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The isomyosins of rabbit middle ear muscles

BY

I. MORARU Jr, G. BECKERS-BLEUKX (1) and G. MARÉCHAL (1)

(Victor Babes Institute, Splaiul Independentei, 76201 *Bucuresti, Romania* and (1) *Département de Physiologie, UCL 5540, B-1200 Bruxelles)*

(1 figure)

Isomyosins of the two middle ear muscles of rabbit have been separated by electrophoresis in non-dissociating conditions. Tensor tympani has four isomyosins called MM1 to MM4 by order of decreasing mobility. Stapedius has two isomyosins, similar to MM1 and MM4. MM1 and MM2 have the same mobility than FM2 and FM3, fast isomyosins observed in psoas. MM4 has the same mobility than IM, a slow isomyosin observed in extensor digitorum longus. MM3 is absent in soleus, psoas or extensor digitorum longus. Slow myosin (SM) of soleus and fast myosin (FM1) of psoas are not observed in middle ear muscles.

Introduction

Myosin, an hexameric molecule, exists in various isoforms that can be separated by electrophoresis in a solution which does not dissociate the molecule into its components (Hoh *et al.,* 1976; d'Albis *et al.,* 1979*a*; Maréchal *et al.,* 1984). Fast muscles contain three isomyosins with different mobilities (rabbit psoas, Srihari *et al.,* 1981), whilst slow muscles contain only one (rabbit soleus, Hoh & Yeoh, 1979). In this work we studied the isomyosins of middle ear muscles. As these muscles undergo continuous mechanical vibrations and contract by rapid reflexes in response to variations in loudness, we surmised that they might have adapted to this peculiar situation by expressing some specialized type of myosin. We have indeed observed that the type and distribution of isomyosins of middle ear muscles is very different from that observed either in psoas, or in soleus or in extensor digitorum longus.

Methods

Muscle preparation. — Seven rabbits of various origins approximately 3 kg in weight have been anaesthetized with ether and bleeded. After decapitation and removal of the lower jaw, the middle ear was opened. The tensor tympani and stapedius muscles were dissected under a binocular microscope equipped with remote controls for focus and magnification. Samples of soleus, psoas and extensor digitorum longus were also obtained. The muscles were frozen in liquid nitrogen and kept at $-90°C$ until use.

Myosin analysis. — A crude extract of muscle was made in a Guba-Straub buffer containing sodium pyrophosphate (MARÉCHAL *et al.*, 1984). It was electrophoresed under non-dissociating conditions (HOH *et al.*, 1976; d'ALBIS *et al.*, 1979). Myosin of a smooth muscle (taenia coli of guinea pig) was added to all samples. As smooth muscle myosin migrates faster than skeletal muscle isomyosins, it provides a reference zone for R_F measurements (MARÉCHAL *et al.*, 1984). The gels were scanned with a densitometer which allowed the computation of R_F values and relative surfaces of each peak using an interactive program (MARÉCHAL *et al.*, 1984).

Results

Figure 1 shows densitometric records of the isomyosins of the two middle ear muscles, stapedius and tensor tympani, as well as of soleus (a typical slow muscle) and of psoas (a typical fast muscle), the last two examples being shown for comparison purposes. The large signal at the left side of the densitograms is an optical artefact due to the entry of the reading beam of the scanner into the gel. The signal on the right side is due to the myosin of the smooth muscle taenia coli (guinea pig) which has been added to the samples. Soleus muscle (Fig. 1C) has a single isomyosin which migrates quite slowly. This observation is in agreement with an earlier work (HOH & YEOH, 1979). This isomyosin has been called SM (for slow myosin, HOH & YEOH, 1979). Psoas muscle (Fig. 1D) has three isomyosins. This finding is also in agreement

