

Reviews

Millisecond Time-Resolved Low-angle X-ray Fibre Diffraction: A Powerful, High-Sensitivity Technique for Modelling Real-Time Movements in Biological Macromolecular Assemblies

John M. Squire, Carlo Knupp, Hind A. AL-Khayat & Jeffrey J. Harford*

Biological Structure & Function Section, Biomedical Sciences Division and *Biological Sciences Department, Imperial College London, London SW7 2AZ, UK.

Fibre Diffraction Review **11**, 28-35, 2003

ABSTRACT

The modern post-Genomic era heralds the elucidation of many thousands of protein and nucleic acid structures by X-ray crystallography and other techniques. But knowledge of individual macromolecular structures is often not enough. In some cases such macromolecules function as components of much larger molecular assemblies, some of which are filamentous in nature. Striated muscle is a particularly well-ordered example of an organised macromolecular assembly and, in addition, it is dynamic; it functions as a mechanical motor using molecular movements which occur in a millisecond timescale. It, therefore, provides a good test case for the development of structural methods. Here we show that time-resolved, low-angle, X-ray fibre diffraction can be used to follow the molecular movements in contracting muscle in real time and with high spatial sensitivity. More generally, the techniques discussed here can be applied to any uniaxially ordered macromolecular assembly.

INTRODUCTION

One of the current aims of structural biologists is not just to determine the atomic arrangements in proteins, nucleic acids and other biological macromolecules, but also to see how these molecules move or change their shape as they carry out their normal biological functions. Ways to attempt to do this with, for example, a typical enzyme involve following structural changes by protein crystallography in a time-resolved mode (Helliwell & Rentzepis, 1997; Helliwell, 2002). For example Laue diffraction or instantaneous monochromatic diffraction methods can be used to take rapid X-ray diffraction 'snapshots' of a protein crystal at a prescribed time after reaction initiation (Hajdu & Johnson, 1990; Duke *et al.*, 1991; Helliwell & Rentzepis, 1997; Helliwell, 2002). Alternatively, by 'snap-freezing' the enzyme-substrate complex at various times during a reaction, the structure can be determined at leisure by conventional crystallographic methods (Taubes, 1994). However, these approaches not only require synchronous initiation of reactions by, for example, photolysis of caged

compounds (e.g. Kaplan *et al.*, 1978), they also demand the maintenance of good 3-D crystalline order giving diffraction to high resolution throughout the process. Systems amenable to these approaches are, as yet, few in number (see Helliwell & Rentzepis, 1997). Even so, these techniques generally apply only to single molecules, or small molecular aggregates, whereas *in vivo* many functional proteins are parts of large macromolecular assemblies that may carry out their normal function in a concerted or cooperative manner. In many cases these assemblies are not such that crystallisation and X-ray crystallography is a realistic approach. How can the structures of such assemblies be solved and how can changes in such large assemblies be followed in a dynamic way? If protein crystallography cannot do it, are there other approaches that can be tried? If so, do these other approaches have a spatial sensitivity that approaches that of conventional crystallography?

A few years ago a 'Ways and Means' article by Holmes (1994) presented the powerful case for combining structures determined by protein

crystallography with results from aggregates studied by cryo-electron microscopy or by high-angle fibre diffraction. In the case of electron microscopy, this is now a common approach (e.g. Wriggers *et al.*, 1999). However, this approach is never likely to be a method for studying dynamic changes in macromolecular assemblies other than as a time series of instantaneous 'snapshots' processed individually from separate experiments. Here we present the case for a dynamic, high sensitivity, X-ray diffraction approach to studying macromolecular assemblies, namely that of using time-resolved low-angle X-ray diffraction from fibres or other assemblies, as a means of following structural changes in biological systems with a time-resolution of microseconds to milliseconds and a spatial sensitivity (not resolution) of a few Å.

