SEPARATION OF TWO HMM-S1 SPECIES FROM WHITE MUSCLE MYOSIN*

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SUMMARY : Heavy meromyosin subfragment 1 was resolved by chromatography on DEAE-cellulose into two fractions characterized by the nature of the alkali light chains present. It was shown that even in an HMM-S1 preparation with an extensive fragmentation of the heavy chain a polyacrylamide gel electrophoresis analysis differentiates alkali light chains among the light fragmentation components. A non-fragmented HMM-S1 was obtained from a papain digest of myofibrils and the chromatographic analysis supplied further evidence of the separation of the two species of HMM-S1 present in rabbit white muscle myosin.

INTRODUCTION. The presence of two kinds of alkali light chains (1) (A-1 and A-2) in rabbit white muscle myosin indicates that either both are constituents of the same myosin molecule or two kinds of myosin molecules (isozymes) are present in the same muscle. Each HMM-S-1 contains one alkali light chain, therefore an HMM-S-1 preparation must be considered as an heterogenous population of two varieties of HMM-S-1 : S-1 (A-1) and S-1 (A-2). A comparison of the characteristics of these two varieties is apt to shed some light on the signification of this heterogeneity. Following earlier observations a chromatographic separation study was undertaken.

In a previous report we mentioned that a tryptic preparation of myosin subfragment-1 was partly resolved by column chromatography on DEAE-cellulose into two kinds of HMM-S-1 species (2). However HMM-S-1 prepared by proteolytic digestion with tryp-

* Oral reports of this work were given at two meetings of the European Muscle Club (10).

Abbreviations used : HMM : heavy meromyosin EDTA : ethylene-diamine-tetraacetic acid - DEAE : diethyl-aminoethyl - SDS : sodium dodecylsulfate - DTT : dithiotreitol - DTNB : bis-(5-carboxy-4-nitrophenyl)disulphide - PAGE : polyacrylamide gel electrophoresis. S-1 (T) HMM-S-1 subfragment obtained by proteolysis with trypsin; (P) : papain; (C) : chymotrypsin.

sin, and to a lesser extent with papain, are usually more or less fragmented depending upon the concentration of the proteolytic agent and the time of proteolysis (2,3,4). This situation leads to complicated patterns in SDS-polyacrylamide gel electrophoresis and makes the analysis considerably more difficult.

We wish to show here that a careful study of the low molecular weight components observed by polyacrylamide gel electrophoresis of HMM-S1 under dissociating conditions supports the conclusion that the two species are fractionated as mentioned earlier according to their alkali light chain.

MATERIAL AND METHODS. Myofibrils from rabbit psoas muscle were obtained according to Perry's method (5) in a final suspension of 10-15 $mg\text{-}ml^{-1}$ in 0.1 M KCl, 0.02 M sodium borate buffer (pH 7.2).

Tryptic proteolysis of myofibrils. EDTA was added to a final concentration of 0.01 M, the pH was adjusted to 8.2 with 0.1 M NaOH, then DTT was added to a final concentration of 0.001 M.

The suspension was incubated with trypsin (bovine pancreas, Worthington, 1 mg enzyme/500 mg myofibrils) for 20 min at 24°C. The proteolysis was stopped by adding a solution of trypsin inhibitor (Soja bean, 2 mg inhibitor/ 1 mg trypsin). All subsequent operations were as described earlier (2).

Myofibril proteolysis with papain. (Papaya latex, Boehringer). The general scheme was essentially the same, with the following details : proteolysis was carried out in a 20 mM Tris-maleate buffer, pH 7.0, EDTA : 0.5 mM, β-mercapto-ethanol : 5 mM, KCl : 20 mM, papain to myofibrils ratio : 1/500 (w/w), temperature 25°C, 4 min. After washing, dissociation and centrifugation to remove actin, the supernatant (crude HMM-S-1) was dialyzed against a 5 mM Tris-HCl buffer, pH 7.3, DTT : 0.5 mM and chromatographed on a DEAE-cellulose column (350 x 15 mm; Whatman DE-52).

The proteins were eluted with a 0-0.12 M KCl gradient in the same solution.

Polyacrylamide gel electrophoresis PAGE was performed in the presence of urea (6.6 M or 8 M) or SDS (1%) (6).

Enzymatic activity, ATPase activity was measured with a pH-stat at 25°C under a nitrogen atmosphere.

