Labeling Muscle Actin with N-(1-pyrene)iodoacetamide

Day 0 and 1

Materials

1. 0.5 mM ATP, 0.2 mM $CaCl_2$, 2 mM Tris-HCl, pH 8.0 at 4^{o}C, 250 ml for day 0.

2. 0.5 mM ATP, 2 mM $MgCl_2$, 100 mM boric acid, pH 8.3 at 4^{o}C, 10 ml.

3. N-(1-pyrene)iodoacetamide (Molecular probes P-29, m.w. 385)

4. 50Ti tubes, small vial.

Procedure (perform under reduced light, 4^{o}C unless otherwise noted)

1. Resuspend 10 mg lyophilized actin in 1 ml buffer 1. Be careful not to introduce bubbles.

2. Add DTT (100 mM stock) to 5 mM.

3. Dialyze against 250 ml buffer 1 overnight.

4. Collect actin from dialysis tubing. Measure volume and bring the concentration of actin to 1.7 mg/ml with buffer 1.

5. Add 100 mM KCl and 2 mM $MgCl_2$ to induce polymerization. Let sit at room temperature for 30 min.

6. Weigh about 1 mg pyrene iodoacetamide and place in a test tube. Dissolve in 100 µl DMSO.

7. Pipet buffer 2, with a volume equal to the volume of actin, into a small vial containing a stirring bar. Calculate the volume of pyrene iodoacetamide stock that contains 0.67 mg of the dye. While stirring, slowly add the calculated volume of pyrene iodoacetamide stock into the vial with a Pipetman.

8. Add the dye solution to actin and mix gently with a Pasteur pipet.

9. Wrap the container with aluminum foil and shake/rotate at room temperature for 16 hr.

Day 2

Materials

1. Buffer 1 as for day 1, 4°C, 2000 ml.

2. G-25-150 column, ~30x1.5 cm. A G-150 column could be used for the simultaneous removal of protein contaminants.

3. 50Ti tubes, volumetric conical tube.

Procedure

1. Pellet actin filaments in a 50Ti rotor at 40,000 rpm, 4°C for 2 hr.

2. Soak the pellet for 1-2 hr in 0.5 ml of buffer 1. Dialyze overnight against 1500 ml of buffer 1.

3. Equilibrate G-25 column with Buffer 1.

Day 3

Materials

1. Ultrapure sucrose.

Procedure

1. Clarify dialyzed actin in a 50Ti rotor at 40,000 rpm, 4°C for 1 hr, or in a 42.2Ti rotor at 30,000 rpm, 4°C for 30 min.

2. Run supernatant through the G-25 column, collect 10 drop fractions.

3. Collect fluorescent fractions in the void volume, measure volume in a volumetric conical tube.

4. Actin can be concentration by an additional cycle of polymerization-depolymerization.

5. Measure concentration and dye/protein molar ratio. Read the OD at 344 nm in an UV spectrophotometer.

D/P = $\{OD_{344} / 22{,}000\} / \{(mg/ml) / 43{,}000\}$, should be 0.9-1.0.

6. Calculate total mg of actin. Store as aliquots in liquid N_2 after dissolving 2 mg sucrose per mg actin.

Reference

J.A.Cooper, S.B.Walker and T.D.Pollard (1983) Pyrene actin: documentation of the validity of a sensitive assay for actin polymerization. *J. Mus. Res. Cell Motil.* 4:253-262.

J.A.Cooper (1992) Actin filament assembly and organization in vitro. in The Cytoskeleton: A Practical Approach (K.L.Carraway and C.A.C.Carraway, eds), IRL Press, Oxford, pp.47-71.