Studies of the diffuse x-ray scattering from contracting frog skeletal muscles

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ABSTRACT Using x-rays from synchrotron radiation, we studied diffuse scattering, sometimes together with the myosin layer lines. With an area detector, sartorius muscles and a time resolution of 150 ms, earlier results from semitendinosus muscles contracting isometrically at 6°C (Lowy, J., and F. R. Poulsen. 1987. *J. Mol. Biol.* 194:595–600) were confirmed and extended. Evidence from intensity changes both in the diffuse scattering and in the myosin layer lines showed that the majority of the heads become disordered at peak tetanic tension. With a linear detector and a time resolution of 5 ms, it was found that during tension rise the intensity increase of the diffuse scattering (which amounted maximally to 12% recorded near the meridian) runs ~20 ms ahead of the mechanical change, comparing half-completion times. This suggests that an appreciable number of heads change orientation before peak tension is reached. In quick release experiments the diffuse scattering intensity showed very little change. Recorded near the meridian during rapid shortening, however, it decreased progressively with a half-time of ~40 ms. This change amounted to ~35% of that observed during the initial tension rise. We interpret this to indicate that during rapid shortening a certain number of heads assume an orientation characteristic of the relaxed state. Viewed in the context of the behavior of the first myosin layer line and the (1, 1) equatorial reflection in similar experiments (Huxley, H. E., M. Kress, A. R. Faruqi, and R. M. Simmons. 1988. Molecular Mechanism of Muscle Contraction), the present results provide further support for the view that the diffuse scattering is mostly due to disordered myosin heads; whilst ordered heads produce the myosin layer lines (Poulsen, F. R., and J. Lowy. 1983. *Nature [Lond.]*. 303:146–152).

INTRODUCTION

Traditionally, x-rays have been applied to structural analysis of biological systems in two very different ways. The crystallographic diffraction method is used to study crystal structures which have a high degree of static long-range order in the arrangement of the molecules. Here there is diffuse scattering which is overlain by sharp peaks (Bragg scattering). The other method is used to investigate molecules in solution. In this case there is no, or very little, order in the arrangement of the molecules. Here the pattern shows only diffuse scattering and there are no sharp peaks. Both methods have been extremely successful, the first for the solution of molecular structures at the atomic level, and the second for the determination of the size and shape of macromolecules and their changes.

Our initial studies (Lowy and Poulsen, 1982, 1987) indicated that the myosin filaments in resting and contracting frog skeletal muscles comprise features both of crystals and of molecules in solution, in that the patterns showed sharp peaks as well as substantial diffuse scattering. Both features contain information about the arrangement of the myosin heads. However, during contraction the intensity of the myosin structure peaks is reduced by as much as 80%, and the remaining 20% is considered to be due to heads that have maintained their resting configuration (Huxley et al., 1982). Thus on the face of it the myosin peaks in active frog muscles seem to contain rather little information about the behavior of active heads. This fact and our finding that diffuse scattering is present in both resting and contracting muscles suggested to us that its study might be worthwhile. Although our data (Poulsen and Lowy, 1983; Poulsen et al., 1987) indicate that much of the diffuse scattering is indeed due to myosin heads the exact proportion has yet to be established.

As regards resting muscles (Poulsen and Lowy, 1983), investigation of both the diffuse scattering and of the myosin layer lines indicated that the myosin heads are ordered to varying extents. We interpreted our data as follows. The more ordered heads are close to the thick filament backbone where they are organized in a near-helical arrangement which mainly produces the myosin layer lines. In this case the movements of the head mass-centers from their equilibrium positions will be relatively small. The less ordered heads are further away from the backbone, have very large movements (r.m.s. of at least 9 nm), and are thus responsible for the displacement disorder which accounts for the major part of the diffuse scattering. The more ordered heads will also contribute to the diffuse scattering due to substitution

disorder which is produced because heads away from the backbone will leave "holes" in the helical lattice. But the possible effect of interference between ordered and disordered heads on a filament remains to be investigated. The fact that the distinctly elongated myosin heads give rise to diffuse scattering which is nearly isotropic means that the distribution of orientations including all heads must be very wide, i.e., they must have almost random orientation. Poulsen et al. (1987) discuss in detail the disorder that can exist in the backbone and in the arrangement of the myosin heads.

Supporting evidence for the two population model of heads comes from electron microscope observations of negatively-stained myosin filaments which showed two configurations of heads: ordered ones lying near the backbone and disordered ones far out from it (Knight and Trinick, 1984). However, without further analysis of the x-ray scattering data it is not possible to define the nature of the variation in the order of the heads. At this stage we will therefore simply speak of two populations of heads, one that is thought of as highly ordered, the other as practically totally disordered. We realize of course that this constitutes a very simplified way of formulating the system.

