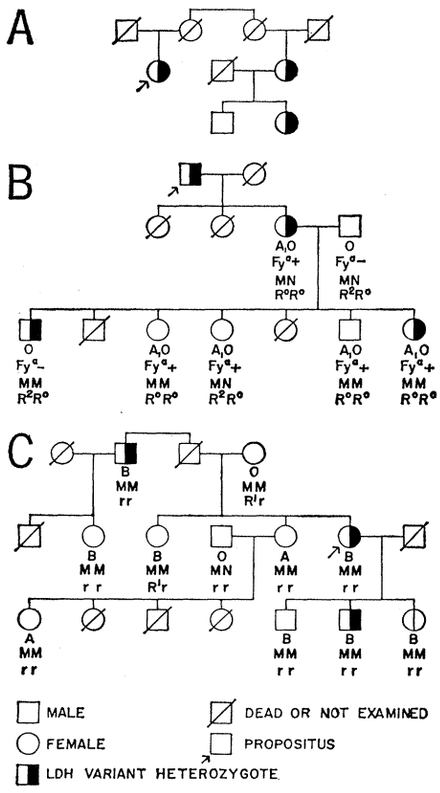

Fig. 2. Pedigrees of families with LDH variants. (*A*) LDH-A_{Mem-2}. (*B*) LDH-B_{Mem-3}; all family members examined were Kell negative and Sutter *a* and *b* positive. (*C*) LDH-A_{Mem-4}; all family members examined were Kell negative.

of LDH-5. The formation of the isozyme bands of LDH-A_{Mem-2} is explained in a similar fashion, except that the altered LDH-A, that is, A″, of this variant moves further toward the anode than that of LDH-A_{Mem-1}. LDH-A_{Mem-4} also consists of a normal LDH-B moiety, but is formed by a normal LDH-A and an altered, more positively charged LDH-A‴. The additional isozyme components of this variant therefore lie closer to the origin in starch gel electrophoresis than the corresponding normal isozymes.

Fritz and Jacobson (*10*) suggested that each unit of LDH-A and LDH-B has attached to it a molecule of nicotinamide-adenine dinucleotide (NAD). These authors postulated that 0.005*M* β-mercaptoethanol partially removes NAD from LDH-A, but not from LDH-B. Mouse tissue extracts thus treated show LDH-5 to be broken up into five components, four components for LDH-4, three for LDH-3, and two for LDH-2. LDH-1 is not altered since it consists of LDH-B units. These findings are reminiscent of the patterns shown by LDH-$A_{Mem-1, -2, and -4}$. However, we do not believe that partial dissociation of LDH-A and NAD play any role in the formation of the variant LDH patterns because (i) the addition of NAD to the starch gel and buffer did not alter the pattern; (ii) electrophoresis of freshly prepared hemolysates of the affected individuals always resulted in identical abnormalities, and these were never seen in normal persons; (iii) all affected relatives of affected individuals always possessed an identical abnormality.

The erythrocyte LDH variant described by Nance *et al.* (*5*) has a fast moving LDH-2 component as observed in LDH-$A_{Mem-1 or -2}$, but without direct comparison identity cannot be established. These authors report that the LDH-3 isozyme of their patient separated into only two bands with the band containing the mutant LDH-A migrating just behind hemoglobin A. As shown in Fig. 1, LDH-3 of LDH-$A_{Mem-1 and -2}$ clearly separates into three bands, the middle band staining more intensely than the other two. This finding supports the concept of random association of LDH-A, variant LDH-A, and LDH-B subunits, a process which in LDH-3 would lead to the formation of three bands in the proportions of 1 : 2 : 1. Boyer *et al.* (*4*) have shown that this indeed occurs in an individual heterozygous for LDH-B and for a variant LDH-B, and it would be reasonable to expect the same phenomenon in persons with an LDH-A variant.

