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# Dependence of the length of the heavy chain of chymotryptic subfragment 1 on the temperature of myosin digestion

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Limited digestion of filamentous myosin with chymotrypsin at 0°C in the absence of divalent cations generates two forms of subfragment 1 (S1), with heavy chains of 95 kDa and 98 kDa. The difference is at the C-terminal end of the chain. The 98 kDa form prevails, in contrast to the preparations obtained by digestion at room temperature which consist of the shorter species and only traces of the longer one. The results support the idea of a temperature-dependent conformational transition at the head-rod junctional region of the myosin heavy chain.

Myosin; Chymotryptic cleavage; Temperature dependence; Subfragment 1

## 1. INTRODUCTION

Recent studies suggest that the structural change which results in generation of force during muscle contraction takes place within the myosin head [1,2]. They do not, however, exclude a contribution of structural transitions in the flexible joint between the head and rod portion of the myosin molecule. One of the approaches to study the function of the head-rod junction is to compare the properties of HMM and of different forms of S1. Preparations of S1 from skeletal muscle myosin are routinely obtained by digestion of filamentous myosin at room temperature, either by chymotrypsin to generate chymotryptic S1 [3], or by papain in the presence or absence of divalent cations to obtain papain Mg-S1 or papain EDTA-S1, respectively [4]. These three forms of S1 differ in the masses of their heavy chains that are longer at their C-terminal end in papain S1 preparations than in chymotryptic S1 [5]. A slight (1 kDa) difference in the heavy chain lengths between papain Mg-S1 (longer) and EDTA-S1 (shorter) has also been reported [2,6]. The difference in the heavy chain lengths between papain S1 and chymotryptic S1 has been successfully used to localize the site of the heavy chain interaction with the light chain LC2 [7]. It has also been shown [2] that papain S1 preparations are more efficient than chymotryptic S1 in supporting in vitro the movement of actin filaments on S1 bound to a nitrocellulose film. The efficiency of the movement supported by chymotryptic S1 increased when S1 was enriched by high performance gel filtration in a fraction with a slightly longer heavy chain which was present in a small proportion in the initial preparation.

In this work we have extended previous investigations on the influence of temperature on the proteolytic susceptibility of the neck region of the myosin head [6,8]. The results demonstrate a simple way to prepare a chymotryptic S1 rich fraction with the longer heavy chain. Such preparations may be useful in further studies on the function of the head-rod junction of the myosin molecule.

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*Abbreviations:* S1, myosin subfragment 1; HMM, heavy meromyosin; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; 1,5-IAEDANS, *N*-iodoacetyl-*N'*-(5-sulfo-1-naphthyl)ethylenediamine



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## 2. MATERIALS AND METHODS

Chymotryptic S1 was obtained from rabbit fast skeletal muscle myosin either according to [3] or by modification of this procedure by carrying out the digestion of myosin at 0°C with TLCK-$\alpha$-chymotrypsin at the enzyme to protein ratio of 1:75 (w/w). The S1 preparations were labelled at the SH1 thiol group with a fluorescent dye 1,5-IAEDANS as described in [6]. Tryptic digestion of S1 (2 mg/ml) was carried out using TPCK-trypsin at the enzyme to substrate ratio of 1:100 (w/w) at 25°C, or 1:10 (w/w) at 0°C in 50 mM KCl, 10 mM imidazole, pH 7.0, and 1 mM dithiothreitol. The digestion was stopped by adding SDS and 2-mercaptoethanol to a final concentration of 1% and incubating for 5 min at 100°C. The digestion patterns were examined on SDS-PAGE according to [9].

## 3. RESULTS AND DISCUSSION

We have recently shown that one of the tryptic cleavage sites in the heavy chain of LC2-deficient myosin, which is close to the site of chymotryptic cleavage yielding the chymotryptic S1, is not susceptible when the digestion is performed at 0°C [6]. Similar temperature sensitivity of the chymotryptic cleavage of the head-rod junction is apparent from Arrhenius plots of the rates of chymotryptic digestion monitored using a pH stat [8]. In the present work, the effect of temperature on the accessibility to chymotrypsin of this region of the myosin heavy chain was investigated by digestion of myosin either at 0°C or at room temperature, followed by isolation of the resulting S1 preparations and their analysis by SDS-PAGE.

