THE ABSENCE OF 3-METHYLHISTIDINE IN RED, CARDIAC AND FETAL MYOSINS

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SUMMARY. Myosin prepared from a muscle containing 98% red fibers (cat soleus) does not contain 3-methylhistidine (3MH)[1]. Cardiac myosin, which is similar to red muscle myosin, is also devoid of 3MH. On re-examining the 3MH content of myosin from a mixed muscle (red and white fibers), there was significantly less than two residues of 3MH per molecule (1.3-1.7 residues per 500,000 grams). This fractional content of 3MH is explained by assuming that myosin from white fibers contains two residues of 3MH per molecule (one residue per heavy chain) whereas myosin from red fibers contains no 3MH. This is the first clear demonstration of a difference in chemical structure in the heavy chains of myosin.

INTRODUCTION

Previous studies from this laboratory (1) and others (2,3) have documented the presence of ϵ-N-trimethyllysine (TML)[1], ϵ-N-monomethyllysine (MML) and 3-methylhistidine (3MH) in rabbit skeletal myosin, specifically in the subfragment I part of the molecule (1).

Trayer et al. (2) showed that in contrast to actin, 3MH appears in rabbit skeletal myosin postnatally. In addition, it has been proposed that cardiac myosin and red muscle myosin are intermediate in content of 3MH, i.e. between fetal and adult rabbit myosin (2,4).

In this paper we present evidence showing that myosins prepared from an essentially pure red muscle, cat soleus, and from various species of cardiac muscle contain no 3MH.

MATERIALS AND METHODS

Myosin was prepared as outlined by Kielley and Harrington (5) with the

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1. Abbreviations used: 3-methylhistidine (3MH); ϵ-N-monomethyllysine (MML); ϵ-N-dimethyllysine (DML); ϵ-N-trimethyllysine (TML); heavy meromyosin (HMM); flexor hallucis longus (FHL).

following modifications: a) in some cases the rabbit skeletal myosin was subjected to a final purification on a column of hydroxyapatite (personal communication Dr. W.W. Kielley); b) in cases where very small quantities of muscle were available (e.g. one day old rabbit),any actomyosin that might have been present following the initial low salt precipitation was dissociated in 0.5M KCl with 5mM Mg-ATP, and the F-actin was removed by sedimentation at 90,000g for 3 hrs. This step was followed by a second low salt precipitation and $(NH_4)_2SO_4$ fractionation; c) the $(NH_4)_2SO_4$ fraction used for fetal, juvenile, and cardiac myosins precipitated between 35-50% saturation (cf 40-50% for adult skeletal myosin).

Bovine cardiac myosin, bovine cardiac heavy meromyosin (HMM) (6) and lobster myosin were gifts of Dr. M. Barany. Cat flexor hallucis longus (FHL) and soleus, and cat cardiac myosins were gifts of Dr. Fred Samaha and were prepared as previously described (7). One preparation of chicken myosin was a gift of Dr. Susan Lowey.

Actin was isolated as F-actin from actomyosin using the dissociation procedure outlined above and then dialyzed against 0.5mM ATP, pH7. The 5000g X 15 min. supernatant was then used for amino acid analysis.

Myosin was dissociated into heavy and light chains in the presence of 5M guanidine hydrochloride. The chains were then separated on a Sephadex G-150 column, 2.5X90 cm., equilibrated with 5M guanidine hydrochloride (8).

Samples for amino acid analysis were hydrolyzed for 72 hrs. at 110°C in 6N HCl, after being sealed under a vacuum. Systems B (pH 5.84. 27°C) and C (pH 5.28, 27°C) have been previously described (1), and both were used to analyze all samples except where noted. The flow rate was 45 ml/hr, and both Beckman UR30 and AA15 resins were used in columns 0.9X50 cm. Analyses on the same preparations were reproducible to within approximately 5%.

Guanidine hydrochloride was Ultra pure quality purchased from Mann Research Labs. All other chemicals were reagent grade.

RESULTS.

