HEAVY MEROMYOSIN- AND ATP-BINDING PROTEIN FROM *ESCHERICHIA COLI*

Akira SOMEYA and Nobuo TANAKA
Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Received 12 February 1979
Revised version received 6 March 1979

1. Introduction

Actin-like protein has been isolated from bacteria [1–5]. Elongation factor Tu from bacteria and actin from mammalian muscles have been compared [6]. EF-Tu polymerizes to form filaments and has certain actin-like properties; and may function as a structural protein of the envelope [2,3]. However, the 2 proteins show differences in the surface lattice structure and antigenicity [3]. Two major proteins from *Escherichia coli* have been isolated [4,5] whose properties are similar to those of skeletal actin.

We have isolated 3 actin-like proteins, capable of binding to heavy meromyosin and ATP, from *E. coli* envelope fraction, using affinity chromatography; and demonstrated on gel electrophoresis that their molecular weights are different from *E. coli* EF-Tu and rabbit skeletal actin. The main protein is mol. wt ~55 000. The results are presented here.

2. Materials and methods

2.1. *Buffers*

Buffer A consisted of 50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris–HCl and 1 mM 2-mercaptoethanol (pH 7.8); buffer B: 2 mM Tris–HCl, 0.2 mM $CaCl_2$ and 1 mM 2-mercaptoethanol (pH 7.8); buffer C: 0.5 M NaCl and 0.1 M $NaHCO_3$ (pH 8.3).

2.2. *Protein preparation*

Actin and HMM were prepared from rabbit skeletal muscle as in [7] and [8], respectively. Elongation factor Tu was purified from *E. coli* JE5506 as in [9]. The envelope fraction of *E. coli* JE5506 was obtained as in [4].

Abbreviations: EF-Tu, elongation factor Tu; HMM, heavy meromyosin

2.3. *Affinity matrix*

ATP–Sepharose was prepared principally as in [10] using AH–Sepharose (Pharmacia).

Conjugation of HMM to Sepharose-4B was as in [11], with a modification: 10 ml cyanogen bromide-activated Sepharose 4B (Pharmacia) in 20 ml buffer C was mixed with 100 mg HMM dissolved in 10 ml of the same buffer. The mixture was kept at room temperature for 1.5 h with gentle shaking. The binding of HMM to the gel was confirmed by eluting out free HMM with buffer C. Most of applied HMM was conjugated onto Sepharose 4B matrix.

The affinity columns were operated at 0–4°C.

3. Results

3.1. *HMM binding proteins from* E. coli

E. coli envelope fraction of 10 ml was mixed with an equal volume of packed HMM–Sepharose 4B, followed by dialysis at 0–4°C overnight against buffer A. The mixture was packed in a column (0.9 × 10 cm) and washed with buffer A until the A_{280} of eluate reached baseline. To separate bound proteins from HMM–matrix, 1 column vol. buffer A containing 10 mM ATP was allowed to flow into the column bed. The fraction, eluted by the subsequent development with the same ATP buffer, was analyzed by SDS–10% polyacrylamide gel electrophoresis. Three protein bands were observed (lane C, fig.1).