In contracting muscle one imagines that more heads will be away from the backbone. Here the diffuse scattering may include contributions from resting heads, from cycling heads in the detached state, and from attached ones which themselves are substantially disordered due to their interaction with actin over a wide range of angles. In spite of these complications we decided to study the diffuse scattering in contracting muscles for two main reasons. First, it is an important feature of the disordered head population that it is still very much in evidence during contraction, while the ordered one practically disappears (as shown by the loss of the myosin layer lines [Huxley et al., 1982]). Second, in unstriated muscles like the anterior byssus retractor of *Mytilus* (ABRM), and the *Taenia coli* of the guinea pig (TCGP) no signs can be detected of an ordered population even in the resting state, i.e., there is large diffuse scattering but no myosin layer lines (Lowy et al., 1970; Lowy and Vibert, 1972).

In designing experiments with contracting frog muscles we anticipated that additional knowledge would come from comparing the configuration of the disordered heads at rest with that of the heads during contraction. The latter appear themselves to be very disordered, as demonstrated not only by the disappearance of the myosin layer lines but also by the nonappearance of any rigorlike layer lines (Huxley et al., 1983), or for that matter of any other kind of new ordered pattern. Our results (Lowy and Poulsen, 1987) showed that during isometric contraction the diffuse scattering elongates meridionally, and this has been confirmed by Amemiya et al. (1987). Our interpretation was that active heads rotate to an average orientation more perpendicular to the long axis. This is in line with Haselgrove's (1980) explanation for the increase in the intensity of the 14.3-nm meridional reflection, and that put forward by Irving (1984) to account for the birefringence changes he observed in isometrically contracting single frog fibers. An alternative interpretation in

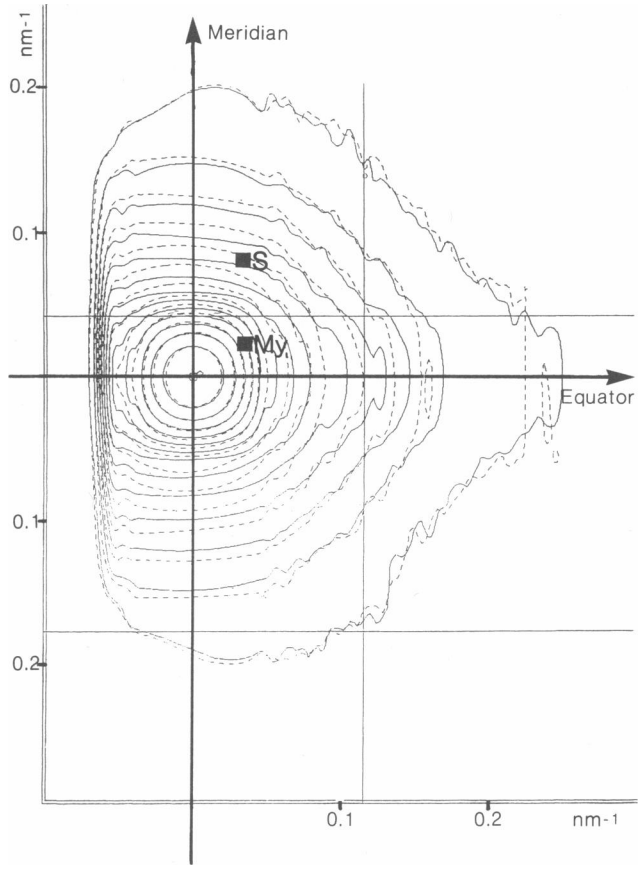

FIGURE 1 Contour lines of the diffuse scattering intensity recorded from isometrically contracting sartorius muscles, using an area detector and a 2.4 m camera. The intensity is shown on a logarithmic scale to give an even distribution of the lines. The sharp actin and myosin reflections have been removed as described previously (Lowy and Poulsen, 1987). X-Ray quanta were collected in seven 150-ms time frames. Here we show only diffuse scattering data recorded at rest (——), and at peak tension (----). Data were accumulated from 50 successive tetani. The results confirm those obtained in similar experiments with semitendinosus muscles (Lowy and Poulsen, 1987). The center of the camera tube was moved sideways from the beam so that the diffuse scattering could be recorded as far out as possible in the equatorial direction where the most pronounced deviation from circular symmetry was seen in the resting muscles. Note that in the transition from rest to peak tension the shape of the diffuse scattering becomes more circularly symmetrical, that is, the intensity increases in the meridional direction and decreases in the equatorial direction. (In another set of experiments illustrated in Fig. 2, a linear detector was placed at location *S* to record the diffuse scattering and at location *My* to record the 42.9-nm myosin layer line.)

terms of increased r.m.s. displacements in myosin (isotropic or anisotropic) cannot be tested using our data, as we showed that, given a r.m.s. displacement of 9 nm, any further increase will cause a change in the diffuse scattering inside the region of the backstop, which is not observable (Poulsen and Lowy, 1983).

