The origin of the series elastic component in single crayfish muscle fibres

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Summary. High-speed cinematographic studies on the length changes of single tetanized crayfish muscle fibres during a step decrease in length revealed a fairly uniform distribution of a highly compliant series elastic component along the fibre length, suggesting that it mainly originates from some structures in each sarcomere other than the cross-bridges.

Although the mechanical behaviour of active muscle can be explained by postulating an elastic component in series with a contractile component[2], the origin of the series elastic component (SEC) in various types of striated muscle is not firmly established. In frog skeletal muscle, the extension of the SEC with the maximum isometric tension (P_0) is about 1% of the slack length (L_0), and the force-extension curves of the SEC are scaled down in proportion to the isometric tension[3-5], suggesting that the SEC largely resides in the cross-bridges. In other striated muscles, however, the extension of the SEC with P_0 seems to be too large for the elastic extension of the cross-bridges (3-7% of L_0, mammalian skeletal muscle[6]; 5-10% of L_0, cardiac muscle[7,8]). Large values of extension of the SEC with P_0 have also been reported on chemically skinned or glycerinated frog fibres[9,10]. Meanwhile, the dependence of the SEC on the isometric tension[4,5] may also be explained by a passive SEC having an exponential force-extension relation[11,12]. The present experiments were undertaken to examine the anatomical origin of a highly compliant SEC in single crayfish muscle fibres.

Material and methods. Single fibres (diameter 80-300 µm) were isolated from the superficial abdominal extensor muscles of the crayfish *(Procambarus clarki)* with a piece of shell attached to each end, and mounted horizontally in an experimental chamber filled with van Harreveld's solution[13]. The fibre was held at L_0 (3.8-7.0 mm) between the force transducer (Aksjeselskapet Mikro-Elektronikk, Inc., AE80, resonance frequency, 3 kHz) and a servo-motor (General scanning, Inc., G-108). The sarcomere length at L_0 (about 9 µm) was almost uniform along the entire fibre length. Since the fibres do not generate action potential, they were maximally stimulated by applying transverse alternating currents (4-6 V/cm, 1000 Hz)[14] through a pair of Pt plates (0.5×2 cm) covering 2 opposite walls of the chamber. The resulting steady isometric tension (4-8 kg/cm^2) was almost as large as the maximum potassium contracture tension, and was taken as P_0. The fibre length was quickly reduced at P_0 (quick release) with the servo-motor, the length changes being completed within 0.6 msec. The length and tension changes were simultaneously recorded on a dual-beam oscilloscope (inset in figure 1). All experiments were performed at room temperature (18-22 °C).

Results. Figure 1 shows a typical force-extension curve of the SEC determined by the above quick release method. The minimum amount of quick release required to reduce the tension to zero, i.e. the extension of the SEC with P_0, was about 2% of L_0. This value seems too large for the elastic extension of the cross-bridges, since it is unlikely that each cross-bridge is elongated by 2% of the length of a half sarcomere or by 900 Å, a value about 1 order of

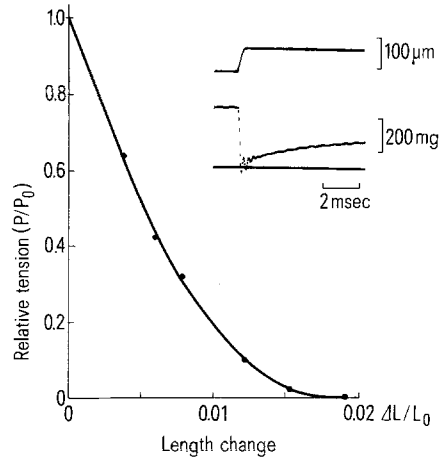

Fig. 1. Force-extension curve of the SEC in crayfish muscle fibres. The tension (P) immediately after a quick decrease in length is plotted against the magnitude of decrease in length (ΔL). P and ΔL are expressed relative to P_0 and L_0 respectively. Inset shows an example of records of length (upper trace) and tension (middle trace) changes. The level of zero tension (lower trace) is also indicated.

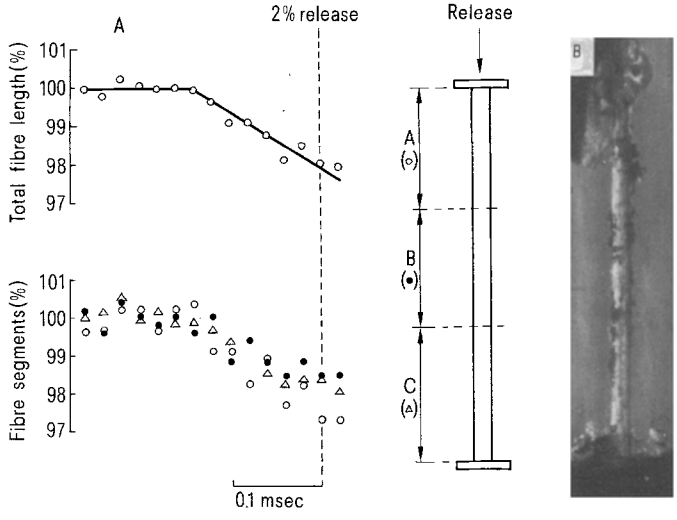

Fig. 2. Length changes of the fibre segments during the course of a quick release. In *A*, length changes of the total fibre length and 3 fibre segments are shown. Note that the shortening takes place fairly uniformly along the fibre length until the moment when the total fibre length is reduced by about 2% (vertical interrupted line), if the accuracy of measurement of segment lengths is taken into consideration. *B* is a frame from a cinefilm of a tetanized muscle fibre during a quick release. Carbon particles attached to the fibre surface can be seen.

magnitude larger than that supposed for frog muscle fibres. Meanwhile, the distance between the thick and thin filaments at L_0 was nearly the same in both crayfish and frog fibres[15].

