

SPECIFIC REASSOCIATION OF THE POLYPEPTIDE SUBUNIT CHAINS OF HELICAL MYOSIN FRAGMENTS

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1. Introduction

A two-stranded rope twisted of 2 polypeptide chains in α -helical conformation is the generally accepted structural basis for most of the fibrous proteins of the α -type [1]. The α -helices of synthetic polypeptides are in general single. The nature bringing about the multiple supercoil structure of the α -proteins is not completely understood.

The different proteolytic fragments of the fibrous part of the myosin molecule provide excellent objects for studying this problem. Up to the present time 7 fragments comprising different parts of the myosin "rod" are known (fig. 1). One of them, light meromyosin (LMM) was shown to be uncoiled and refolded to the original structure when solvent conditions are changed between denaturing and benign, respectively [2, 3]. We have successfully extended these reversibility studies to the urea denaturation of 4 further fragments (LF-1, LF-2, LF-3 and HMM-S-2). Carrying out the "renaturation" experiments with a mixture in urea of all 5 fragments (LMM plus the ones specified above) a surprising specificity according to length has been observed. No molecules consisting of a shorter and a longer chain were formed though a considerable part of the longer chains is identical in sequence with several kinds of shorter chains present (fig. 1).

Abbreviations used: LMM, light meromyosin; LF-1, LF-2 and LF-3, helical subfragments of LMM; HMM-S-2, heavy meromyosin subfragment 2; HMM-S-3, heavy meromyosin subfragment 3; GuHCl, guanidin-HCl.

2. Methods

Myosin was prepared by the method of Portzehl et al. [4] as described by Bálint et al. [5]. LMM was obtained according to Szent-Györgyi et al. [6].

The isolation of HMM-S-2 was carried out as previously described [7].

The "helical mixture" containing 5 different fragments employed in our experiments was prepared on the grounds of the experiments of Bálint et al. [8], in the following way: a suspension of myofibrils, prepared according to Perry [9], or myosin was digested by trypsin (trypsin to myosin = 1:120 w/w) at a low ionic strength, in the presence of 0.01 M EDTA at 25° for 60 min. The digestion was terminated by the addition of 3 vol of 96% ethanol. The collected precipitate was dissolved in 0.5 M KCl + 0.01 M phosphate buffer pH = 7.2 containing trypsin inhibitor in 3-fold weight excess over trypsin and was dialysed for 16 hr against 20 vol 0.5 M KCl + 0.01 M phosphate buffer, pH = 7.2 + 5 mM 2-mercaptoethanol + 1 mM EDTA. The redissolved fraction contained the following components: LF-1, LF-2, LF-3 and HMM-S-2. This preparation was then completed by addition of some 15% LMM.

Disc electrophoresis cannot be carried out in GuHCl, therefore we chose urea as denaturant. As it was shown earlier in this laboratory [3] urea reactivated to some extent the trypsin-trypsin inhibitor complex present as a contamination in LMM, while treatment with concentrated GuHCl completely inactivated all trypsin traces. These considerations led us to a procedure in which the proteins were treated first with 6 M GuHCl for 4-6 hr at room temp, then they were dialysed against a solution of 6.6 M urea at 4° for 48 hr. The

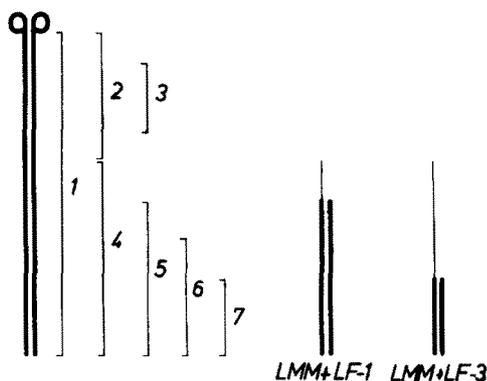


Fig. 1. Proteolytic fragments of the helical "tail" of myosin. Drawings in approximately true proportions with respect to length. 1: "total rod", MW = 220,000 [18]; 2: heavy meromyosin subfragment-2 (HMM-S-2), MW = 60,000 [18] or 72,000 [7]; 3: HMM-S-3, MW = 49,000 [7]; 4: LMM, MW = 130,000–150,000 [3, 12, 13]; 5: LMM-subfragment-1 (LF-1), MW = 112,000 [5]; 6: LF-2, MW = 84,000 [5]; 7: LF-3, MW = 56,000 [5]. To the right: "hybrids" which would be formed when 2 chains of unequal length of LMM and/or its fragments would refold to a 2-chain structure. L + LF-3: hybrid with greatest, L + LF-1 hybrid with smallest possible relative length of unpaired chain segment.