Several other important problems remain unsolved. Thus, on their own, the scattering data from experiments with contracting muscles do not provide direct information about the relative contribution to the diffuse scattering from detached and attached heads, or about the strength and duration of attachment, or about the problem of the presence of one or more orientations (assuming the model of two head populations). If the heads do in fact rotate in isometric contraction, the data do not indicate whether the whole or only part of the head is involved. On the same model, the interpretation of the diffuse scattering intensity changes themselves is a fairly complicated matter. This is because they may involve a change in the number of disordered heads, or a change in their average orientation, or a change in their size or shape, or a combination of all four. For example, isometric contraction experiments (Lowy and Poulsen, 1987) where an area detector was used to investigate events during tension rise, gave results about the diffuse scattering concerning changes in intensity and overall shape as seen in intensity contour plots. Hence we deduced that tension rise was associated with changes in both the number and average orientation of the disordered heads. But from that data we could not determine possible contributions produced by changes in the size or shape of the heads, or due to variations in the distribution of head orientations.

In the investigations of the diffuse scattering to be described here we improved the time resolution in the experiments where an area detector was used. We also extended the scope of the work by recording events with a linear detector not only in isometric tetani but also during imposed mechanical transients. These results were obtained with a time resolution of 5 ms and gave new and useful information about the diffuse scattering, particulary in relation to the behavior of the first myosin layer line.

METHODS

X-Ray data acquisition

Two sets of experiments were carried out on the double focusing monochromator–mirror camera of the European Molecular Biology Laboratory (EMBL) Outstation on the storage ring DORIS of the Deutsches Elektronen Synchrotron (DESY) in Hamburg (Koch and Bordas, 1983), using the standard data acquisition and evaluation systems (Boulin et al., 1986, 1988).

In one set of experiments we used methods described previously (Lowy and Poulsen, 1987) to record the pattern with an area detector and an improved time resolution of 150 ms. In the second set, using the methods of Huxley et al. (1983), the time resolution was 5 ms and the pattern was recorded with a linear position-sensitive multiwire detector (Hendrix et al., 1982).

Sartorius muscles from the frog *Rana ridibunda* (set at a sarcomere length of 2.3 μm) were mounted vertically in oxygenated Ringer at 6°C and attached to a transducer to record isometric contractions developed in response to tetanic stimulation. Intervals of 3 min were allowed between successive tetani to ensure that the muscle did not fatigue.

At the end of the experiment the muscle was removed from the chamber and the total background scatter was recorded for 50 or 75 s in recordings with the area and linear detectors, respectively. In experiments with the area detector, the pattern from the resting muscle was recorded for 1 min before the start and after the completion of the contraction series.

Results during isometric tetanic contractions came from experiments with 11 sartorius muscles where the pattern was recorded in seven time frames of 150 ms each. Only the changes in the transition from rest (frame 2) to peak tension (frame 4) are dealt with below.

Data treatment methods used to obtain intensity contour plots were described previously (Lowy and Poulsen, 1987). Only data outside an axial location of 0.025 nm^{-1} on either side of the equator were used, because in that part of the pattern (to be called the exclusion zone) there are contributions from the backbones of the actin and myosin filaments, from the zero-order layer lines due to the helical arrangement of actin and myosin molecules, and from the S-2 rods (see discussion in Poulsen et al., 1987).

At this point we wish to explain that by the 'shape' of the diffuse scattering we mean its form as it appears in intensity contour plots, for example in Fig. 1.

Results using the linear detector were obtained from 11 sartorius muscles in isometric tetani and also when subjected to transient length changes as described by Huxley et al. (1983).

The muscle was attached to a Ling Dynamic 201 moving coil vibrator and stimulated for 600 ms. After peak tension was reached the muscle was released by ~2.5% (within 1 ms) so that tension dropped to ~1/10 of the maximal value (*Po*). In the subsequent 80 ms, the muscle was allowed to shorten by ~5–10%. This corresponds to shortening velocities of 0.625–1.25 lengths/s. During this period the tension shows a small transient increase but remains at a very low value (Fig. 2). After the end of the shortening the tension redevelops to a value corresponding to the shorter muscle length.