Many important biological systems occur naturally as extended aggregates, some of which are regularly organised as filaments. Actin filaments, myosin filaments, collagen fibrils, microtubules, intermediate filaments and some viruses represent obvious examples. Some of these, such as actin, myosin and microtubules, clearly have a dynamic role in basic cellular processes; cell division, organelle movement, muscle contraction and so on. Provided that such filaments can be obtained in sufficiently large quantities, preferably with good axial alignment, they can give enormously rich fibre X-ray diffraction patterns. Such patterns rarely extend to the kinds of resolution that X-ray crystallographers are used to, but, even so, diffraction in the low to medium-angle region can be extremely informative. Here we use the examples of actin and myosin filaments that occur in abundance and excellent alignment in skeletal muscle tissue to illustrate the power of the low-angle time-resolved X-ray diffraction technique. Note that the arguments also apply, but with less sensitivity, to low-angle scattering from solutions of macromolecular aggregates when the structures of the individual macromolecules are known (Perkins *et al.*, 1993; 2001; Sugimoto *et al.*, 1995; Maruta *et al.*, 2000; Svergun and Koch, 2002). However, in this case only rotationally averaged data are obtainable and there is more ambiguity in the modelling.

LOW-ANGLE X-RAY DIFFRACTION FROM RESTING MUSCLE

Figure 1 shows a low to medium-angle X-ray diffraction pattern from a bony fish muscle (sculpin)

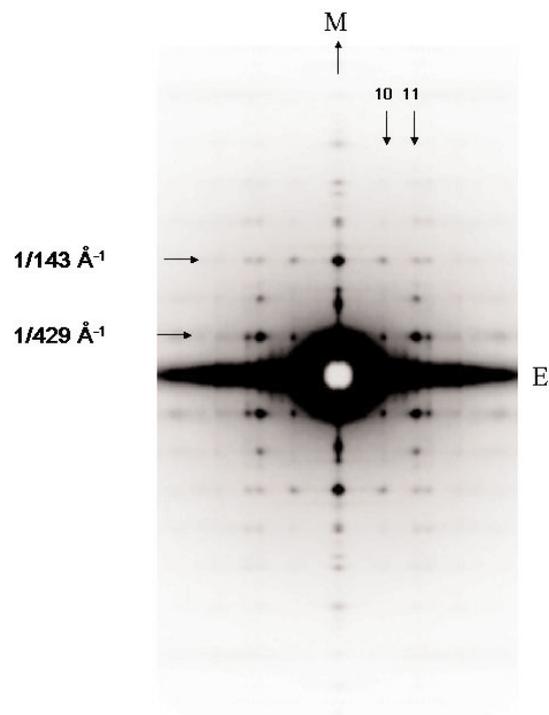


Figure 1. Low-angle X-ray diffraction pattern from the myotomal muscle of the sculpin fish in the resting state recorded at SPring-8. The fibre axis was vertical (M). The layer-line intensities are largely from the quasi-helical arrangement of myosin heads around the muscle myosin filaments which have a 429 Å repeat. The 10 and 11 row-lines (vertical) that arise from the high degree of lateral and axial order in the hexagonal A-band array of myosin and actin filaments are also indicated.

in the relaxed state and recorded at the Spring-8 synchrotron in Japan. The pattern shows the beautifully sampled low-angle layer-lines typical of diffraction from fish muscles (Harford & Squire, 1986), including the characteristic set of layer-lines, orders of an axial repeat of 429 Å, which are due to the quasi-helical arrangement of the globular heads of the myosin molecules in the muscle myosin filaments. Such patterns cannot usually be used in direct Fourier synthesis by solving the phase problem as in protein crystallography. But what if one already knows from protein crystallography the structures of the principal protein components - in this case myosin and actin molecules? We have found that the observed low-angle diffraction patterns from muscle are sensitive not only to the position and orientations of such molecules, but also to the relative positions of their sub-domains. Whole molecule or sub-domain movements of only a few Å can have a marked effect on the low-angle diffraction patterns.

