INHIBITION OF NORMAL AND TRANSFORMED FIBROBLAST AGGREGATION BY SKELETAL MUSCLE F-ACTIN

Avraham OPLATKA, Julian BOREJDO, Hana GADASI and Andras MUHLRAD

Department of Polymer Research, The Weizmann Institute of Science, Rehovot, Israel and Department of Cellular Biochemistry, The Hebrew University Medical School, Jerusalem, Israel

Received 22 January 1976

1. Introduction

The presence of a protein similar to muscle actin in the plasma membrane has been demonstrated in a variety of cells [1–4] and it has been suggested that actin, in combination with myosin, may play a role in cell adhesion and aggregation [5]. Transformation leads to changes in agglutination by plant lectins [6] and in contact inhibition of growth [7] and was shown to involve a decrease in membrane-associated actin in the case of fibroblasts [1,2]. It was therefore interesting to examine whether exogenous actin or myosin could influence surface-dependent properties of cells such as aggregation. In the following, we describe the effect of rabbit skeletal F-actin on the rate of aggregation of normal and transformed fibroblasts. We found that actin may inhibit aggregation and that this is associated with the binding of appreciable amounts of the protein.

2. Materials and methods

The cells used were: secondary cultures of embryonic mice Balb/c, C57 BL/6 and C3H fibroblasts (gifts from Dr M. Small) and the cell lines: mouse Balb/c 3T3 and its SV40-transformed derivative (3T3 SV40) (gifts from T. Koch). Cells were grown in Falcon dishes in Dulbecco's Modified Eagle Medium (DME) supplemented with 10% calf serum, 50 U/ml penicillin and 50 μg/ml streptomycin.

Embryonic mice fibroblasts were suspended by treatment with either EDTA (0.02% EDTA in Ca^{2+}- and Mg^{2+}-free phosphate buffered saline supplemented with glucose) or trypsin (0.25% trypsin in Puck's saline). 3T3 cells were treated with 1:1 mixture of EDTA and trypsin solutions. The resulting cell suspensions were transferred to centrifuge tubes and spun at 100 *g* for 5 min. The supernatant solution was discarded and the cell pellet was gently resuspended by repeated pipetting (Hank's balanced salt solution (HBSS modified to omit phenol red and substitute glucose with dextrose) at a density ranging from 0.5 to 1×10^6 cells/ml.

Cell aggregation was quantitatively estimated using the modifications of turbidimetric [8] and gyrating shaker [9] techniques. Spectrophotometer cuvettes were filled with 2 ml of cell suspension and rotated at 74 rev/min at 37°C in a dry incubator. Decrease in optical density measured at 660 nm and the reduction in the number of single cells in 10 μl aliquots was followed at fixed time intervals.

Actin was prepared from back muscles of white New Zealand rabbits [10]. Myosin and heavy meromyosin were obtained from the same muscles according to Azuma and Watanabe [11] and Lowey and Cohen [12], respectively. Natural tropomyosin was prepared from rabbit skeletal muscle acetone powder [13].

Adsorption of actin to fibroblasts was measured as follows: increasing amounts of actin were added to cell suspension and incubated in a gyrating shaker for 10 min at 37°C. The cells were centrifuged down and the actin content in the supernatant determined by scanning the actin band on SDS-polyacrylamide gels (10% polyacrylamide, 1% SDS, 0.1% phosphate

buffer pH 7.0) by the Gilford Gel Scanner. In control experiments the corresponding quantities of protein were added *after* the cells were centrifuged down. The difference between the two amounts for each initial concentration was taken as the amount adsorbed. This procedure was necessary in view of the fact that centrifugation caused cell lysis.

3. Results

As measured by the turbidimetric method, the process of reaggregation of dissociated control embryonic mice Balb/c fibroblasts was completed during two-h periods (fig.1); the decrease in optical density resulted from the formation of large cell aggregates which eventually sedimented to the bottom of the spectrophotometer cuvette in spite of continuous rotation of the samples.