Using a 10-mm slit width, the linear detector was placed parallel to the meridian at three different locations in the pattern where results with the area detector had shown significant diffuse scattering intensity changes during isometric contraction (Fig. 3 *a* and legend to Fig. 3 *b*). We also studied the behavior of the 42.9-nm myosin layer line which was recorded at the location *My* indicated in Fig. 1.

The x-ray shutter was opened for 1.5 s during each contraction. The beam at the specimen was ~1 mm high and 4 mm wide, and thus the whole of the beam cross-section was covered by the muscle. The pattern was collected during 256 successive time frames each of 5 ms duration. These were synchronized with the stimulation of the muscle and with any length changes that were applied to it. Recordings of the changes in the pattern were repeated until a satisfactory number of counts had been accumulated. This usually required 50 tetani.

Results from all the 11 muscles showed similar diffuse scattering changes. The largest changes occurred in muscles where peak tension, and recovery of tension after a quick release, remained consistently high throughout the 50 tetani contraction series. Results from the best four muscles as judged by these criteria are presented in Figs. 2 and 3 *b*. The data in these two figures were produced by a total of 200 contractions for each detector position. After background subtraction and alignment the data were averaged and normalized to the total number of counts recorded in the three above mentioned positions of the detector. The

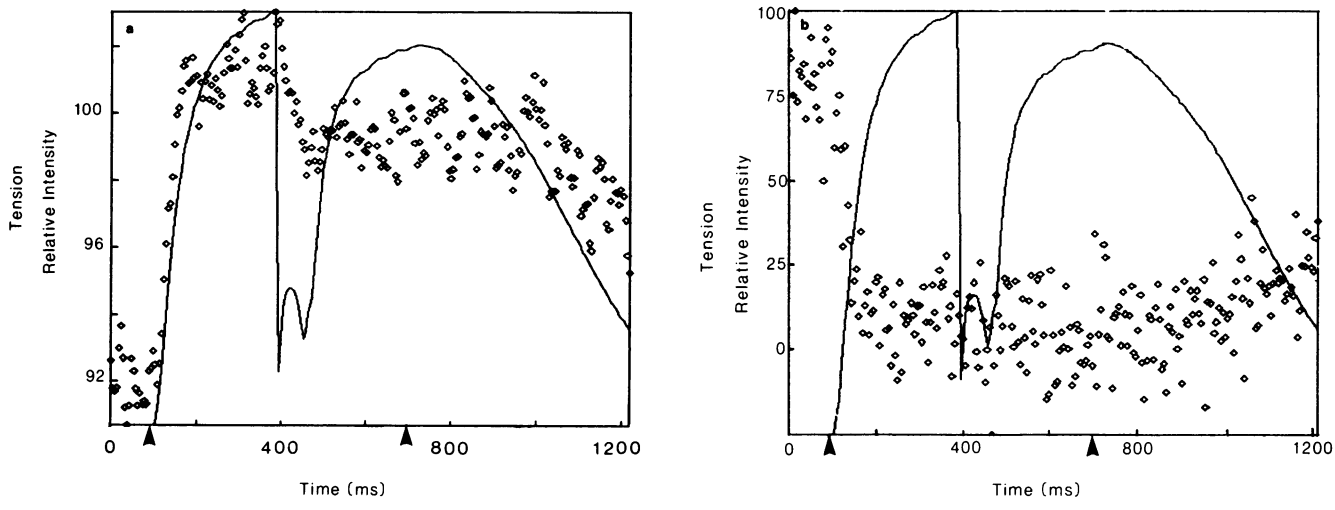

FIGURE 2 Time course of tension (——) and intensity of the diffuse scattering or of the 42.9-nm myosin layer line (°°°°). A linear detector was used to record the pattern with a time resolution of 5 ms. The period of tetanic stimulation is indicated by arrows. Tension values on the ordinate range from 0 to 100% within the limits of both diagrams. At peak tension (*Po*) the muscle was released by 2.5% (in 1 ms) to about *Po*/10; this was followed by a second release of 5–10% (in 80 ms). During the second release the tension shows a small transient increase but remains at a very low value. The divisions on the ordinate refer to the intensity of the diffuse scattering which was normalized to the total intensity collected all along the length of the detector at the three different lateral positions shown in Fig. 3 *b*. (*a*) The intensity of the diffuse scattering was recorded at the location marked *S* in Fig. 1. (*i*) Transition from rest to peak tension. As expected from the area detector pattern at that location the scattering intensity increases. In addition, the linear detector recording reveals that the increase reaches its half-completion time ~20 ms ahead of half-peak tension. (*ii*) Quick release. No changes can be detected. (*iii*) Rapid shortening. Although the shortening velocity is already maximal at the beginning, the decrease that takes place in the scattering intensity is not instantaneous but develops progressively with a half-time of ~40 ms. (*b*) The intensity of the 42.9-nm myosin layer line was recorded at the location marked *My* in Fig. 1. (*i*) The intensity *decrease* during tension rise is much faster than the intensity *increase* in the diffuse scattering. (*ii*) Quick release. No changes can be detected. (*iii*) Rapid shortening. Very little change is seen.

intensity of the diffuse scattering was taken as the average of three channels in each minimum between the myosin layer lines.