RESULTS AND DISCUSSION. Tryptic digestion of myofibrils is a relatively simple way to obtain myosin subfragment-1. HMM-S-1 is easily

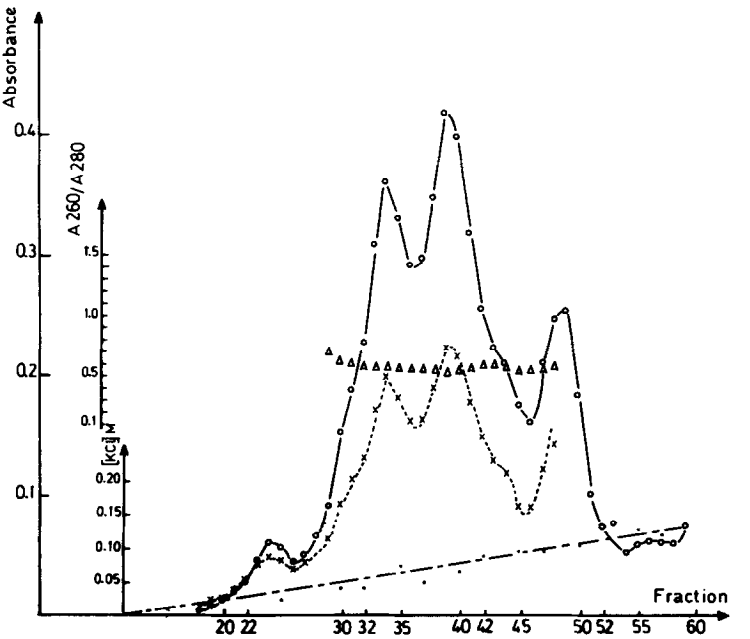

Fig. 1 DEAE-cellulose chromatography of S-1 (T). Elution with a KCl gradient 0-0.15 M. ⊙: absorbance read at 280 nm ; x : absorbance read at 260 nm ; Δ : ratio of absorbance 260/280 ; 6 ml fractions ; flow-rate 20-22 ml hr^{-1}.

purified on a DEAE-cellulose column. Fig. 1 shows a very distinct heterogeneity of the elution profile as previously described (2). When the various fractions were analyzed by urea-PAGE we could regularly confirm (fig. 2a) the presence of two fast-moving components (U_A and U_B). Component U_A was almost exclusively present in the first eluted fractions whereas the second, U_B, appeared later and progressively replaced U_A. When these same fractions were submitted to SDS-PAGE (fig.2b) a rather complicated pattern was usually observed as a consequence of proteolytic fragmentations undergone by the heavy subunit of HMM-S1. In fig.3 the amount of material present in the various bands was compared for the two electrophoretic analyses. It can be seen that component U_A in urea-PAGE corresponds to band L_2 (21000 daltons) in SDS-PAGE. Similarly U_B can be identified to B_2 (15000 daltons).

The apparent molecular weights of L_2 and B_2 are close enough to the usually accepted values for the two alkali light chains A-1 and A-2. Moreover by varying the proteolysis conditions it was possible to induce a significant variation in the electro-