Table I presents the methylated lysine and histidine content of myosin

TABLE I

ADULT SKELETAL MYOSIN

Muscle and (No. Preps.)	Moles per 500,000 grams protein MML	DML	TML	3MH
Rabbit Psoas (7)	1.4 (0.6)[a]	<.1	4.0	1.5
Chicken Pectoralis (4)	1.5	<.2	3.8	1.5
Sheep[c] (1)	1.0	NR[b]	4.1	1.7
Cat Flexor Hallucis Longus (2)	1.1	<.2	3.6	1.3
Cat Soleus (3)	<.05	.5	5.0	<.05

Methods of analysis are described in the text.
[a]. Two preparations had a lower content of MML than five others.
[b]. NR=not resolved; this preparation was analyzed only with system C, in which DML and TML co-elute.
[c]. Lateral muscles of thigh including Semitendinosus, Semimembranosus, Vastus Lateralis.

from four different species of mammals. Recent studies by Samaha et al. (9,10) have distinguished three different types of muscle fibers on the basis of histochemical stains for myosin ATPase. These three fiber types include white (α), red (β), and intermediate ($\alpha\beta$). Histochemical studies have shown that cat soleus is composed of 98% β fibers, whereas the cat FHL is composed of approximately 60%α, 35%$\alpha\beta$, and 5%β fibers; rabbit psoas and chicken pectoralis are similar to the cat FHL in containing predominantly α and $\alpha\beta$ fibers, but very few β fibers (personal communication, Dr. Lloyd Guth).

Whereas the content of TML was between four and five residues per molecule for the various myosins (see Discussion), 3MH was notably absent ($<.05$ residues) in cat soleus myosin. Thus the content of 3MH (1.3-1.7 residues per 500,000 grams) in the first four myosins of Table I is consistent with white myosin containing two moles of 3MH per molecule and red myosin containing zero moles of 3MH per myosin molecule. It is of note that the content of MML, which is similar to 3MH in FHL myosin, is also markedly reduced in cat soleus myosin. The significance of this

correlation is not clear, however, since the content of MML varied both between species and even among the rabbit psoas preparations themselves. As shown in Table I, two of seven preparations of rabbit psoas myosin had markedly lower contents of MML compared to the other five presented in Table I and reported elsewhere (1).

When rabbit skeletal myosin was chromatographed on Sephadex G-150 in the presence of 5M guanidine hydrochloride, the light and heavy chains were well separated. All of the MML (as well as TML and 3MH) was found associated with the heavy chains. Furthermore, when the single heavy chain peak was divided into three fractions, the relative content of the methylated amino acids remained constant across the peak.

ε-N-dimethyllysine (DML) was absent in cat FHL, rabbit and chicken myosins, but measurable levels (0.5 residues) were noted in cat soleus myosin.

Table II presents the values for methylated lysine and histidine in cardiac myosin. Cardiac myosin, which is considered to be a red type myosin (11), is similar to cat soleus in that both contain essentially no 3MH.

TABLE II

CARDIAC MYOSIN

Species and (No. Preps.)	Moles per 500,000 grams MML	DML	TML	3MH
Rabbit (1)	1.1	nil	4.3	<.10
Sheep (2)	1.5	NR[a]	4.2	nil
Cat (1)	1.1	NR[a]	3.8	<.15
Bovine (1)	<.10	NR[a]	4.2	nil
Bovine HMM[b] (1)	0.2	nil	4.5	nil

Methods of analysis are described in the text.
a. These preparations were analyzed only with system C, in which DML and TML co-elute.
b. For HMM, results expressed as moles per 340,000 grams.

Cardiac myosin does contain approximately four residues of TML/molecule (cf five residues in cat soleus myosin), and in agreement with previous results with skeletal myosin all the TML in bovine cardiac myosin is present in the HMM part of the molecule (1). The MML content is again variable but not enough preparations are available to know if the low content in bovine cardiac myosin reflects species variation.

Table III relates content of methylated amino acids in myosin to animal maturity. Myosins from fetal sheep and one day old rabbits do not contain significant amounts of 3MH, confirming the results of Trayer et al. (2) for 28 day fetal rabbit skeletal myosin. Moreover, in contrast to 3MH, TML is present at essentially adult levels (see Table I) in both fetal sheep and one day rabbit myosins. The 3MH contents of myosin from one day, 10 day, 23 day (2), and adult rabbits are 0, 0.4, 1.2, and 1.8 residues respectively, indicating that the time course of methylation in the neonatal period is gradual rather than abrupt. Myosin from two day old chickens, which are clearly more mature and more mobile than rabbits of the same age, contains 3MH in essentially the same quantity as adult

TABLE III

SKELETAL MYOSIN FROM FETAL AND POSTNATAL ANIMALS

Muscle	Animal Age & (No. Preps.)	Moles per 500,000 grams protein MML	DML	TML	3MH
Sheep hind leg & back	130 days gestation (1)	.7	NR[a]	3.5	<.10
Rabbit hind leg	1 day (2)	.2	.6	4.0	nil
	10 day (1)	<.1	NR[a]	4.5	0.4
	adult (1)	1.6	NR[a]	4.2	1.8
Chicken leg	2 day (1)	1.7	NR[a]	3.7	1.5

Methods of analysis are described in the text.
a. These preparations were analyzed only with System C, in which DML and TML co-elute.

chicken myosin. Trayer and Perry (12) noted a direct correlation between myosin specific ATPase activity and animal maturity.

