An Automated Apparatus for Isometric Force Analysis of Skinned Muscle Fibers

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Abstract

Skinned muscle fibers provide a powerful means to assess the functional effects of compounds that modulate the sarcomere. The main drawback of this preparation as an assay system is its low-throughput nature. As part of an effort to optimize small molecule activators of the skeletal sarcomere for therapeutic applications in conditions where muscle weakness is a feature, an automated system was designed that can simultaneously run multiple types of isometric force assays. Six identical units, controlled through a single software interface, run a variety of assay protocols. Each unit independently measures the force of a single suspended fiber as it is submerged into various solutions in a temperature controlled block. Assay protocols are unique for each tissue type and desired measurement. Fiber quality is automatically assessed by switching between fully contracting and fully relaxing pCa solutions. If sufficiently robust, fibers are tested by indexing between solutions of varying pCa or compound concentration. In each new solution, the software monitors the rate of force generation and when the fiber has reached a force plateau, automatically moves to the next solution. Control pCa profiles of rabbit psoas fibers measured over a year and a half period show typical variation of < 0.1 pCa unit from historic values. This capability has allowed characterization of several hundred compounds aiding with the selection of a troponin activator as a development candidate for diseases characterized by muscle weakness.

Introduction

Small molecule activators of the skeletal sarcomere are a potential therapeutic approach in diseases in which muscle weakness is prominent. Activators are initially identified in a high throughput screen using detergent-treated skeletal myofibrils. Compounds of sufficient potency and specificity progress to functional assays on glycerol-treated skinned skeletal muscle fibers. Although isolated myofibrils preserve an intact sarcomere, they do not provide a functional readout (contractile force) and are not under tension as in intact muscle. Measurements of skinned fibers address these issues but are inherently low-throughput. In order to provide sufficient skinned fiber assay capacity, a higher-throughput means of testing compounds on skinned fibers was developed.

Background

Basic Isometric Skinned Fiber Apparatus

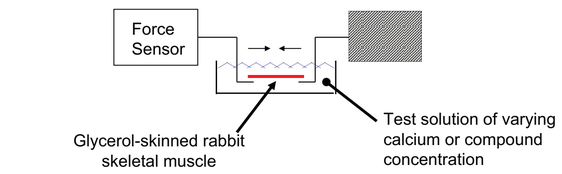
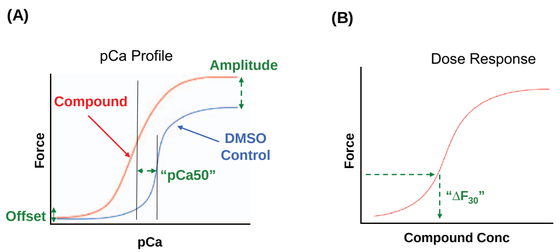


Figure 1. Basic Isometric Skinned Fiber Measurement. Isolated, glycerol-treated skinned muscle fibers are mounted between a fixed post and a force transducer and sequentially exposed to varying calcium or compound concentrations. The tension of the fiber is monitored as it contracts under isometric (fixed length) conditions.



pCa Profile

> Fixed compound concentration (10µM or 60µM)
> Vary calcium
>"pCa_{50}" = midpoint of the response curve
> Allows analysis of compound activity over full calcium range

Dose Response

> Fixed calcium concentration (pCa 6.0)
> Vary compound concentration
> "ΔF_{30}" = concentration required to reach 30% of the range between DMSO and the fully contracted state
> Allows determination of relative potency

Figure 2. Isometric Skinned Fiber Experiments. Two basic experiment types are used to classify and rank-order compounds. pCa Profile assays vary calcium at fixed compound concentrations (Figure 2A). This allows analysis of a compound's activation response over a full range of calcium concentrations. The tension generated by the fiber is analyzed relative to that of DMSO control fibers. This assay measures shifts in pCa and changes in shape of the pCa curve. The second type of assay is a Dose Response (Figure 2B) and measures the concentration dependent activation effect of compounds at a fixed calcium concentration. The standard metric from this assay is a ΔF_{30} or the concentration of compound that increases force to 30% of the response range between DMSO controls at the test pCa and the fully contracted state.

