Journal of Muscle Research and Cell Motility **5**, 657–663 (1984)

Crossbridge order and orientation in resting single glycerinated muscle fibres studied by linear dichroism of bound rhodamine labels

THOMAS P. BURGHARDT, MARK TIDSWELL and JULIAN BOREJDO

Cardiovascular Research Institute, University of California, San Francisco, California 94143, U.S.A.

Received 19 December 1983 and in revised form 24 April 1984

Summary

Linear dichroism of iodoacetyl-rhodamine labels attached to the highly reactive thiol of the myosin heads was measured in order to infer the spatial orientation and the degree of order in myosin crossbridges in single glycerinated rabbit psoas fibres at rest. We have previously shown that in rigor the chromophoric labels are well ordered and that in the presence of MgADP and during isometric contraction a large fraction of probes is also ordered but at an attitude different from that of rigor. Here we show that in relaxed muscle the probe order is dependent on total ionic strength: at and above 0.180 M there is little evidence for any preferred probe orientation, implying a high degree of crossbridge disorder. Below 0.160 M there is progressively more order with decreasing ionic strength down to 0.100 M, below which no measurements could be taken at room temperature (because the fibres would not relax). The dichroism observed under these conditions resembles that of the rigor state in that the dichroism peaks at the same polarization of excitation light, implying that the average probe attitude relative to the fibre axis is larger than 54.7°. Stretching the muscle beyond the point of overlap between actin- and myosin-containing filaments does not affect the ionic strength dependence of the amount of order present in relaxed muscle, suggesting that the observed order is due to ionic interactions of crossbridges with the thick filament surface.

Introduction

The refinement of models of muscle contraction requires the elucidation of the three-dimensional arrangement of the crossbridges in muscle in different physiological states. The development of methods of studying the orientation of the dipolar (fluorescent or paramagnetic) probes embedded in the crossbridge has intensified this interest (Aronson & Morales, 1969; Borejdo & Putnam, 1977; Thomas & Cooke, 1980; Yanagida, 1981; Borejdo *et al.*, 1982; Wilson & Mendelson, 1983). From the outcome of these studies, and also from electron microscopic and X-ray diffraction data (Reedy *et al.*,

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1965; Huxley & Brown, 1967) there emerged a general consensus that in the rigor state the crossbridges all assume a well-defined inclination with respect to the myofibrillar axis, that in the presence of ATP analogues a significant fraction of crossbridges becomes disorganized, and that under relaxing conditions the degree of crossbridge disorder is even greater.

It is in regard to the amount of crossbridge disorder in the relaxed state that ambiguity still remains. Electron paramagnetic resonance (e.p.r.) and our earlier linear dichroism data (Thomas & Cooke, 1980; Borejdo *et al.*, 1982) imply a high degree of crossbridge disorder in resting glycerinated muscle fibres. Recent polarization of fluorescence measurements (Wilson & Mendelson, 1983) suggest that the embedded probes may have a small amount of order. In this work we reinvestigate the question of probe orientation and order in single resting muscle fibres. We find that the degree of probe order is strongly dependent on the ionic strength of the relaxing solution. At sufficiently high, but physiological, ionic strength all order disappears. Lowering the ionic strength introduces progressively more order and is consistent with the ordered probes assuming an orientation similar to that present in rigor muscle. We also find that the ionic strength dependence of the observed order is identical in fibres with and without overlap between thin and thick filaments, strongly suggesting that the order is imposed on the probes through crossbridges having ionic interactions with the thick filament surface.

Methods

Chemicals

ATP and ADP were obtained from Sigma and iodoacetamidotetramethyl rhodamine (IATR) from Research Organics. All other chemicals were of analytical grade.

Solutions

The standard rigor solution contained 80 mM KCl, 5 mM $MgSO_4$, 2 mM EGTA, 5 mM potassium phosphate buffer, pH 7.0, and 1 mM DTT. The relaxing solution had the same composition as the rigor solution except that ATP was added at 4 mM concentration. The ionic strength of bathing solutions was changed by altering the concentration of KCl: it equalled the concentration of KCl plus 58 mM.