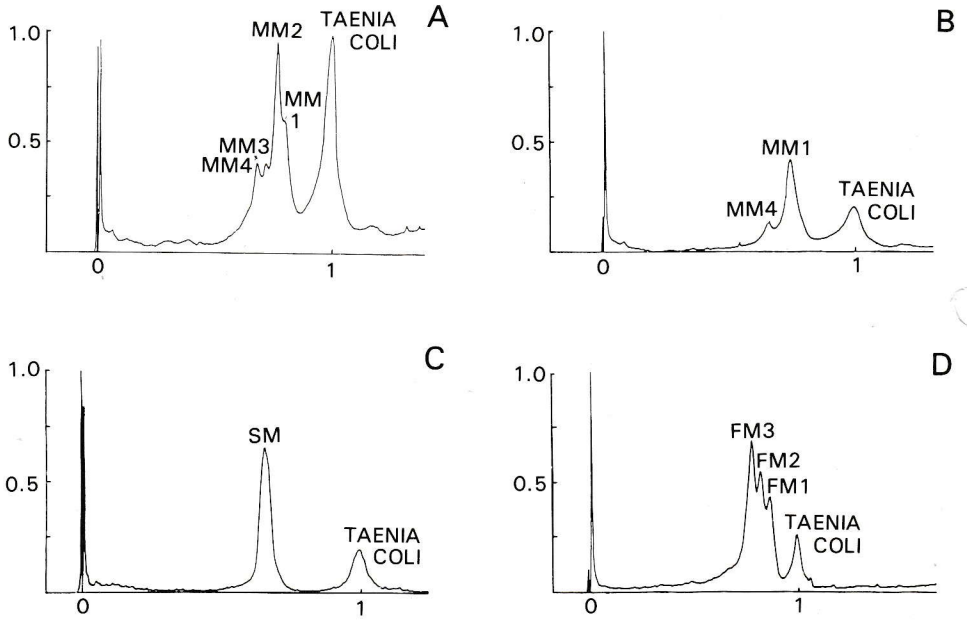

FIG. 1. *Isomyosins of rabbit muscles.* The isomyosins have been separated by electrophoresis in a non dissociating solution. The gels have been scanned at 536 mμ (absorbance in ordinate). The mobility (in abscissae) is measured in unit of the mobility of taenia coli myosin, added to the myosin extract to provide an internal standard.
A : Tensor tympani : MM1 to MM4 : isomyosins of middle ear muscles; B : Stapedius; C : Soleus : SM : slow isomyosin; D : Psoas : FM3, FM2, FM1 : fast isomyosins.

with an earlier report (SRIHARI *et al.*, 1981). The three fast isomyosins were called FM1, FM2 and FM3 by order of decreasing electrophoretic mobilities (SRIHARI *et al.*, 1981). We have also analysed a mixed muscle, extensor digitorum longus (EDL). This muscle contains three fast myosins, similar to those observed in psoas muscle, and a slow isomyosin, which migrates slightly faster than SM, and which is called IM (intermediary myosin) (densitogram not shown). Stapedius muscle has two isomyosins (Fig. 1A) and tensor tympani muscle has four isomyosins (Fig. 1B). We have called MM1 to MM4 (for middle ear muscle myosin) the four isoforms observed in tensor tympani, by order of decreasing mobilities.

Accurate measurements of the mobilities of the various forms allow a comparison between the isomyosins. The mobility of an isoform is expressed as R_F, *i.e.*, the ratio of the mobility of the isoform to the mobility of taenia coli myosin (which is set equal to 1). Table I reports all the measurements.

TABLE I. *Mobilities* (R_F) *of rabbit isomyosins*

Muscle	*n*	Name of isomyosin					
		SM	MM4 IM	MM3	MM2 FM3	MM1 FM2	FM1
Stapedius	11		0.705 ± 0.004			0.788 ± 0.004	
Tensor tympani	14		0.688 ± 0.003	0.722 ± 0.003	0.766 ± 0.004	0.804 ± 0.003	
Soleus	8	0.650 ± 0.003					
Psoas	5				0.784 ± 0.007	0.825 ± 0.005	0.866 ± 0.004
EDL	4		0.683 ± 0.020		0.763 ± 0.011	0.800 ± 0.009	0.840 ± 0.001

R_F values are ratio's of the mobility of the isomyosins to the mobility of taenia coli myosin; IM : intermediary myosin; FM3, FM2, FM1 : fast isomyosins of psoas and EDL; MM1 to MM4 : isomyosins of middle ear muscles. EDL : Extensor digitorum longus.

