 2-fold excess of 2-mercaptoethanol.

S-carboxymethylated myosin heavy chains were

 Elsevier/North-Holland Biomedical Press

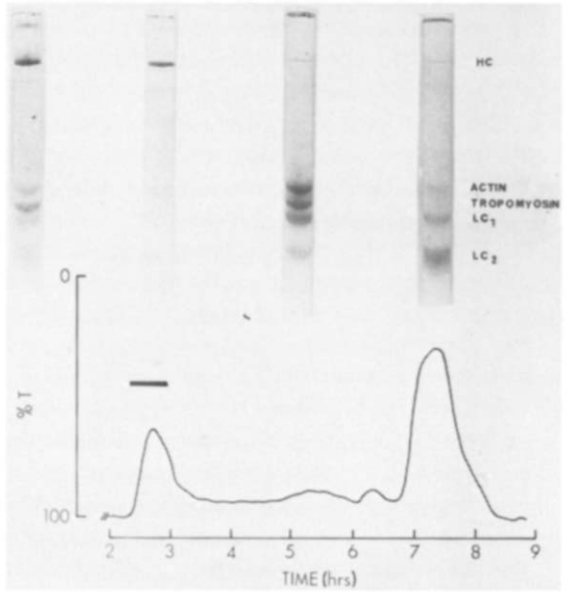

Fig.1. Sepharose 4B chromatography of *S*-carboxymethylated heavy chains of ventricular myosin in 6 M urea, 0.025 M Tris, 0.19 M glycine and 0.01% sodium azide. Flow rate, 20 ml/h. The myosin heavy chain (HC) appears in the first peak. The horizontal bar indicates the fractions pooled for heavy chain analyses. SDS–polyacrylamide gel electrophoretic analysis of this and other fractions are shown above the elution profile. The gel on the extreme left is that for the unchromatographed material.

purified by gel chromatography at 4°C through a column of Sepharose 4B equilibrated with 6 M urea in 0.025 M Tris, 0.19 M glycine and 0.01% (w/v) sodium azide. The urea (AR grade) solution used for preparing the column buffer was deionized by passing through an ion-exchange column. Figure 1 shows the elution profile. The first peak, which was virtually pure heavy chain as indicated by SDS–gel electrophoresis, was collected and dialysed for 48 h with 2 changes of 0.1% (v/v) acetic acid and 0.01% sodium azide at 4°C, then dried by rotary evaporation.

2.3. *Cyanogen bromide digestion*

For cyanogen bromide digestion [2] purified heavy chain was suspended in 2 ml 70% (v/v) formic acid. To this was added an equal volume of a freshly prepared solution of 0.2 M cyanogen bromide in 70% formic acid. The mixture was allowed to react in the dark at 4°C for 60 h, at the end of which 10 vol. distilled, deionized water was added and the peptides dried by rotary evaporation. The peptides were suspended in a solution (0.01 M sodium phosphate, 0.1% 2-mercaptoethanol, 8 M urea, 6% (w/v) sucrose, pH 7) for subsequent analyses.

2.4. *SDS–polyacrylamide gel electrophoresis*

One-dimensional peptide maps of myosin heavy chains were run in gels of 15% T, 3% C (notation from [3]) in 0.1% SDS in 50 mM sodium phosphate buffer, at pH 7.0, for 6 or 12 h with recirculation of buffer between upper and lower baths. Gels were cast in 6 mm (i.d.) × 7.0 cm tubes. Electrophoresis was carried out at room temperature at 80 V. Unhydrolysed myosin heavy chains were analysed in SDS–gels (fig.1) of 4% T, 3% C for 1 h. Gels were stained and scanned as in [4].

2.5. *Two-dimensional peptide mapping*

Peptides were separated by isoelectrofocussing in the first dimension and by SDS–gel electrophoresis in a gradient gel slab in the second dimension [5]. Peptides were focussed in 3 mm × 7 cm gels at 500 V for 12 h. The gradient gel slabs (3–27% acrylamide, measuring 7 cm × 7 cm × 3 mm, obtained from Gradipore, Sydney) were run for 6 h at 80 V in a 3 cell Gradipore electrophoresis unit. Gels were stained in 0.25% Coomassie brillant blue R for 1 h at 70°C and destained by diffusion.

2.6. *Amino acid analysis*

V_1 and V_3 heavy chains for amino acid analysis were prepared as in section 2.2 except that the *S*-carboxymethylation step was omitted. The myosin samples were hydrolysed in constant boiling HCl in an evacuated sealed tube at 110°C for 24 h. Amino acid analysis was done by the Spackman method [6] with a Jeol amino acid analyser model JLC-6AH.