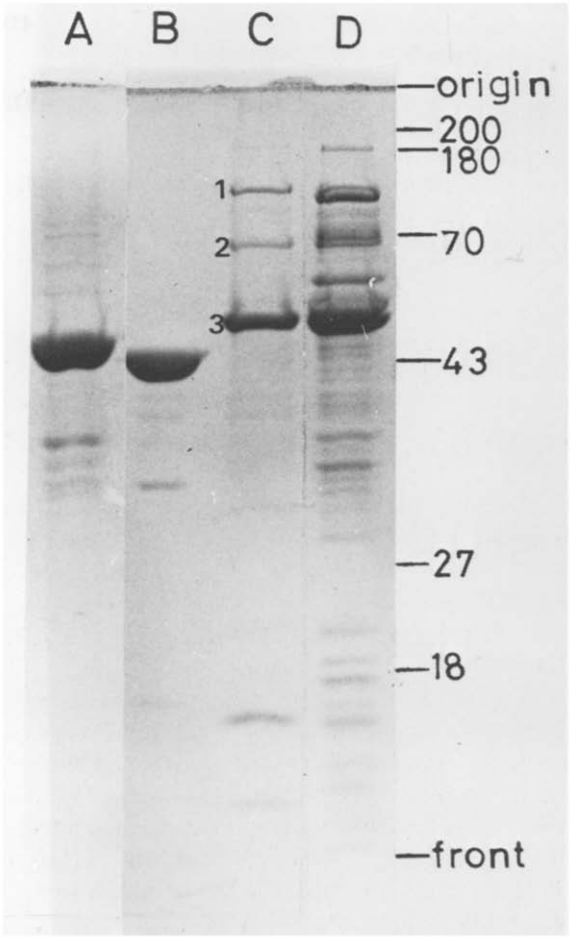

Fig.1. SDS–10% polyacrylamide gel electrophoresis of HMM binding and ATP binding proteins. (A) *E. coli* EF-Tu. (B) Rabbit skeletal actin. (C) Eluate of HMM–Sepharose column with buffer A containing 10 mM ATP. (D) Eluate of ATP–Sepharose with buffer B containing 10 mM ATP, after washing with 0.6 M ammonium acetate buffer. The gels were stained with Coomassie brilliant blue. The positions and mol. wt × 1000 of rabbit skeletal actin and myosin on the gel are indicated as standards in the right margin.

3.2. *ATP binding proteins from* E. coli

E. coli envelope fraction of 10 ml was absorbed to an equal volume of ATP–Sepharose, followed by dialysis against buffer B; and the mixture was packed in a column as in the case of HMM affinity. The column was washed with buffer B containing 0.6 M ammonium acetate until proteins were not detected in eluate [12]. The fraction, subsequently eluted with buffer B containing 10 mM ATP, was analyzed on gel electrophoresis; and several protein bands were detected (lane D, fig.1).

3.3. *Comparison of HMM and ATP binding proteins with EF-Tu and skeletal actin*

As shown in fig.1, three proteins (bands 1–3 of lane C) were observed to possess ability of binding to HMM and ATP, and therefore seemed to be actin-like protein. However, their molecular sizes were different from *E. coli* EF-Tu (lane A) and from rabbit muscle actin (lane B). These HMM and ATP binding proteins were estimated to be mol. wt ~125 000, 70 000 and 55 000 by comparison of mobilities on SDS–10% polyacrylamide gel electrophoresis with those of standards of rabbit skeletal myosin and actin [13].

4. Discussion

Actin-like proteins, capable of binding to HMM and ATP, have been isolated from *E. coli* envelope fraction. Their function in bacterial cells remains to be determined. They may participate in maintainance of envelope structure, cell movement and/or active transport.

The protein, obtained in this experiment, did not polymerize in 0.6 M KCl, but aggregated in 35% sat. $(NH_4)_2SO_4$; and did not significantly stimulate HMM-ATPase activity (data not shown). These properties are different from those of actin-like proteins or EF-Tu [2–5]. EF-Tu is reported [2] to be identical to actin-like protein and polymerize in the presence of 0.6 M KCl. EF-Tu has been shown [3] not to polymerize in 0.6 M KCl buffer, but forms fibrous paracrystals in 30–70% $(NH_4)_2SO_4$ buffer. EF-Tu neither binds to myosin nor enhances myosin-ATPase activity [2]. Actin-like protein, prepared [4,5], promotes myosin-ATPase activity. The discrepancy in properties of actin-like proteins, obtained by various investigators, suggests the existence of unknown complementary component(s) of actin-like protein in *E. coli*.

References

[1] Mincoff, L. and Damadian, R. (1976) J. Bacteriol. 125, 353–365.
[2] Beck, B. M., Arscott, P. G. and Jacobson, A. (1978) Proc. Natl. Acad. Sci. USA 75, 1250–1254.

[3] Wurtz, M., Jacobson, G. R., Steven, A. C. and Rosenbusch, J. P. (1978) Eur. J. Biochem. 88, 593–597.
[4] Nakamura, K. and Watanabe, S. (1978) J. Biochem. 83, 1459–1470.
[5] Nakamura, K., Takahashi, K. and Watanabe, S. (1978) J. Biochem. 84, 1453–1458.
[6] Rosenbusch, J. P., Jacobson, G. R. and Jaton, J.-C. (1976) J. Supramol. Struct. 5, 391–396.
[7] Spudich, J. A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[8] Yagi, K. and Yazawa, S. (1966) J. Biochem. 60, 450–458.
[9] Jacobson, G. R. and Rosenbusch, J. P. (1977) FEBS Lett. 79, 8–10.
[10] Dilham, P. T. (1971) Methods Enzymol. 21, 191–197.
[11] Cuatrecasas, P. T., Wilchek, M. and Anfinsen, C. B. (1968) Proc. Natl. Acad. Sci. USA 61, 636–643.
[12] Trayer, H. R. and Trayer, I. P. (1975) FEBS Lett. 54, 291–296.
[13] Siemankowski, R. F. and Dreizen, P. (1978) J. Biol. Chem. 253, 8648–8658.