

Electric charge changes in cross-striated barnacle and rat muscle during contraction

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Abstract: *Background:* A change in the fixed electrical charge between the rigor and the relaxed condition has been observed previously in the A-band of skeletal muscle. The aim of this study was to observe possible charge changes in the A-band during contraction and relate these to the contractile processes. *Methods:* Donnan potentials were measured in the relaxed state and during Ca-stimulated contractions in skinned rat and barnacle muscle fibres. From the potentials, the fixed electric charges in the A- and the I-bands were calculated. *Results:* A rise in negative charge was seen when moving from the relaxed to the contracted state. The A-band charge was more negative during contraction than the charge observed previously in rigor. The I-band charge follows the A-band charge during contraction. *Conclusion:* The difference in A-band charge in rigor and contracted state may be due to a charge change in both the rod and the head part of the myosin molecule during contraction, but only of the rod part in rigor. This is probably due to the effect of the increasing Pyrophosphate on the I-filament charge, as ATP decreases during contraction.

Key words: skeletal-muscles, protein charge concentration, muscle contraction

INTRODUCTION

A change in the fixed electric charge of the A-band has been observed between the rigor and relaxed states of muscle [1, 2]. The protein charge concentration was calculated from the Donnan potential, measured with KCl-filled microelectrodes in the vicinity of the filaments, in a muscle specimen without binding membranes. The Donnan potential method for determination of protein charges in muscle was also applied in earlier studies by Collins & Edwards [3], Pemrick & Edwards [4], Elliott et al. [5], and Naylor et al. [6]. A thorough theoretical validation of the method was given by Elliott & Bartels [7], where possible errors were also discussed. The method was later reviewed by Elliott & Hodson [8], and verified by comparing the Donnan method with charge measurements using gel electrophoresis [9]. Today, the method is therefore a well-established way of determining the electric charge concentrations of proteins.

The main protein in the A-band is myosin, and the charge change taking place in the A-band, when moving from rigor to relaxed state, is shown to be due to a change of the myosin charge [10]. The main protein of the I-band is actin, but the proteins troponin and tropomyosin also play a role in the contraction process. During contraction there is interaction between the myosin and actin via the head part of some of the myosin molecules [11].

If the electric charge change seen between the rigor and relaxed states of muscle [1, 2] bears any relationship to contraction, then charge changes between relaxed state and contraction ought to be observed.

In the present study, we investigated fixed electric charges on the muscle filaments during Ca-stimulated contraction of skinned cross-striated muscle fibres from barnacle and

rat, using the Donnan potential method. The choice of the two preparations was based on the following: rat muscle is a mammalian muscle and directly comparable with muscle in humans; studies of charge changes between rigor and relaxed states are mainly studied in rat muscle; therefore rat muscle is the obvious first choice. Contraction of even slow mammalian muscle, however, is very fast when trying to measure changes at various sites along a contracting muscle fibre with a microelectrode. To obtain more reliable results based on a higher number of measurements, giant fibres from the barnacle *Balanus nubilis* were therefore also studied. These fibres are cross-striated and have a diameter of 0.5-3.0 mm [12]. Due to their size, they skin easily, are often used for experiments, and are well described physiologically [13, 14, 15]. With a slower contraction speed than fibres from warm-blooded animals, and a well-described contraction pattern, these fibres are ideally suited for the present study.

The aim of our investigation was to study charge changes observed during contraction and relate these to the contractile processes.

MATERIALS AND METHODS

Muscle preparations. Giant barnacle (*Balanus nubilis*) muscle fibres. Single fibres were dissected, together with their baseplate and tendon attachments, as described by McLaughlin & Hinke [16]. A fibre was then transferred to a petri dish, and the baseplate fixed to the bottom of the dish with plasticine; the fibre was soaked for 30 min in relaxing solution containing (mM): K-propionate 200, MgCl₂ 5, K₂EGTA 9, CaCl₂ 1, Na₂ATP 1, Tris/HCl buffer (pH 7.1) 20. The fibre shortened during this procedure but was easily stretched, and following the stretch, the tendon was attached with silk to a small fixed hook in the dish. The fibre was skinned manually by cutting a small hole in the membrane with a piece of broken razor blade, and then pulling off the membrane – away from the

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hole – with an insect pin. When the skinning was completed, the tendon was fixed to a transducer.