The electrophoretic patterns of the two chymotryptic S1 preparations, when inspected on 12% polyacrylamide gels, do not substantially differ (fig.1a). Both preparations comprise the intact heavy chain and the light chains LC1 and LC3; the absence of divalent cations during digestion results in degradation of the LC2 light chain. Unlike in the papain S1 preparations, the LC1 is not degraded to its derivatives. Electrophoresis on 6% gels (fig.1b) shows that the mobility of the heavy chain of S1 obtained by chymotryptic digestion at 0°C is intermediate between those of S1 routinely prepared by digestion of myosin with chymotrypsin and papain, respectively, at room temperature.

To determine more accurately the molecular mass and the degree of homogeneity of the heavy chain, chymotryptic S1 preparations obtained by digestion of myosin at either 0°C or at room temperature, fluorescently labelled with 1,5-IAEDANS at the SH1 thiol group to enable identification of the C-terminal portion of the chain, were digested with trypsin at 0°C. We have previously shown that tryptic digestion at 0°C of LC2-deficient papain S1 generates the C-terminal heavy chain fragment of 26 kDa instead of a 20 kDa fragment formed at room temperature [6]. The latter seems to correspond to the C-terminal 20 kDa tryptic fragment of the heavy chain of chymotryptic S1 routinely prepared by digestion of myosin at room temperature [10]. As shown in fig.2, tryptic cleavage of S1 prepared by digestion of myosin at 0°C, in addition to the N-terminal 27 kDa and central 50 kDa fragments, generated two fluorescent C-terminal fragments, of 20 kDa and 23 kDa, the latter being the predominant species. On the other hand, in the tryptic digest of S1 prepared by digestion of myosin at room temperature, only a faint band of the 23 kDa peptide can be seen. Fig.2 also shows that the 23 kDa fragment is fast degraded to the 20 kDa one when the tryptic digestion is carried out at 25°C.

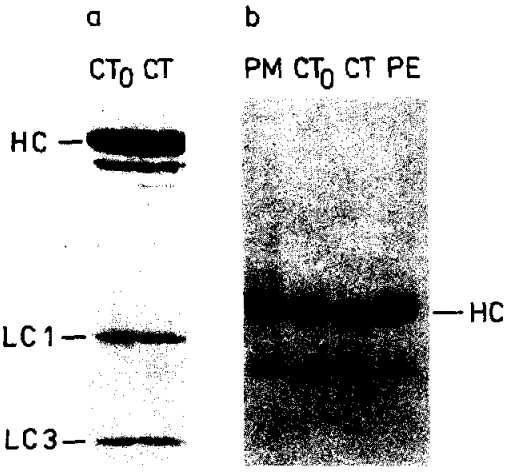


Fig.1. SDS-PAGE of the myosin S1 preparations on a 12% (a) and 6% (b) gel. Chymotryptic S1 was obtained by digestion of myosin at 0°C ($CT_0$) or at room temperature (CT). For comparison, papain Mg-S1 (PM) and EDTA-S1 (PE) are also shown.