3. Results

3.1. *Homogeneity of myosins used for heavy chain analysis*

V_1 was obtained uncontaminated by V_2 and V_3 in the 30 week 3–4 rats as well as the 15 triiodothyronine treated adult rats. Only a few of the 16 hypothyroid

rats showed exclusively V_3, but most of the others showed trace amounts of V_2 and V_1. Only hearts showing > 95% V_3 were used for V_3 heavy chain analyses.

3.2. *Amino acid composition of V_1 and V_3 heavy chains*

The amino acid compositions of V_1 and V_3 heavy chains are shown in table 1. Significant differences in the content of arginine and methionine are seen. The arginine content of V_3 is 15% higher than that of V_1. This difference, by reducing the net negative charge on V_3 relative to V_1 probably contribute significantly to the lower electrophoretic mobility of V_3 in pyrophosphate gels [1].

The profile of the amino acid composition for V_1 and V_3 heavy chains reported here is very similar to that in [7] for heavy chains of euthyroid and hypothyroid rat ventricular myosin heavy chains. These authors did not have sufficient data to demonstrate a difference in amino acid composition in their myosins. Furthermore, their euthyroid adult rat probably contained significant amounts of all 3 forms of myosin, as shown [1] and confirmed in this study.

Table 1

	V_1 – HC (mol/mol HC)	V_3 – HC (mol/mol HC)
Lys	159 ± 4	159 ± 5
His	32 ± 1	33 ± 1
Arg	99 ± 3	114 ± 3
Asp	174 ± 5	174 ± 3
Thr	83 ± 1	82 ± 1
Ser	94 ± 3	92 ± 2
Glu	309 ± 2	308 ± 3
Pro	49 ± 1	49 ± 3
Gly	99 ± 5	99 ± 5
Ala	153 ± 2	152 ± 1
Cys	29 ± 3	28 ± 1
Val	91 ± 3	89 ± 1
Met	50 ± 3	42 ± 1
Ile	78 ± 2	79 ± 1
Leu	182 ± 4	184 ± 2
Tyr	40 ± 1	39 ± 1
Phe	54 ± 1	54 ± 1

Amino acid composition of the heavy chains (HC) of V_1 and V_3 components of rat ventricular myosin. Values given are means ± SD of 4 determinations. Values for arginine ($2P < 0.001$) and methionine ($2P < 0.01$) are significantly different for V_1 and V_3 heavy chains using the *t*-test

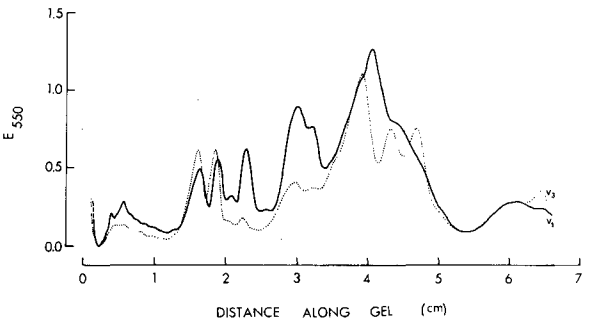

Fig.2. Densitometer profiles of CNBr-peptides of V_1 (continuous line) and V_3 (dotted line) heavy chains of ventricular myosin after electrophoresis for 6 h in SDS–polyacrylamide gels.

3.3. *One-dimensional maps of CNBr-peptides of V_1 and V_3 heavy chains*

Analysis of CNBr-peptides of V_1 and V_3 heavy chains in SDS–gels revealed significant differences between them. Figure 2 compares the profiles of these peptides revealing differences over a wide range of molecular size. The peptides at the large molecular weight end of the spectrum has been resolved further by a more-prolonged electrophoretic run. This is shown in fig.3, in which differences between V_1

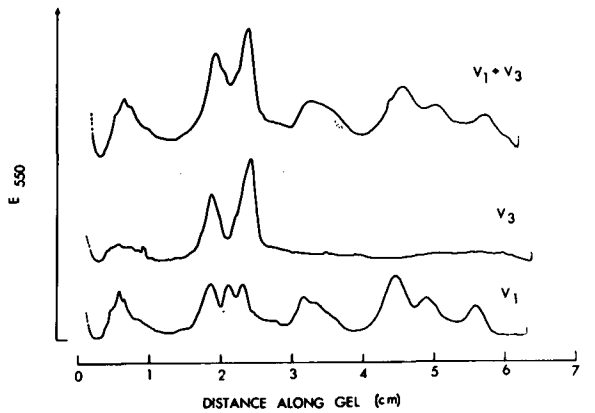

Fig.3. Densitometer profiles of CNBr-peptides of V_1 heavy chains, V_3 heavy chains and a mixture of these (V_1 + V_3) after electrophoresis for 12 h in SDS–polyacrylamide gels.