It is unlikely that the diffuse scattering change during the slow shortening (Fig. 2 *a*) could arise from an increase of muscle mass in the beam (due to muscle movement) because at that time the 42.9-nm myosin layer line shows very little change (Fig. 2 *b*).

Some measure for the significance of the intensity changes in the diffuse scattering was provided by taking as the base line the value at rest where no such changes are expected. Measurements from Fig. 2 show that at rest the average variation is ~2.5% peak-to-peak, whereas during tension production the maximal diffuse scattering intensity changes recorded near the meridian amount to ~12%.

RESULTS

1. Scattering changes in the transition from rest to peak tension

To ensure that the muscles we used behaved like those studied in similar experiments by Huxley et al. (1982, 1983), we looked at the layer lines in isometric contractions, as well as during transient mechanical changes. The latter are described in sections 4 and 5.

Regarding the myosin reflections and the 5.9-nm actin layer line, we confirmed that in the transition from rest to peak tension the 42.9-nm myosin layer line usually loses ~80% of its resting intensity (over 90% in some experiments, see Fig. 2 *b*); that the intensity of the 14.3-nm meridional myosin reflection increases as does that of the 5.9-nm actin layer line; that the intensity of the 21.5-nm meridional myosin reflection diminishes substantially; and that there are no signs of rigorlike layer lines.

When we recorded the diffuse scattering from isometrically contracting sartorius muscles with an area detector, we obtained results (Fig. 1) similar to those in our previous experiments with semitendinosus muscles (Lowy and Poulsen, 1987): the asymmetric shape of the diffuse scattering seen in the resting state becomes more circularly symmetrical in that its intensity increases in the meridional direction and decreases in the equatorial direction.

The present results also confirm out previous observation (Lowy and Poulsen, 1987) that in the transition from rest to peak tension the total number of counts changes by <2%. This means that the sum of the intensities of the diffuse scattering and myosin layer lines remains practically constant, assuming that the contributions from these two components are of a similar order of magnitude and that no changes occur elsewhere in the pattern.

Recording the diffuse scattering with a linear detector at the locations indicated in Fig. 3 *a* provided further

