The effect of non-muscle tropomyosin on the interaction of filamin with F-actin

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1. INTRODUCTION

High-M_r proteins with filamin activity to crosslink F-actin filaments have been found in various types of muscle [1–3] and in a number of non-muscle cells [4–7]. In cultured non-muscle cells these proteins were localized in the stress fibers as well as in the microspikes and the membrane ruffles [8]. Tropomyosin is present in the stress fibers as well as F-actin [9,10] and can influence the interaction of filamin-like proteins with F-actin.

There is competition between smooth muscle filamin and the skeletal or smooth muscle tropomyosin for the binding sites on F-actin [11,12]. Here, we show that non-muscle tropomyosin isolated from bovine brain competes much less effectively with filamin for binding to F-actin and only partially inhibits the filamin–F-actin interaction.

2. MATERIALS AND METHODS

2.1. *Preparation of the proteins*

Actin was isolated from rabbit skeletal muscle as in [13] and additionally purified by gel-filtration on Sephadex G-150 column [14]. Chicken gizzard filamin was prepared as in [15]. Tropomyosin from bovine brain and rabbit skeletal muscle was prepared as in [16]. Proteins were > 95% pure as determined by SDS–polyacrylamide gel electrophoresis.

2.2. *Binding assays*

Prior to the binding experiments the proteins were dialysed overnight against 100 mM KCl, 8 mM $MgCl_2$ and 10 mM Tris–HCl (pH 7.4). The samples containing 20 μM rabbit skeletal muscle F-actin, 0.8 μM chicken gizzard filamin and/or 9 μM tropomyosin, were gently mixed, incubated for 30 min at 20°C and then centrifuged at 100000 × g for 20 min at 20°C. After removal of the supernatant, pellet was dissolved in 0.1% SDS. Protein composition in pellet and supernatant fractions were analysed in SDS–polyacrylamide gel electrophoresis as in [17] using 7.5–12% gradient of acrylamide.

The M_r-values used were 42000, 70000, 60000 and 500000 for rabbit skeletal muscle actin, rabbit skeletal muscle tropomyosin, bovine brain tropomyosin and chicken gizzard filamin, respectively.

3. RESULTS AND DISCUSSION

As shown in the control experiments, F-actin alone sediments but filamin is soluble and remains in supernatant after centrifugation (fig.1a–d). In the presence of F-actin, > 90% of filamin can be found in the pellet (fig.1e,f). Incubation of F-actin

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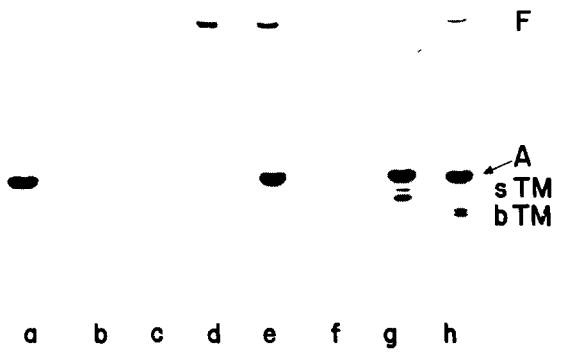

Fig.1. Interaction of chicken gizzard filamin with rabbit skeletal muscle F-actin containing bovine brain or rabbit skeletal muscle tropomyosin: (a,c,e,g,h) pellets; (b,d,f) supernatants; (a,b) F-actin (A) alone; (c,d) filamin (F) alone; (e,f) F-actin and filamin; (g) F-actin, skeletal muscle tropomyosin (sTM) and filamin; (h) F-actin, bovine brain tropomyosin (bTM) and filamin. SDS–polyacrylamide gel electrophoresis was done as in [17].

