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Molecular movements in contracting muscle: towards "muscle – the movie"

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Abstract

The recent publication of the crystal structures of G-actin and of myosin subfragment-1, together with analysis of a time-resolved series of well sampled low-angle 2D X-ray diffraction patterns from bony fish muscle permits the study of the molecular movements in muscle that are associated with generation and regulation of contractile force. Here it is shown that even though low-angle (i.e. low resolution) X-ray diffraction patterns are being used, these patterns are sensitive, for example, to sub-domain movements of as little as 3 Å or 4° within the actin monomers of actin filaments. Actin filament diffraction patterns from whole muscle are being used to define actin domain and tropomyosin movements involved in regulation. Myosin and actin filament diffraction patterns are being used together to start to show how the complete "quasi-crystalline" unit cell in the bony fish muscle A-band can be modelled as a series of time-slices through a typical tetanic contraction of the muscle. In this way, the time sequence of images can be used to create "muscle – the movie".

Key words: Actin filament structure; Regulation; Tropomyosin shift; Myosin cross-bridge mechanism; Time-resolved X-ray diffraction

1. Introduction

One of the driving ambitions of structuralists studying muscle contraction is to be able to follow the molecular movements involved both in force generation and in the regulation of activity. Two main approaches to this are being used; electron microscopy of muscle rapidly frozen at particular stages of the contractile cycle [1–5] and time-resolved X-ray diffraction from contracting muscle [6–8]. Electron microscopy has the great advantage that direct images of the tissue are obtained. However, there are disadvantages in that (i) freeze-fixation of a single specimen can obviously only trap one stage of the cycle and a whole series of such images from different specimens is needed to produce a picture of what is happening, and (ii) electron microscopy, even using freeze-fixation, is prone to preparative and imaging artifacts. X-ray diffraction has the problem that it is not a direct method; it does not directly lead to an image of the diffracting object and tricks are needed to get round the well-known phase problem. However, the technique can be applied to living, contracting muscle, and by time-slicing the observed X-ray diffraction data it is possible to monitor structural events in the same intact muscle at different stages of its con-

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tractile cycle. Here we report on progress that is being made in using time-resolved X-ray diffraction data to reveal the molecular events involved in actin filament regulation and in the movement of myosin cross-bridges during force production in bony fish muscle. The picture is not yet complete, but the progress to date is substantial and the prospects for being able to produce "muscle – the movie" [9] are good.

Muscular force is produced by the repetitive interaction of the cross-bridges on myosin filaments with the adjacent actin filaments [10,11]. The actin filaments in vertebrate striated muscles also contain the regulatory proteins troponin and tropomyosin on the actin filaments [12]. The myosin cross-bridges (or myosin heads) are ATPases which are activated by actin interaction [13]. The most recent, generally accepted, working hypothesis is that myosin heads with the ATP hydrolysis products ADP and Pi (inorganic phosphate) bound can attach transiently to actin in a so-called weak-binding state, after which transition to a strong binding state (or states) is associated both with product release and force generation [14]. The force-generating step has been ascribed to a "swing" of the elongated myosin heads on actin, thus producing a working stroke of each head of about 100 to 120 Å [10,11]. However, this is only a working hypothesis and evidence that unambiguously substantiates or disproves this idea has been rather hard to find. Two main questions therefore dominate the work of structural biologists working on muscle: (1) do the myosin heads swing on actin to produce movement and, if not, how is movement produced? and (2) how is the production of force regulated (i.e. how is muscular activity switched on and off)?