It shows that the slow form observed in stapedius has an electrophoretic mobility (0.705) intermediate between that of MM4 (0.688) and that of MM3 (0.722). On the other hand, the mobility of the fast isoform of stapedius (0.788) is intermediate between that of MM2 (0.766) and that of MM1 (0.804). Comigration experiments of stapedius myosins with tensor tympani myosins gave four zones identical to those observed in tensor tympani isomyosins. This result supports the conclusion that stapedius isomyosins are not different from tensor tympani isomyosins. On the other hand, the data do not allow a precise identification of the stapedius isomyosins with the tensor tympani isomyosins. There is some logical argumentation in favour of the opinion that the slow form of stapedius might be identical with MM4 and that the fast form is identical with MM1 (see Discussion). For the time being we propose to accept provisionally this identification.

It is also interesting to compare the isomyosins of tensor tympani with those observed in other striated muscles. Table I demonstrates that there is no slow myosin of soleus type present in middle ear muscles. The intermediary myosin of EDL is most likely present in stapedius and tensor tympani (MM4). It is problematical to equate the fast forms of middle ear muscles myosins with those of psoas, since the fast forms MM1, MM2 and MM3 have not the same respective mobilities as the fast forms FM1, FM2 and FM3. However, MM1 may be identical to FM2, and MM2 may be identical to FM3 (see Discussion). In any case MM3 is an isoform observed neither in soleus, nor in EDL, nor in psoas.

These conclusions have been further tested by comigration experiments. Addition of soleus myosin to stapedius or tensor tympani myosin resulted in a supplementary zone which migrates more slowly than the slowest form of middle ear muscle isomyosin. Thus SM is absent in middle ear muscles. Addition of psoas myosin to middle ear muscle myosin also gave a supplementary zone, which now, however, is faster than the fastest middle ear isomyosin. Thus FM1 is absent in middle ear muscles. Addition of EDL myosin to middle ear muscle myosin had the same effect than adding psoas myosin. Thus there is no observable difference between IM and MM4. In summary, the comigration experiments fully support the conclusions drawn from the analyses of the isomyosin R_F's values.

TABLE II. *Relative proportions of rabbit isomyosins* (percent of total myosin)

Muscle	*n*	Name of isomyosin					
		SM	MM4 IM	MM3	MM2 FM3	MM1 FM2	FM1
Stapedius	11		16 ± 1			84 ± 2	
Tensor tympani	14		15 ± 2	26 ± 3	39 ± 3	20 ± 2	
Soleus	8	100					
Psoas	5				44 ± 2	37 ± 1	19 ± 2
EDL	4		18 ± 7		37 ± 7	27 ± 6	18 ± 3

SM : Slow myosin; IM : intermediary myosin; FM3, FM2, FM1 : fast isomyosins of psoas and EDL; MM1 to MM4 : isomyosins of middle ear muscles. EDL : extensor digitorum longus.

Table II reports the relative proportions of the various isomyosins expressed in percent of total myosin. They have been obtained by computer integration of densitograms similar to those shown in Figure 1. Both middle ear muscles contain the same proportion of the slowest migrating form, MM4 : approximately 15 %. The only middle ear isomyosin which is certainly absent in psoas or limb muscle, *i.e.* MM3, is present in tensor tympani in a sizeable proportion (26 %). It is interesting to point out that the relative proportions of the four isomyosins of tensor tympany are approximately equal to those of EDL, if the isoforms are ranked by order of increasing mobility.

Discussion

The significance of the isomyosins observed in middle ear muscle cannot be fully appreciated until we known the distribution of their light chains and the composi-

tion of their heavy chains. Until such work is performed we can only propose the most likely interpretation of our data, obtained after consideration of three hypotheses. We shall first consider the case of the tensor tympani muscle.