In previous work (Hudson *et al.*, 1997; Squire *et al.*, 1998) we have modelled the array of myosin heads in resting bony fish muscle based on diffraction

patterns such as that in Figure 1 and the result is shown in Figure 2(a). Note that here the filament long axis has been turned 90° relative to Figure 1. It is these myosin heads that interact with actin filaments to produce muscular force and movement. The current working model for muscle action involves the so-called crossbridge cycle. The myosin heads (crossbridges) start off loaded with the molecule adenosine triphosphate (ATP) that has been hydrolysed to adenosine diphosphate (ADP) and inorganic phosphate (Pi). In this state the heads can bind to actin, provided that the muscle machinery has been switched on by Ca²⁺ (Squire and Morris, 1998). Actin then activates the attached myosin heads to release the 'products' Pi and ADP during which the heads undergo a structural change on actin thus generating force and movement (sliding) of the actin filaments relative to the myosin filaments (see Squire, 1997; Harford *et al.*, 1998). An attached myosin head (crossbridge) remains attached to actin until a fresh ATP molecule binds to the head and releases it from actin. The detached head can then hydrolyse the bound ATP to ADP and Pi and reset itself ready for another crossbridge cycle further along the actin filament.

This mechanism is a working model. The key structural question here is 'how can one actually see and monitor the structural events that take place in the crossbridge cycle?'. It should also be noted that in typical muscles each head goes through several attachment/ detachment cycles on actin in a second; interesting events are in fact taking place in muscle on a millisecond timescale. A technique is needed to monitor changes in muscle structure with very fast time resolution and with considerable structural sensitivity.

LOW-ANGLE X-RAY DIFFRACTION FROM ACTIVE MUSCLE

That very big intensity changes occur in the low-angle diffraction patterns from muscle in different physiological states is illustrated in Figure 3. This shows a **difference** diffraction pattern obtained by subtracting a pattern from fully relaxed muscle (as in Figure 1) from a pattern from fully active muscle. In this case the muscle being used was the fin muscle of plaice (*Pleuronectes platessa*) and the patterns were recorded with a camera length of about 2.5 m on the high intensity beamline 16.1 at the CLRC Daresbury Synchrotron (Bliss *et al.*, 1995).

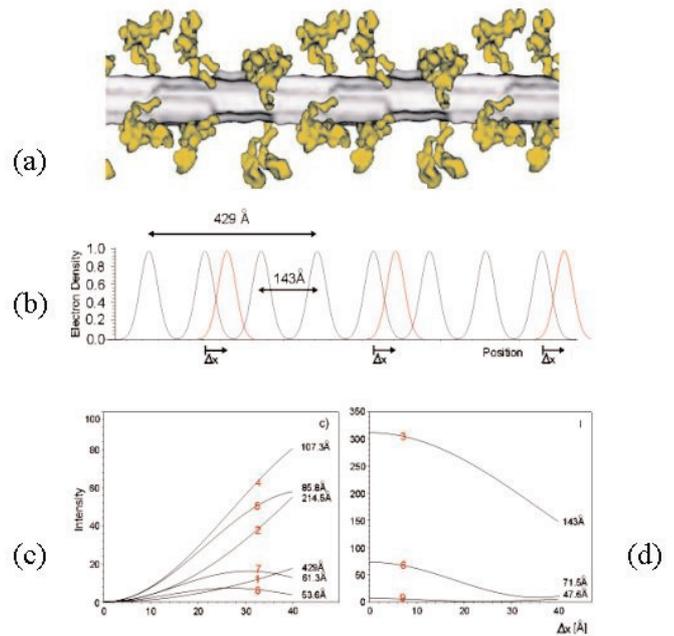


Figure 2. (a) The crossbridge array on myosin filaments in relaxed bony fish muscle determined by Hudson *et al.* (1997). (b) Example of an axial shift by Δx of every third level of heads in (a) and the effect of this on various low-angle meridional X-ray reflections from muscle (c,d), all orders of 429 Å. The usual strong meridional orders of M3 (labelled 3) at 143 Å are shown in (d) and the so-called 'forbidden' reflections in (c) are meridional reflections that become apparent when a true 429 Å repeat is established.