To examine the anatomical origin of the above highly compliant SEC, fine carbon particles were firmly attached to the fibre surface, and the length changes of the fibre segments divided by the particles were recorded during the course of a quick release of a tetanized fibre with a 35 mm ultra high-speed cine-camera (Beckman, Inc., Model 165) at 40,000–50,000 frames/sec (figure 2B). As shown in figure 2A, the time course of length change of each fibre segment was fairly uniform until the total fibre length was shortened by 2%, i.e. the magnitude of quick release required to reduce the isometric tension from P_0 to zero (figure 1). This implies a uniform distribution of the highly compliant SEC along the entire fibre length. Microscopic observation (up to $\times 400$) and cinematographic recording (Redlake Corp., Locam, 500 frames/sec) of isometrically contracting fibres excluded the possibility that the SEC originates from extremely stretched sarcomeres distributed along the fibre length; though some fibre segments shortened by stretching others during the development of isometric tension, the resulting variation of sarcomere length along the fibre length was not very large.

The present results strongly suggest that the highly compliant SEC in crayfish muscle fibres results not only from the elastic elongation of the cross-bridges but also from the elongation of some other structures in each sarcomere such as the thick and thin filaments and the Z-disc. Much more experimental work is needed on the origin of the SEC which may be essential for the understanding of muscle contraction.

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The fate of choline in the circulating plasma of the rat

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Summary. Labelled free choline injected into the peritoneum failed to enter the brain but preferentially entered the liver. Subsequently labelled phospholipid was found in the plasma with a concurrent increase in the brain. This labelled plasma injected by cardiac puncture caused a rapid incorporation of the choline labelled phospholipid into the brain.

Diamond[1] has shown that a pulse label of radioactive choline could within a short time label the ACh pool in the brain. However some authors have argued that the choline found in the brain is supplied under normal conditions in the blood not as free choline but as a choline containing phospholipid. (For a recent review see Ansell and Spanner[2].) This paper reports the fate of labelled choline injected into the peritoneal cavity.

Methods. Injection of [methyl-^{3}H] choline: 50 µCi of [^{3}H] choline were injected into the peritoneal cavity of male rats, which were then killed at intervals of 7.5 to 120 min following the initial injection. Plasma samples were prepared by centrifuging heparinized blood at 3000 rpm for 5 min in a bench centrifuge. Tissue samples were homogenized in a 1% solution of Triton X-100 and the radioactivity calculated in dpm/g fresh tissue.

Labelled free choline in the plasma was separated from the labelled phospholipid by passing a sample of plasma through a 5×0.63 cm column of Amberlite CG50 resin in the sodium form. The compounds having a net neutral or negative charge passing through the resin were identified as phospholipids[3], the free choline was eluted from the column with 0.1 M HCl.

Preparation of plasma containing labelled phospholipid: Female rats were injected with 200 µCi of [methyl-^{3}H] choline. After 4–5 h the blood was removed by cardiac puncture and the labelled plasma obtained. Choline free plasma was prepared by running the plasma through columns of Amberlite CG50 resin. The plasma containing labelled phospholipid was then injected into male rats of 300 g, again by cardiac puncture.

Extraction of labelled choline, ACh, and phosphatidyl choline from brain: The labelled products in the forebrain 60 min after an i.p. injection of 200 µCi of [^{3}H] choline were examined in rats weighing 300 g. The cerebrum was removed by the freeze blow method and the material obtained extracted with absolute ethanol at −70 °C for 20 h to reduce post mortem release of choline[4].

The extract was run on a 12×1 cm column of Amberlite IRF97[5] in 0.02 M Na_2HPO_4-citric acid buffer at pH 4.3. Under these conditions neutral and negatively charged molecules came through the column with the void volume while choline and ACh followed later.

Results. Choline injected into the peritoneal cavity finds its way into the blood, liver and brain[6]. The distribution of isotope in the tissues with time shows a rapid uptake into the liver (figure 1) but very little isotope is found in the brain (the concentration is only 0.5% of that in the liver). When the total weight of the tissue is taken into consideration it was found that 50% of the injected isotope is in the liver but less than 0.05% in the brain. The isotope level in the kidney reached a peak at 15 min and was accompanied by an increase of isotope in the urine.

By separating the free choline in the plasma it was found that the amount of labelled free choline in the plasma falls steadily, while the labelled phospholipid in the plasma