Rat soleus muscle. Fibre bundles containing up to 10 fibres were dissected out and chemically skinned in a petri dish in a solution containing mM: KCl 150, MgCl₂ 5, K₂EGTA 4, Na₂ATP 5, Imidazole/HCl buffer (pH 7) 20 with 50 µg ml⁻¹ saponin added, as described previously in detail [2]. During measurements, one end of the fibre bundle was attached to a fixed hook in the dish; the other end attached to a transducer. M. soleus, which in rat is mainly slow, was chosen because attempts to use the fast semitendinosus muscle failed. It was not possible to obtain reliable potential recordings (see later) during the faster contraction, although the general trends in the results from the fast muscle were the same as seen for the slow muscle.

Electric measurements and observation of contraction. Donnan potentials were measured in the A- and I-bands with microelectrodes under a high power light microscope (Zeiss 405 inverted, ×400 magnification) with phase and polarization contrast combined [1, 2]. The protein charge concentration was calculated from the Donnan potentials, as described by Naylor *et al* [6], using the Perrin programme [17]. The theoretical background for these measurements has been given by Elliott and Bartels [7].

The microelectrodes used had an outer diameter of ca. 0.1 µm and a resistance of about 20 MΩ. A combined system of phase contrast and polarization contrast was used to enable a clear view of the position of the tip of the electrode in either the A- or I-band during measurement. The electrode was inserted by hand; if there was any doubt about the localization of the tip, the reading was discarded. The shortest sarcomere length measured in the rat muscle was 2.7 µm, which gave an A-band width of 1.65 µm and an I-band width of 1.05 µm. It is therefore possible, even during shortening, to locate a 0.1 µm tip in both the A- and I-bands. The barnacle muscle had longer sarcomere lengths, typically increasing from 4.5 µm in any particular fibre [12], and thus created less of a problem than the rat muscle.

The contraction could be followed clearly in the microscope, and readings were only taken as readings during contraction as long as shortening was taking place. An accurate measurement of force was not possible in the setup, since the microelectrode measurement setup occupied the space in the microscope viewing field, and demanded all the concentration of the person carrying out the measurements. To confirm that the contraction observed was in agreement with earlier measurements of Ca²⁺-stimulated rat and barnacle muscle, a small robust RCA 5734 mechano-electronic transducer (Radio Corporation of America, New York, USA) was fixed to the microscope base, and one end of the muscle was connected to the transducer via a 12 mm long piece of silk. The transducer was calibrated to give 130 mV/mN. With this arrangement, as explained above, the force measurements were not accurate enough to deduce anything about velocities due to the large compliance of the system, but were taken anyway to confirm that the force obtained was size- and duration-wise in agreement with earlier studies of skinned rat fibres [18] and of barnacle fibril bundles [19, 20, 21].

Solutions. The relaxing solution used in all experiments was (mM): KCl 100, MgCl₂ 6.5, K₂EGTA 4, Na₂ATP 5, Imidazole/HCl buffer (pH 7) 20.

The contraction solution consisted of the relaxing solution where Ca was added to give a pCa of 4.5 (calculated using the Perrin programme as described in Naylor *et al.* [6]).

Creatine phosphate (phosphocreatine (PCr)) (5 mM) and creatine phosphate kinase (Sigma, 64-69 units ml⁻¹) were added to all the experimental solutions.

Experimental procedures. All experiments were carried out at 21°C. Following the fixing of the muscle ends, the muscle was left to equilibrate in relaxing solution for 30 min. 15 Donnan potentials in the A- and 15 Donnan potentials in the I-bands were recorded.

The relaxing solution was quickly sucked out of the dish and replaced with contraction solution using two 30 ml syringes. During the following contraction, Donnan potentials were recorded in the A- and I-bands (as many as possible) until no further shortening was observed.

When contraction had ceased, a further 15 potentials were recorded in both the A- and I-bands before the bathing solution was changed back to relaxing solution.

15 potentials in each band were again recorded following 15 min equilibration in relaxing solution. In most cases, it was possible to take the muscle through 2, and sometimes 3, reproducible contraction-relaxation cycles.

Statistics. Since the data followed a normal distribution, Student's t-test was used for comparisons with P < 0.05 showing a significant difference.

RESULTS

Barnacle muscle. The protein charge concentrations in the A- and I-bands of barnacle muscle are given in Table 1. The A- and I-bands showed the same fixed electric charge during the relaxed state, and the same applied during contraction. The negative fixed electric charge of both I- and A-band increased when, following Ca-stimulation, a relaxed muscle went into contraction (P<0.001). This was reversed by transferring the muscle back to the relaxed state. When the muscle, still in contracting solution, had ceased to contract, the fixed electric charge was lower than in the relaxed state (P<0.005).

