Preparation of Heavy Meromyosin and Light Meromyosin from Myosin II

Day 1

Materials

1. 2 M KCl, 50 mM PIPES, 4^{o}C, 10 ml.

2. Digestion buffer: 0.5 M NaCl, 50 mM (KH_2PO_4+K_2HPO_4, first make a 100 mM stock at pH 6.5), pH 6.5, 4^{o}C, 250 ml.

3. Myosin stored as precipitates in ammonium sulfate.

4. Narrow dialysis tubing.

Procedure

1. Mix myosin suspension in ammonium sulfate and take appropriate amount of myosin.

2. Pellet myosin at 12,000 rpm, 4^{o}C, for 15 min in a SS34 rotor.

3. Resuspend pellet in a very small volume of buffer 1 (the concentration should be at least 20 mg/ml).

4. Dialyze against the digestion buffer overnight.

5. Make sure saturated ammonium sulfate is available.

Day 2

Materials

1. 0.5 mg/ml alpha-chymotrypsin (Cooper Biomedical) in 0.001 N HCl, 1 ml. Prepare immediately before use.

2. 100 mM PMSF in 70% EtOH. Highly toxic.

3. Narrow dialysis tubing.

4. Stopwatch.

5. 20 mM KCl, 10 mM (KH_2PO_4+K_2HPO_4), 1 mM DTT, pH 6.5, 4^{o}C, 500 ml. 6. Saturated ammonium sulfate.

Procedure

1. Estimate myosin concentration by measuring OD^{280} after appropriate dilution of a small aliquot to ~1 mg/ml into 0.6 M KCl (e.g. 25 µl in 500 µl). Concentration (mg/ml) = OD / 0.53 x dilution factor.

2. Dilute to 20 mg/ml with the buffer used for dialysis.

3. Equilibrate myosin solution to ~25^{o}C by submerging in a waterbath. Add chymotrypsin to 0.05 mg/ml while stirring.

4. After exactly 10 min incubation at room temperature, stop the reaction by adding PMSF to 0.1-0.3 mM while stirring. Incubate for another 1 min.

5. Dialyze overnight against buffer 5.

Day 3

Materials

1. 0.6 M KCl, 50 mM (KH_2PO_4+K_2HPO_4), pH 7.0, 4^{o}C, 1 liter (for light meromyosin prep only).

2. Saturated ammonium sulfate (for heavy meromyosin prep only).

3. 95% EtOH, 4^{o}C (for light meromyosin prep only).

4. 20 mM KCl, 10 mM (KH_2PO_4+K_2HPO_4), 1 mM DTT, pH 6.5, 4^{o}C, 1 liter (for heavy meromyosin prep only).

Procedure

1. Centrifuge in a 50Ti rotor at 40,000 rpm for 1 hr at 4^{o}C.

2. Supernatant contains heavy meromyosin. The yield of heavy meromyosin should be 40-50% of the theoretical yield (~3 mg from 10 mg myosin). Heavy meromyosin can be further purified by performing ammonium sulfate cut between 40 and 55%. Dialyze exhaustively against buffer 4. Collect supernatant after centrifugation in a 50Ti rotor at 40,000 rpm, 4^{o}C for 1 hr.

3. Pellet contains light meromyosin. Resuspend in a small volume of buffer 1 (typically 3-5 ml).

4. Add 3 volumes 95% ETOH while stirring on ice. Use a 1 ml Pipetman and add slowly around the side of the beaker.

5. Stir at 4^{o}C for 2-3 hr.

6. Transfer to a plastic centrifuge tube with a 1 ml Pipetman. Centrifuge in a SS34 rotor at 10,000 rpm, 4^{o}C for 30 min.

7. Carefully discard supernatant. Resuspend pellet (as a slurry) in 1 ml buffer 1 with a Pipetman or a plastic transfer pipet.

8. Dialyze extensively against ~1000 ml buffer 7, change buffer once to ensure removal of EtOH.

Day 4 on

Materials

1. 0.03 M KCl, 10 mM (KH_2PO_4+K_2HPO_4), pH 7.0, 4^{o}C, 2 liter.

2. 2 M KCl, 2 mM PIPES, 4^{o}C, 10 ml.

Procedure

1. Centrifuge in a 50Ti rotor at 40,000 rpm, 4^{o}C, for 1 hr.

2. Collect supernatant. Dialyze overnight against 1000 ml buffer 1.

3. Centrifuge in a 50Ti rotor at 40,000 rpm, 4^{o}C, for 1 hr.

4. Resuspend pellet in buffer 2 and dialyze into desired buffer. Clarify in the ultracentrifuge before use.

Reference

S.S.Margossian and S.Lowey (1982) Preparation of myosin and its subfragments from rabbit skeletal muscle. *Methods Enzymol.* 85:55-77.