Preparation of Skeletal Muscle Myosin

Day 1

Materials

1. Large supply of glassware (4 liter beakers, graduated cylinders, stirring rods, rubber scraper, beakers, GSA or GS3 bottles, large stirring bars, large vessel for high volume dialysis).

2. Pre-tared plastic pan for weighing muscle.

3. Meat grinder, prechilled in a cold room and rinsed with 20 mM EDTA, pH 7.0 immediately before use.

4. Large carboy of cold distilled H_2O.

5. 1 M (KH_2PO_4 + K_2HPO_4), pH 6.5, 400 ml.

6. 100 mM EDTA, pH 7.0, 500 ml.

7. 0.3 M KCl, 0.15 M KPO_4, 20 mM EDTA, 5 mM $MgCl_2$, 1 mM ATP, pH 6.5, 4^oC (extraction buffer). Need 3 ml per gram of ground muscle or 1500 ml per rabbit (for solutions 7, 8, and 9, either use EDTA stock solution or start with room temperature water for dissolving EDTA).

8. 1 M KCl, 25 mM EDTA, 60 mM KPO_4, pH 6.5, 4^oC. Need 0.25 ml per gram of ground muscle, or 100 ml per rabbit.

9. 0.6 M KCl, 25 mM KPO_4, 10 mM EDTA, 1 mM DTT, pH 6.5, 4^oC. Need at least 6 ml per gram of ground muscle, or 3000 ml per rabbit.

10. 0.5 N Acetic acid, 50 ml.

11. 1 M KCl for rinsing pH electrode, 100 ml.

12. Dialysis tubing, large size.

Procedure (avoid air bubbles thorought the protocol)

1. Obtain fresh rabbit or chicken muscles and cool on ice.

2. Grind twice in meat grinder, use the fine mesh at least for the second grinding. Weigh (typically 300-400 g per rabbit).

3. Extract with 3 volumes of extraction buffer for exactly 10 min with constant stirring. Do this in the cold room. Make sure the GSA rotor is chilled.

4. Separate muscle residue by spinning in a GSA rotor for 15 min at 12,000 rpm, 4^{o}C. The pellet may be used for the preparation of acetone powder.

5. Adjust pH to 6.6 slowly with 0.5 N acetic acid. This should be done carefully with adequate stirring to avoid myosin precipitation. Leave the pH electrode in 1 M KCl for several hrs before cleaning up.

6. Measure the volume. Dilute supernatant with 10 volumes of cold distilled H_2O. Let cold water flow very slowly into the beaker while stirring. Check pH and readjust to 6.6 if necessary.

7. Let precipitates settle and siphon off supernatant (optional).

8. Pellet precipitated myosin by centrifuging for 5-15 min at 7,000 rpm, 4^{o}C, in a GSA or GS3 rotor.

9. Resuspend pellets in buffer 8 with stirring rods and rubber scraper. Use a total of 0.25 ml for each gram of ground muscle. Save a small portion of the buffer for rinsing out the bottles.

10. Dialyze overnight against buffer 9. Pour the solution directly through a funnel into a dialysis tubing (put a beaker under the tubing, in case of an accident). The volume should be at least 24-fold higher than the volume of myosin.

Day 2

Materials

1. Glass stirring rods.

2. Large supply of glassware as for Day 1. Also SS34 tubes.

3. Saturated ammonium sulfate with 10 mM EDTA. Add 390 g ultrapure ammonium sulfate to 500 ml distilled H_2O (final volume larger than 500 ml) and heat until dissolved. Add EDTA to a concentration of 10 mM. Cool in cold room overnight, then adjust pH with ammonium hydroxide to an apparent pH of 8.2. Dilute a small amount 1:10 with water and read pH. It should be ~7.0. If there are no ammonium sulfate crystals at botttom of beaker, add solid ammonium sulfate until this occurs.

4. Cold distilled H_2O.

5. 2 M KCl, 200 ml, 4^{o}C.

6. 0.5 M KCl, 100 ml, 4^{o}C.

Procedure

1. Measure volume of dialyzed myosin solution. Add very slowly, with constant stirring, equal volume of cold distilled H_2O.

2. Let stir on ice for 1/2 hr.

3. Centrifuge in a SS34 rotor for 1/2 hr at 18,000 rpm, 4^{o}C.

4. Measure the volume of supernatant (the pellet contains actomyosin). Dilute carefully with 7 volumes of cold distilled H_2O.

5. Centrifuge in a GSA rotor for 15 min at 12,000 rpm, 4^{o}C. Discard supernatnat.

6. Resuspend pellets in a small amount (10-15 ml) of 2 M KCl. Keep track of the volume added. Use glass stirring rods to disperse pellets.

7. Transfer the slurry into a graduate cylinder and calculate the volume of the pellet.

8. Add more 2 M KCl to bring the KCl concentration to 0.5 M.

Volume = 0.307 x volume of pellet - volume of added KCl

9. Use a small volume of 0.5 M KCl to get pellets into solution.

10. Slowly add saturated ammonium sulfate to 40% saturation (2/3 of current volume). Maintain constant stirring.

11. After 15 min, centrifuge in a SS34 rotor for 10 min at 13,000 rpm, 4^{o}C.

12. Collect Supernatant. Measure volume. Add saturated ammonium sulfate to 50% satuation (1/5 of measured volume).

13. Myosin can be stored in 50% saturated ammonium sulfate for several months at 4^{o}C. Expect a yield 1.0-1.5 g from 300-400 g muscle.

References

T.D.Pollard (1982) Myosin purification and characterization. Methods*Cell Biol.* 24:333-371.

S.S.Margossian and S.Lowey (1982) Preparation of myosin and its subfragments from rabbit skeletal muscle. *Methods Enzymol.* 85:55-71.