Cardiac muscle sample preparation for X-ray diffraction

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Small Angle X-ray Diffraction Study:
Equatorial reflections: 1,0 – myosin filaments; 1,1 – actin & myosin filaments.
The $I_{1,1}/I_{1,0}$ ratio represents the distribution of cross-bridge mass.
What can you do with skinned cardiac muscle?


3. Colson BA, Bekyarova T, Locher MR, Fitzsimons DP, Irving TC and Moss RL. **Protein kinase A-mediated phosphorylation of cMyBP-C increases proximity of myosin heads to actin in resting myocardium.** *Circulation research.* 2008;103:244-51.


Lattice structure alterations in DCM HCM and RCM mouse models associated with mutations in myosin regulatory light chain
Hypercontractility in D166V-HCM, and hypocontractility in D94A-DCM hearts

Inner ventricular septum

Posterior wall

Inner ventricular diameter

Ejection fraction (EF %)

Fractional shortening (FS %)

D166V-HCM
RLC-WT
D94A-DCM
Decreased maximal tension and increased Ca$^{2+}$ sensitivity in D166V-HCM
No change of maximal tension but decreased Ca$^{2+}$ sensitivity in D94A-DCM
Small angle X-ray data acquired at serial pCa solutions
Rat cardiac muscle fiber  diffraction: Meridian

ATP pCa9 170mM SL:2.3µm

M6 7.2 nm
M3 14.3 nm
Collagen 20nm
Tn3 12.9 nm

dATP pCa9 170mM SL:2.3µm
Sample Types-skinned cardiac muscle bundle

• Mouse:
  Left ventricular papillary muscle
  Cross section diameter: ~200-300 µm
  3-4 samples from one heart

• Rat:
  Right ventricular trabeculae and papillary muscle
  Cross section diameter: ~200-400 µm
  3-4 samples from one heart
Heart dissection-solutions

- **Mice**
  1. Wash heart with 0.9% NaCl
  2. Dissect the bundle in Solution A (pCa8 solution + 30mM BDM + 15 units per mL of creatine phosphokinase (CPK) + Protease inhibitors (sigma p8340) + 15% glycerol)
  3. Muscle bundle samples will transfer to solution A without 15% glycerol for 15 min on ice to remove glycerol.

- **Rat**
  Krebs–Henseleit (KH) : 118.5 mM NaCl, 5 mM KCl, 2 mM NaH2PO4, 1.2 mM MgSO4, 10 mM glucose, 25 mM NaHCO3, and 0.1 mM CaCl2, as well as 20 mM (BDM)

BDM, Protease inhibitors and CPK freshly add everyday
**Skinned protocol**

- Skinned overnight with 1% Triton-X 100 in 4°C and stored in 50% of glycerol.
- Freshly skinned in room temperature for 1.5 to 2 hours.
**Sample preparation**

- Isolated muscle fibers out from the heart
- Using insect pin to fix muscle length
- Stored in 1.5ml of tubes with skinning solution
- Put on the shaker and skinning for 1.5 to 2 hours
- Change freshly skinned solution ones during the process
- Washing 3X 5min with solution without tritonX-100 on ice
- Stored skinned muscle fibers in 4 °C and use same day for x-ray experiment

**Skinned solution:**

**Miami for mice**

Solution A + 1% of tritonX-100. pH adjust by K-propionate to 7 (IS:150mM)

PS. Washing with pCa8 +CPK + protease inhibitor solution without BDM.

**Washington for Rat**

Relaxing solution: 0.1 M KCl, 5mM K$_2$EGTA, 9mM MgCl$_2$, 4mM Na$_2$ATP

with 1% of TritonX-100 and Protease inhibitor (sigma P8340), pH adjusted by KOH to 7

Note: many people prefer K-propionate to KCl, but sample looks similar in my hand
How to check the quality of preparation - Laser

D94A DCM mouse cardiac muscle SL=2.1μm
Freshly skinned in room temperature without BDM treatment and 2% of TritonX-100 for 2 hours

Rat EDL skeletal muscle
Summary

• Freshly prepare fiber sample
• Freshly add BDM, protease inhibitor into the skinning solution
• Check the quality skinning sample by laser before coming to Argonne
• Well trained and energetic student, postdoc...... better data more data. Optimal your protocol will bring more success.

Question? Discussion
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