Fibre labelling and manipulations

Single fibres were dissected from glycerinated rabbit psoas muscle and labelled at the highly reactive thiol (SH_1) with IATR as described previously (Burghardt *et al.*, 1983). We investigated the effect of the modification of SH_1 with IATR by measuring the active tension in single fibres. The tension was measured as a function of ionic strength and for several ratios of modified to unmodified crossbridges; the labelling ratio was measured by the decrease in EDTA-activated ATPase in the modified fibres. Over the range of ionic strength used in our experiments and for labelling ratios of 0.0–0.30 the force per unit area generated by modified fibres was not significantly different from that generated by unmodified fibres. This observation indicates that the labelled crossbridges retain their native properties under the conditions imposed on them in these experiments (the labelling ratio for fibres used in the relaxation experiments was 0.16).

Single fibres were mounted between two glass rods on a microscope slide. The distance between the rods (and therefore the sarcomere length) could be varied by moving the glass slide to which one of the rods was attached. Sarcomere length was measured by comparison with a calibrated ocular graticule in a microscope under high magnification. Stretched fibres often showed nonhomogeneous sarcomere length distribution along the fibre length. For the measurements at nonoverlap between thick and thin filaments we were careful to collect the data only from fibre segments which had a sarcomere length in excess of 4.0 μm.

Dichroism measurements

Dichroism measurements were performed as described earlier (Borejdo *et al.*, 1982; Burghardt *et al.*, 1983). The results are quantified in terms of a model-independent variable b defined by the equation

$$A = 1 + b\cos^2 \Psi \quad (1)$$

where A is the normalized absorption and Ψ is the angle between the fibre axis and the incident electric field polarization vector. Equation 1 holds for fibres with azimuthal symmetry. Variable b is directly related to moments of the angular distribution function of the probes and, through a model, to parameters such as the probe latitudinal angle θ and the degree of order (Burghardt *et al.*, 1983). In general for a fixed θ, larger absolute value of b means an increase in order.

Results

Fig. 1 shows a typical dichroic curve of relaxed fibres at two selected ionic strengths. Superimposed is the dichroism of a rigor fibre at intermediate ionic strength. Under the

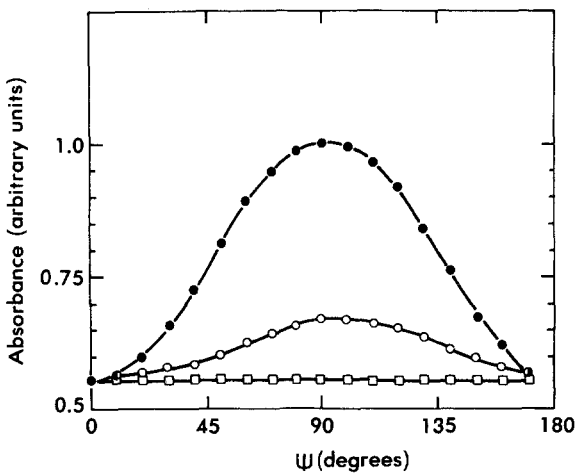

Fig. 1. Comparison of typical absorption curves from muscle fibres in relaxation at different ionic strengths (open symbols) and in rigor (closed circles; μ = 0.138 M). The ionic strength of the relaxed fibre was either 0.258 M (open squares) or 0.078 M (open circles). The fibres were held at rest length. Ψ is the angle between the fibre axis and the direction of polarization of the exciting light. The data were normalized so that the absorbance at 90° in rigor was unity and the absorbance at 0° was equal for all three curves.

assumption that the average probe orientation is constant, the greater concavity of the lower ionic strength curve implies better probe order within the fibre. This degree of order is quantified by the model-independent parameter b. In general, when b retains its algebraic sign in a physiological transition an increase in the absolute value of b indicates an increase in order provided that the probe orientation remains constant. Using Equation 1 the model-independent parameter b was measured to be -0.034 and -0.20 in a relaxed fibre at 0.258 M and 0.078 M, respectively, and -0.44 in a rigor fibre. Assuming the crossbridges in these fibres to be subdivided into two populations, one perfectly oriented at a polar angle θ relative to the fibre axis and the other random, the parameter b can be expressed as a function of the probe angle θ and the fraction of oriented crossbridges h. We have shown (Burghardt *et al.*, 1983) that