LDH-A_{Mem-1} was found in three Negroes, one of whom had sickle cell trait (Hb A-S). Family studies could not be performed. LDH-A_{Mem-2} was found in two Negroes, one of whom also had the high fetal hemoglobin trait. His family unfortunately was not available for study. The pedigree of the other family with LDH-A_{Mem-2} is shown in Fig. 2*A*. The family of one of the two Negroes with LDH-B_{Mem-3} was studied and is shown in Fig. 2*B*. LDH-A_{Mem-4} was found in a single Caucasian and her family (Fig. 2*C*). All affected individuals of one family possessed the same erythrocyte LDH variant. The LDH variants appeared to be inherited as autosomal codominant characteristics. All the individuals found are considered to be heterozygotes either for LDH-A or for LDH-B. The study of the family with LDH-B_{Mem-3} indicates that there is no linkage of the locus for LDH-B with either the ABH system or the Fy^a gene. Hemoglobin electrophoresis and blood typing for the Rh system and the MNS, Kell, and Sutter groups were performed on all family members, but the results do not permit any conclusions as to linkage between those characteristics and the LDH loci. The finding of eight individuals with variant LDH enzyme patterns in a group of 940 randomly selected persons indicates that this variation may not be as rare as heretofore suspected.

ALFRED P. KRAUS
CHARLES L. NEELY, JR.
Division of Hematology and Laboratory Medicine, University of Tennessee College of Medicine, Memphis

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Myosin Substructure: Isolation of a Helical Subunit from Heavy Meromyosin

Abstract. *A highly α-helical subunit has been isolated from the heavy-meromyosin portion of myosin. This molecule has a helix content of about 73 percent and an amino acid composition similar to that of light meromyosin. The presence of this subunit supports the current view that the myosin molecule consists of a long helical rod with a globular region at one end.*

Muscle fibers owe their striation to two sets of interdigitating filaments: the thick, or myosin-containing filaments, and the thinner, actin-containing filaments (*1*). Interactions between the filaments seem to be mediated by projections or "bridges" periodically arranged along the myosin filaments. In

Table 1. The α-helix content of the fibrous muscle proteins and their subunits.

Protein	$-b_0$	α-helix (%)
Paramyosin	700	100
Tropomyosin	640	91
Light meromyosin fraction 1	640	91
Light meromyosin (LMM)	530	76
Myosin	400	57
Heavy meromyosin (HMM)	300	43
HMM *p*H 2	260	37
HMM top fraction	320	46
HMM pellet	200	29
HMM fraction 1	510	73
HMM fraction 2	190	27

order to understand how myosin and actin interact at these sites during contraction, a detailed picture of the morphology of the myosin molecule and the organization of the myosin filament is essential.

When trypsin is added to a solution of myosin, a water insoluble, fibrous protein, light meromyosin, and a more soluble, globular protein, heavy meromyosin, are formed (*2*). The high α-helix content of light meromyosin fraction 1, a more highly purified form of light meromyosin (*3*), enables one to interpret the light scattering and hydrodynamic data in terms of a two-stranded α-helical rod about 800 Å long by 20 Å wide (*4, 5*). Although the wide angle diffraction pattern of heavy meromyosin fibers indicates

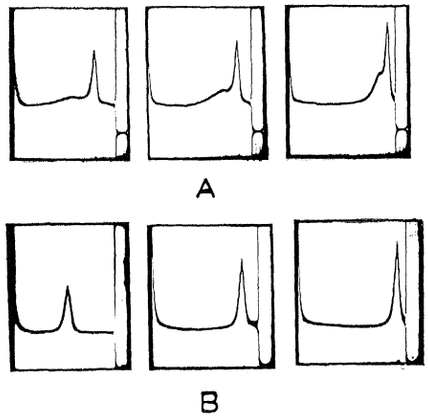

Fig. 1. Sedimentation pattern of heavy meromyosin before and after fractionation. The speed is 59,780 rev/min.; the temperature is 20°C; sedimentation proceeds to the left. The solvent is 0.02*M* KCl, 0.01*M* HCl. *A*, 0.83 percent heavy meromyosin (HMM); the bar angle is 60 degrees. *B*, 0.04 percent HMM fraction 1, after centrifugation and gel filtration; the bar angle is 50 degrees.

some regions of coiled-coil α-helix (*6*), the relatively low α-helix content of the protein in solution implies that a major portion of heavy meromyosin is nonhelical. These x-ray diffraction and physical-chemical studies led to the proposal that myosin consists of an α-helical rod with a more globular portion or portions attached to it (*5, 7*). This globular region, which presumably contains the actin binding site(s) and adenosine triphosphatase activity, was postulated as the "bridge" seen in the electron micrographs of myofibrils.