Analyses of lobster myosin demonstrated the following molar contents of methylated amino acids per 500,000 grams of protein: MML=5.0, DML=3.1, TML=16.6, and 3MH=1.8.

Previous reports have indicated that smooth, cardiac, red skeletal, invertebrate, and fetal rabbit skeletal actins contain the same quantity of 3MH as adult rabbit skeletal actin (i.e. 1 mole per 45,000 grams) (4,13). In agreement with this, actins isolated from rabbit cardiac, fetal sheep skeletal, and cat soleus muscles contained 0.7-0.8 moles of 3MH per 45,000 grams of protein.

DISCUSSION

The results presented suggest a well-defined pattern of methylation in mammalian myosins. Since myosin is known to be composed of two heavy subunits (and two or more light subunits) (14), it appears that white, cardiac, and fetal myosins contain two residues of TML per heavy chain[2]. In contrast, red myosin (cat soleus) contains significantly more TML (2.5 residues/heavy chain) as well as a small amount of DML (.2-.3 residues/heavy chain). Inasmuchas calf thymus histone IV contains a mixture of MML and DML at residue 20 (15), it is possible that pure red myosin contains three residues of DML plus TML per heavy chain.

MML is present in much more variable and often in less than stoichiometric amounts, with no clear pattern of variation discernible presently. Even in cardiac muscle (ventricle) which is presumably a homogeneous tissue, the MML content is less than stoichiometric (per heavy chain) and variable. Although MML may be present in a contaminant protein, the more or less constant occurrence of this residue in different muscle types and species, plus the fact that it co-elutes with the heavy chain of myosin on Sephadex

2. This represents an average value per subunit and does not necessarily imply equal contents in each subunit.

G-150 chromatography in 5M guanidine, suggests that it is present in the myosin molecule. As noted above fractional methylation has been reported for other proteins (15,19).

Mixed skeletal myosins (Table I) contain about 0.7-0.9 residues of 3MH per heavy chain whereas red (cat soleus), cardiac, and fetal myosins contain no 3MH. The presence of less than stoichiometric amounts of 3MH in mixed skeletal myosins suggests that these muscles contain a mixture of red myosin (no 3MH) and white myosin (one 3MH/heavychain)[2] but mainly the latter. As discussed above mixed skeletal myosins are composed predominantly of white (α) and intermediate ($\alpha\beta$) fiber types. Although pure white muscles are not available, it is presumed that white fibers (α) contain only white heavy subunits of myosin. Intermediate fibers may then contain both red and white myosin heavy subunits or, alternatively, may contain a third type of myosin heavy subunit. Preliminary studies on the mouse hind limb, which contains primarily the intermediate ($\alpha\beta$) fiber type (Dr. Lloyd Guth, personal communication) indicate that in this case .35-.5 residues of 3MH are present per heavy chain subunit. This result is consistent with intermediate fibers being composed of a mixture of red and white heavy subunits.

It is worth noting that both cardiac and red myosin differ from white myosin in their alkali lability (16), lower specific ATPase activity, and decreased sensitivity to trypsin (6,11). Except for possible qualitative differences in light subunit composition (7,11) the absence of 3MH in cardiac and red myosin demonstrates the first clear difference in chemical structure of red-type myosins from white myosin. It is not clear whether the absence of 3MH in red-type myosin is due to a failure to methylate histidine or to absence of the histidine residue(s) which is methylated in white myosin.

Although reported results for the 3MH content of mixed skeletal myosins are in agreement with those presented here, it is not clear why significant

amounts of 3MH were found by others in red (0.4-1.0 moles/500,000 grams) or cardiac (0.4-1.4 moles/500,000 grams) myosin unless it is assumed that actin was not completely removed from the myosin preparations examined (2,4).

Despite the apparent constancy of the methylation of histidine for both actin (one residue of 3MH per mole) and myosin (none or one residue of 3MH per heavy chain), spanning mammals to the ameba (actin) (17) or lobster (myosin), methylated lysines are more variable. Examples of the latter include the variability of MML in mammalian myosin, the unusually high levels of MML, DML, and TML in lobster myosin, and the occurrence of DML in ameba actin (18).

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