Alpha-Actinin–containing Aggregates in Transformed Cells Are Highly Dynamic Structures

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Abstract. Normal rat kidney cells infected with a Rous sarcoma virus (strain LA23) were used to study the dynamics of alpha-actinin–containing aggregates in transformed cells. Experiments were performed by microinjecting living cells with iodoacetamidotetramethylrhodamine alpha-actinin and allowing the fluorescent analogue to incorporate into cellular structures. Subsequent time-lapse recording indicated that the alpha-actinin–containing aggregates can undergo rapid formation, movement, and breakdown. In addition, experiments using the photobleaching recovery technique indicated that alpha-actinin molecules associated with the aggregates have a very high rate of exchange, whereas those associated with adhesion plaques in normal cells exchange much more slowly. The dynamic properties of alpha-actinin–containing aggregates may be closely related to the changes in cellular behavior upon oncogenic transformation.

Materials and Methods

Cell Culture and Protein Preparation

NRK epithelial cells (NRK-52E; American Type Culture Collection, Rockville, MD) and Rous sarcoma virus–infected NRK cells (LA23; gift of R. L. Erikson, Harvard University) were grown in F12-K medium (KC Biological Inc., Lenexa, KS) supplemented with 10% FCS (KC Biological Inc.), 50 U/ml penicillin, and 50 µg/ml streptomycin. The cells were maintained in a 5% CO2 environment at 34°C. Clones of LA23 cells were screened after temperature shift according to their morphological changes (Wang, 1984) one day before experiments were performed.

Muscle alpha-actinin from frozen turkey gizzards and nonmuscle alpha-actinin from frozen calf thymus (Pel-Freez Biologicals, Rogers, AR) were purified and subsequently labeled with iodoacetamidotetramethylrhodamine (IATR; Molecular Probes Inc., Eugene, OR) staining. Cells were plated onto injection dishes (Wang, 1984) and temperature shifted 1 day before experiments were performed.

Microinjection

Microinjection was performed as described by Graessmann et al. (1980) and cells were maintained on the microscope during the experiment as described previously (Wang, 1984). Experiments with LA23-NRK cells were performed as described by Graessmann et al. (1980) and cells were maintained on the microscope during the experiment as described previously (Wang, 1984). Experiments with LA23-NRK cells were performed in a 5% CO2 environment at 34°C.

1. Abbreviations used in this paper: IATR, iodoacetamidotetramethylrhodamine; NRK, normal rat kidney.
formed as soon as 10 min after microinjection because of the immediate incorporation of the analogue. No difference in results was detected with prolonged incubation after microinjection. For NRK-52E cells, the culture dish was returned to a regular CO2 incubator for 2 h to allow incorporation of injected molecules.

**Fluorescent Microscopy and Photobleaching**

Fluorescent microscopy techniques were described previously (Wang, 1984). A 100 X Neofluar objective (NA 1.30) was used for all experiments. For time-lapse sequences, images were recorded every 1-3 min by averaging
Figure 3. An 8-min time-lapse sequence which shows aggregates in an IATR alpha-actinin-injected LA23-NRK cell. Times of image recording are shown in the lower left-hand corner of each micrograph. Dramatic changes occur in this group over an 8-min period. Two aggregates (arrowheads) show both intensity changes and relative movement during the course of recording. Arrows indicate an aggregate that disappears totally. Bar, 10 μm.

Results

NRK cells, infected with a temperature-sensitive mutant of Rous sarcoma virus (strain LA23) and cultured at a permissive temperature, were used to study the dynamics of alpha-actinin-containing aggregates. Although alpha-actinin-containing aggregates are present in many types of transformed cells, the cell line used shows an excellent spreading capability which greatly facilitates microinjection and fluorescence microscopy. At the permissive temperature, adhesion plaques and stress fibers are either absent or greatly diminished, whereas aggregates are present in at least 80% of the cells and are often clustered into groups both in the central region and near the edge of the cell (Figs. 1 and 2; see also Carley and Webb, 1983). After microinjection, IATR alpha-actinin becomes incorporated into aggregates almost immediately. Although most experiments were performed with gizzard alpha-actinin, identical results were also obtained with nonmuscle (thymus) alpha-actinin.

By careful focusing, all fluorescent aggregates appeared to be located on the ventral (bottom) surface of the cell. This was further verified by marking the dorsal surface with red fluorescent beads, which had an appearance easily distinguishable from the aggregate. When focusing on the beads (Fig. 1 a), it was obvious the aggregates were present on a different focal plane. Sharp images of the aggregates appeared only after the microscope was refocused on the ventral surface (Fig. 1 b).

Using time-lapse recording, we found these aggregates to be highly dynamic. They are capable of movement (arrows, Fig. 2; arrowheads, Fig. 3), as well as changes in shape and size (arrowheads, Fig. 3). The rate of movement varied between 0 and 0.8 μm/min. In addition, one aggregate may pinch to form two or three (arrowheads, Fig. 2) or may appear or disappear (arrows, Fig. 3). These activities often resulted, within 5 min, in dramatic changes in the morphology of the entire group of aggregates (Fig. 3).

To determine whether the dynamic changes in morphology are coupled to a high rate of exchange of protein components, microinjected cells were photobleached to study the apparent mobility of alpha-actinin associated with the aggregates.
Figure 4. Photobleaching recovery of an aggregate in a IATR alpha-actinin-injected LA23-NRK cell. Times of image recording are indicated in the lower left-hand corner of each micrograph. An aggregate (arrow) is shown before (a), immediately after (b), 5 s after (c), and 11 s after (d) photobleaching. Extensive recovery is reached by the end of the period of recording. Bar, 10 μm.