The myosin layer-lines in Figure 1, based on the 429 Å repeat, largely disappear when the muscle is activated and this shows up in the difference diffraction pattern (Figure 3) as black regions. In addition various parts of the pattern increase in intensity (green and yellow regions). In particular, layer-lines which come from the actin filaments and have spacings which are orders of about 360 Å generally become stronger. This is partly due to activation-related changes produced by Ca²⁺ within the actin filaments themselves and partly due to the increased mass on actin produced by the attaching myosin heads. The reflection A2 indicated in Figure 3 is the second order layer-line of the 360 Å actin repeat that occurs at a spacing of about 2/360 Å⁻¹. It changes intensity as a function of muscle Ca²⁺-activation (Squire & Morris, 1998). These actin layer-lines will be discussed more in a later Section.

We show below that intensity changes of the kind in Figure 3 are sensitive to rather small movements of the myosin heads or actin sub-domains. Modelling

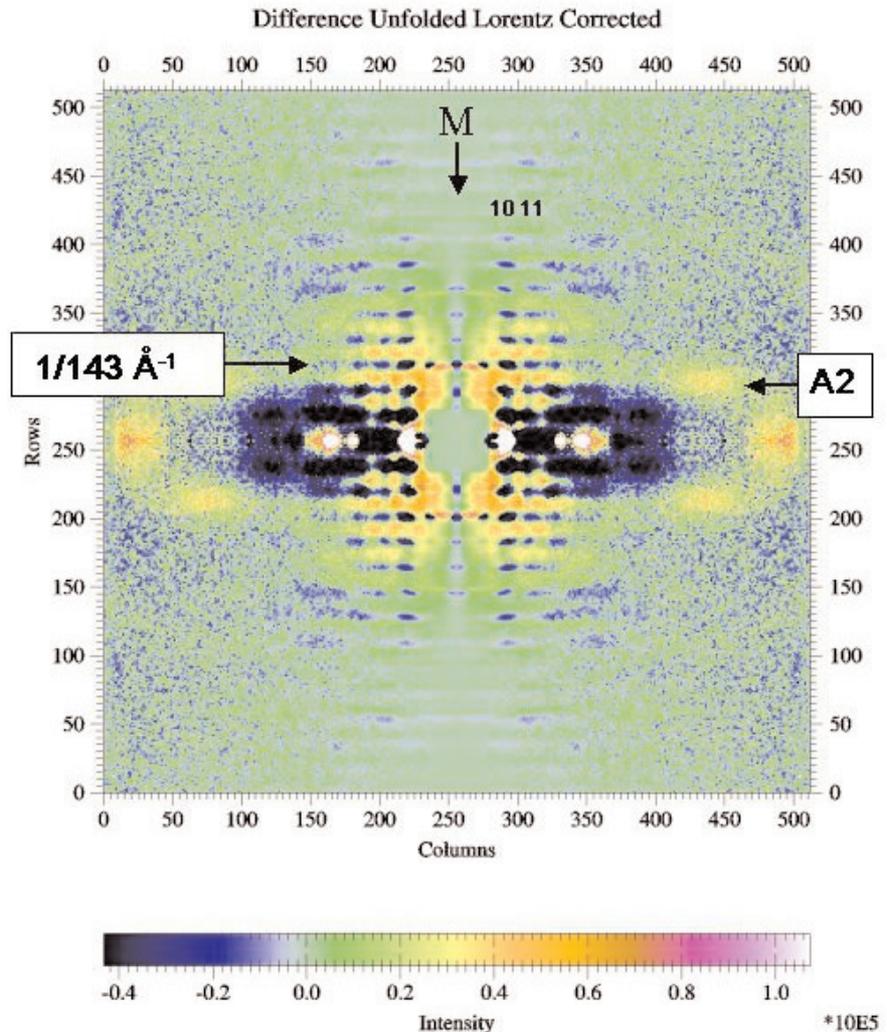


Figure 3. Difference diffraction pattern between resting and active plaice fin muscle (active-resting) recorded using the RAPID detector on beamline 16.1 at the Daresbury SRS (muscle long axis vertical). For details see text.

with known protein structures the intensity changes observed in time-resolved low-angle X-ray diffraction patterns is in fact very sensitive to quite subtle molecular changes.