Recent electron micrographs of individual myosin molecules have in fact shown some molecules composed of a rod with a globular region at one end (*8, 9*). Because of the strong tendency of myosin to aggregate and the experimental difficulties of electron microscopy (such as possible changes in dimension on drying and breakage of molecules), it is not surprising that a wide variety of lengths for the molecule has been observed. Furthermore, rods without globules and rods with two globules have also been seen. Despite the varying lengths, it is most probable that the rod portion of myosin is greater than 1100 Å long. Since light meromyosin is considerably shorter than this length, these results indicate that part of heavy meromyosin has a rodlike structure. Rice (*8*) has shown electron micrographs of heavy meromyosin molecules which have a globular portion similar to that found in intact myosin, but the rod is thinner (5 to 10 Å) than in myosin and light meromyosin (15 to 20 Å).

In order to elucidate further the structure of myosin, we have attempted to isolate the rodlike molecule predicted to be in heavy meromyosin. The isolation of such a subunit would help to answer the questions whether the light meromyosin molecule, or a modified form of it, extends all the way to the "bridge" of myosin, and what the composition and size of this bridge is. In this communication the preparation and some properties of a new helical fraction are described.

The rationale behind the fractionation procedure was that a helical core, if it exists, might be stable in acid, whereas the more globular region would be largely denatured at low *p*H. Acid stability is characteristic of paramyosin, tropomyosin and light meromyosin. Although light meromyosin breaks into low molecular weight fragments (protomyosins) in urea and guanidine hydrochloride (*10*), its viscosity and helix content remain unchanged in 0.01*M* HCl (*11*). In contrast, it has been shown that globular proteins, such as bovine serum albumin, tend to expand in acid and lose helix content (*12*). The globular portion of heavy meromyosin, a subunit which has had numerous peptide bonds split by proteolysis (*13*), and which lacks cystine bonds for added stability, would be expected to disintegrate readily under highly charged conditions.

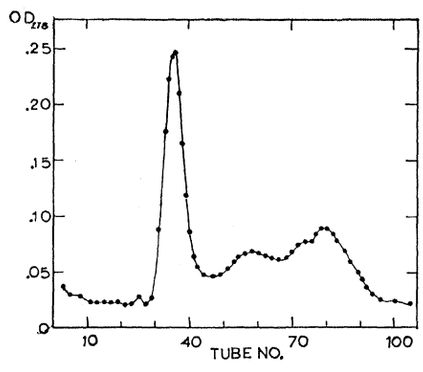

Fig. 2. Separation of the top fraction of heavy meromyosin on a column of Sephadex G-200 (2 by 80 cm). The eluant is 0.02*M* KCl, 0.01*M* HCl. Each tube contains 2.5 ml of solution.

When heavy meromyosin was titrated to below *p*H 4, two peaks were observed in the analytical ultracentrifuge in place of the former single, sharp peak at neutral *p*H. The intrinsic sedi-

Table 2. The amino acid composition of heavy meromyosin fraction 1 compared to other α-proteins (expressed as residues per 10^5 g).

Amino acid (*18*)	HMM (*5*)	HMM Fr. 1	LMM (*5*)	Tropomyosin (*19*)
Lysine	86	110	90	107
Histidine	14	14	20	6
(NH_2)	90	96	107	64
Arginine	34	41	57	42
Aspartic acid	82	75	82	89
Threonine	44	37	37	26
Serine	39	40	38	41
Glutamic acid	137	197	198	213
Proline	32	8	7	2
Glycine	50	27	22	12
Alanine	73	74	79	110
½ Cystine	(11)	3	7	7
Valine	48	26	41	27
Methionine	26	29	15	16
Isoleucine	44	41	41	30
Leucine	73	95	92	95
Tyrosine	21	10	10	15
Phenylalanine	36	20	6	4
Tryptophane	—	—	—	0

mentation constant, $S^0_{20,w}$, of the slower moving peak was 3.3*S* and that of the faster peak, about 10*S*. To eliminate artifacts arising from charge effects, $S^0_{20,w}$ was measured at an ionic strength of 0.01 and 0.03*M*. As expected, when $S^0_{20,w}$ was plotted against concentration, the slope was markedly different at each ionic strength, but the intercept, $S^0_{20,w}$, remained constant. All the rodlike proteins, paramyosin, tropomyosin and light meromyosin, have sedimentation constants of about 3*S*. From the theoretical treatment of the flow properties of rods, it can be predicted that the sedimentation rate is more dependent on the diameter of the molecule than the length (*14*). Thus it appeared plausible that the 3*S* component we had observed might consist, at least in part, of a rodlike molecule. Furthermore, upon heating the solution to 70°C, the sedimentation rate of the slow peak remained unchanged, but the rate of the leading peak increased. This kind of behavior is expected from the very stable α-helical molecules as distinct from more labile proteins.