Current answers to question (2) are based on work in the early 1970s which showed a characteristic change in the low-angle X-ray diffraction patterns from actin filaments (cf. Fig. 1a) in muscle in the active or relaxed states [15–18]. This change was interpreted by modelling [17–19] in terms of a shift of tropomyosin molecules across the face of the actin monomers, thus exposing the site on actin to which the myosin heads can bind. The shift was thought to be brought about as a result of a nerve signal-induced action potential across the muscle fibre membrane causing the intracellular release of Ca^{2+} ions which bind to troponin through the troponin-C subunit on the actin filaments. This so-called "steric blocking model" has been a useful working model of thin filament regulation, but the modelling on which it was based was rather simple [17–19] and it has had its setbacks [20]. The particular problem in the 1970s was that the crystal structure of G-actin was not available. However, the landmark work of Kabsch et al. [21] provided the crystal structure of the G-actin/DNase I complex at atomic resolution. Since then other actin structures have been published [22,23]. It is therefore very timely to reconsider the steric blocking model armed with this new knowledge.

In an analogous way, it has been very difficult in past years to make unambiguous deductions about myosin cross-bridge behaviour using the low-angle X-ray diffraction patterns from myosin filaments [24,25] because the precise shape of the myosin heads was not known and this is needed to model the X-ray diffraction data. Once again, this problem has now been overcome due to the more recent landmark work of Rayment et al. [26], who have solved the crystal structure of myosin subfragment-1 (S-1).

With the myosin head shape and actin monomer shapes available, very detailed modelling of the low-angle diffraction X-ray diffraction patterns from contracting muscle can be carried out. In this group we have been making extensive use of a particular muscle type that has advantages for structural studies. In the A-bands of most muscles, the myosin filaments can have one of two orientations (i.e. 180° apart) around their long axes. The problem is that these two orientations do not occur in a systematic arrangement, but in a statistical superlattice structure [27]. This means that the low-angle X-ray diffraction patterns from skeletal muscles from animals such as frog, rabbit, chicken or human are sampled in a complicated way (a mixture of sampled and unsampled layer-lines) that is not straightforward to analyse [25]. On the other hand it has been found that the A-bands of the muscles of bony fish have their myosin filaments all oriented

identically to give a quasi-crystalline A-band array [27–29]. This in turn leads to low-angle X-ray diffraction patterns (Fig. 1b) in which the layer-lines are beautifully sampled by a simple set of row-lines. Here we show how such fish muscle X-ray diffraction patterns can be analysed to produce a movie of myosin cross-bridge action in a contractile cycle.

2. Actin filament structure and regulation

Actin filaments are helical structures that give rise to a characteristic set of layer-lines in X-ray diffraction patterns from whole muscle (see ref. [25]). Fig. 1a shows a diffraction pattern from fish muscle in rigor. In this state the myosin heads, in the absence of nucleotide, are strongly bound in a stereospecific way to actin. For this reason the actin layer-lines are enhanced in intensity and the diffraction patterns obtained show up the main features of the actin filament diffraction pattern very well. Actin filaments in vertebrate skeletal muscles have an axial repeat (R) of about 350 to 370 Å and layer-lines are observed at orders of this repeat. The strongest of these are usually the 1st and 2nd layer-lines (corresponding to spacings of R and of $R/2$ at about 180 Å) and the 6th and 7th layer-lines (corresponding to spacings $R/6$ at 59 Å and $R/7$ at 51 Å). It is the 2nd layer-line that was found to change substantially in intensity, from very weak to quite strong, as a result of activation and it was this layer-line in particular that was modelled [17–19] to give rise to the idea of the tropomyosin shift and the steric blocking model. In a recent publication [30], we have shown that armed with a knowledge of the actin monomer shape and structure and the way postulated by Holmes et al. [31,32] of putting such actin monomers together to create a filament, the

