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Review Article

Mechanical analysis of *Drosophila* indirect flight and jump muscles

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ABSTRACT

The genetic advantages of *Drosophila* make it a very appealing choice for investigating muscle development, muscle physiology and muscle protein structure and function. To take full advantage of this model organism, it has been vital to develop isolated *Drosophila* muscle preparations that can be mechanically evaluated. We describe techniques to isolate, prepare and mechanically analyze skinned muscle fibers from two *Drosophila* muscle types, the indirect flight muscle and the jump muscle. The function of the indirect flight muscle is similar to vertebrate cardiac muscle, to generate power in an oscillatory manner. The indirect flight muscle is ideal for evaluating the influence of protein mutations on muscle and cross-bridge stiffness, oscillatory power, and deriving cross-bridge rate constants. Jump muscle physiology and structure are more similar to skeletal vertebrate muscle than indirect flight muscle, and it is ideal for measuring maximum shortening velocity, force–velocity characteristics and steady-state power generation.

1. Introduction

The use of *Drosophila* as a model organism has had a major impact on many fields of biology. Over the past couple of decades, progress has been made to expand its utility in muscle physiology by developing preparations from two *Drosophila* thoracic muscles, the indirect flight muscle (IFM) and jump muscle for mechanical evaluation. These two muscles are very interesting as they possess informative and adaptive physiological characteristics [1–3], but combined with the power of *Drosophila* genetics, their use to answer many interesting and relevant muscle biology questions is greatly enhanced. These muscles have been used to investigate the structure–function characteristics of muscle proteins [4–6], determine mechanisms behind muscle and cardiac diseases [7] and study the molecular basis of muscle fiber type diversity [1,8]. In this review I focus on the preparations, equipment, and mechanical analysis techniques that have been developed to investigate the functional properties of IFM and jump muscles.

2. Drosophila indirect flight muscle

The IFM is composed of 12 dorsal longitudinal muscle (DLM) fibers and 14 dorsal ventral muscle (DVM) fibers inside the thorax. The fibers are about a millimeter in length and have an oval shape when viewed in cross-section. The fibers range between 100–200 μm in diameter. The IFM fibers do not directly attach to the wings, but instead attach to the cuticle. The deformation of the cuticle acts on a specialized hinge that causes the wings to move [9]. The two sets of IFM contract out of phase with each other to set up a resonance vibration in the thorax, similar to that of a tuning fork [10]. The stiffness of the IFM, plus the stiffness of the cuticle and inertia of the wings are the major influences on wing beat frequency and wing stroke amplitude with further adjustments possible from some of the small direct flight muscles [11,12] and perhaps by modulating the stiffness of the IFM through calcium concentration [13]. This is in contrast to most other animal muscle types where the firing rate of the motor nerve sets the muscle contraction frequency. Instead, the IFM fibers of *Drosophila* are asynchronous, meaning they do not contract synchronously with each nerve action potential from the flight muscle motor nerve [14]. The IFM has a very prominent stretch activation response which, along with shortening deactivation, allows for net work and power generation at relatively high calcium concentrations [14]. These adaptations enable the very high contraction frequencies required for flight without incurring the high cost of calcium pumping with each contraction cycle. These high speed adaptations also influence the types of muscle mechanical measurements that can be made with *Drosophila* IFM.

2.1. IFM dissection and skinned fiber preparation

We prepare IFM fibers for mechanical evaluation from female *Drosophila* that have been raised at 18 °C as the females are larger than the males and flies raised at colder temperatures are slightly larger than flies raised at higher temperatures. The larger size makes fiber dissection easier, but is not essential for a good skinned fiber preparation. Fibers are normally dissected from the

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thoraces of 2 to 3-day-old *Drosophila*. The IFMs of younger flies are still growing, adding thick and thin filaments [15], thus fibers from younger flies generate less force and power [16]. However, we are sometimes forced to use younger flies as some muscle protein mutations cause myofibril ultrastructure deterioration once the flies start using their IFMs. For example, we use fibers from newly eclosed to 2-h-old flies for our embryonic myosin based chimeric mutants [16].

2.1.1. Dissection of IFM

The first step in IFM dissection is removal of the head, abdomen and wings using fine spring scissors (Vannas 4 mm spring scissors, Fine Science Tools) and forceps (Dumont #5, Fine Science Tools) (Fig. 1A). The thorax, with the legs still attached, is placed in a Plexiglas chamber. This chamber is made by drilling a 2 cm diameter hole through a 0.5 cm high, 2.5 cm by 7.5 cm Plexiglas rectangle and gluing it to a standard glass microscope slide of the same length and width. The chamber contains dissection solution (pCa 8.0, 5 mM MgATP, 1 mM free Mg^{2+}, 0.25 mM phosphate, 5 mM EGTA, 20 mM *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES, pH 7.0), 175 mM ionic strength, adjusted with Na methane sulfonate, 1 mM DTT, 50% glycerol and 0.5% Triton X-100). The dissection chamber is kept cold by using it on a custom designed aluminum stage cooled by a cold water/radiator fluid mixture at 4 °C from a chiller (ThermoFlex900, Thermo Electron Corporation, Waltham MA) flowing through milled out channels in the stage. The stage has a 2 cm diameter hole in the center to allow light in from the bottom if desired. Two cuts, using very fine spring scissors (Minivannas, 2 mm, Fine Science Tools), are made to split the thorax open (Fig. 1B), one through the ventral cuticle between the two sets of legs and the other through the dorsal cuticle. The dorsal cut can be made off to one side to ensure that the opposite side's set of DLM fibers are not damaged. At this step, the dissection solution can access the fibers and skinning begins. The fibers should remain in this solution for 1 h.