MONITORING AXIAL MOVEMENTS OF MYOSIN HEADS.

We have tested the sensitivity of the myosin filament low-angle diffraction pattern in two ways. In one case (Figure 2) we have represented the axial positions of myosin heads as a 1-dimensional array of Gaussian density profiles and have tried moving some of the elements of this array. In the other case (Figure 4) we have used the whole modelled diffraction pattern and have tested the layer-line sensitivity to head position by Fourier difference synthesis.

Figure 2(b) is a simple approximation to what is seen in Figure 2(a). The myosin head arrangement in Figure 2(a) has a true axial repeat of 429 Å and a near sub-repeat of this at 143 Å. What has been done in Figure 2(b) is to start with an array of myosin head levels with a true 143 Å repeat and then move every third of these levels by a small distance (Δx ; Fig.

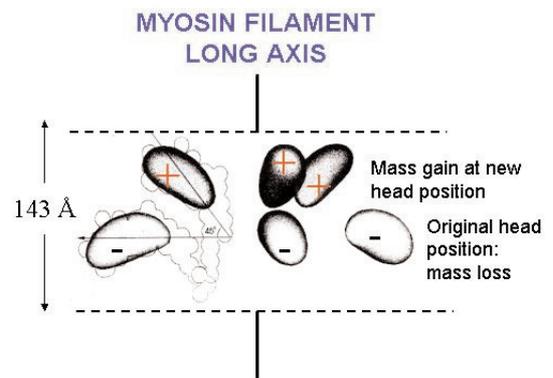


Figure 4. Illustration of the sensitivity of the low-angle X-ray diffraction method to single head movements in one of each of the three myosin head pairs in only one crown out of three myosin head crowns in a 429 Å repeat as in Fig. 1(a). A very clear shift of mass is observed from the lower densities (mass loss: -ve peaks) to the upper densities (mass gain: +ve peaks) using Fourier difference synthesis. The peaks coincide exactly with the original and new positions of the heads (fine outlines).

2(b)). The resulting Fourier transform has been computed as a function of Δx for the first few orders of the 429 Å repeat (Figure 2(c)). It can be seen that very large intensity changes in these very low-angle reflections occur even when Δx is only 10 or 20 Å. The spacings of the layer-lines themselves are clearly 'low' resolution in the crystallographic sense, but

they are nevertheless sensitive to movements measured in a few Å.

The second test, illustrated in Figure 4, is to take one level of the myosin heads in Figure 2(a), a so-called 'crown' of heads in which there are three pairs of heads separated by 120° around the filament backbone, and then within each head pair in the crown move one of the heads by tilting it axially to a new position, as might occur in a putative crossbridge cycle.

We computed Fourier transforms based on either the 'original' model in Figure 2(a) or on the 'modified' model in which one head in each pair was moved only in one of the three crowns separated axially by 429 \AA . The amplitudes in the two Fourier transforms were then subtracted and this difference was used with the model phases from the original structure to give a Fourier difference electron density map (Figure 4). Only the affected crown level is shown in Figure 4, which represents the density difference between the modified and original structures computed using layer-lines of spacings ranging from the first order at 429 \AA to the highest order used, the 6th, at 72 \AA . Fourier difference maps show peaks of negative density where an object has moved away from and peaks of positive density where objects have moved to. Figure 4 is no exception; there are clear negative densities where the myosin heads originally were and there are clear positive densities at the positions to which the heads were moved in the modified model. Clearly the low-angle diffraction pattern, despite its apparently low-resolution, can be used to monitor myosin head movements such as those that might occur between different muscle states.