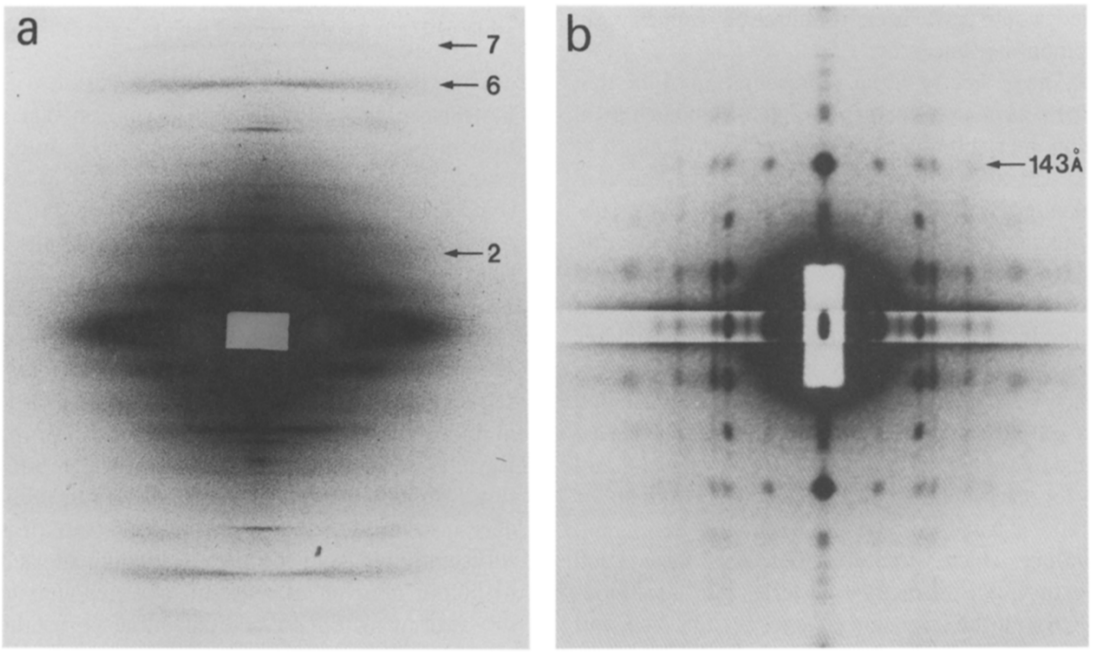


Fig. 1. (a) Diffraction pattern from a plaice fin muscle in the rigor state recorded on a mirror/ monochromator camera on a GX-18 rotating anode X-ray generator. The actin layer-lines are enhanced in this pattern due to myosin head labelling of the actin filaments. Layer-lines 2, 6 and 7 are indicated. (b) Low-angle X-ray diffraction pattern from plaice fin muscle in the relaxed state recorded on line 2.1 at the Daresbury Synchrotron Radiation Source with the beam size slitted down to give optimal separation of the diffraction peaks. The beautiful sampling of the myosin layer-lines (arrow indicates the 3rd layer-line related to a spacing of 143 Å in the myosin filaments) is evident. This sampling remains in patterns from active muscle, with very little reduction of order [42].

changes in the actin diffraction pattern involved in activation could be modelled with quite high sensitivity. The approach was to try using global search methods to define the relative positions of the four sub-domains of the actin monomer and the position of tropomyosin in the actin filament and to investigate the molecular movements needed to explain the X-ray diffraction data. Results from many previous studies of actin X-ray diffraction patterns were used [15–18,33–36], but in particular, full use was made of the intensity differences reported by Yagi and Matsubara [34] to occur in X-ray diffraction patterns from frog semitendinosus muscle stretched to very long sarcomere lengths to remove any overlap (and hence interaction) of the myosin and actin filaments. Since such global searches require prodigious amounts of computing time, we chose to simplify the problem by representing the four individual sub-domains of the actin monomer as uniform spheres of appropriate size. To carry out the same global search with each atom position specified would have been prohibitively expensive in computing time.

The geometry of our representation of the thin filament model is shown in Fig. 2, with each actin monomer sub-domain numbered as in Kabsch et al. [21] and the spheres located as in the thin filament model of Holmes et al. [31]. Thus, sub-domains 3 and 4 of actin were close to the thin filament axis (Figs. 2, 3 and 4), sub-domains 1 and 2 were at higher radius, and the tropomyosin strands, represented as small overlapping spheres [19,30], were taken to be on the actin surface. In each case the sphere positions were represented in cylindrical coordinates as radius r, azimuthal angle θ, and axial position z. By varying r, θ and z for each subdomain and varying r and θ for tropomyosin (no z value is needed here), and finding which structures not only gave good "actin-like" diffraction patterns but also modelled well the layer-line changes seen by Yagi and