The next and subsequent steps in the dissection require custom designed probes. Tungsten wire probes are made by electrochemical etching in a saturated solution of $NaNO_3$. A direct current power supply is connected to a carbon rod, the negative terminal, that is immersed in the nitrate solution and the other end of the circuit, the positive terminal, is connected to a banana clip. We connect the clip to the shaft of a hypodermic needle into which 0.005 inch diameter tungsten wire (Small Parts, Inc.) has been inserted and crimped into place. The wire is bent appropriately for the specific probe and shaped by repeated immersions into the etching solution.

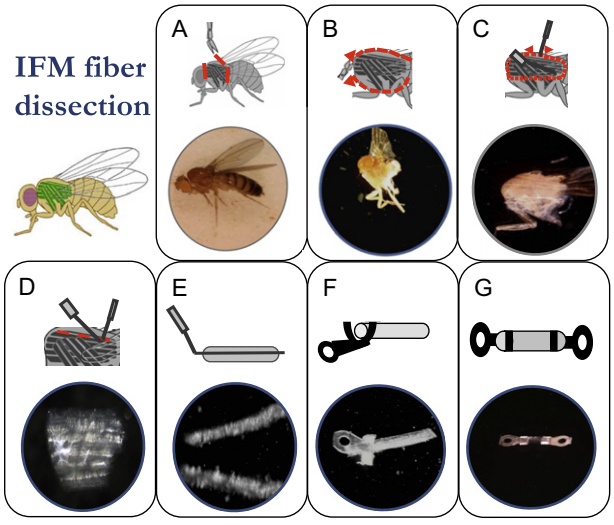

Fig. 1. Dissection and T-clipping of an IFM fiber. The steps of preparing an IFM fiber for mechanical measurements are (A) isolation of the thorax by removing the head, abdomen and wings, (B) splitting the thorax in half and immersing it in skinning solution, (C) removing the bundle of DLM fibers, (D) separating the 6 fibers in the bundle from each other, (E) splitting one of the fibers in half, (F) clamping the tabs of the T-clips around the ends of the fiber to produce (G) a clipped IFM fiber preparation.

One key to successful IFM fiber preparation is careful extraction of the fibers from the half thorax (Fig. 1C). There are no tendons to cut as the fibers attach directly to the cuticle, so the fibers must be carefully detached at their insertion points. Detachment is performed using a tungsten wire probe that has a 45 degree bend prior to an approximately 200 μm long and 50 μm tip that rapidly narrows to a sharp point. A combination up and down sewing machine-like motion and a slight side to side motion works best to detach a bundle of DVM fibers (Fig. 1D bottom). The fibers can be transferred to 0.5 ml of storage solution (dissection solution minus Triton X-100) in another dissection dish once the fibers have soaked for 1 h in dissection solution (or alternatively moved after one of the next two dissection steps). A wire loop small enough to support a drop of solution such that the fiber bundle is supported by surface tension makes a good transfer device. The fibers are held together by tracheal tubes that must be severed, using a straight pair of tungsten wire probes that have a fine but not sharp point to separate the fibers (Fig. 1D). The IFMs are directly oxygenated through these tubes. A scissors-like motion and side to side motion are used to separate the fibers, taking care not to stretch or stab the fibers with the probes. The individual fibers have an oval shape when viewed in cross-section with a definite long and short axis. The best fibers are split longitudinally down their middle, as parallel to the myofibrils as possible, to produce fibers with a diameter of approximately 125 μm. The probe for splitting the fiber in half is bent at about a 45 degree angle. The portion after the bend is slightly longer than the length of the fiber. This probe should have a very thin, non-tapered tip, ~20 μm in diameter. This probe is easily damaged, so new ones need to be made frequently. A half-fiber enables better diffusion of MgATP and other solution components into and out of the fiber.

Fibers left in the dissection solution longer than one hour gradually become softer and more compliant. If left overnight in dissection solution, they produce less force and power than fibers used immediately or stored in storage solution (dissection solution without Triton-X 100). Fibers transferred to storage solution can be stored for at least a week at −20 °C without any performance degradation.

2.1.2. T-clipping

The IFM fibers are attached to the force transducer and length change motor using aluminum foil T-clips (Fig. 1F and G). The T-clips are laser cut from food grade aluminum foil (MicroConnex, Snoqualmie, WA). Due to their small size, the clips are not completely cut free from the foil, but are left attached to rectangular strips so we end up with the clips arranged in rows of about 50 per strip. The clips can be scaled and cut at various sizes to match different fiber diameters. The size of the hole is important to enable the T-clip to fit snugly on the force and length hooks so it does not move during some of the more abrupt mechanical perturbations. The tabs of the T-clips are pre-bent into a U-shape while still attached to strips of aluminum foil. This makes wrapping them around the fiber ends easier (Fig. 1F). The clips are cut off the strips and stored in a large drop of glycerol in a Petri dish until needed.

Proper attachment of the clips to the fibers is a balance between clamping the ends of the fiber tightly enough so the fiber does not slide around in the clip, but not so tight that the ends of the fiber are crushed (Fig. 1G). A good guide is that the fiber should bulge slightly where it emerges from the clip. Poor clipping is the most