Extraction of Muscle Actin from the Acetone Powder

Materials

1. 0.5 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM DTT, 0.02% NaN_3, 2 mM Tris-Cl, titrated at 4^oC to pH 8.0 with KOH (Buffer A). 1 liter each day.

2. KCl, 3 M stock.

3. $MgCl_2$, 100 mM stock.

4. Mortar and pestle.

5. Glass wool.

6. Glassware: SS34 tubes, 50Ti tubes, 50.2Ti tubes, funnel, 500 ml graduate cylinder.

7. 40 ml Dounce homogenizer.

8. Medium dialysis tubing.

9. Ultrapure sucrose.

10. Lyophilizer.

Procedure

1. Grind 10 g acetone powder in a mortar. Collect powder in a beaker and add 200 ml buffer A. Stir in a ice bath for 30 min.

2. Centrifuge in a SS34 rotor for 30 min at 11,000 rpm, 4^oC.

3. Filter supernatant through glass wool stuck in a funnel into a chilled graduate cylinder. Wear gloves when handling the glass wool. Measure the total volume.

4. While stirring slowly in a beaker at 0^oC, carefully bring the solution to 100 mM KCl (3.3 ml of 3 M stock per 100 ml filtrate) and then to 2 mM $MgCl_2$ (2 ml 100 mM $MgCl_2$ per 100 ml filtrate). Stir for 1 min.

5. Seal the beaker with parafilm and let it sit at 4^oC for about 30 min. The viscosity should increase significantly.

6. While stirring gently, add additional 3 M KCl to bring final KCl concentration to 0.8 M (33 ml of 3 M stock per 100 ml filtrate). Stir very slowly on ice in the cold room for 60 min.

7. Centrifuge in a 50.2Ti rotor for 2 hours at 40,000 rpm, 4^{o}C.

8. Rinse, briefly, tubes and pellets with a small volume of 0.8 M KCl in buffer A and discard the fluid. Soak the pellets in a total of 15-20 ml buffer A (use a smaller volume if actin is to be column purified) for ~ 2 hr.

9. Pick up pellets with a glass rod and collect them in a Dounce homogenizer, use the supernatant to rinse the bottom of the tube and transfer it to the homogenizer.

10. Homogenize carefully, avoid bubbles.

11. Transfer homogenized solution into medium dialysis tubing. Dialyze against 1 liter of Buffer A for 12-24 hr.

12. Replace buffer A twice. Continue the dialysis for 12-24 hr after each change.

13. Clarify solution in a 50Ti rotor at 40,000 rpm, 4^{o}C for 2 hr.

14. Collect supernatant, measure volume and determine concentration of actin.

mg/ml = (OD290 - OD320)/0.62. Lowry assay is much more reliable.

15. Calculate the total amount of actin. Proceed to column purification with a G-150 column or add 2 mg ultrapure sucrose per mg actin and stir gently at 0oC until sucrose dissolves completely.

16. Lyophilize, store dessicated at -20^{o}C.

17. To use actin, resuspend lyophilized actin in Buffer A, dialyze against Buffer A overnight, clarify before use, e.g. at 40,000 rpm in 50Ti rotor, 1 hr. If sulfhydryl group is to be labeled, add 0.5-5 mM DTT before dialysis.

Reference

J.A.Spudich and S.Watt (1971) Regulation of rabbit skeletal muscle contraction. *J. Biol. Chem.* 246:4866-4871.

Column Purification of Actin

Materials

1. Buffer A (see actin extraction procedure), 1 liter

2. 2.5x50 cm Sephadex G-150-120 column, pre-equilibrated with buffer A.

3. (optional) Rolling-ball viscometer

Procedure

1. Load ~12 ml G-actin solution onto the column. Flow rate should be ~20 ml/hr at a maximum of 36 cm pressure. Collect 1.5-2.0 ml fractions. Sensitivity of the UV monitor is set at 1.0 OD for a loaded concentration of 5 mg/ml.

2. There should be 2 peaks; one after ~80 ml and the other after ~160 ml. Collect the second peak, starting about halfway to two thirds up the rising part of the peak.

3. If necessary, fractions can be assayed using rolling-ball viscometry. Pool fractions with > 20 cp apparent viscosity.

4. Column purified actin can be stored by lyophilization as for non-CP actin, after adding 2 mg sucrose per mg actin. The volume of lyophilization per tube should be no larger than 2 ml.