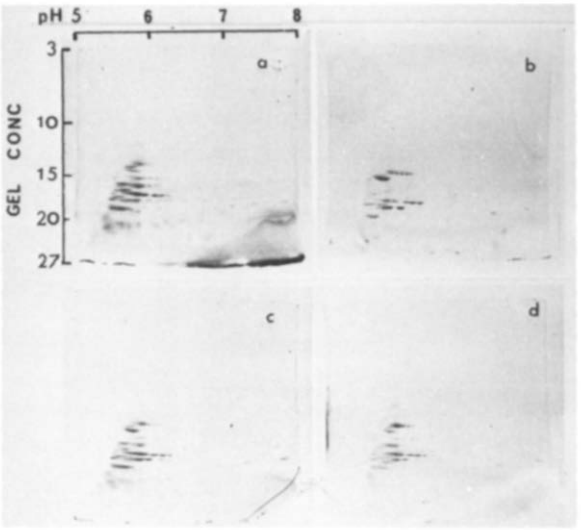

Fig.4. Two-dimensional CNBr-peptide maps of heavy chains of ventricular myosin. The first dimension is isoelectrofocussing and the second dimension is SDS–gradient gel electrophoresis. (a) V_1 heavy chain peptides, (b) V_3 heavy chain peptides, (c) mixture of V_1 and V_3 heavy chain peptides, (d) peptides from myosin with V_1, V_2 and V_3 components. Molecular weight markers ran in the gradient gel under similar conditions indicate that most of the peptides are between 14 000 dalton (21% gel) and 68 000 dalton (13% gel).

peptides and V_3 peptides are more clearly shown. These differences have been consistently observed in 3 different preparations of V_1 and V_3 heavy chains digested for 60 h. Other preparations, digested for 24 h and 48 h under the same conditions, showed essentially the same profiles.

3.4. *Two-dimensional CNBr-peptide maps of V_1 and V_3 heavy chains*

Figure 4a and 4b show, respectively, the two-dimensional CNBr-peptide maps of V_1 and V_3 heavy chains. These maps can be seen to differ significantly from each other. There appears to be significantly more spots in the V_1 map, and this correlates well with the significantly higher content of methionine in V_1 heavy chain compared with V_3 heavy chain. The characteristic differences between these maps have been consistently observed in 3 different peptide preparations of V_1 and V_3 heavy chains.

3.5. *Analysis of CNBr-peptides of V_1, V_2 and V_3 heavy chains*

Since it has not been possible to obtain V_2 heavy chains in sufficient quantities for peptide analysis, the heavy chain peptides from adult euthyroid rat ventricle, which has been verified to contain V_1, V_2 and V_3 in approximately equal proportions, were used. One-dimensional maps of two different mixed-heavy chain CNBr-peptide preparations show a profile indistinguishable from that of a mixture of V_1 and V_3 heavy chain peptides illustrated in fig.3. Two-dimensional maps of these 2 preparations of mixed-heavy chain peptides (fig.4d) are similar to those of a mixture of V_1 and V_3 heavy chain peptides (fig.4c). No spots can be seen in the former other than those present in the latter.

4. Discussion

The results of amino acid analysis and CNBr-peptide mapping of the heavy chains of V_1 and V_3 reveal structural differences between them. Peptide mapping of mixed (V_1, V_2 and V_3) heavy chains showed apparently no new peptides in addition to those in V_1 and V_3. These experiments establish that the three electrophoretically distinct forms of ventricular myosin are isoenzymes which differ in heavy chain structure. The simplest model of the heavy chain structure of these isoenzymes consistent with the present results would be $(HC_\alpha)_2$, $HC_\alpha HC_\beta$, $(HC_\beta)_2$ for V_1, V_2 and V_3, respectively, where HC_α and HC_β are the two structurally distinct heavy chains. Further characterization of these peptides are necessary to rule out the possibility that the heavy chains of isoenzymes V_1 and V_3 may be more heterogeneous than suggested by this model. The hybrid nature of V_2 is suggested by peptide mapping and by the intermediate nature of its electrophoretic mobility and Ca^{2+}-activated ATPase activity [1].

In the light of these results, the action of thyroid hormones in modulating ventricular isoenzyme distribution can be interpreted as an action on the expression of genes coding for ventricular myosin heavy chains: these hormones favour the expression of the gene coding for HC_α, while their absence favours the expression of the gene coding for HC_β.

Acknowledgements

This work was supported by grants from the National Heart Foundation and the National Health and Medical Research Council of Australia.

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