By varying the *p*H and the ionic strength, the relative proportions of the slow and fast peaks could be changed. Raising the ionic strength much above 0.1 caused the 3*S* component to disappear and to be replaced by one fast-sedimenting, broad peak. When the ionic strength was lowered to 0.03 or less, the area of the fast peak diminished and became highly polydisperse (Fig. 1*A*). These experiments indicate that the formation of the slow peak is due to the electrostatic repulsion of the positively charged fragments in the heavy meromyosin molecule.

The slow component, if indeed the rod, would be expected to have a high helix content, a property which could be used to judge the success of the fractionation procedure in isolating the 3*S* component. Helix content was determined by measuring the optical rotatory dispersion between the wavelengths of 365 and 578 mμ. A value of −700 for the coefficient b_0 in the Moffitt-Yang equation (*15*) was taken to indicate 100 percent α-helix (*16*). The percentage of α-helix on this scale is listed for the various muscle proteins in Table 1.

Acidification of heavy meromyosin causes only a 6 percent loss in α-helix content. The value of b_0 remains unchanged between *p*H 2 and *p*H 3, and is unaffected by the amount of salt present. Apparently, when the *p*H drops to about one unit below the *p*K of the carboxyl groups, the charge is sufficiently great to disrupt the less stabilized α-helical regions, and there is no subsequent change in the secondary structure of the molecule.

A solution of heavy meromyosin in 0.01*M* HCl was centrifuged in the model L ultracentrifuge at 50,000 rev/min until the faster sedimenting component was effectively removed. The helix contents of the top fraction and of the redissolved pellet are given in Table 1. The top fraction shows slightly more α-helix than the bottom pellet, but far less α-helix than light meromyosin. Although the evidence at this stage of the fractionation suggested that parts of the heavy meromyosin molecule differed in helix content, no clear-cut evidence for a helical rod such as light meromyosin existed.

Ultracentrifuge patterns of the concentrated upper fraction help to explain the low helix content by demonstrating the presence of slowly sedimenting material behind the major 3*S* peak. In order to remove these low molecular weight fragments, the top fraction was passed through a Sephadex G-200 column equilibrated with 0.01*M* HCl. Shortly after the void volume (about tube No. 23), a sharp, symmetrical peak appeared followed by a series of broad, skewed peaks (Fig. 2). The leading peak also appeared homogeneous in the ultracentrifuge (Fig. 1*B*).

The helix contents of these two fractions, which will be referred to as heavy meromyosin fraction 1 (tubes Nos. 31-47) and heavy meromyosin fraction 2 (tubes No. 53-90) are listed in Table 1. The fact that the isolated fraction is less helical than light meromyosin fraction 1 may indicate that small amounts of polypeptide contamination are still present (as was the case with light meromyosin), or that the molecule has nonhelical regions near the broken ends.

Summarizing the results, a highly helical subunit with a b_0 value of 510 has been isolated from heavy meromyosin. The amino acid composition of this subunit is given in Table 2.

For comparison, the composition of heavy meromyosin, light meromyosin and tropomyosin has been included. The strong resemblance of this molecule to the α-helical fibrous proteins is striking. The low proline content, characteristic of highly α-helical molecules (*17*), and the high proportion of basic and acidic groups, is particularly noteworthy.

Although the characterization of this new helical fragment is incomplete, the isolation of heavy meromyosin fraction 1 does seem to support the earlier lines of evidence that myosin consists of a helical rod which extends all the way from one end of light meromyosin to the globular region of myosin. This globular region, by virtue of its low α-helix content, may have the necessary flexibility to engage in cyclic interactions with the actin filaments.

SUSAN LOWEY
Children's Cancer Research Foundation and *Harvard Medical School, Boston, Massachusetts*

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