

Cryo-EM Sample Preparation Of Native Myosin Filament From Striated Muscle

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The detailed structure of filaments formed from myosin II is poorly understood. In so far as it is known, vertebrate thick filaments follow a single structural model; invertebrate thick filaments show high variability between species and between muscles of the same species. To date, high resolution structures have been reported only for myosin filaments isolated from invertebrates and no structures have been reported for myosin filaments in which one or more thick filament proteins have been mutated. A structure of thick filaments from vertebrates would be the most useful for understanding the effects of mutations on muscle function in humans. Despite numerous cryo-EM advances, structures of vertebrate thick filaments are limited to low resolution negatively stained specimens. The main problem seems to arise from difficulties in preserving the apparently more fragile vertebrate thick filaments using techniques that work well for invertebrates. Here, we report progress in the cryo-EM sample preparation of thick filaments which preserves the structure of both backbone and myosin heads. We are using rabbit psoas myofibrils as the model system to utilize the sensitivity of the ordered head arrangement to biochemical conditions. The approach uses an overnight incubation of myofibrils in a calcium-free relaxing buffer plus 1% Triton X-100 and calcium-insensitive gelsolin to remove as much of the sarcomeric actin as possible. All subsequent steps incorporate calcium-insensitive gelsolin in the buffers. Calpain-digested myofibrils were sheared by pulling the suspension through a 26-gauge needle with a syringe only 5 times to release filaments. Thick filaments were applied to a 1.2/1.3 Quantifoil grid and washed with relaxing buffer plus 0.5% glutaraldehyde to preserve ordered heads which otherwise disorder rapidly in vertebrate thick filament cryo-EM samples. This same method without the glutaraldehyde works well with invertebrate thick filaments. Supported by NIH.