Developing an Automated Solution

As part of the assay progression paradigm, it was necessary to develop methodology to provide sufficient capacity for skinned fiber experiments. Several key features drove hardware and software design decisions. The system needed to be able to run multiple types of experiments simultaneously and asynchronously. It needed to run experiments unattended by automatically determining when fibers reached tension plateaus and include fiber quality metrics. Test solutions needed to be chilled and maintained at constant temperature, and the system needed to be robust and to provide a means of reviewing data before aggregating and publishing to a chemically intelligent relational database.

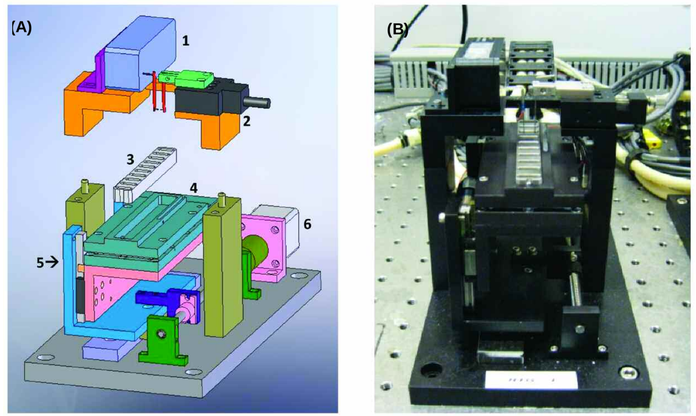


Figure 3. Automated Fiber Test Apparatus. A custom automated fiber test platform was designed and built in house. Figure 3A shows the solid-edge engineering model for the device created in SolidWorks and Figure 3B an assembled device. The apparatus was built using a commercially available force transducer (1) (Model 403A, 5mN range, Aurora Scientific, Ontario, Canada). The transducer is mounted on a removable stand (2) which references to the rest of the rig through alignment pins and is removable for ease of mounting fibers. Test solutions are held in a removable 10-well stainless bath block (3) (250µl wells) which fits into a Peltier-cooled platform (4). The height of the platform is controlled by a servo motor (5) and its axial position controlled by a stepper motor (6). To move to a new test solution, the platform is lowered and the stepper motor indexes to a new position and then raises the bath around the fixed fiber position.

Figure 4. Fiber Apparatus Control Software. Six fiber rigs are controlled from a common application interface written in LabVIEW. Each instrument can independently and asynchronously run any of the defined protocols; protocols differ by experiment type and by fiber type (for example pCa Profile vs. Dose Response assays or fast vs. slow skeletal fibers). Parameters which govern each respective protocol (defined below) are stored in user-editable configuration files. Once a protocol is selected, the user specifies the test article and assay conditions (compound ID, concentrations) before initiating a run. During the assay, force data are displayed graphically (1) along with the commensurate well position (2). A common window (not shown) allows all rigs to be simultaneously monitored and notifies the operator when an assay completes. XML data files containing test conditions and acquisition parameters along with raw data are written to a file server for subsequent processing.

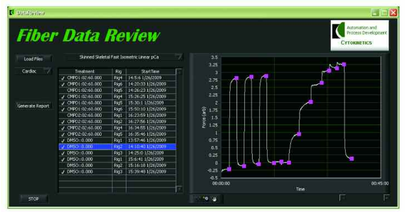


Figure 5. Fiber Data Review. Fiber data acquired during a run instance are loaded into an application for review and quality assessment. Each trace can be viewed and selected for inclusion into an aggregate report. Data are grouped by experiment type and by test article, internally normalized based on algorithm type, and written to a report along with DMSO control data. The data report is formatted for import, curve fitting and storage into a chemically intelligent database which enables fiber metrics to be queried and reported alongside compound structure.

Example Data

The skinned fiber rigs have enabled hundreds of compounds to be tested in support of lead development over an approximate one and a half year period. The instrument has proven robust and stable with daily control pCa profiles of rabbit psoas fibers showing typical variation of < 0.1 pCa unit from historic values.