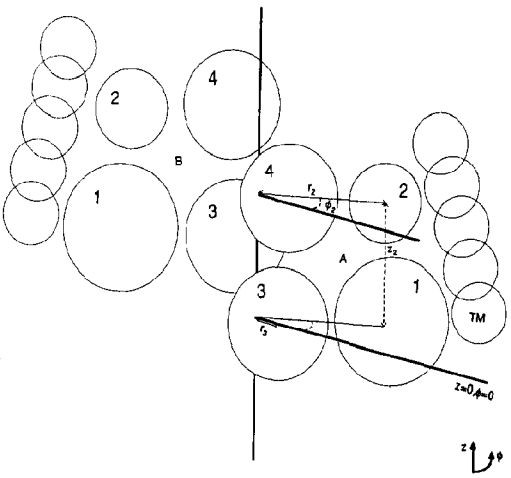
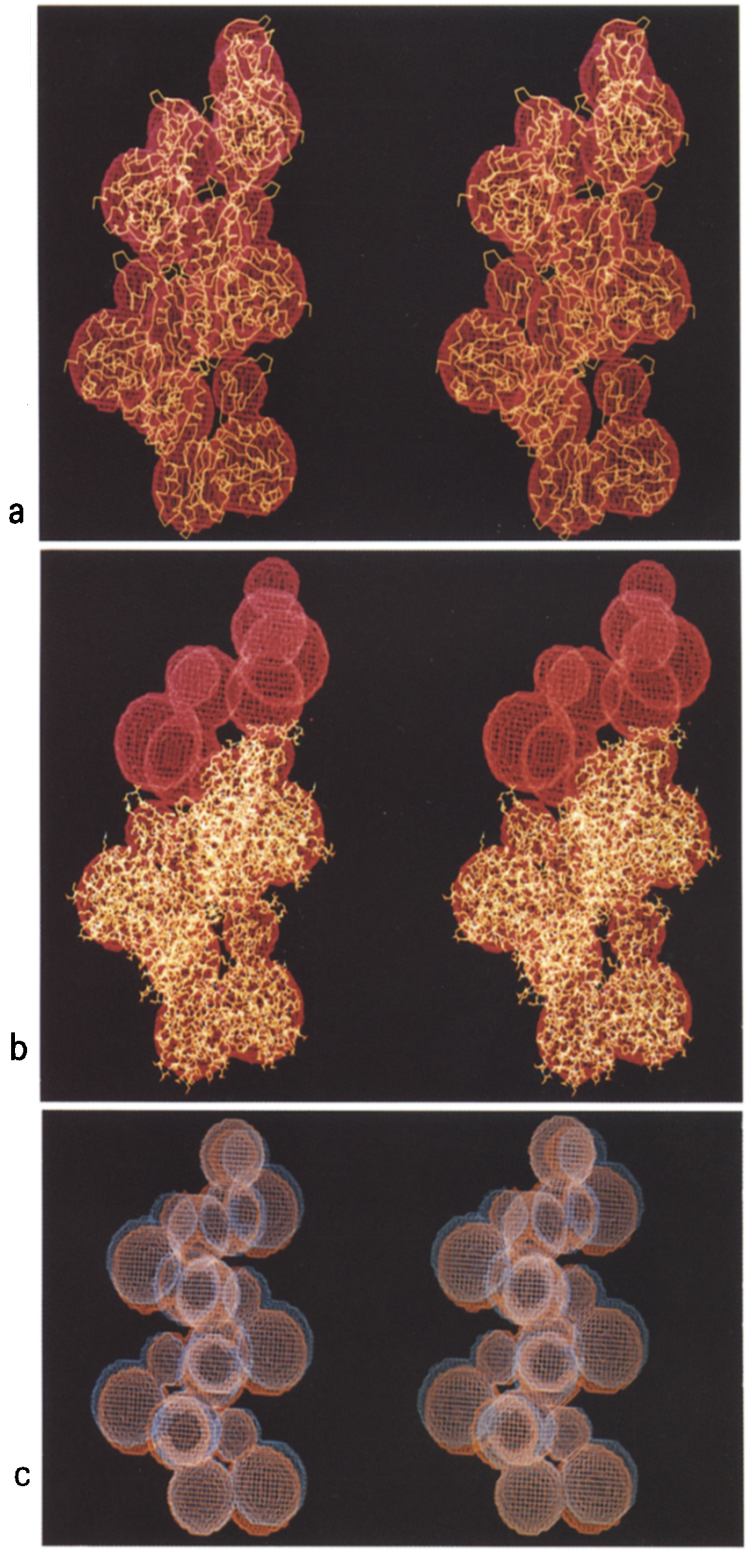