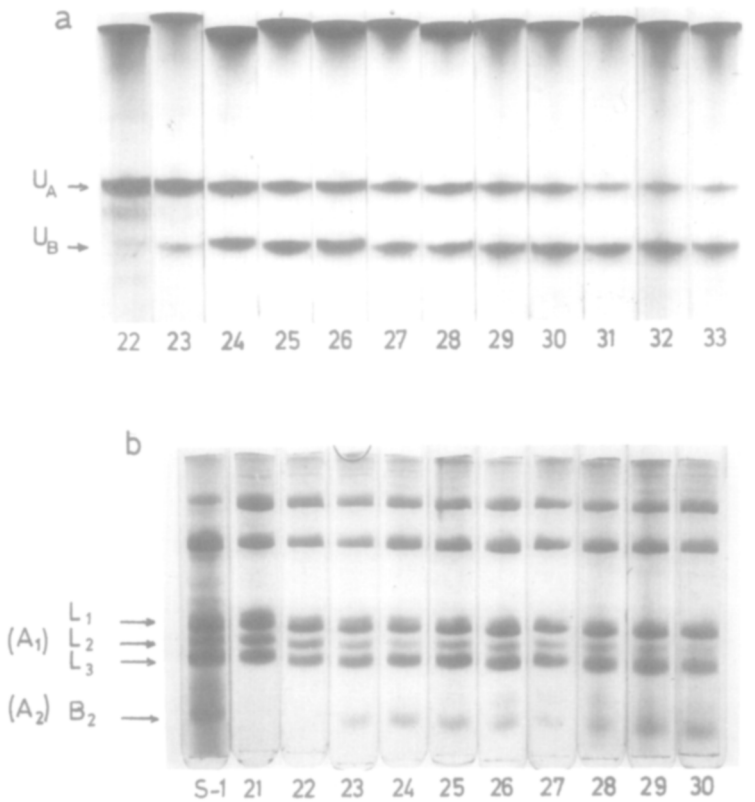

Fig. 2 a) urea-PAGE of samples taken from each fraction of a chromatography as in fig. 1. Urea 6.6 M, 10 % gels stained with amido-black.
b) SDS-PAGE of same fractions. SDS : 1 %, 10 % gels stained with Coomassie Brilliant Blue.

phoretic pattern (table 1). High concentrations of the proteolytic agent increased considerably the relative amount of L_1 and L_3 whereas L_2 was maximum for preparations in which the extent of fragmentation was minimum. (The analyses were performed on early fractions of the elugrams where B_2 was virtually absent). The high value of L_1 for certain preparations, together with its apparent molecular weight, indicate that it cannot be one of the light chains.

These observations are coherent with the hypothesis that L_2 is one of the light chains (A-1) and we note that it can be markedly deficient under certain preparation conditions (table 1) since the expected value would be of the order of 18%.

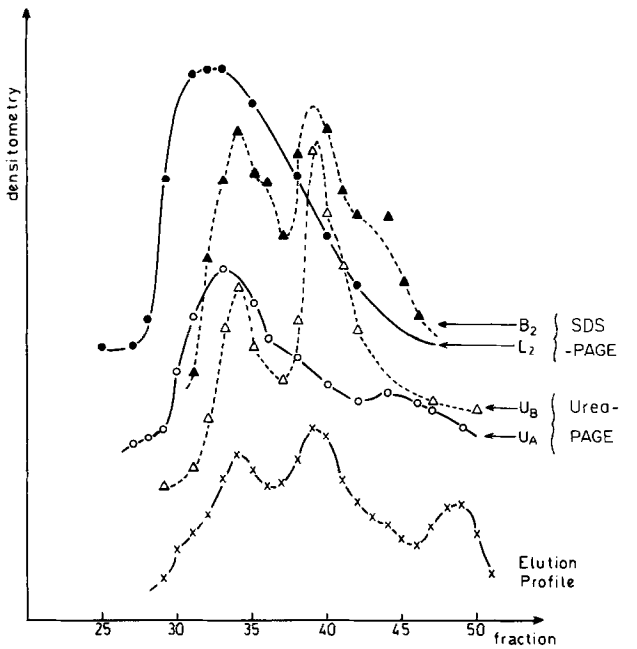

Fig. 3 Comparison of amounts of U_A and U_B in urea-gels with L_2 and B_2 in SDS-gels, showing a correlation between U_A and L_2 (A-1 light chain) and U_B and B_2 (A-2 light chain). Intensity of the bands in arbitrary units.

Further evidence was obtained by two successive electrophoreses. The two components U_A and U_B were first separated by urea-PAGE, the corresponding zones were cut and the slices were incubated at 95°C for 5 min in a solution of 1% SDS then placed separately on top of SDS-gels. These electrophoreses (fig.3) indicate that the components U_A and U_B have the same mobility as L_2 and B_2 respectively, while these mobilities are also respectively identical to those of the two alkali light chain A-1 and A-2. It can be observed also that in 6.6 M urea no component other than the light chains leaves the bulk of peptides found near the origin of the gel. This system may thus be particularly useful to study the stoichiometry of light chains in various myosin, HMM and HMM-S-1 preparations including those giving complex patterns in SDS-PAGE.

For further confirmation of the suggested assignment for L_2 (A-1) and B_2 (A-2), an attempt was made to prepare a non-fragmented HMM-S-1. The use of papain instead of trypsin for a proteolytic digestion of myofibrils was first suggested by Cooke (7).