urea solution was changed 3 times; the last dialysing solution contained 6.6 M urea + 5 mM 2-mercaptoethanol + 2 mM EDTA.

"Renaturation" of samples containing urea was achieved by dialysis against a buffer containing 0.5 M KCl + 0.01 M phosphate buffer, pH 7.2, + 5 mM 2-mercaptoethanol + 2 mM EDTA, carried out at 4° for 48 hr or 7 days. All the preparations remained completely dissolved during the whole procedure of denaturation and return to benign solvent.

Protein concentration was measured by the biuret method in 0.5 M KCl and spectrophotometrically in the presence of 6.6 M urea (at 280 nm, $A = 0.53 \text{ mg/cm}^2$).

Optical rotatory dispersion was measured with an OPTON REPM spectropolarimeter. Helix content was calculated from the Cotton-effect at 233 nm according to [10].

Disc electrophoresis of native and renatured samples was carried out in the modified Davis' system [5], that is, the composition of the running gel was 5.66% acrylamide with 1% crossbinding. For electrophoresis in 6.6 M urea we used buffers half as concentrated as those free of the denaturant.

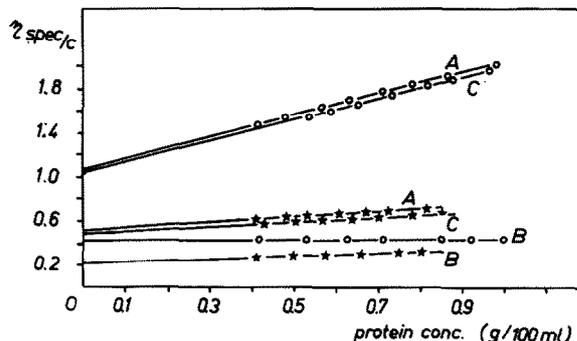


Fig. 2. Concentration dependence of reduced viscosity. LMM: (○—○—○), "helical mixture": (★—★—★); A: untreated; B: in 6.6 M urea; C: after elimination of urea. Measurements were made with Ubbelohde viscosimeters at 20.0°. Flow-times for solvents were 90–120 sec. Solvent for A and C was 0.5 M KCl + 0.01 M phosphate (pH = 7.2) + 5 mM 2-mercaptoethanol + 2 mM EDTA; for B 6.6 M urea + 0.01 M phosphate (pH = 7.2) + 5 mM 2-mercaptoethanol + 2 mM EDTA.

Table 1.
Summary of physical data.

	Helix content (%)	Intrinsic viscosity (dl/g)
LMM untreated	93	1.08
In 6.6 M urea	0	0.42
Reassociated	81	1.02
"Mixture" untreated	82	0.52
In 6.6 M urea	5	0.22
Reassociated	77	0.49

Gels were stained by boiling them at 90–95° in a solution of 7% acetic acid containing 1% Amidoblack 10 B. Destaining was carried out electrophoretically.

3. Results and discussion

The uncoiled or folded state of these helical rod-like molecules can be well followed by intrinsic viscosity and ORD measurements. Fig. 2 shows the reduced viscosity vs. concentration curves for the preparations before urea-treatment, in urea and after its