Fig. 2. Illustration of the "sphere" model used to represent an actin filament. Positions of the four sub-domains of monomer A and the position of the tropomyosin strand (TM), represented as five small spheres associated with each actin subunit, were defined by parameters r, θ and z relative to the actin filament axis (central vertical line) and a radial "origin" through the centre of the sphere representing sub-domain 3. Actin subunit B is rotated from A by $-167°$ and shifted axially by 27.5 Å. (Slightly modified from Squire et al. [30].)

Matsubara [34], we were able to confirm that a tropomyosin shift appears to be essential. Interestingly, tropomyosin position in the "off" state (Fig. 4) is on sub-domain 3, but close up to sub-domain 1 of actin, and it is sub-domain 1 which is known to be the main myosin binding site [31,37]. In the "on" (active) state the tropomyosin moves to a position that is still on sub-domain 3 but is now much further away from sub-domain 1. By placing the myosin head (S-1) in the position on actin modelled recently by Rayment et al. [37], it is clear that, in the "off" state, tropomyosin is in a good position to influence myosin binding, and, in the "on" state, it is sufficiently far from the myosin binding site not to hinder attachment, but still close enough to the edge of myosin to have an influence on the

Fig. 3. Various stereo representations of actin structures used in this analysis. (a) The Holmes et al. [31] actin model represented as spheres and as a C_α skeletal model. The two representations are set with the sub-domain centres of mass coincident. (b) As in (a) except that all the main atoms (C, N, O) are shown. (c) Comparison of the sphere models for actin in the "on" (light blue) and "off" (deep yellow) states, as modelled in this study.


a
b
c


a
b
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cross-bridge cycle. In addition to this tropomyosin shift, there are also movements of the actin sub-domains; in particular there is a fairly large movement of sub-domain 2 (Fig. 3c). It appears that, in the "off" state, sub-domain 2 and tropomyosin may be close enough to interact; sub-domain 2 may, in fact, stabilise the "off" position of tropomyosin. In the "on" state, tropomyosin moves one way and sub-domain 2 in the opposite direction, perhaps being released by tropomyosin and then reverting to its natural lowest energy position.

To summarise, the steric-blocking model appears to be in good shape; tropomyosin does seem to exert its influence by virtue of its position on the actin filament surface. In addition, there are movements of the actin sub-domains that may provide insights into the factors that stabilise different thin filament states. However, although the story so far seems fine and it appears that low-angle X-ray diffraction data can be modelled with quite high precision to reveal small sub-domain movements within protein molecules, the question remains about the validity of using simple spheres to represent complex protein structures. Is the sphere approximation reasonable? We have attempted to test this using representative models. The results of one test are shown in Figs. 3 and 5. What has been done in Fig. 3 is to superimpose the actin filament atomic model of Holmes et al. [31] and our sphere model of the same structure [30]. The centre of each sphere was located at the calculated centre of mass of the atoms in the appropriate sub-domain. The radius of each sphere was chosen to give a total volume appropriate to the known molecular weight of the sub-domain and using a standard protein density. The images in Fig. 3 serve to demonstrate the close similarity of the distributions of mass in the sphere representation and in representations with either just the C_α positions