MONITORING MOVEMENTS OF THE SMALLEST SUB-DOMAIN OF ACTIN

The sensitivity of the low-angle region of the muscle diffraction pattern can also be illustrated by changes in actin filament structure that may be involved in Ca^{2+} regulation of muscular activity. Figure 5 illustrates the principle.

As proposed by Holmes *et al.* (1990), the actin monomer, with its four sub-domain structure (Kabsch *et al.*, 1990) can be positioned and oriented with appropriate helical symmetry to give a sensible model for the actin filament (Figure 5(a)). Although this model has been subsequently refined (Lorenz *et al.*, 1993), it appears to be substantially correct. Our own analysis supports this conclusion (AL-Khayat *et*

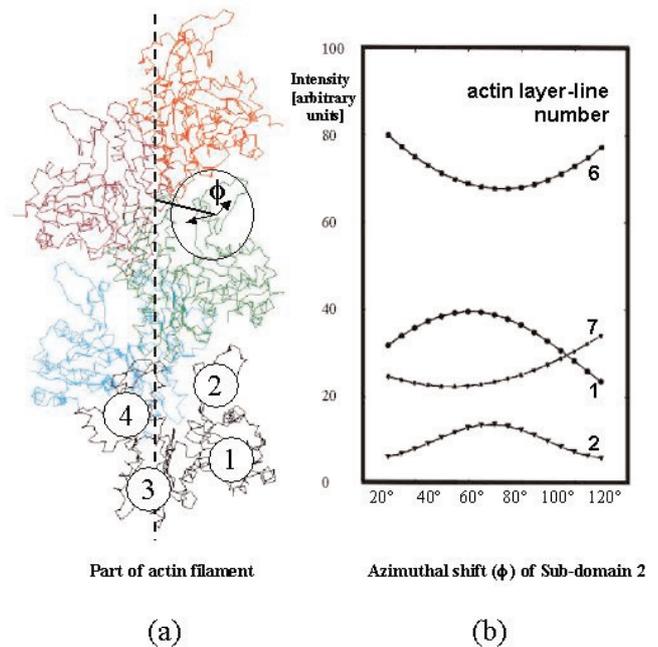


Figure 5. (a) Representation of the actin filament structure proposed by Holmes *et al.* (1990), where each actin monomer is shown as a ribbon diagram through the α -carbon atoms in the G-actin crystal structure of Kabsch *et al.* (1990). The lowest monomer has the actin sub-domains numbered and an upper monomer shows the azimuthal movement of sub-domain 2 used in (b) and discussed in the text. The myosin binding site on actin is mainly located on sub-domain 1. (b) The computed peak intensities on some of the low order actin layer-lines (1 at $1/360 \text{ \AA}^{-1}$; 2 at $1/180 \text{ \AA}^{-1}$; 6 at $1/59 \text{ \AA}^{-1}$ and 7 at $1/51 \text{ \AA}^{-1}$) shown as a function of the azimuthal position of the smallest actin sub-domain, sub-domain 2. The azimuthal angle is measured around the actin filament axis as in (a), where the 70° position is that in the Holmes *et al.* (1990) structure of the actin filament.

al., 1995). On the whole, the remaining uncertainties in the structure have been to do with the position of the smallest of the four sub-domains, namely sub-domain 2. This is because its small mass will make only a relatively small contribution either to observed X-ray diffraction patterns or to helical reconstructions from electron micrographs of actin filaments. The position of sub-domain 2 will therefore be relatively difficult to determine.