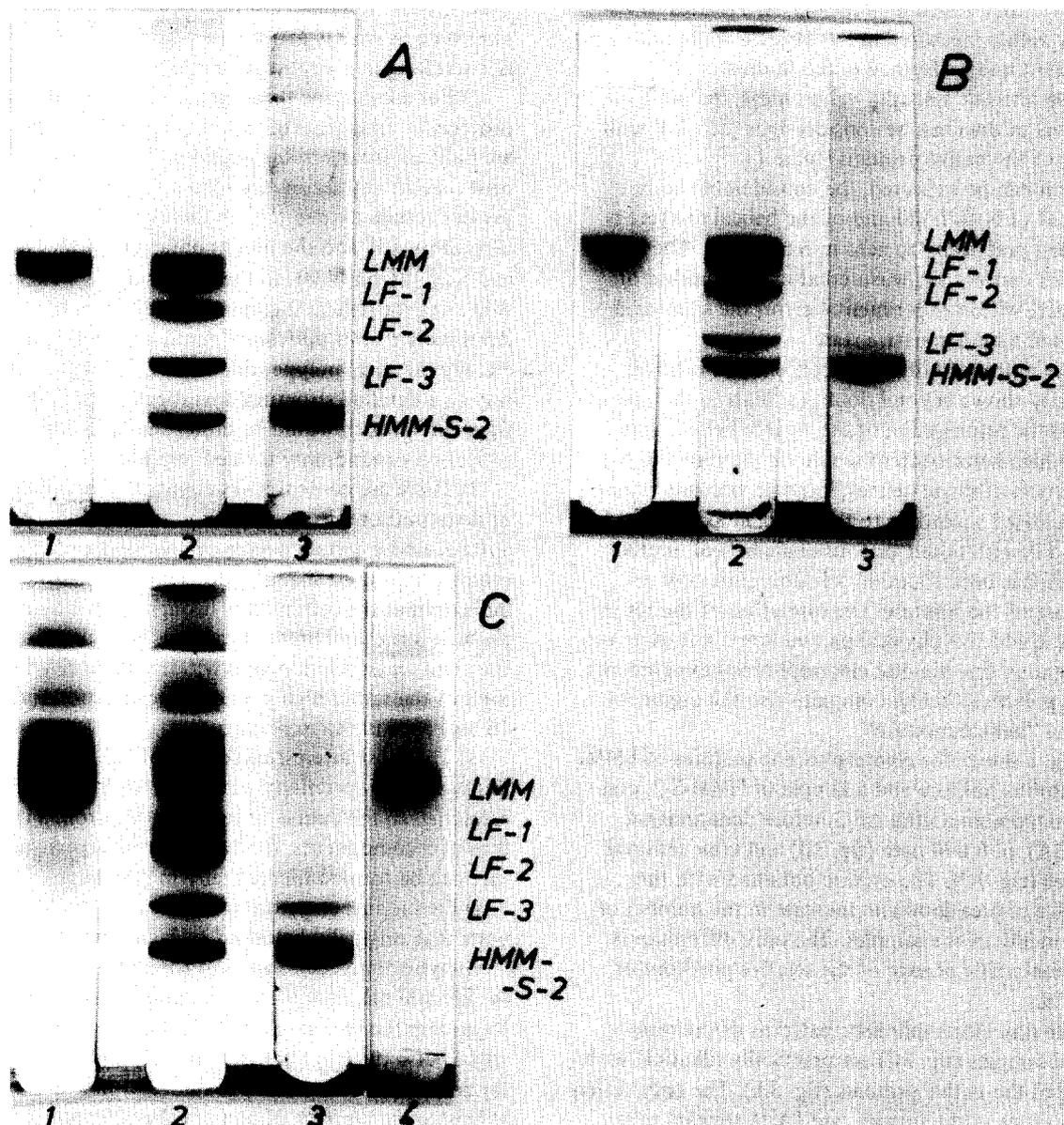


Fig. 3. Disc electrophoresis of helical protein fragments. A: untreated samples, B: electrophoresis in the presence of 6.6 M urea, C: after elimination of urea. In each photograph: 1: LMM; 2: "helical mixture"; 3: HMM-S-2 contaminated with LF-3; 4 (in C): LMM as reassociated by 7 days dialysis.

removal. In table 1 the results of ORD measurement and intrinsic viscosities are summed up.

Calculating according to Tanford [11] the intrinsic viscosity values found have given a molecular weight of 65,100 for the LMM subunit, or presuming 2

identical subunits, 130,000 for the molecular weight of LMM. This value is similar to the value arrived at by viscosity measurement in GuHCl [3], but somewhat lower than the one accepted in the literature: 151,000 [12], 146,000 [13]. It must be noted,

however that Tanford's formula used relates to viscosity values measured in 8.0 M urea, while our measurement was performed in 6.6 M urea.