The first hypothesis is the most straightforward. It considers that isomyosins of identical R_F's are identical. In this case MM4 is identified to IM, and MM2 and MM1 are identified to FM3 and FM1. In this view, MM3 is an isoform peculiar to middle ear muscle, perhaps an hybrid form of IM heavy chain with some fast alkaline light chain, or, alternatively, a slow type of isomyosin specific to middle ear muscles. A difficulty with this hypothesis is to explain the absence of FM1 in middle ear muscle. Indeed, it is known that FM1 is an homodimer of LC_{3f} (HOH, 1978; d'ALBIS *et al.*, 1979*b*), which is present in all fast muscles so far analysed (HOH, 1978; d'ALBIS *et al.*, 1979*a* & *b*; SRIHARI *et al.*, 1981; WHALEN *et al.*, 1981; MARÉCHAL *et al.*, 1984).

A second hypothesis is that there are two varieties of myosin heavy chains specific to middle ear muscles. A slow type, which would correspond to MM4 and a fast type. The fast type would combine with the two fast alkaline light chains, giving three isozymes. In this view, MM3 would be a LC_{1f} homodimer, MM2, a LC_{1f}-LC_{3f} heterodimer, and MM1, a LC_{3f} homodimer, in analogy with the fast isomyosins where a specific fast heavy chain is combined with the alkaline light chains, forming FM3, a LC_{1f} homodimer, FM2, a LC_{1f}-LC_{3f} heterodimer or FM1, a LC_{3f} heterodimer (BILLETER *et al.*, 1981; HOH, 1978; d'ALBIS *et al.*, 1979*b*). In a softer version of this hypothesis one would admit that MM4 is identical to IM. This hypothesis seems likely since one expects that middle ear muscles would have no special need for a special type of slow myosin to sustain tonic contraction, but would have great need for a special type of very fast myosin in order to rapidly contract during the reflex responses to loud sounds.

A third hypothesis must be examined. Middle ear muscles are submitted to nearly continuous mechanical vibrations, which might conceivably damage the muscles. If it is the case, there would occur a continuous process of muscle regeneration. It is known that regenerating adult muscles express transiently embryonic myosins and neonatal myosins (MARÉCHAL *et al.*, 1984). Neonatal myosins migrate faster than isomyosins of adult fast muscles in rat (MARÉCHAL *et al.*, 1984) and in rabbit (HOH & YEOH, 1979). As middle ear muscles do not contain isomyosins faster than psoas isomyosins, it follows that they do not contain any neonatal myosins. They might contain embryonic myosins since these myosins migrate somewhat more slowly than the fast forms, at least in rats (MARÉCHAL *et al.*, 1984). In this case MM1 and MM2 could be the embryonic forms called eM3 and eM4 (see MARÉCHAL *et al.*, 1984). This hypothesis seems unacceptable, since it would require that MM3 be the fast myosin form of middle ear muscle, a form absent in stapedius. Moreover, it does not seem likely that neonatal forms could be absent when both adult and embryonic forms are present.

The three hypotheses discussed must now be considered in the frame of the stapedius muscle. The most likely hypothesis is that stapedius muscle contain one slow isomyosin identical to IM and one fast isomyosin identical to FM3. It seems unlikely that the fast form is identical to MM2 in the frame of the second hypothesis, *i.e.* because it would correspond to an heterodimer existing alone; however it could be identical to MM1. The statistical accuracy of the data is not such that this hypothesis can be excluded. To the contrary, if it is accepted we are led to the following quite simple conclusion, which is in our view the best interpretation of the data. Middle ear muscles contain a slow form of myosin which is probably identical to IM. This

myosin accounts for the slow tonic contractions of these muscles. They also contain a fast myosin : its heavy chain is specific for middle ear muscles. It is combined to one alkaline light chain (probably LC_{3f}) in stapedius, but with the two alkaline light chain (LC_{3f} and LC_{1f}) in tensor tympani, distributed in the three isomyosins typical of most fast muscles.

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G. MARÉCHAL
Département de Physiologie, UCL 55.40
Avenue Hippocrate 55, B-1200 Bruxelles.