Table 1

Proteolysis conditions		L_1 (%)*	L_2 (%)*	L_3 (%)*
trypsin/ myofibril (w/w)	time (min)			
1/600	40	11.2	18.6	6.7
1/2000	60	11.4	19.0	4.7
1/130	10	19.8	18.0	14.7
1/130	20	21.0	15.0	14.8
1/130	35	24.5	14.9	9.4
1/130	60	25.2	12.8	16.9
1/130	120	26.4	10.0	16.3

Compared amounts of components L_1, L_2, L_3 in SDS-PAGE for various preparations. Samples were taken from fractions at the beginning of the elution profile where the amount of A-2 (U_B in urea-PAGE or B_2 in SDS-PAGE) was negligible. Gels were stained with Coomassie Brilliant Blue and intensity was recorded with a Vernon densitometer equiped with an integrator.

* values expressed as percent of protein present on gels (SDS-PAGE as described in Material and Methods).

Papain preparations of HMM-S-1 (fig.4) were significantly less fragmented, and the low molecular weight components are the two alkali lights chains almost exclusively. In spite of the poor separation achieved in this chromatography the same pattern was observed whereby first fractions contain essentially HMM-S-1 with alkali light chain A-1, whereas A-2 is predominant in the last fractions. A faint band with a molecular weight of ca 17000-18000 was often present in the first eluted fractions. It is likely but not definitely proved that it is a residual DTNB light chain material.

ATPase activity. In an early study (2) we observed essentially no difference in EDTA-ATPase specific activity between the various fractions of S-1 (T) along the elution profile. On the contrary Yagi et al. (8) reported differences of a factor 3 with

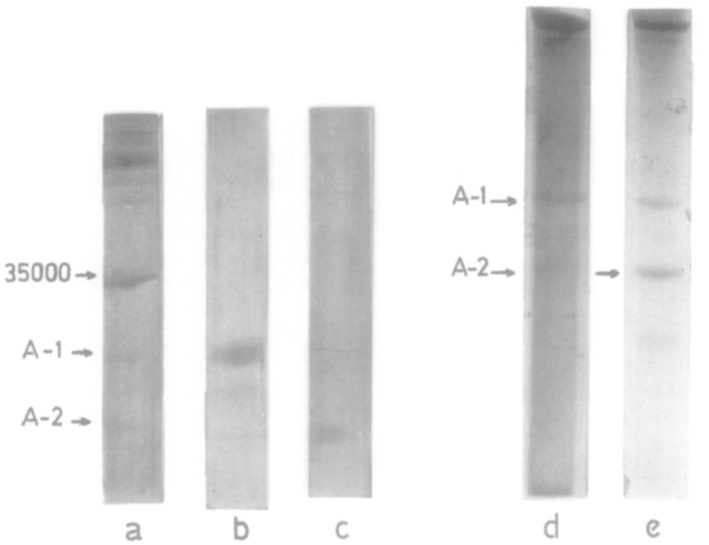

Fig. 4 Polyacrylamide gel electrophoresis : 10 % gels, 1 % SDS.
a) S-1 (P) before DEAE-cellulose chromatography. In addition to the two alkali light chains ; a 35 000 MW component is regularly found at this stage of the preparation.
b) U_A component from a urea-PAGE (see text).
c) U_B component from a urea-PAGE (see text)
d) and e) samples of S-1 (P) taken at the beginning and end of the elution from a DEAE-cellulose-column.

S-1 (C). In the present study the EDTA-ATPase activity of S-1 (P) was of the order of 8 μmole Pi $min^{-1}.mg^{-1}$ while Ca-ATPase gave 2-3 μmole Pi $min^{-1}.mg^{-1}$ throughout. Although low values were sometimes recorded in the first fraction it is not unlikely that they were due to a light chain deficiency. During the final writing of this report, Weeds and Taylor (9) published an excellent separation of the two kinds of S-1 (C). They found no difference in ATPase activities in the absence of actin.

Thus it is established that the variously prepared HMM-S1: S-1 (C) (Yagi and Otani (8) ; Weeds and Taylor (9), S-1 (P) and S-1 (T) (this study) can be separated according to their alkali light chain. A rapid comparison of characteristics is in favour of S-1 (C) which is not fragmented and can be easily fractionated by the chromatographic procedure described by Weeds and Taylor. S-1 (T) is almost invariably fragmented and S-1 (P) seems to be more difficult to resolve. Nevertheless these last two preparations may still be useful in structure-function studies.

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