Volume 211, number 1, 31–34 FEB 04359 January 1987

A method to exchange alkali light chains on myosin subfragment 1

M. Crasnier

Department of Biochemistry, University of Birmingham, PO Box 363, Birmingham B15 2TT, England

Received 7 October 1986; revised version received 21 November 1986

Exchange of bound alkali light chains on myosin by free alkali light chains is described. It was found that the yield of hybrid obtained was dependent on the incubation time in 4.7 M NH_4Cl at pH 9.5. 60% recovery of S1(A1) from S1(A2) was obtained using only a 2-fold molar excess of A1 over S1(A2).

Actin; Myosin; Myosin subfragment 1; Alkali light chain; Hybridization

1. INTRODUCTION

The interactions of actin with myosin are essential to the molecular events associated with muscle contraction. Such interactions have been characterized in vitro with fluorescent labelled proteins by using fluorescence energy transfer experiments [1,2]. Thus fluorescent probes have been selectively introduced into skeletal muscle myosin subfragment 1 (S1) prepared by chymotryptic digestion of myosin and containing either the alkali 1 light chain (A1) or the alkali 2 light chain (A2). The probes can be covalently attached to the unique fast reacting residue (SH1) of the heavy chain [1] or to the unique SH group of the light chain [2]. In the latter case the labelling requires a hybridization procedure usually in which labelled A1 is added to S1(A2) to give a labelled S1(A1) species. The preparation of hybrids is thus an essential part of the fluorescence energy transfer experiments.

Attempts have been made to obtain hybrids with similar ATPase activity and F-actin binding characteristics to the 'native' S1 [3,4]. High salt concentrations (4.7 M NH_4Cl [3], 4 M LiCl [4,5]) have been used in the presence of an excess of free light chain. However the exchange yields obtained were relatively low even with about a ten molar excess of free light chain over S1 [6]. Ueno and Morita [7] showed that there was not a clear dissociation of S1 in 4.7 M NH_4Cl at pH 7.0 and proposed to perform the exchange in 4.7 M NH_4Cl at pH 9.9 as both alkali light chains were known to dissociate from the heavy chain at high pH [8].

Here we report that the dissociation of the light chain from the heavy chain in 4.7 M NH_4Cl at pH 9.5 is complete as described [7] but about 60% of hybrid obtained under these conditions is inactive. We are attempting to improve the experimental conditions in order to increase the yield of active hybrid.

Correspondence address: M. Crasnier, Department of Biochemistry, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01605, USA

Abbreviations: S1, myosin subfragment 1; S1(A1), S1(A2), rabbit muscle myosin subfragment 1 containing either the alkali 1 light chain (A1) or the alkali 2 light chain (A2); Bicine, *N,N*-bis(2-hydroxyethyl)glycine; resin SE-53, sulphoxyethyl cellulose

2. MATERIALS AND METHODS

2.1. *Preparation of proteins*

Fast twitch muscle myosin from the longissimus dorsi muscle of New Zealand rabbits was prepared

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

as described in [1]. Subfragment 1 was prepared by chymotryptic digestion of myosin [9] as modified in [10]. Pure S1(A1) and S1(A2) were obtained as in [1]. Light chains were extracted from myosin by the urea method and fractionated as cited in [11]. Protein concentrations were estimated by absorbance measurements at 280 nm; the absorption coefficients used were: for S1, 0.8 $mg \cdot ml^{-1} \cdot cm^{-1}$; for A1 light chain, 0.31 $mg \cdot ml^{-1} \cdot cm^{-1}$; and for A2 light chain, 0.45 $mg \cdot ml^{-1} \cdot cm^{-1}$ [11]. Protein purity was routinely analysed by polyacrylamide gel electrophoresis under denaturing conditions in 12% polyacrylamide gels in the presence of 0.1% SDS in Tris-Bicine buffer at pH 8.3 [12]. Gels were stained with 0.4% (w/v) Page blue 83 in 50% (v/v) methanol and 10% (v/v) acetic acid.

Rabbit skeletal muscle actin used for actin-activated ATPase measurements was prepared as described in [1]. G-Actin concentration was determined by absorbance at 290 nm using an absorption coefficient of 0.63 $mg \cdot ml^{-1} \cdot cm^{-1}$ [13]. Prior to any measurements the G-actin was polymerized as described in [1].

2.2. *ATPase measurement*

Actin-activated ATPase activity was followed by measuring the amount of P_i released as a function of time [14]. The standard incubation medium (1 ml) contained 25 mM triethanolamine-HCl, 2.5 mM $MgCl_2$, 2.0 mM ATP, pH 7.5, F-actin (5-fold molar excess over S1) and S1. After incubation at 25°C, the reaction was stopped by addition of 1 ml of 10% trichloroacetic acid. The phosphate concentration was estimated as in [14]. A unit of ATPase activity was defined as the amount of P_i released ($\mu mol \cdot min^{-1}$) under standard conditions.