Table 1 Protein charge concentration in mM in mechanically-skinned barnacle muscle fibres. pCa = 4.5 in the solution inducing the contraction, and the number of fibres measured was 29. 15 measurements were taken in the A- and in the I-bands of each fibre in the relaxed condition, in the contraction solution after the sarcomere shortening has ceased, and in the relaxing solution subsequent to exposure to contraction solution. The total number of readings was included when calculating the average value given in the Table. During sarcomere shortening, n is the total number of readings taken from all 29 measured fibres in the described state; s.d. is given in all cases.

	Relaxed condition	During sarcomere shortening	In contraction solution after the sarcomere shortening has ceased	In relaxing solution subsequent to exposure to contraction solution
A-band	-54 ± 3	-88 ± 12 (n = 127)	-47 ± 8	-54 ± 3
I-band	-54 ± 3	-83 ± 10 (n = 112)	-42 ± 8	-53 ± 3

Table 2 Protein charge concentration in mM in chemically-skinned soleus muscle from rat. pCa in the contraction solution was 4.5. The number of fibre bundles measured was 13. Each bundle consisted of 7-12 fibres with a sarcomere length between 2.8 and 3.2 μm . 15 measurements were taken in the A- and in the I-bands of each fibre bundle in the relaxed condition, in the contraction solution after the sarcomere shortening had ceased, and in relaxing solution subsequent to exposure to contraction solution. The total number of readings is included when calculating the average values in the Table. During sarcomere shortening, n is the total number of readings taken from the 13 fibre bundles; s.d. is given in all cases.

	Relaxed condition shortening	During sarcomere solution after sarcomere shortening has ceased	In contraction subsequent to exposure to contraction solution	In relaxing solution
A-band	-48 ± 4	-77 ± 11 (n = 51)	-30 ± 4	-48 ± 4
I-band	-48 ± 4	-78 ± 15 (n = 51)	-30 ± 4	-48 ± 4

When the contraction solution was exchanged for relaxing solution, the A- and I-band charge returned to the values seen prior to contraction.

Rat muscle. The protein charge concentration for the A- and I-band of rat muscle is given in Table 2.

Rat muscle showed a similar charge pattern to barnacle muscle when changing between relaxation and contraction with $P < 0.001$ when comparing relaxed state with contraction, and $P < 0.025$ when comparing relaxed state with contraction solution after contraction has ceased.

DISCUSSION

We have shown that during contraction, the fixed electric charge on the A- and I-filaments do change over a short time interval, and that it is possible to observe stages in which the charge is in a quasi-steady state. The Donnan theory may therefore be applied in each case. It is apparent that the state with the highest negative charge is an average over a series of peak values, and the actual peak value may be even greater than calculated here.

The rise in negative electric charge in the A-band, when a muscle is transferred from the relaxed to the contracted state, is greater than the rise seen when the muscle changes from relaxed to rigor condition [1, 2]. Since the observed charge change is independent of sarcomere length, and because the microelectrode method used is lacking in time resolution, one can only speculate about the mechanism behind this charge change, and about how it is related to the molecular processes involved in the excitation-contraction coupling, and in the contraction itself. There is also a significant difference between the contraction state and the rigor state in the behaviour of the I-filament charge. In rigor, the I-filament charge is lower than the A-filament charge. During contraction, the I-filament charge is the same as the A-filament charge.

On examining the A-filament charge, it was found earlier that ATP in the absence of Ca^{2+} causes the muscle A-band to be in a state of low negative charge by binding/adsorption to the rod part of the myosin molecule, possibly at a site close to the hinge region [2, 10]. This low-charge state is probably maintaining

the A-filament in readiness to perform a contraction as soon as Ca^{2+} has reached the troponin sites on the I-filaments. Subsequent events, including the attachment of the crossbridge to the I-filament, could cause the conformational or other change that brings the myosin molecule into the high charge state until the contraction is over. A mechanism for producing a higher negative charge would be to produce or activate more 'Saroff sites' [2, 22], where negative ions can be absorbed by the surface of the filaments. This might involve 'melting' of the helical protein structure, as suggested by Harrington [23], but it may also occur in the manner suggested by Bartels & Elliott [2] and Elliott *et al.* [24], with a change in the ion-binding capacity of a part of the myosin molecule.