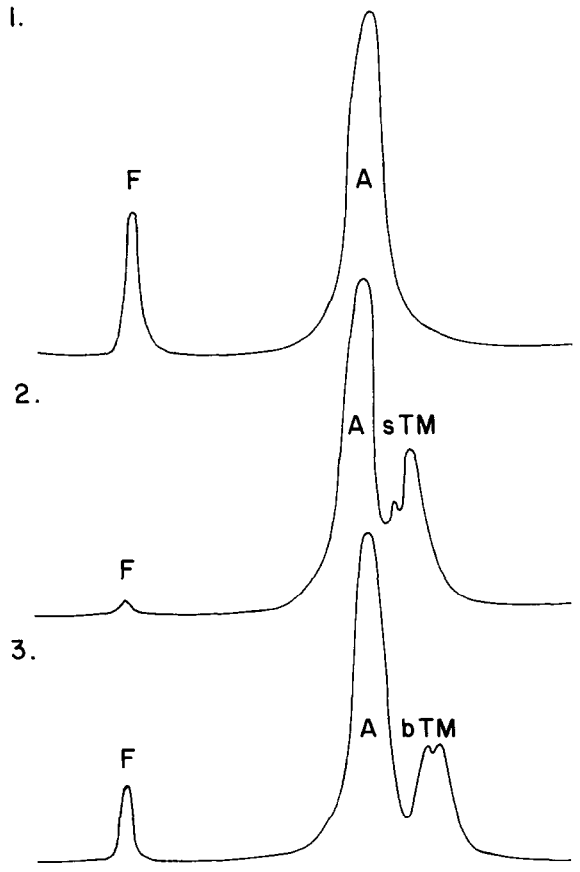

Fig.2. Densitometric scans of the gels e(1), g(2) and h(3) in fig.1.

together with bovine brain or rabbit skeletal muscle tropomyosin prior to addition of chicken gizzard filamin caused significant reduction in binding of the latter protein to F-actin (fig.1g,h). Densitometry of the gels showed that in case of rabbit skeletal muscle tropomyosin the amount of filamin in the pellet was negligible (< 5–10% of filamin added). Much more filamin (30–50%) was found in the pellet when instead of tropomyosin from skeletal muscle, tropomyosin isolated from brain was used (fig.2). This phenomenon seems to be a consequence of much weaker binding of brain tropomyosin than that from skeletal muscle to F-actin. Non-muscle tropomyosin, which is 1/7 shorter than its muscle counterpart and does not polymerize, binds very poorly to F-actin under most preferable conditions for binding of polymerizable, muscle tropomyosin to F-actin [16,18,19]. This property of non-muscle tropomyosin may allow filamin to be an integral component of native microfilaments as shown in case of cultured mammalian cells [6,20].

REFERENCES

[1] Wang, K.J., Ash, F. and Singer, S.J. (1975) Proc. Natl. Acad. Sci. USA 72, 4483–4486.
[2] Bechtel, P.J. (1979) J. Biol. Chem. 254, 1755–1758.
[3] Koteliansky, V.E., Glukhova, M.A., Shirinsky, V.P., Babayev, V.R., Kandalenko, V.F., Rukosuev, V.S. and Smirnov, V.N. (1981) FEBS Lett. 125, 44–48.
[4] Hartwig, J. and Stossel, T. (1975) J. Biol. Chem. 250, 5696–5705.
[5] Wallach, D., Davis, P. and Pastan, I. (1978) J. Biol. Chem. 253, 3328–3335.
[6] Schloss, J.A. and Goldman, R.D. (1979) Proc. Natl. Acad. Sci. USA 76, 4484–4488.
[7] Hartwing, J.H. and Stossel, T.P. (1979) J. Mol. Biol. 134, 539–553.
[8] Heggeness, M.H., Wang, K. and Singer, S.J. (1977) Proc. Natl. Acad. Sci. USA 74, 3883–3887.
[9] Lazarides, E. (1976) J. Supramol. Struc. 531, 383–415.
[10] Wehland, J. and Weber, K. (1980) Exp. Cell Res. 127, 397–408.
[11] Maruyama, K. and Ohashi, K. (1978) J. Biochem. (Tokyo) 84, 1017–1019.
[12] Zeece, M.G., Robson, R.M. and Bechtel, P.J. (1979) Biochim. Biophys. Acta 581, 365–370.
[13] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.

[14] Rees, M.K. and Young, M. (1967) J. Biol. Chem. 242, 4449–4458.
[15] Koteliansky, V.E., Glukhova, M.A., Shirinsky, V.P., Smirnov, V.N., Bushueva, T.L., Filimonov, V.V. and Venyaminov, S.Yu. (1982) Eur. J. Biochem. 121, 553–559.
[16] Dabrowska, R., Nowak, E. and Drabikowski, W. (1983) J. Musc. Res. Cell Motil. in press.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Dabrowska, R., Prochniewicz, E. and Drabikowski, W. (1983) J. Musc. Res. Cell Motil. in press.
[19] Cote, G.P. and Smillie, L.B. (1981) J. Biol. Chem. 256, 7257–7261.
[20] Schloss, J.A. and Goldman, R.D. (1977) J. Cell Biol. 75, 256a.