3. RESULTS

The exchange reaction of the light chains was realized using both freeze-dried S1(A2) and A1 with a 2-fold molar excess of A1 over S1(A2). S1(A2) and A1 were mixed together with a solution of 4.7 M NH_4Cl, 2 mM EDTA, 2 mM DTT with pH adjusted to 9.5 by addition of NH_3. The next steps were as in [7]. The mixture was gently stirred at 4°C for 30 min and then dialysed against 50 mM triethanolamine-HCl, 2 M NH_4Cl, 0.5 mM DTT, pH 8, for 2 h. The dialysis was further continued against the same buffer with 1 M NH_4Cl and then against 10 mM Mops, 0.5 mM DTT, pH 8, without NH_4Cl both for 2 h. After further dialysis overnight the solution was applied to a SE-53 column equilibrated with the Mops buffer. The elution was performed with two linear gradients of NaCl from 0 to 0.1 M and then from 0.1 to 0.5 M. The elution profile is shown in fig.1.

Different fractions were analysed by polyacrylamide gel electrophoresis. Fractions 10 (peak 1, fig.1), 70 (peak 2, fig.1) and 95 (peak 3, fig.1) corresponded respectively to (i) a mixture of A1 and A2, (ii) a S1(A1) hybrid species and (iii) a S1(A1) hybrid species slightly contaminated by S1(A2) (fig.2).

The fractions of peaks 2 and 3 were pooled in order to measure the actin-activated ATPase activity of both hybrids. When assayed under standard conditions, the specific activity of S1(A1) of peak 2 was 0.98 unit/mg and similar to the one of a native S1(A1) which was 1 unit/mg. On the other hand, S1(A1) of peak 3 which represented 60% of

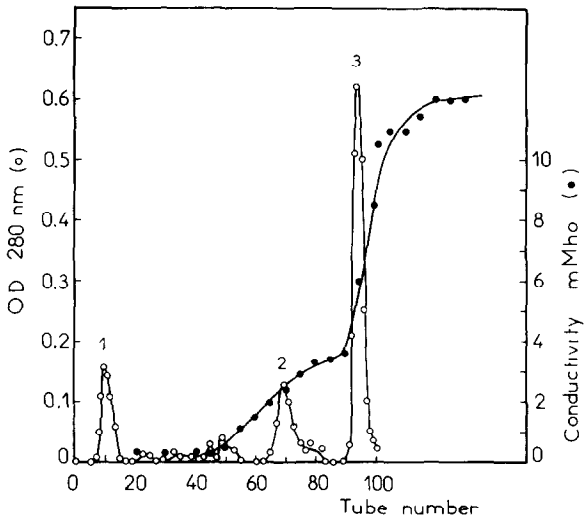

Fig.1. SE-53 chromatography of a mixture of S1(A2) and A1 after incubation in 4.7 M NH_4Cl at pH 9.5 for 30 min. The sample (11 mg protein) was applied to the column (20 × 2.5 cm) in 10 mM Mops, 0.5 mM DTT, pH 8, at a flow rate of 40 ml/h. 2 ml fractions were collected. The elution was performed with two linear gradients of NaCl from 0 to 0.1 M and then from 0.1 to 0.5 M.

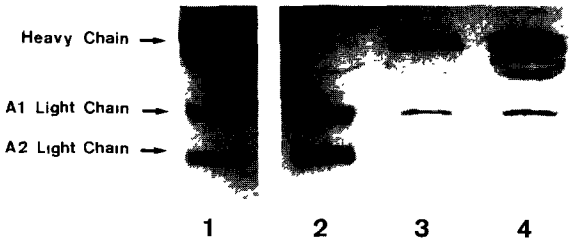

Fig.2. Polyacrylamide gel electrophoresis in the presence of SDS of different fractions from the SE-53 column. Lanes: 1, sample loaded on the SE-53 column; 2, fraction 10 (peak 1, fig.1); 3, fraction 70 (peak 2, fig.1); 4, fraction 95 (peak 3, fig.1).

total S1(A1) was inactive and precipitated when dialysed.

The exchange reaction was performed with the following change. After having been stirred at 4°C for 30 min the mixture was immediately applied to a Sephadex G-25 column equilibrated with 10 mM Mops, 0.5 mM DTT, pH 8, and then to the SE-53 column as described above. In that case, the inactive hybrid represented 40% of total S1(A1). The time of incubation at 4°C was then reduced to 15 min and the sample applied to G-25 Sephadex and SE-53 as above. The SE-53 elution profile obtained under those conditions is shown in fig.3.

