Video Article

Actin Co-Sedimentation Assay; for the Analysis of Protein Binding to F-Actin

Jyoti Srivastava[1], Diane Barber[1]

[1]Department of Cell and Tissue Biology, University of California, San Francisco - UCSF

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Abstract

The actin cytoskeleton within the cell is a network of actin filaments that allows the movement of cells and cellular processes, and that generates tension and helps maintains cellular shape. Although the actin cytoskeleton is a rigid structure, it is a dynamic structure that is constantly remodeling. A number of proteins can bind to the actin cytoskeleton. The binding of a particular protein to F-actin is often desired to support cell biological observations or to further understand dynamic processes due to remodeling of the actin cytoskeleton. The actin co-sedimentation assay is an in vitro assay routinely used to analyze the binding of specific proteins or protein domains with F-actin. The basic principles of the assay involve an incubation of the protein of interest (full length or domain of) with F-actin, ultracentrifugation step to pellet F-actin and analysis of the protein co-sedimenting with F-actin. Actin co-sedimentation assays can be designed accordingly to measure actin binding affinities and in competition assays.

Video Link

The video component of this article can be found at https://www.jove.com/video/690/

Protocol

1. Preparation of actin

The source of actin used in this assay was non-muscle human platelets (β-actin) obtained from Cytoskeleton Inc. Stock aliquots at 10 mg/ml are kept in the -70ºC freezer. For the assay, the actin is diluted to 0.4 mg/ml in a buffer containing 5 mM Tris pH 8, 0.2 mM $CaCl_2$, 0.2 mM ATP and 0.5 mM DTT, centrifuged at 20,000g for 10 mins at 4ºC. The supernatant, which is monomeric actin is ready to be polymerized. Actin polymerization is induced by the addition 50 mM KCl, 1 mM ATP and 2 mM $MgCl_2$. The polymerization occurs at room temperature for 1 hour.

2. Preparation of protein

In this assay, we are using the C-terminus of talin, which has been purified as a GST-tagged protein. The protein to be used in the assay is subjected to a high-speed centrifugation to pre-clear of aggregates prior to incubation with F-actin.
The protein is spun at 100,000g in a Beckman ultracentrifuge for 20 mins at 22ºC.

3. Actin-protein Incubation

The amount of actin and protein in an assay will vary. In this example we are using actin in excess. Normally, a molar ratio is calculated, eg. a 4:1 molar ratio of actin to protein.
In the following assay, the final reaction volume is 150 µl. The buffer in which the protein and F-actin are incubated will depend on the protein to be tested and on the conditions required. In this example, we use a buffer containing 10 mM Tris pH 7.0, 1 mM ATP, 0.2 mM DTT, 1 mM EGTA, 0.1 mM $CaCl_2$ and 2 mM $MgCl_2$. The protein and F-actin are incubated in the buffer for 1 hour at room temperature.

4. Sedimentation of F-actin

Following the incubation, the samples are spun at 100,000g at 22ºC. We use a Beckman ultracentrifuge in the video. The rotor is carefully removed so as not to disturb the pellets.

5. Analysis of protein co-sedimentation with F-actin

The supernatants are carefully removed by pipetting into an eppendorf tube and 5 X Laemmli SDS-PAGE sample buffer is added. An appropriate volume of 1 X Laemmli SDS-PAGE sample buffer is added to the pellets remaining, pipetted up and down, and transferred to new eppendorf tubes. The relative amounts of protein in the pellets and supernatants are analyzed following their separation by SDS-PAGE and Coomassie Blue staining of the gel or Western blotting.

In order to determine the specificity of protein interaction with actin, actin concentration-dependent co-sedimentations can be performed. For this sort of experiment, a fixed amount of the protein and a series of increasing amounts of actin are incubated, and analysis is carried out as described above.

Discussion

Actin co-sedimentation is a simple in vitro assay to analyze specfic proteins binding to F-actin. In this video, we demonstrate one example of how a simple actin co-sedimentation can be carried out. We show how to prepare F-actin, prepare the protein to be tested and the procedure of actin co-sedimentation. A number of points should be considered when carrying out actin co-sedimentation assays. For instance, the buffer components for the incubation can vary depending on the protein to be tested and pH, temperature and salt concentration may affect the binding to F-actin. We have demonstrated a simple binding to F-actin, however this type of assay can be elaborated upon and used to determine binding specificity of a protein or protein domain to F-actin.

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