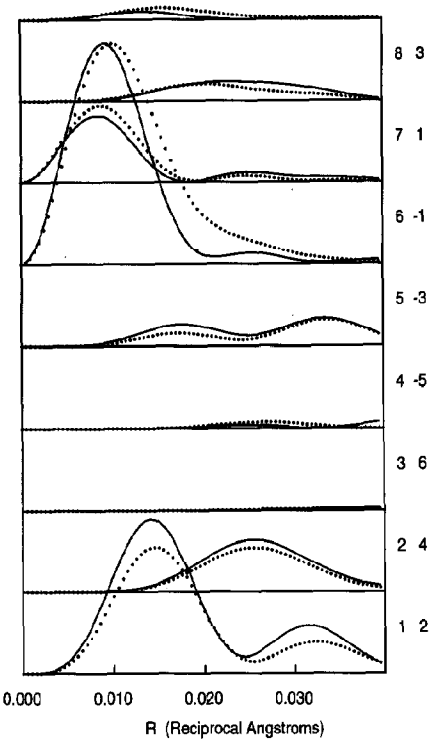

Fig. 5. Comparison of the computed Fourier transforms of the representations of the Holmes et al. [31] actin model shown in Fig. 3b as either spheres (dotted lines) or atoms (solid lines). Intensity is plotted against reciprocal space coordinate R (i.e the distance along the layer-line). The general agreement is good. The numbers on the right show the layer-line number (orders of $R = 360$ Å) and the order (n) of Bessel function used in the computation. An additional term ($n = -7$) was used on layer-line 3.

Fig. 4. The "off" (a) and "on" (b) states of the actin model with the tropomyosin strands included. Between (a) and (b) the tropomyosin strand at the front moves to the left away from sub-domain 1 (blue), but remains at all times on the surface of sub-domain 3 (green). The main movement in the actin appears to be that of sub-domain 2 (light grey) which is close to tropomyosin in the "off" state (a), but moves in the opposite direction to tropomyosin in the "on" state. (c) Representation of the full fish muscle A-band unit cell, viewed down the muscle long (c) axis, and showing four complete unit cells. The actin filaments are modelled as in (a) with the tropomyosin appearing light green and the actin sub-domains white and red. The backbones of the myosin filaments are not shown here, but the three different crowns of myosin heads at different axial levels within a 429 Å axial repeat are shown as light pink on one level, blue on the next level and mauve or dark blue on the third level. An axial perturbation of the crossbridges has been included in the model. The model was displayed using AVS on a DECstation 5000. The computed 2D diffraction pattern from a model such as this is shown in Fig. 6.

shown (Fig. 3a) or with all the C, O or N atoms shown (Fig. 3b). In addition, the corresponding Fourier transforms can be computed for the low-angle layer-lines from the sphere model and from the full atomic model. The computed patterns are shown in Fig. 5, where it can be seen that, at least for layer-lines 1 to 7, the agreement is satisfactory. We conclude that, despite its simplicity, the four sphere model for the actin monomer is quite good for the purpose of this kind of calculation.

3. Modelling the A-band unit cell in fish muscle

The actin filament structure discussed above, and still represented as spheres, can be incorporated into a computer model of the full unit cell of the A-band in bony fish muscle. This structure (Fig. 4c) comprises on average one myosin filament and two actin filaments in the unit cell with the two actins at the trigonal points (fractional unit cell coordinates 1/3, 2/3 and 2/3, 1/3) of the hexagonal lattice. The myosin filaments are known to have the myosin head pairs of each myosin molecule arranged approximately helically along the A-band length around a central thick filament backbone. The cross-bridge array can be described as a 3-start 9 residue per turn helix [25,38], thus giving the myosin filaments 3-fold rotational symmetry at each level (crown) of cross-bridges. Successive crowns are spaced on average at axial separations of about 143 Å and the true axial repeat of the array is 429 Å (i.e. 3×143 Å). However, the crown spacing is not exactly 143 Å; it is known that a periodic axial perturbation in this array occurs so that inter-crown spacings can be 20 to 30 Å away axially from their ideal helical positions [24,39]. Finally, because the actin and myosin filaments have different axial repeats (we have taken them to be 429 Å and 715 Å; $R = 715/2$ Å) the c axis repeat of the fish muscle unit cell is taken to be 2145 Å. The a and b axes were set at 473 Å.