Figures 1-3. Photobleaching recovery of an aggregate in a IATR alpha-actinin-injected LA23-NRK cell. Times of image recording are indicated in the lower left-hand corner of each micrograph. An aggregate (arrow) is shown before (a), immediately after (b), 5 s after (c), and 11 s after (d) photobleaching. Extensive recovery is reached by the end of the period of recording. Bar, 10 μm.

Figures 4-6. Photobleaching recovery of an aggregate in a IATR alpha-actinin-injected LA23-NRK cell. Times of image recording are indicated in the lower left-hand corner of each micrograph. An aggregate (arrow) is shown before (a), immediately after (b), 5 s after (c), and 11 s after (d) photobleaching. Extensive recovery is reached by the end of the period of recording. Bar, 10 μm.

Discussion

Based on available information on protein composition and location, there appears to be a strong resemblance between aggregates in transformed cells and adhesion plaques in normal cells. First, aggregates, which are seen on the ventral cell surface (Fig. 1), have been shown to correspond to gray plaques in interference reflection microscopy images (David-Pfeuty and Singer, 1980; also our unpublished observation). This indicates that they are probably involved in the association of the cell with the substrate, although, based on the darkness of these plaques, the contact appears to be weaker than that mediated by adhesion plaques (Carley and Webb, 1983). Second, many proteins found in adhesion plaques, including actin, alpha-actinin, vinculin, talin, and fimbrin, are also concentrated in the aggregates in transformed cells (David-Pfeuty and Singer, 1980; Burridge and Connell, 1983; and Carley et al., 1985). Notably, vinculin and talin are concentrated specifically at adhesion plaques and cell–cell contact sites in normal cells (Geiger, 1979; Burridge and Feramisco, 1980; and Burridge and Connell, 1983).

However, compared to adhesion plaques, aggregates have a much higher rate of morphological reorganization and protein exchange. Adhesion plaques in normal cells are known
to be relatively stationary structures. They appear to assemble near the leading edge of the cell, show little movement during cell locomotion while most other structures move forward, and disassemble when they lag far behind the leading edge (Izzard and Lochner, 1980). In contrast, aggregates can appear and disappear, even far away from the edge of the cell, within a 5-min period. Furthermore, they also undergo rapid movement and splitting during their life span.

These differences between adhesion plaques and aggregates may be related to the stability of their proteins. Indeed, using fluorescence recovery after photobleaching, we found that essentially 100% of alpha-actinin molecules in the aggregates are highly mobile, with a recovery kinetic curve indistinguishable from that of the surrounding cytoplasm. Therefore, alpha-actinin and possibly other protein components can exchange at a very high rate in and out of the aggregates, and the rate-limiting step for the exchange may be the diffusion of molecules in the cytoplasm. On the contrary, similar experiments on adhesion plaques show very limited recovery over a prolonged period of time, consistent with the previous report that at least 60% of alpha-actinin molecules have a very low mobility (Geiger et al., 1984).

Several factors may contribute to the difference in stability between aggregates and adhesion plaques. It has been shown that the aggregates contain a high concentration of protein tyrosine kinase (e.g., David-Pfeuty and Singer, 1980; Chen

Figure 5. Time course of fluorescence recovery after photobleaching an aggregate. Time 0 represents the first time point measured after photobleaching. Halftime of fluorescence recovery for this aggregate is estimated to be 4.0 s.

Figure 7. Time course of fluorescence recovery after photobleaching of an adhesion plaque in NRK-52E cell. Time 0 represents the first time period measured after photobleaching. At the end of 50 s, fluorescence recovery has reached only 45%.

Figure 6. Photobleaching recovery of an adhesion plaque in a nontransformed NRK-52E cell injected with IATR alpha-actinin. Times of image recording are indicated in the lower left-hand corner of each micrograph. The adhesion plaque (arrow) is shown before (a), immediately after (b), 11 s after (c), and 52 s after (d) photobleaching. Even at the end of the sequence, recovery is still far from being complete. Bar, 10 μm.
An increase in the level of protein phosphorylation may, therefore, affect the stability of the structure. Alternatively, destabilization of structures may be due to extracellular proteases, which are highly active in transformed cells under the aggregates and cause the degradation of the extracellular matrix (Chen et al., 1984). This may result in a decrease in the adhesion of cells and an increase in the mobility of proteins in the adjacent cytoplasm. Future experiments, such as studying the effect of exogenously added fibronectin on the mobility of proteins in aggregates, may shed some light on the actual mechanism involved.

If the aggregates are indeed related to adhesion plaques, the question arises as to the exact relationship between them. David-Pfeuty and Singer (1980) have suggested that aggregates do not simply represent residues of disrupted stress fibers and adhesion plaques in the transformed cell, but rather, weakened (and possibly destabilized) adhesion plaques. Alternatively, Bershadsky et al. (1985) reported that Rous sarcoma virus transformation affects the formation of mature adhesion plaques, but not the formation of nascent contact sites with the substrate. Therefore, it is also possible that aggregates represent nascent adhesion structures that are unstable and incapable of developing into adhesion plaques.

The disruption of strong adhesion sites and the formation of weak, unstable substrate contacts can clearly account for the less spread and less stable morphology of transformed cells. In addition, since adhesion plaques are normally formed near the active lamellipodia, the rapid assembly and disassembly of aggregates at random sites of transformed cells implies that transformed cells have either lost or greatly altered the control mechanism for the locomoting machinery.

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