Here we have tested this idea by using a four sphere model of the actin monomer, where each actin subdomain is represented by a uniform sphere of appropriate volume (AL-Khayat *et al.*, 1995). The diffraction pattern on the first few layer-lines (orders 1, 2, 6 and 7 of a 360 \AA repeat) has then been computed for various models in which the only structural change introduced is in the azimuthal position of sub-domain 2 around the actin filament axis. Compared with the Holmes structure, sub-

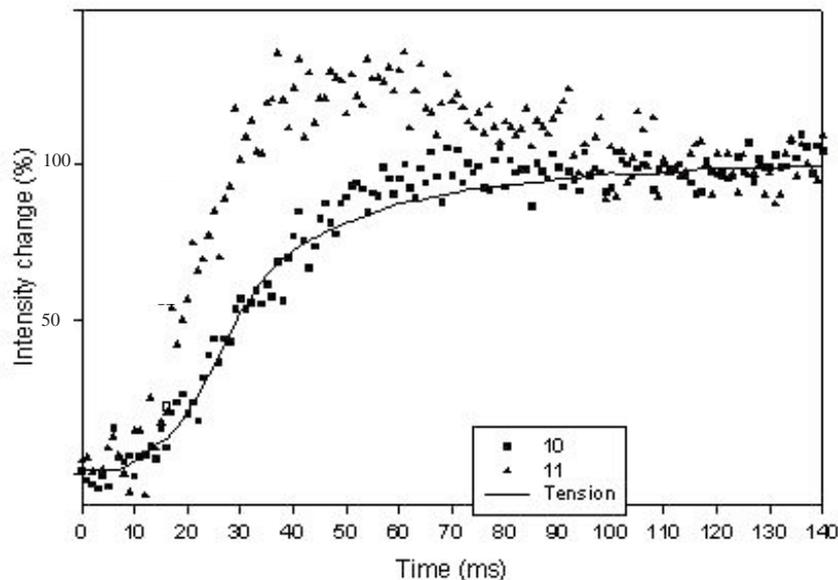


Figure 6. Time-courses of the change in intensity of the equatorial 10 and 11 reflections from plaice fin muscle during tetanic contractions and recorded on a 1 ms timescale using the fast multiwire "RAPID" area detector (Lewis *et al.*, 1996) on beamline 16.1 (Bliss *et al.*, 1995) at the CLRC Daresbury Laboratory. The continuous line shows the recorded tension in the muscle (arbitrary scale).

domain 2 has been moved azimuthally by up to a maximum of $\pm 40^\circ$ around the actin filament axis. Figure 5(b) shows the result. Very marked changes of relative intensity occur on the computed low-angle layer-lines (spacings $1/360 \text{ \AA}^{-1}$ for $l = 1$, $2/360 \text{ \AA}^{-1}$ for $l = 2$, $6/360 \text{ \AA}^{-1}$ for $l = 6$, $7/360 \text{ \AA}^{-1}$ for $l = 7$) as sub-domain 2 alone is moved away from the Holmes position. The azimuthal movements may appear to be quite large, but the helical radius of sub-domain 2 from the actin filament axis in the Holmes model is only about 21 \AA . Azimuthal swings of up to 30° therefore correspond to shifts in the centre of mass of the sub-domain of only up to 7 \AA ; the pattern is very sensitive to quite small movements. Compared with the Holmes structure ($\emptyset = 70^\circ$), which has I_6/I_2 equal to about 5, the structure with sub-domain 2 at $\emptyset = 100^\circ$ (i.e. 7 \AA away) has this same ratio equal to about 13; well over twice the Holmes value.

It is instructive to note that the 2nd actin layer-line has an axial spacing in reciprocal space of about $1/180 \text{ \AA}^{-1}$ and the peak is at a radial position of about $1/50 \text{ \AA}^{-1}$. Layer-line 6 has an axial spacing of about $1/59 \text{ \AA}^{-1}$ and peaks at a radial position of about $1/110 \text{ \AA}^{-1}$. Clearly the 'resolution' of the data in the conventional crystallographic sense is very limited and the structure cannot be solved to high resolution. But these same layer-lines are remarkably sensitive to sub-domain movements of only a few \AA once the structures of the component domains in the molecule have been solved crystallographically. This

represents an excellent example of two apparently disparate techniques, protein crystallography and low-angle X-ray fibre diffraction, coming together to provide useful insights that each technique on its own cannot provide. In conclusion, rather subtle relative movements of the centres of mass of protein sub-domains can be defined quite well by low-angle X-ray fibre diffraction.

