Analysis of Structure Function Relationship on Skinned Muscle Fibers by Combination of Different Tests: Ca and Sr Affinities, Slack Test (V_{max}), Myosin and Troponin Electrophoresis

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Abstract

Ca and Sr affinities, slack test (W_{max}) and gel electrophoresis of contractile proteins are convenient techniques for analyzing structure-function relationship on skinned muscle fibers. The experimental conditions for all these analyses are here briefly reported.

Key words: skinned muscle fibers, Ca and Sr affinities, slack test (V_{max}), myosin, troponin electrophoresis.

BAM 3 (3): 219-220, 1993

Determination of fiber type by Ca and Sr affinities

The experimental procedure is as follows: a single fiber segment of about 2 mm long is isolated from EGTA skinned muscle biopsies. The fiber is mounted in the experimental chamber between a fixed forceps and a force transducer (Kulite BG 10). The diameter of the fiber is measured with a binocular micrometer. The optimal length for force development is obtained by adjusting the sarcomere length using a He-Ne laser beam.

The criterion for fiber identification is based on the difference of Ca and Sr activation characteristics (Tension/pCa and Tension/pSr relationships) between slow and fast skeletal muscle fibers (Fink et al., 1986).

As previously described (Mounier et al., 1989), the fiber is activated in solutions containing various concentrations of Ca (expressed in pCa values, pCa = -log [Ca]). The tension recorded at the various pCa is expressed as the ratio of this tension (P) to the maximal tension (P_0) obtained in a high Ca concentration (i.e. pCa 4.2) and the Tension/pCa (T/pCa) relationship is established. Different parameters of the T/pCa are studied: the threshold for activation, i.e. the lowest Ca concentration required to obtain a tension development; the pCa_{50} parameter, i.e. the pCa inducing 50% of the maximal tension, indicator of the Ca affinity of the troponin C; and the steepness of the relationship; by fitting the data to the Hill equation, the following coefficients are calculated: n_H for the whole curve, or n_1 and n_2 for P/P_0 higher and lower than 50%, respectively.

For the Sr activation characteristics, the same procedure is used but Ca is replaced by Sr and the T/pSr relationship is established.

The difference (pCa_{50} - pSr_{50}) is used to reflect the relative affinity of a fiber to Ca and Sr. It is generally assumed that fast skeletal muscle fibers are less sensitive to Sr than to Ca when compared to slow fibers (Takagi and Endo, 1977). Thus a fiber exhibiting little or no difference between Ca and Sr affinities is identified as slow-type. On the contrary, a fast-type fiber can be identified by a high difference between the pCa_{50} and the pSr_{50}.

Determination of V_{max}: Slack test

V_{max} of single skinned fibers is determined by the slack test method (Edman, 1979; Moss, 1986). By means of cellulose acetate dissolved in acetone, a fiber segment is glued between the hook of a force transducer (AME 801 element, Aksjeselskapet, Horten, Norway) and the arm of a length step generator (GWV_2 vibrator, Electronics Ltd).

The fiber is maximally activated in the pCa 4.2 solution. When the maximal tension (P_0) has reached its steady state, the fiber is rapidly released to a shorter length such that tension fell to zero. The fiber shortens under zero load until the slack is taken up, after which tension begins to redevelop. The fiber is then reextended to its original length and relaxed. Each fiber is activated and slackened four to six times by use of different length steps. The duration of unloaded shortening, or time between onset of slack and redevelopment of tension, is measured for each step length. The amount of length change is plotted as a function of the

duration of unloaded shortening. V_{max} [fiber length (FL/s)] is calculated by dividing the slope of the fitted straight line by the fiber segment length. The data are normalized to a SL corresponding to that obtained in the maximally activated fiber (SL_{max}).

$$V_{max} = \text{slope} \times 1/\text{segment length} \times SL/SL_{max}$$

SDS PAGE analysis

The procedure used to analyse the myosin composition of the skinned muscle fibers was described by Danieli-Betto et al. (1990).

After physiological measurements (T/pCa, T/pSr, V_{max}) the fiber segment is transferred into a capillary tube containing 20 µl of Laemmli's buffer (10% v/v glycerol, 5% v/v β-mercapto ethanol, 2.3% w/v SDS, 62.5 mM Tris/HCl buffer, pH 6.8), and left incubated 3 hours at room temperature.

Myosin heavy chain (MHC) isoforms are separated on a 6% polyacrylamide slab gel using the method described by Danieli-Betto et al. (1986). The concentration of the stacking gel is 4%. Both gels contain 40% (v/v) glycerol. The running buffer is 32.5 mM Tris, 288 mM glycine, 0.1% (w/v) SDS. 2 µl of the sample preparation are sufficient to identify MHC isoforms in the fiber.

The rest of the sample is loaded for myosin light chain (MLC) content on a 10-20% polyacrylamide linear gradient slab gel, according to the method described by Salviati et al. (1982). The stacking gel and the running buffer are identical to those used for MHC separation.

After the gel run, the gel slabs are silver stained according to Ansorge's procedure (1983) or by the commercialized silver stain kit (Biorad- 161 0443).

Relative amounts of the different isoforms of MHC and MLC can be determined by comparing the silver staining intensities of the photographs of the electrophoretic bands, using an image processor (Alcatel-TITN) connected with "gel pro" software.

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