The results clearly show that the relative rates of chymotryptic cleavage of the myosin heavy chain at two sites, generating a 95 kDa and 98 kDa heavy chain fragment (90 kDa and 93 kDa according to [2]), change depending on the temperature. At room temperature the rate of

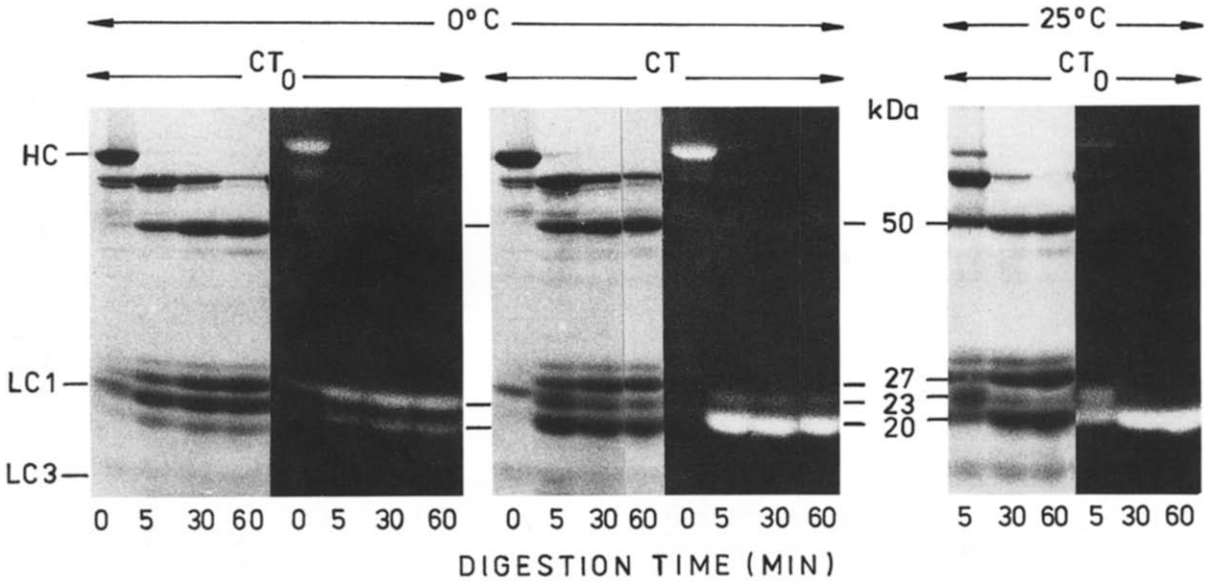


Fig.2. Electrophoretic patterns of tryptic digestion of chymotryptic S1 preparations. S1 obtained by chymotryptic digestion of myosin at either 0°C ($CT_0$) or room temperature (CT) were digested with trypsin at 0°C or at 25°C as indicated in the figure. On the left, gels stained with Coomassie blue; on the right, fluorescence of the IAEDANS-labelled fragments. SDS-PAGE was carried out on 12% gels.

cleavage at site A, closer to the N-terminus of the heavy chain (see fig.3), must be nearly equal to the cleavage rate at site B, as judged from the generation of only traces of the fluorescent 23 kDa peptide during subsequent digestion of the obtained S1 with trypsin at 0°C. Lowering of the temperature seems to decrease the rate of the chymotryptic cleavage at site A relative to that at site B, resulting in the appearance of considerable amounts of the longer S1.

These observations support the suggestion of a temperature-dependent conformational transition in the head-rod junction [6,8] that may be relevant

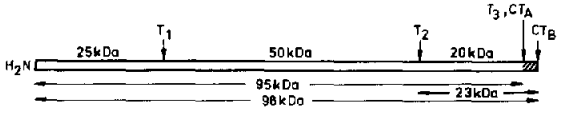


Fig.3. Sites of chymotryptic ($CT_A$, $CT_B$) and tryptic (T) cleavage at the head-rod junction and in the S1 portion of the myosin heavy chain. The accessibility of sites $CT_A$ and $T_3$ diminishes as the temperature is lowered, resulting in the generation by chymotrypsin of a longer (98 kDa) S1 heavy chain portion through the cleavage at site $CT_B$, and in the formation of a 23 kDa C-terminal fragment during subsequent tryptic digestion of the longer S1 at 0°C.

to the temperature dependence of force generation during muscle contraction. Moreover, they demonstrate a simple way of preparation of the 98 kDa heavy chain chymotryptic S1 rich fraction.

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