In experiments with whole myosin in the presence and absence of ATP under low Ca^{2+} conditions [10], the charge measured in the absence of ATP is about 30% higher than in its presence in micromolar to millimolar quantities. We suggest that this is due to the cycling of each myosin molecule through a charge-discharge process, with the discharge caused by the momentary binding of ATP to the active sites on the myosin head, when ATP is present in the solution. In the absence of ATP, the molecule remains in the charged state. If the addition of Ca^{2+} causes the charging process to take place more rapidly, then the measured time-averaged charge would be higher, in keeping with our current A-band observations.

Our results are therefore consistent with the electrical model for the origin of the contractile impulse suggested by Elliott & Worthington [25], where force is generated by the 'snap back' of the myosin head after it has been deformed by the electric fields during the charging process, and when it is then discharged by the binding of ATP to the active site.

Another important question is: how do the charge changes observed here relate to the known metabolic processes? The muscles studied are all skinned by methods that leave the sarcoplasmic reticulum largely intact. Following the skinning, the muscles are left to equilibrate in relaxing solution, and it is assumed that all free Phosphocreatine (PCr) is washed out while the PCr bound to the intercellular structures is left. The ATP-concentration is high at the start of the contraction, and the ATP-breakdown must be the domineering process at this stage. ATP-breakdown is proton generating, but the buffering capacity of the muscle cell ought to be high enough not to show a significant effect of H^+ on the protein charges during the first phase of the contraction. Furthermore, PCr-breakdown is proton absorbing, and at the start of the contraction there must be some breakdown of the bound PCr which counteracts the proton production. During the ATP-breakdown, the inorganic phosphate (P_i^{2-}) concentration will increase rapidly and these anions may absorb to Saroff-sites, as described by Bartels & Elliott [2] and Bartels *et al.* [10], creating a higher negative charge on the filaments. ADP will also increase and start to compete with ATP for the ATP-binding sites. These processes may cause changes of the filaments towards rigor structure. The overall effect of these binding processes would be as seen during contraction in our experiments, an increase in negative electric charge which easily could reach values higher than the rigor value due to the P_i^{2-} binding. As ATP-breakdown continues, acidosis will occur, and H^+ may compete with Ca^{2+} for the Ca-binding sites, as suggested by Allen *et al.* [26]. A pH-decrease is also known to have a strong decreasing effect on the negative electric charge [27]. Furthermore, an increase in Mg^{2+} has been reported during fatigue by Westerblad & Allen [28]. If this also happens in the skinned muscles, Mg^{2+} will bind to

the Saroff sites, and the effect will be an even further decrease in the negative electric charge [27]. The overall picture would be a decrease in the negative electric charge as the contraction reduces and stops, as seen in our experiments. Our results are also in accordance with the experiments by Caillé [29, 30], where the charge was measured in a whole piece of muscle matrix after 30 min equilibration in solution containing ATP and Ca^{2+} , and a drop in negative electric charge was seen in both ATP- and Ca-containing solutions.

However, the question remains: why is the I- and A-band charge concentration the same during the contraction, while it is different in the rigor condition? In rigor and in high ADP concentrations (5 mM), or similar ATP-analogue solutions, the charge concentrations follow the rigor pattern. In ATP and in Pyrophosphate (PP_i) solutions, the relaxed charge concentration pattern is seen [31]. As long as the ATP concentration is sufficiently high to keep the contraction cycling running, the PP_i produced may be absorbed to the filaments and create a higher negative charge. In the I-filament, the binding is likely to be at the low affinity nucleotide site [32] with following changes in the cation binding, if it is absorption of PP_i to the I-filaments which creates the increased negative charge. As the PP_i concentration increases further, the cross-bridge formation becomes weaker [33], and the negative charge decreases [31]. In a PP_i concentration of 5 mM and no ATP present, a relaxed charge pattern is seen, and the charge concentration is about 2/3 of the concentration in the relaxed condition [31]. This agrees well with our present study where the protein charge concentration in contraction solution, after the shortening has finished, is around 2/3 of the charge concentration in relaxing solution.

We may therefore conclude that:

- 1) The A-band charge in a cross-striated muscle during contraction shows a rise in the negative electric charge, compared to the relaxed muscle. This charge change is similar to the change seen between rigor and relaxed muscle, but the contracting muscle has a higher negative charge than the rigor muscle, which may be explained by a further binding of PP_i to the filaments during contraction.
- 2) The I-band charge follows the A-band charge during contraction and relaxation, and the difference from the rigor charge pattern is probably due to ATP or PP_i binding/absorption to the I-filaments during contraction and relaxation.
- 3) The difference between rigor and contracting muscle may be due to a charge change on the myosin head during contraction caused by the different ion-binding to the I-filament in both cases.

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