The intrinsic viscosity values measured after the removal of urea can be considered as identical with those of the native proteins (table 1).

As it can be expected, the considerable helix content of both LMM and of the helical mixture is reduced practically to zero in 6.6 M urea. The values of helix content as measured after the removal of urea are, within experimental error, the same as the values obtained in native state.

The return of both helix content and intrinsic viscosity shows beyond doubt (at least in the case of LMM) the reformation of the double helical rope. If the high helix content would be the result of the formation of single helices, intrinsic viscosity would be increased according to the doubled axial ratio. It should be also noted that no single-helical, rodlike protein has been found in solution until now. In the case of the mixture, interpretation of the return of these additive physical parameters is not quite unambiguous, but the disc electrophoresis experiments to be presented below complete also the argument for the "helical mixture".

Fig. 3 shows the electrophoretic pictures of LMM, the fibrous mixture and a sample of HMM-S-2, contaminated with a little LF-3 before denaturation (fig. 3A), in 6.6 M urea (fig. 3B) and after removal of urea (fig. 3C). The picture obtained with the samples in urea shows no increase in the number of discs in any of the samples. The only difference is a characteristic change of the relative positions of the discs.

The disc electrophoretic patterns of the reassociated samples (fig. 3C) are practically identical with those of the native proteins (fig. 3A). The only difference is that in the former case LMM appears as a diffuse spot (see fig. 3C). If, however, a more prolonged dialysis is carried out the picture of reassociated LMM becomes nearly as sharp as that of native LMM (see fig. 3C, gel 4). The difference in the pictures of native LMM and of LMM renatured by a relatively short dialysis cannot be the expression of a considerable structural difference, as the physical data presented in table 1 pointing to complete renaturation were obtained after dialysis for 48 hr only.

The complete reversibility of the unfolding of

these myosin fragments, shown beyond doubt in pure state as well as in a mixture by these experiments, is interesting in many respects:

1) For a long time it was generally assumed that proteolytic fragments of myosin, especially LMM, are built up from smaller peptides, "protomyosins", held together by secondary forces only [14]. The present results extend former findings from our laboratory [3] and the results obtained by McCubbin and Kay [2] on LMM, to the LMM subfragments as well as to the helical fragment derived from HMM. All these are built up from 2 continuous, electrophoretically identical polypeptide chains. This opens the way to a detailed structural investigation of all these fragments, which would be rather senseless with a collection of randomly formed peptides.

2) There are controversies about the reversibility of denaturation of the whole myosin molecule. Some authors find a certain degree of reversibility [15], others doubt these claims [16]. Our results seem to indicate that the difficulty is brought about by the globular structural units of myosin. With the fibrous fragments reassociation goes without difficulty in spite of the rather high protein concentrations (13–16 mg) used in our experiments.

3) The most interesting feature of the present findings is the specificity according to length of the refolding. In the course of reassociation from a mixture of 5 components, the two-chained superstructure can be formed theoretically in two ways:

i) the original structure is restored, that is, subunits that originally belonged to each other are folded;
ii) hybrid molecules are also formed (see fig. 1).
In this case maximally 15 species ought to be found. Regarding the resolving power and the great sensitivity of disc electrophoresis, these "hybrids" when formed would cause the appearance of new discs or at least some blurring of one or other component. The results of disc electrophoresis of renatured mixtures clearly exclude the possibility of the formation of hybrid molecules. It follows from all this that only polypeptide chains of the same length unite to reform the superhelical structure.

To explain this fact we must rule out the assumption that finding of the proper match is based simply upon structural specificity, like in the case of the renaturation of enzymes consisting of several subunits [17], because in the case discussed here the

sequence of any subunit is identical with that of a considerable segment of all the longer chains present. As until now no single helical protein rod free in solution has been described, one must suppose that in the course of reassociation the α -helical and coiled-coil structures develop simultaneously. We assume that hybrid molecules are not formed, because if they were, the part of the longer subunit not participating in the coiled-coil structure could not take the α -helical conformation either. Such a structure would be less stable thermodynamically than a perfect double rope, hence in a system in equilibrium it would be eliminated.

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