While the addition of 0.01 to 10 μg/ml actin to the secondary cultures of embryonic mice cells did not exert observable effects, the O.D. decreased by 27% only after 2 h at 100 μg/ml as compared to practically 100% in the controls (fig.1). A parallel measurement of the number of single cells/ml in withdrawn aliquots showed an equivalent effect: after 2 h the number of single cells in the control dropped from 720×10^3 to 40×10^3 cells/ml while in the presence of 100 μg/ml actin the corresponding decrease was from

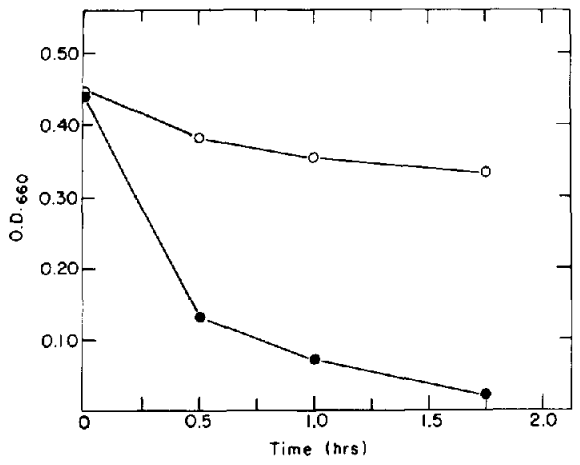

Fig.1. Optical density (O.D.) as function of time of a secondary culture of Balb/c cells in the absence (•) and in the presence (○) of actin (100 μg/ml). Volume 2 ml.

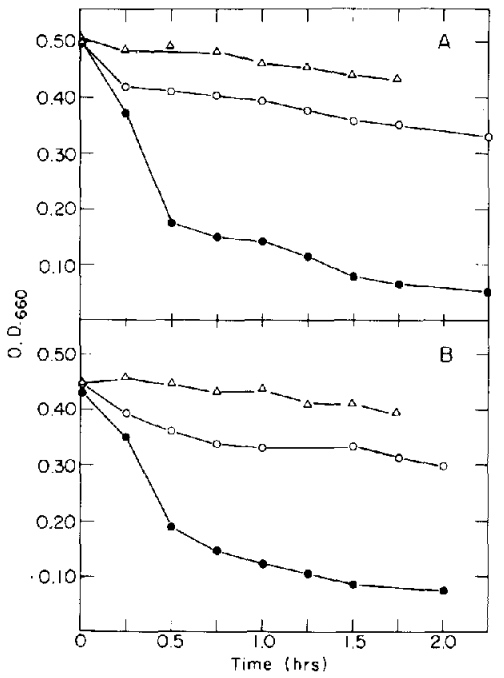

Fig.2. Optical density as function of time for 3T3 cell line fibroblasts in the absence (•) and in the presence of actin (○ – 100 μg/ml, △ – 200 μg/ml). (A) Untransformed cells; (B) SV40 transformed cells. Volume 2 ml.

660×10^3 to 240×10^3 cells/ml. Actin at a concentration of 200 μg/ml was sufficient for really complete inhibition of aggregation over this time period. Similar results were obtained with 3T3 and 3T3-SV40 fibroblast cell lines (fig.2). At 100 μg/ml actin the

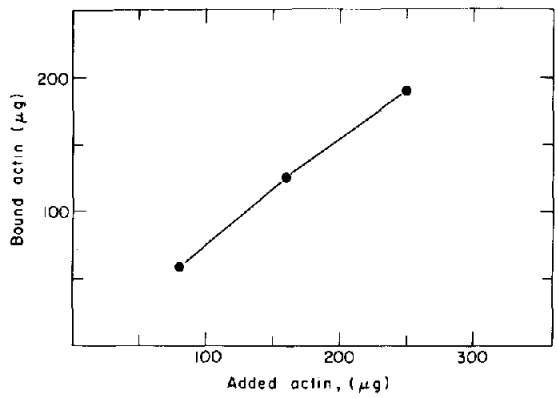

Fig.3. Adsorption of actin by a secondary culture of Balb/c cells. Volume 2 ml.

behavior of the transformed cells did not differ significantly from that of the untransformed cells.