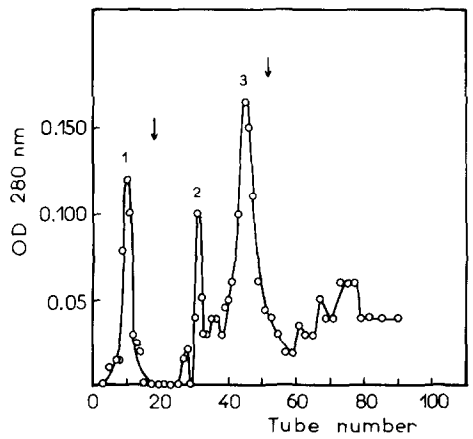

Fig.3. SE-53 chromatography of a mixture of S1(A2) and A1 after incubation in 4.7 M NH_4Cl at pH 9.5 for 15 min. The sample (5 mg protein) was applied to the column (20 × 2.5 cm) in 10 mM Mops, 0.5 mM DTT, pH 8, at a flow rate of 40 ml/h. 2 ml fractions were collected. The first and second arrows show the start of the linear gradients of NaCl from 0 to 0.1 M and from 0.1 to 0.5 M, respectively.

Two main peaks were obtained, the first one (peak 1, fig.3) corresponded to the mixture of light chains and the second one (peak 3, fig.3) to the active hybrid S1(A1). A S1(A2) species was also eluted with the first linear gradient (peak 2, fig.3). When the second gradient was performed, no inactive hybrid was obtained.

Under these conditions the yield of active hybrid obtained represented 60% of the native S1(A2) used.

4. DISCUSSION

Ueno and Morita [7] showed that the A2 light chain is preferentially dissociated from the heavy chain when S1 is incubated in the presence of 4.7 M NH_4Cl at pH 9.9. This result is not surprising as it was known from previous studies that the A2 interaction with the heavy chain is weaker than that occurring with the A1 light chain [15,16]. For this reason the exchange of the light chains on the heavy chain was performed using A1 light chain and S1(A2).

When S1(A2) and a 2-fold molar excess of A1 over S1(A2) were incubated in 4.7 M NH_4Cl at pH 9.5 for 30 min and then dialysed against decreasing concentrations of NH_4Cl, A2 was fully dissociated from the heavy chain and a S1(A1) hybrid species was obtained. However, when performed under those conditions, part of the S1(A1) hybrid was inactive. Both active and inactive species could be separated on a SE-53 column and we showed that 60% of the total hybrid obtained was inactive. This result was indeed not surprising as it was known from previous studies [17] that the removal of light subunits at alkaline pH (or in the presence of 2 M guanidine) was accompanied by the aggregation of the heavy chain with an irreversible inactivation of S1. When the hybridization was performed for 15 min and NH_4Cl removed immediately by gel filtration, no inactive hybrid was obtained and we found 60% recovery of S1(A1) from S1(A2) using only a 2-fold molar excess of A1.

Whatever the method used to obtain hybrids, denaturing conditions leading to light subunit dissociation resulted in an inactivation of the ATPase activity of S1 even in the presence of ATP which has been reported to protect the ATPase activity of myosin [18]. The present results show that

the exchange of the light chains is a rapid process when compared to the time-dependent denaturation which occurred even after the reassociation of light subunits.

ACKNOWLEDGEMENTS

We are very grateful to Dr I.P. Trayer and Dr H.R. Trayer for helpful discussions during the course of this work. We are indebted to the NATO for financial support.

REFERENCES

[1] Trayer, H.R. and Trayer, I.P. (1983) Eur. J. Biochem. 135, 47–59.
[2] Marsh, D.J. and Lowey, S. (1980) Biochemistry 19, 774–784.
[3] Dreizen, P. and Gershman, L.C. (1970) Biochemistry 9, 1688–1693.
[4] Stracher, A. (1969) Biochem. Biophys. Res. Commun. 35, 519–525.
[5] Gershman, L.C. and Dreizen, P. (1970) Biochemistry 9, 1677–1687.
[6] Wagner, P.D. and Weeds, A.G. (1977) J. Mol. Biol. 109, 455–473.
[7] Ueno, H. and Morita, F. (1984) J. Biochem. 96, 895–900.
[8] Gaetjens, E., Barany, K., Bailin, G., Oppenheimer, H. and Barany, M. (1968) Arch. Biochem. Biophys. 123, 82–96.
[9] Weeds, A.G. and Taylor, R.A. (1975) Nature 257, 54–56.
[10] Prince, H.P., Trayer, H.R., Henry, G.D., Trayer, I.P., Dalgarno, D.C., Levine, B.A., Cary, P.D. and Turner, C. (1981) Eur. J. Biochem. 121, 213–219.
[11] Henry, G.D., Trayer, I.P., Brewer, S. and Levine, B.A. (1985) Eur. J. Biochem. 148, 75–82.
[12] Weeds, A.G., Hall, R. and Spurway, N.C. (1975) FEBS Lett. 49, 320–324.
[13] Lehrer, S.S. and Kerwar, G. (1972) Biochemistry 11, 1211–1217.
[14] Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–380.
[15] Burke, M. and Sivaramakrishnan, M. (1981) Biochemistry 20, 5908–5913.
[16] Burke, M. and Sivaramakrishnan, M. (1981) J. Biol. Chem. 256, 8859–8862.
[17] Gershman, L.C., Stracher, A. and Dreizen, P. (1969) J. Biol. Chem. 244, 2726–2736.
[18] Sivaramakrishnan, M. and Burke, M. (1981) J. Biol. Chem. 256, 2607–2610.