The information contained in the well-sampled myosin layer-lines in the low-angle X-ray patterns from resting fish muscle (Fig. 1b) can be used, in principle, to determine the relaxed configuration of the myosin cross-bridges. Both the off-meridional intensities and the meridional reflections, which provide information about the axial perturbation of the cross-bridges, need to be fitted. This fitting procedure is now in progress; here we show the approach that is being used. Our initial computer model of the fish muscle A-band unit cell was created before the full details of the myosin head shape were published [26], but after the earlier electron microscopy study of the same S-1 crystals was reported [40,41]. The initial model therefore used a simple representation of the early published myosin head shape. The kind of model that was used is shown in figs. 4c and 4d. Here the actin filament structure is as in Figs. 2, 3 and 4a and the myosin filaments have been modelled to give at least a qualitatively reasonable fit to the myosin layer-line data (Fig. 1b). A simple representation of the computed 2D transform from the model in Fig. 4c is shown in Fig. 6. It should be compared with Fig. 1b, remembering that no attempt has yet been made to optimise the fit. Despite this, the general correlation of key features of the observed and computed pat-

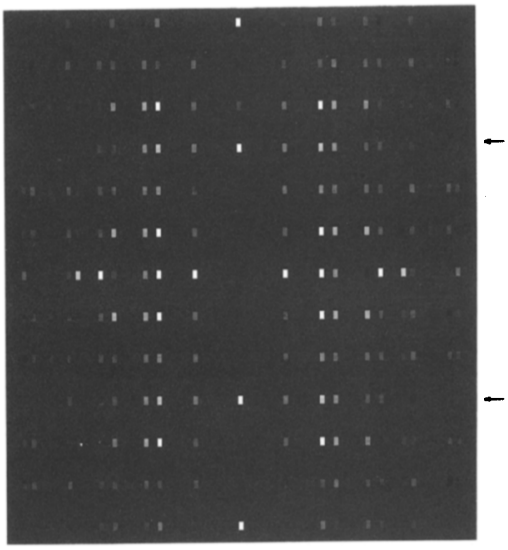

Fig. 6. Representation of the output of a program developed to create models of the fish muscle A-band unit cell (such as Fig. 4c) and to compute the expected diffraction pattern. This distribution of intensity can be compared with Fig. 1b, although no attempt has yet been made to optimise the fit. The 143 Å layer-line is indicated by the arrow.

terns is encouraging. Fortunately, courtesy of Dr. Ivan Rayment [26], we now have available to us the recently determined myosin head (S-1) shape based on the full crystal structure determination. This shape is now being incorporated into our computer model of the fish muscle unit cell and the whole unit cell structure is being refined. By searching for the best agreement between the observed and calculated intensities we should soon have a good model of the structure of resting fish muscle. This model can then be refined by combining observed amplitudes and model phases in a conventional refinement procedure.

4. Generation of "muscle – the movie"

Once the resting structure of fish muscle has been defined, it can be used as a starting point to create a movie of the molecular events involved in contraction [9]. Using the high-intensity synchrotron X-ray source at the Daresbury Laboratory (line 2.1), we have been able to record several 5 ms time-resolved series of 2D diffaction patterns from bony fish muscle undergoing typical tetanic contractions [7]. Fortunately, in the case of this muscle but not other vertebrate skeletal muscles, the diffraction patterns remain well sampled throughout the contractile cycle [42]. This means that we have a series of diffraction patterns like Fig. 1a from successive 5 ms-spaced times throughout a tetanus, in which the main changes are in the intensities of the diffraction peaks. The hope is that the model for relaxed fish muscle can be used as a starting point for phasing later stages of the cycle. A series of model images of each of these steps will provide the required movie of the contractile event.

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