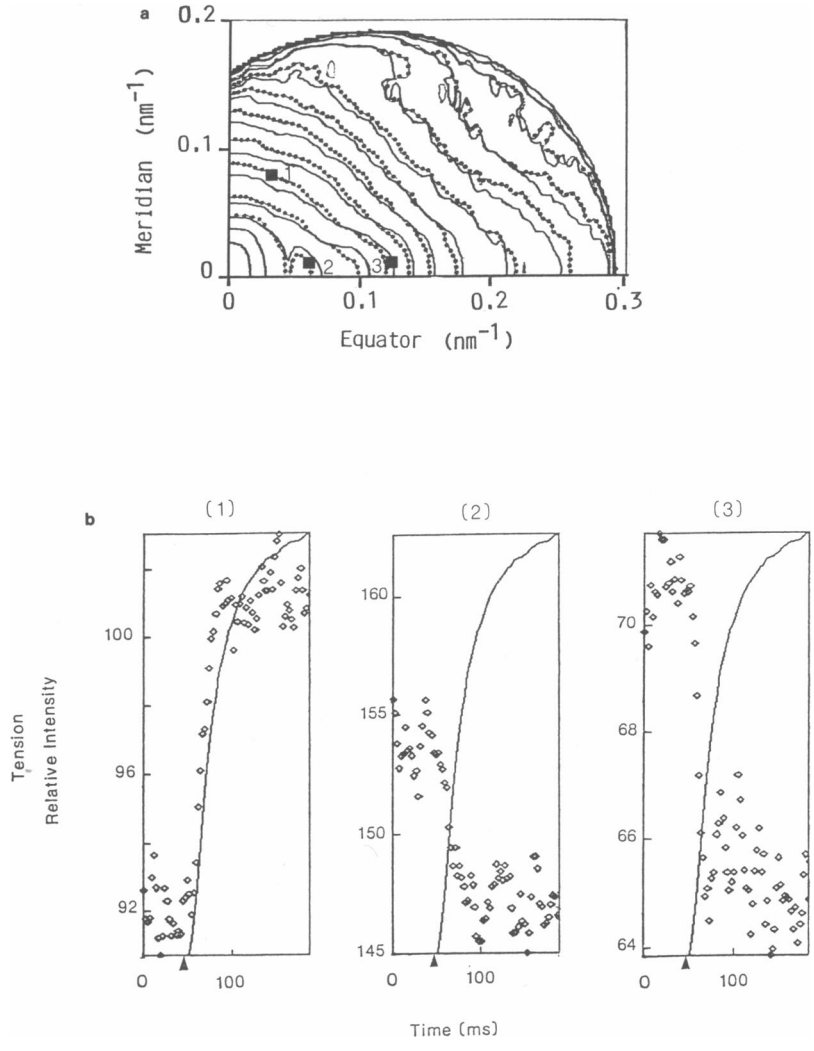

FIGURE 3 (*a*) Area detector pattern recorded in isometric contractions showing changes similar to those seen in Fig. 1. (*b*) Time course of tension (——) and intensity of diffuse scattering (°°°°). The diffuse scattering was recorded with a linear detector at the locations marked *1*, *2*, and *3* in *a*. These were at 12, 24, and 48 mm from the meridian and in reciprocal space correspond to lateral positions 0.03347, 0.06694, and 0.13387 nm^{-1}, respectively. As expected from the area detector pattern in *a*, the scattering intensity increases at *1*, and decreases at *2* and *3*. The time course of the scattering changes is the same at all three locations. For further details see text. Tension values on the ordinate range from 0 to 100% within the limits of all three diagrams.

confirmation of the area detector results in that the scattering intensity showed an increase in the meridional direction (Fig. 3 *b*, at 1), and a decrease some way out along the equator (Fig. 3 *b* at 2 and 3). To establish that these intensity changes are representative of what happens in the entire reciprocal space, recordings would have to be made at many more locations.

2. Correction of a previous result

Lowy and Poulsen (1987) reported that, in isometric experiments, the shape of the diffuse scattering is practically circularly symmetrical at rest, becomes asymmetric at peak tension, and remains (slightly less) asymmetric for at least 3 min after a tetanic contraction (a period we called the 'relaxation phase'). Further experiments with isometrically contracting muscles have confirmed that the shape of the scatter does indeed always change in the same way from rest to peak tension (Fig. 1). But contrary to our previous observation we now find that the shape of the diffuse scattering in the relaxation phase does not differ from that seen at rest. The explanation for this discrepancy is as follows.

In our previous work (Lowy and Poulsen, 1987) the resting patterns that produced nearly circularly symmetrical contour plots came from auxotonic contraction

experiments where the muscle shortens against an increasing load. The majority of these patterns showed an artifact in that the intensity on one side was stronger than on the other. We therefore devised a method to correct for this. In the corrected patterns the diffuse scattering was found to be nearly circularly symmetrical, and thus closely resembled the shape seen in our earlier work with resting muscles (Poulsen and Lowy, 1983). We therefore assumed that the result of a circular shape from the auxotonic patterns would also be valid for the resting muscles used in the isometric contraction experiments. However, data from resting muscles recorded in a more recent series of isometric and auxotonic contraction experiments gave contour plots that were clearly asymmetrical in that they showed some extra intensity spreading from the equator (Fig. 1). Accordingly, we examined the few resting patterns from the 1987 auxotonic experiments where the intensity artifact was not present; it turned out that these had the same kind of asymmetry as the more recent ones. It is therefore obvious that our method for correcting the resting patterns in the 1987 auxotonic contraction experiments was not adequate and this means that the nearly circularly symmetrical contour plots in Fig. 4 *b* of Lowy and Poulsen (1987) are in error.

In the light of these results we reexamined our 1987 patterns from the isometric contraction experiments and found that the shape of the diffuse scattering in the resting muscles is asymmetric and identical with that seen in the relaxation phase. Therefore, during that phase, the heads return to their resting configuration and do not maintain a different orientation as we claimed in our 1987 paper.

The more recent experiments with auxotonically contracting muscles confirm out 1987 observation that there is very little difference in the shape of the diffuse scattering as seen in resting and shortening muscles.