$$b = \frac{h[3\cos^2\theta(2\cos^2\eta - \frac{1}{2}) - \frac{1}{2}\cos^2\eta]}{\frac{1}{3} - \frac{1}{3}h[\cos^2\theta(2\cos^2\eta - \frac{1}{2}) - \frac{1}{2}\cos^2\eta]} \quad (2)$$

where η is the cone half angle of the independent motion of the rhodamine group. η has been measured to be 20° (Borejdo *et al.*, 1982). We have also shown previously that for rigor fibres $\theta = 69°$, $h = 0.63$*. Assuming that θ remains unchanged from rigor to relaxation (Wilson & Mendelson, 1983) and using Equation 2, we now find at $\mu = 0.258$ M that $h = 0.042$ and at $\mu = 0.078$ M, $h = 0.27$.

In an alternative model, the crossbridges are assumed to possess an average orientation $\bar{\theta}$ while they are evenly distributed in a cone of half-angle γ. The relationship of b to $\bar{\theta}$ and γ has the form of Equation 2 with the following replacements: $h = 1$, $\theta = \bar{\theta}$, and $\eta = \gamma$. In this model if $\bar{\theta}$ is again assumed to be 69° then at $\mu = 0.258$ M, $\gamma = 56°$ and at $\mu = 0.078$ M, $\gamma = 45°$. Since we know the probe moves independently from the crossbridge these values of γ include the half-angle of the independent probe motion.

Fig. 2 summarizes the effect of ionic strength on the parameter b for relaxed muscle. Lowering the ionic strength has the effect of imposing progressively more order on the crossbridges. The maximum value of the parameter b that we observed in a series of 162 experiments on 18 different muscle fibres was 0.256 at $\mu = 0.098$ M (about half the magnitude of b for rigor muscle). Below this ionic strength the absolute value of b often declined; we explain this finding by noting that muscle fibres in relaxing solution contracted at low ionic strength at room temperature. The failure of striated muscle fibres to relax under these conditions has been observed previously (Brenner *et al.*, 1982; Gordon *et al.*, 1973; Gulati, 1983; Loxdale & Tregear, 1983). Contracting muscle exhibits a large dichroism in the opposite sense to that observed in rigor and relaxed muscle

*In our earlier work (Burghardt *et al.*, 1983) we were able to unambiguously determine the model parameters θ and h because we measured the dichroic curve for both labelled fibres in rigor and unlabelled fibres in rigor that were decorated with labelled S1. In the latter experiment, b is measured from the dichroic curve, $h = 1$ and θ can be determined using Equation 2. Given this θ value (69°) the fraction h is determined for labelled fibres in rigor. The decorated fibre experiment is impossible to perform on relaxed fibres so that ambiguity in θ and h for relaxed fibres remains.

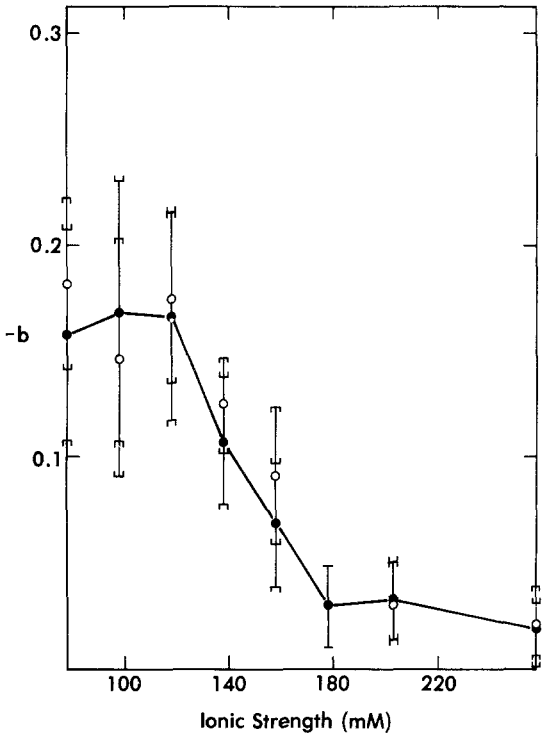

Fig. 2. The effect of ionic strength of relaxing solution on the model-independent parameter b of a single muscle fibre. The closed circles indicate the average b value taken from 18 different muscle fibres at resting length. The open circles indicate the average b value taken from six different muscle fibres that are stretched beyond overlap between the thick and thin filaments. The vertical bars with outward facing brackets (][) indicate the standard deviation for closed circles, the inward facing brackets ([]) indicate the standard deviation for open circles.

