AMINOACID COMPOSITION OF MYOSIN AND ACTIN OF MUSCLES IN ANIMALS WITH HEREDITARY MYOPATHIA

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An increase in the content of leucine with isoleucine, valine, and alanine, and a decrease in the content of tyrosine and histidine were found in the aminoacid spectrum of myosin from line 129/RE mice with hereditary myopathia. The content of valine was increased, and that of tyrosine and histidine decreased, in mice with the heterozygotic state. The glycine content was increased and the histidine content decreased in the aminoacid composition of actin from affected and heterozygotic mice.

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Progressive muscular dystrophy (PMD) is accompanied by the breakdown of muscle protein and by increasing atrophy of the skeletal muscles. Investigations [2, 6-9] have shown changes in the physicochemical properties of the principal contractile protein (myosin) in human PMD and in hereditary muscular dystrophy in animals. Radiochemical studies of the biosynthesis of muscle proteins [1] have revealed qualitative differences in the incorporation of certain amino acids into these proteins in mice of line 129/RE with hereditary myopathia. Barany and co-workers [5], who investigated chickens with hereditary muscular dystrophy, found no changes in the aminoacid spectrum or physicochemical properties of the myosin.

Because of the contradictory information on the aminoacid composition of myosin and the lack of information on the aminoacid composition of the other contractile proteins of muscle tissue in PMD, experiments were carried out to study the aminoacid spectrum of myosin and actin.

EXPERIMENTAL METHOD AND RESULTS

Twelve mice with hereditary myopathia, aged from 2.5 to 3.5 months, with the 129/RE dy dy genotype, and 12 mice carrying the pathological gene of the dystrophy with the 129/RE+ dy genotype, aged from 4 to 8 months, were used in the experiments. Ten healthy mice of the same line acted as controls. Heterozygosis was confirmed by the existence of animals with myopathia among the progeny of the individuals studied. Samples of myosin and actin were obtained by the method of Ivanov and co-workers [3]. The protein was hydrolyzed for 32 h in ampules with 10 volumes of 6N HCl solution, sealed after evacuation of the air, in an incubator at 105°. The aminoacid spectrum of the hydrolyzed proteins was studied by ascending paper chromatography [4]. The concentration of each amino acid was determined from specially plotted calibration curves relating to standard solutions of amino acids.

Fifteen amino acids were clearly separated by chromatography. As the results in Table 1 show, the aminoacid spectrum of myosin from the diseased animals differed from that of the healthy animals by its higher content of leucine with isoleucine, valine, and alanine and its lower content of tyrosine and histidine. The change in the content of glycine and serine was not statistically significant. In the heterozygotic mice the same characteristic changes in aminoacid content were found, but the change in the content of leucine with isoleucine, alanine, and serine was not statistically significant. The aminoacid spectrum of actin from

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TABLE 1. Amino Acid Composition (in %) of Myosin from Skeletal Muscles of Healthy, Affected, and Heterozygotic Mice of Line 129/RE with Hereditary Muscular Dystrophy (M±m)

Amino acid	Healthy mice (10)	Affected mice (12)	Heterozygotic mice (12)
Leucine with isoleucine	12,74± 0,21	13,6± 0,26 $P<0,02$	13,32± 0,37
Phenylalanine	3,46± 0,23	3,15± 0,17	3,46± 0,26
Valine	4,21± 0,20	5,43± 0,33 $P<0,01$	5,56± 0,47 $P<0,05$
Methionine	3,41± 0,11	3,50± 0,17	3,40± 0,19
Tyrosine	2,26± 0,15	1,23± 0,17 $P<0,001$	1,50± 0,29 $P<0,05$
Alanine	7,82± 0,21	9,13± 0,20 $P<0,001$	8,95± 0,66
Threonine	4,37± 0,22	4,48± 0,20	4,29± 0,13
Glutamine	16,45± 0,22	16,12± 0,26	16,20± 0,27
Glycine	5,85± 0,31	5,30± 0,28	5,47± 0,35
Serine	3,98± 0,12	4,39± 0,20	4,30± 0,16
Asparagine	8,85± 0,10	9,20± 0,41	8,81± 0,16
Arginine	7,16± 0,21	7,46± 0,18	7,25± 0,14
Histidine	4,25± 0,10	2,93± 0,11 $P<0,001$	3,16± 0,30 $P<0,01$
Lysine	14,16± 0,37	13,96± 0,27	14,17± 0,32
Cysteine	Not determined quantitatively		

TABLE 2. Amino Acid Composition (in %) of Actin from Skeletal Muscles of Healthy, Affected, and Heterozygotic Mice of Line 129/RE with Hereditary Muscular Dystrophy (M±m)

Amino acid	Healthy mice (10)	Affected mice (12)	Heterozygotic mice (12)
Leucine with isoleucine	13,52± 0,32	13,00± 0,36	13,37± 0,41
Phenylalanine	3,97± 0,18	4,01± 0,25	4,10± 0,40
Valine	4,33± 0,21	4,17± 0,17	4,38± 0,28
Methionine	4,11± 0,25	3,87± 0,20	4,00± 0,29
Tyrosine	4,50± 0,33	4,80± 0,30	4,65± 0,24
Alanine	8,74± 0,31	9,20± 0,30	9,10± 0,35
Threonine	6,02± 0,33	5,94± 0,30	5,44± 0,12
Glutamine	12,76± 0,31	12,46± 0,29	12,66± 0,20
Glycine	6,72± 0,39	9,23± 0,42 $P<0,001$	8,30± 0,45 $P<0,02$
Serine	5,49± 0,23	5,52± 0,28	5,63± 0,21
Asparagine	8,81± 0,35	9,13± 0,24	9,00± 0,51
Arginine	7,00± 0,24	6,79± 0,25	7,20± 0,39
Histidine	4,89± 0,24	2,71± 0,19 $P<0,001$	2,56± 0,46 $P<0,001$
Lysine	9,26± 0,26	9,17± 0,26	9,18± 0,44
Cysteine	Not determined quantitatively		

the diseased mice (Table 2) differed from that of actin from healthy animals by its higher content of glycine and its lower content of histidine. The changes in the content of alanine, and of leucine with isoleucine, were not statistically significant. An increase in the glycine content and a decrease in the histidine content were observed in the heterozygotic mice.

The changes discovered in the amino acid composition of the two principal contractile proteins of muscles could be evidence of changes in their structures. This suggests that in line 129/RE mice with hereditary muscular dystrophy, pathological changes take place not only in the lyosin molecule, but also to some extent in the actin molecule. The disturbances of the amino acid composition in heterozygotic animals also lead to changes in the protein molecule. This may be linked with the absence of stable synthesis of the contractile proteins, in conjunction with their more rapid breakdown in the muscles of the mice with hereditary muscular dystrophy.

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