3. Tension rise

Fig. 2 *a* reveals that the meridional intensity increase of the diffuse scattering starts at about the same time as tension rise, and reaches its half-completion time ~20 ms ahead of half-peak tension. The intensity decrease of the 42.9-nm reflection also runs ahead of tension (Huxley et al., 1982) but its lead is much greater than that of the diffuse scattering (compare Fig. 2, *a* and *b*). The fact that the 42.9-nm reflection falls quicker than the diffuse scattering rises does not imply that the total intensity is not conserved. It is likely that in other parts of the pattern where we did not make recordings with the linear detector (for example on the equator and the 14.3-nm meridional reflection), rapid increases occur in the Bragg scattering which cancel out the observed difference between the 42.9-nm reflection and the diffuse scattering.

Inspection of Fig. 3 *b* demonstrates that the decrease in the diffuse scattering intensity recorded away from the meridian and further out along the equator has the same time course as that of the intensity decrease in the meridional direction. The extent of the decrease cannot be evaluated from these patterns as the recordings were made within the exclusion zone.

4. Quick release

No changes can be detected in the intensity of the diffuse scattering (Fig. 2 *a*) nor in that of the 42.9-nm reflection (Fig. 2 *b*).

5. Rapid shortening after a quick release

During such shortening at about $Po/10$, the diffuse scattering decreases progressively. This change is in the direction toward the relaxed level, has a half-time of ~40 ms, and amounts to ~35% of that observed during the initial tension rise (Fig. 2 *a*). The intensity remains at the diminished level until tension has decayed to about half-maximum and then returns very slowly to its resting level.

Very little change can be detected in the intensity of the 42.9-nm myosin layer line (Fig. 2 *b*).

DISCUSSION

In what follows we take the presence of two myosin head populations as a working hypothesis. We deal first with the somewhat unexpected absence of a change in the diffuse scattering during a quick release. To discuss this, certain results regarding the layer lines have to be considered. Huxley and Brown (1967) found that in rigor at maximal overlap the myosin layer lines practically disappear and are replaced by a new set that looks somewhat weaker and more diffuse, and has a repeat that corresponds to the pitch of the actin helix. Their interpretation was that most myosin heads have attached to actin at a fixed angle, thus generating the characteristic 'decorated actin layer lines'. The rigor configuration of the heads was assumed to correspond to the end of the working stroke. However, in isometrically contracting muscles, Huxley and Brown (1967) failed to detect any sign of decorated actin layer lines. This was somewhat unexpected on the assumption that during peak tension an appreciable number of heads might be attached to actin at the same angle

as in rigor. Huxley and Brown (1967) proposed that the active heads would attach over a wide range of angles as a consequence of the asynchronous functioning of the heads that occurs along a given stretch of overlap in order to generate a steady sliding force. Moreover, the number of heads attached to actin at any given time during isometric contraction would be much smaller than in rigor. Discussing the same problem, Huxley et al. (1983) pointed out that, taking the first actin layer line, the increase in intensity seems to be <5–10% of that recorded in rigor muscles. This corresponds to no more than 20–30% of heads attached in rigor, where 95–100% are believed to be attached (Thomas and Cooke, 1980). As there are great technical difficulties in recording so small an increase in the actin layer lines during isometric contraction, Huxley et al. (1983) took advantage of the much shorter exposure times that can be achieved with synchrotron sources and position-sensitive detectors to reinvestigate the problem. An isometrically contracting muscle was released very rapidly (within 1 ms) by an amount corresponding to the presumed working stroke (~12 nm), the idea being that this would change the configuration of the heads without detaching them from actin. As pointed out above, during the isometric force-generating state the heads are supposed to have a wide range of angular configurations due to their asynchronous action. The quick-release maneuver was designed to bring them all momentarily to the end of their working stroke, that is into the same fixed angle rigor configuration. This should have produced a glimpse of the rigor pattern long enough to be recorded by the methods used. In the event, no sign of such a pattern was detected by Huxley et al. (1983). Thus it would appear either that the rigor configuration in the active cross-bridge cycle has an extremely short life time, or that it may not in fact be part of that cycle.

The results from the diffuse scattering experiments indicate that on average the myosin heads adopt a preferred orientation during isometric contraction (Lowy and Poulson, 1987). Thus the quick-release maneuver would be expected to upset or change this orientation rather than to create a new one from the wide range of orientations. Our failure to detect any sign of a change is puzzling, but this cannot be taken as an unequivocal result until the experiments have been repeated with a better time resolution.