(Burghardt *et al.*, 1983), and therefore the contraction of even a few myofibrils within the observed volume will result in a decrease in an absolute value of b. In contrast to the strong dependence of the dichroism on ionic strength exhibited by relaxed muscle (Fig. 2), fibres bathed in rigor or MgADP solution showed no sensitivity to ionic strength (data not shown).

The dependence of the dichroism on ionic strength for a single muscle fibre at rest, at a sarcomere length at which there is no overlap between thin and thick filaments is shown in Fig. 2. In these experiments on six muscle fibres there was no significant difference in the dichroic curves at various ionic strengths from the fully overlapped muscle fibres. Similarly, for fibres with no overlap between thick and thin filaments, there was no difference in dichroism when the fibres were bathed in relaxing, rigor or MgADP-containing solution. This was true for any ionic strength tested within the range indicated in Fig. 2 (data not shown).

Discussion

The order in chromophoric probes observed at low ionic strength in resting muscle fibres can be imposed on the crossbridges to which the probes are attached by either thin or thick filaments. Brenner *et al.* (1982) have obtained evidence for crossbridge attachment to actin in relaxed muscle at low ionic strength and at low temperature (5° C). However, the fact that the relaxed fibres stretched beyond the point of overlap between thick and thin filaments have the same dichroism (at any ionic strength) as unstretched fibres is a powerful argument in favour of the idea that the order is imposed on the crossbridges by interactions with the thick filaments. Consistent with this idea is our finding that in nonoverlap fibres at any given ionic strength, dichroism was not dependent on the type of the bathing medium (relaxing, rigor or MgADP-containing). A similar observation was made earlier by Nihei *et al.* (1974). Our conclusions do not preclude the idea of interactions between crossbridges and actin at low ionic strength, but suggest that such interactions do not contribute to crossbridge order.

In contrast to our results, in their measurement of polarization of fluorescence from relaxed fibres, Wilson & Mendelson (1983), working at a fixed ionic strength of 0.15 M, observed a difference in $P_\perp$ for fibres at short and long (3.5 μm) sarcomere lengths. However, their interpretation of these data (see their Table 2) that this difference in P_1 translates into a small difference in probe randomness is consistent with our observation of no measurable difference in the state of the crossbridge in relaxed fibres at full overlap and at no overlap.

The molecular mechanism by which myosin heads interact with the thick filaments to give order at low ionic strength can only be conjectured upon at this time. Because the high ionic strength abolishes the order, ionic interactions between charged regions of the myosin rod and the S1 are the most likely possibility. The physiological significance of our observation is equally unclear at present: extrapolating to the 'physiological' ionic strength in muscle cells (0.2 M) our results suggest little crossbridge order under these conditions. At the same time X-ray diffraction from live resting frog muscles shows a clear meridional reflection at 143 Å and an off-meridional reflection at 429 Å. Because these reflections may be interpreted as reporting crossbridge periodicity along the fibre axis (Huxley & Brown, 1967; Poulsen & Lowy, 1983), the absence of any chromophoric probe order as reported by linear dichroism is not necessarily a conflicting observation.

Acknowledgements

We thank Professor M. F. Morales for helpful suggestions and Ms Carroll Flynn for technical assistance. T.P.B. is a postdoctoral fellow and J.B. is an Established Investigator of the American Heart Association. M.T. was supported by the N.L. Tartar Research Fund. This research was funded by USPHS grant HL-16683, NSF grant PCM-7922174, an American Heart Association Grant-In-Aid 81-612 and a grant from the Muscular Dystrophy Association.

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