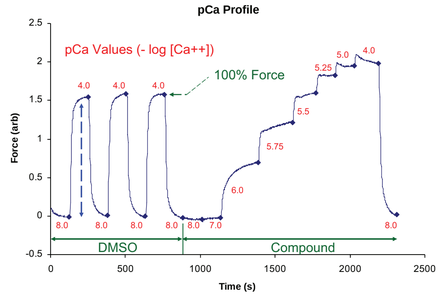


Figure 6. pCa Profile. pCa Profiles are used to assess how compounds change the fiber tension response over a wide calcium range. At the initiation of a pCa profile experiment, the fiber quality is assessed by iterating between control wells of pCa 8 (fully relaxing) and pCa 4 (fully contracting) solutions. Data are collected in each control well for a fixed interval (typically 120 seconds). The fiber is deemed stable if two sequential fully relaxed and two sequential fully contracted force values are within a fixed percentage (typically 2.5%) of the initial full response. Once stable, the fiber is submerged into successively increasing calcium concentrations containing fixed compound concentrations (typically 10 or 60uM). After a minimum period of time in the new condition, the acquisition protocol monitors the rate of force development to determine when the fiber has reached a response plateau. Stability is assessed by fitting the slope of a moving window (typically previous 30 seconds) and comparing it to the initial full response range; the slope must be less than a percentage of the maximum range (typically < .025%). At the end of the assay the fiber is returned to fully relaxing conditions to determine the reversibility of the measurement and confirm fiber integrity. All responses are normalized to the full response range (difference between last two pCa 4 and pCa 8 tension values determined at start of experiment).

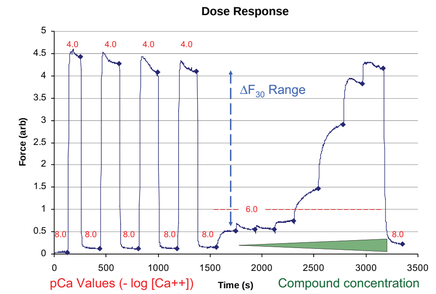


Figure 7. Dose Response. Dose Response experiments are used to determine relative compound potency. Fiber quality is assessed at the start of the experiment as described above for the pCa Profile. Once stable, the fiber advances through a series of pCa_{25} solutions (calcium concentration eliciting 25% contractile response) starting with a DMSO control and then through increasing compound concentrations. The compound concentration which increases fiber tension by 30% compared to DMSO pCa_{25} control is the ΔF_{30} value and is useful as a relative potency metric for rank-ordering compounds.

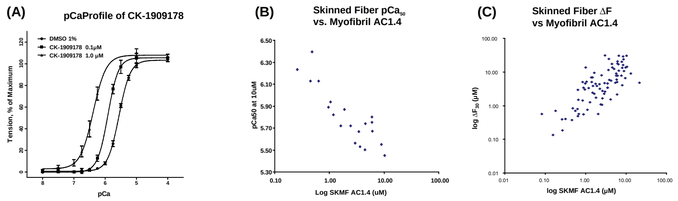
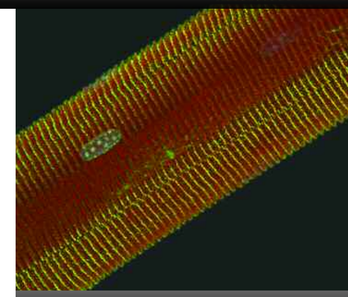


Figure 8. Data for CK-1909178, a representative compound from a chemical series that activates the sarcomere through interaction with the troponin complex are shown in Figure 8A. The data show how the compound sensitizes the sarcomere to calcium without changing the maximum fiber tension. Figure 8B and 8C show data from additional compounds in this chemical series comparing respectively pCa_{50} and ΔF_{30} values measured on skinned fibers vs. biochemical activity (AC1.4) in myofibrils (compound concentration activating isolated skeletal myofibrils by 40%). Within this series, there is good correlation between myofibril activity and data derived from skinned fiber measurements.



Conclusions

As part of an assay paradigm to optimize small molecule activators of the skeletal sarcomere, a higher-throughput system for testing compounds in isolated skinned muscle fibers was developed. The system consists of multiple rigs controlled independently through a common application interface which runs several assay protocols. Each protocol runs unattended and has built-in fiber quality and stability metrics. A separate application for reviewing data was also developed that allows fiber traces to be examined and selected for aggregation into a consolidated report ready for upload into a relational database. This system has allowed characterization of several hundred compounds as part of a lead-optimization program.

CYTOKINETICS