The inhibition of aggregation by actin was found to be accompanied by appreciable binding of F-actin by the cells. The results for embryonic mice Balb/c fibroblasts are shown in fig.3. Thus, at an actin concentration of 125 μg/ml the amount adsorbed per cell was about 1.6×10^{-4} μg. No significant differences could be observed with respect to adsorption between 3T3 and 3T3-SV40 fibroblast cell lines.

Actin was specific in its ability to affect the aggregation rate of suspended fibroblasts. Other proteins derived from striated muscle: myosin, heavy meromyosin and natural tropomyosin were ineffective and so were bovine serum albumin and polylysine at concentrations of up to 1 mg/ml. ATP, at up to 10 mM concentration, was ineffective in influencing the aggregation of all cells tested, both in the presence and in the absence of actin.

In view of the recent findings [14] that the initial rates of fibroblast aggregation differ in EDTA- and trypsin-suspended cells we checked for the possibility that actin is effective only in freshly trypsinized cells. Embryonic mice Balb/c fibroblasts were dissociated from dishes by a brief treatment with EDTA solution and the resulting monolayer broken down into individual cells by repeated pipetting. The aggregative behavior of such cells was the same as that of trypsin dissociated Balb/c fibroblasts both in the absence and in the presence of added actin. However, it is possible that the rates of aggregation in EDTA-dissociated contact-inhibited and transformed 3T3 cells would differ in the presence of actin just as they differ in its absence [14].

To check for the possibility that the inhibition of aggregation results from an increase in viscosity associated with the addition of actin we measured the rate of aggregation of Balb/c fibroblasts in BSS to which varying amounts of carboxymethyl cellulose have been added. The aggregation was not affected in up to 1% (final concentration) of carboxymethyl cellulose (specific viscosity of 2.04). The specific viscosity of BSS containing 125 μg/ml actin was 0.104, indicating that the increase in viscosity of medium does not contribute to the effect observed.

4. Discussion

Taking the diameter of the embryonic fibroblasts as 10 μm and assuming uniform coating of the surface then, at an actin concentration of 125 μg/ml, the amount adsorbed (1.6×10^{-4} μg/cell), yields 0.5 μm as the thickness of the actin layer. Since the length of actin filaments is usually about 1 μm it appears that the filaments form a 'brush' which is dense and long enough to prevent aggregation through contact between the cell surfaces. Since ATP did not abolish the inhibitory effect of actin and in view of the relatively large amount of actin bound to the cells it appears unlikely that actin binds to the surface through interaction with myosin, but rather that it binds to a relatively abundant receptor, possibly glycoprotein.

References

[1] McNutt, N. S., Culp, L. A. and Black, P. H. (1973) J. Cell. Biol. 56, 412.
[2] Wickus, G., Gruenstein, E., Robbins, P. W. and Rich, A. (1975) Proc. Nat. Acad. Sci. USA, 72, 746.
[3] Tilney, L. G. and Detmers, P. (1975) J. Cell. Biol. 66, 508.
[4] Cohen, I., Gabbay, J., Glaser, T. and Oplatka, A. (1975) Brit. J. Haematol. 31, 45.
[5] Jones, B. M. and Kemp, R. B. (1970) Expt. Cell. Res. 63, 301.
[6] Inbar, M. and Sachs, L. (1969) Proc. Nat. Acad. Sci. USA, 63, 1418.
[7] Nicolson, G. L. (1971) Nature New Biol. 233, 244.
[8] Jones, B. M. (1965) Nature 205, 1280.
[9] Moscona, A. A. (1952) Expt. Cell. Res. 3, 535.
[10] Lehrer, S. S. and Kerwar, G. (1972) Biochemistry 11, 1211.
[11] Azuma, N. and Watanabe, S. (1965) J. Biol. Chem. 240, 3847.
[12] Lowey, S. and Cohen, C. (1962) J. Mol. Biol. 4, 293.
[13] Hartshorne, D. J. and Mueller, H. (1969) Biochim. Biophys. Acta 175, 301.
[14] Cassiman, J. J. and Bernfield, M. R. (1975) Expt. Cell. Res. 91, 31.