We now turn to a consideration of events during rapid shortening at about $Po/10$ where the intensity of the diffuse scattering recorded near the meridian decreased (Fig. 2 *a*). This could mean that some heads assume an orientation more parallel to the long axis, i.e., an orientation characteristic of the resting state. It remains to be shown whether these heads (as they move from actin towards the backbone of the myosin filament) still cycle or have somehow become deactivated by the preceding two shortening maneuvers. In similar shortening experiments the intensity of the (1, 1) equatorial reflection and the spacing of the 14.3-nm meridional myosin reflection were found to return progressively (over 20–30 ms) to about half their resting value whereas, in contrast, the intensity of the 14.3-nm reflection decreased very rapidly at the beginning of the shortening (Huxley et al., 1988). Of course, the equatorial data can also be interpreted in terms of a movement of heads from actin toward the thick filament backbone.

To establish that the decrease in the intensity of the diffuse scattering during rapid shortening (Fig. 2 *a*) is representative of what happens in the entire reciprocal space, recordings would have to be made at many more locations. On the present data, one would expect the fall in the diffuse scattering to be accompanied by some increase in the intensity of the 42.9-nm myosin layer line: but our patterns recorded at 6°C show very little evidence of such a change. Regarding the 42.9-nm reflection, Huxley et al. (1988) obtained the same result in similar shortening experiments at 7°C, but at 15°C found a significant increase in the intensity of that layer line. This led Huxley et al. (1988) to suggest that the return to the relaxed configuration is faster at the higher temperature. It is possible that some clues could come from experiments where the diffuse scattering is also recorded at different temperatures.

In conclusion, we wish to discuss the origin of the diffuse scattering. Previous evidence that an appreciable amount is due to myosin heads was obtained from studies of resting and rigor muscles (Poulsen and Lowy, 1983), observations on synthetic myosin filaments (Poulsen et al., 1987), and recordings of shape changes in the diffuse scattering during the transition from rest to peak isometric contraction (Lowy and Poulsen, 1987). Our new evidence comes from comparing certain aspects of the behavior of the diffuse scattering and of the first myosin layer line (Fig. 2, *a* and *b*).

They behave similarly in that no intensity change can be detected during a quick release. (For the myosin reflection this was first demonstrated by Huxley et al., 1982, 1983.)

They also behave similarly in that both have a substantial lead over tension rise. In the case of the diffuse scattering this suggests that the relation between tension and the associated reorientation of the myosin heads may be far from linear; that is, even at a small tension level an appreciable amount of reorientation of the heads might take place. Another feature evident from Fig. 2 is that the first myosin layer line has a much greater lead over tension than the diffuse scattering. This could be because the layer line is affected by an early breakdown of

interfilament order which we believe does not influence the diffuse scattering in the regions of the pattern we have studied. (Changes in the interfilament order could affect the intensity distribution on the equator and this was one of the reasons why most of that region was excluded from the analysis of the data.)

Our hypothesis of two myosin head populations in frog skeletal muscles (Poulsen and Lowy, 1983) has received support from experiments using a wide variety of other techniques. Many of these investigations were reviewed by Offer (1987), who has spoken of ordered 'heads-in' and disordered 'heads-out' configurations. Taken together, all the available data are consistent with the idea that the myosin heads exist in a dynamic transition between these two configurations. The arrangement of the heads is affected by the degree of overlap with the thin filaments, pH, temperature, ionic strength, divalent cation concentration, phosphorylation of light chains, and nucleotide binding. Evidently the arrangement of the heads is quite labile, as it can be altered by relatively minor changes in the environment.

The relative proportion of heads in the two configurations seems to vary considerably from one type of muscle to another. Judged by the intensities of the diffuse scattering and myosin layer lines, one extreme is represented by the apparently complete absence of the latter in unstriated muscles like the ABRM and TCGP; the diffuse scattering in such muscles is very strong indeed. There is evidence for the presence of some ordered heads in a muscle whose structure closely resembles that of the ABRM namely, the pedal retractor of *Mytilus* where Castellani et al. (1983) recorded weak myosin layer lines. These reflections are also weak in mammalian heart muscle (Matsubara and Millman, 1974), but considerably stronger in frog skeletal muscle (Huxley and Brown, 1967). In all three of these cases the diffuse scattering is moderately strong. On the other hand, myosin layer lines are dominant and the diffuse scattering is very weak in fish muscle (Harford and Squire, 1986) which may therefore be placed at the other end of the spectrum.

We wish to dedicate this paper to the memory of Marcus Kress. Our thanks are due to Drs. H. E. Huxley, M. H. J. Koch, R. M. Simmons, and A. R. Faruqi for their enthusiastic help with the experimental work. We are also most grateful to Dr. Murray Stewart for his constructive criticisms of the manuscript, and to Prof. G. F. Elliott, Mr. C. R. Staddon, and Ms. Meryl Prestidge for many useful discussions.

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