DYNAMIC EXPERIMENTS WITH FAST TIME RESOLUTION

Time-resolved low-angle diffraction studies, which are now carried out routinely at Daresbury and elsewhere, can provide information about continuous structural changes in macromolecular assemblies at millisecond time resolution or better (Harford and Squire, 1992; Irving *et al.*, 1992; Martin-Fernandez *et al.*, 1994; Squire *et al.*, 1994; Lombardi *et al.*, 1995 and many others). As an example, Figure 6 illustrates results from dynamic studies of contracting fish muscle on beam line 16.1 at the CLRC Daresbury Laboratory. These were obtained using the very fast multiwire area detector known as RAPID. RAPID was developed by the Daresbury detector group (Lewis *et al.*, 1996) and is still the fastest readout area detector for time-resolved low-angle X-ray diffraction studies at any synchrotron. The traces in Figure 6 show the intensity changes of the innermost 10 and 11 equatorial reflections (d-spacing about 420 \AA and 364 \AA respectively) from the hexagonal lattice of myosin and actin filaments in the muscle A-band as they change when the

muscle is activated to develop its maximum tension (in an isometric tetanus). These reflections lie on the equator (E) of the diffraction pattern in Figure 1 and on the vertical 10 and 11 row-lines indicated there, but they are overexposed in this particular pattern. Usually in experiments of this kind it has been necessary to subject the muscle to a series of similar contractions and to pool the results in appropriate time bins after the initiation of contraction. However, in most previous studies it was also necessary to pool results from several different muscles in order to accumulate sufficient counts. Unfortunately this leads to degradation in the signal; there is variability between different muscles. With new technical developments it is now possible to acquire enough signal from a few contractions of a single muscle recorded in 1 ms time bins. What is seen in Figure 6 confirms previously reported 'pooled' results from several contractions of several different muscles at lower time resolution (Harford and Squire, 1992). Of particular note is that the timecourses of the 10 and 11 intensity changes are clearly different. Changes in the 10 intensity (actually a drop shown inverted in Fig. 6) are roughly in step with the tension, whereas the 11 reflection changes much faster than tension, overshoots and then returns to a steady level at the tension plateau. This behaviour would not be produced by a single attached conformation of myosin heads on actin. Something much more complicated involving at least two structurally different actin-attached states must be involved.

CONCLUSIONS

We have shown that, although small atomic movements cannot be studied this way, modelling from low-angle X-ray diffraction patterns can provide an indication of mass movements of a few Å within and between proteins and protein domains and can be monitored on a millisecond timescale. These movements can be modelled provided that there is not a total structural reorganisation within the protein. Small changes in the relative atomic positions within a sub-domain cannot be determined by this method. The example of the actin filament shown above has used simple spherical shapes to model the actin sub-domains. We have shown elsewhere (Squire *et al.*, 1994; AL-Khayat *et al.*, 1995) that, at the resolution being considered, the computed diffraction pattern from such a model compares well with patterns computed using all the atomic positions. The subtleties of the changing

interactions at the atomic level can be studied by other approaches, including molecular graphics and modelling, in some cases based on the sub-domain movements determined from low-angle diffraction data.

Protein crystallography is an immensely powerful method in structural biology. Time-resolved low-angle X-ray fibre diffraction can build on and complement this technique to show the general arrangement of proteins in oriented macromolecular assemblies and can reveal molecular and sub-molecular movements on a microsecond or millisecond timescale. Improving facilities at synchrotron radiation sources (e.g. the ESRF at Grenoble, France; SPring-8 in Japan; the APS in the USA, etc.) are now opening up even greater opportunities to capitalise on the complementary powers of the macromolecular crystallography and time-resolved low-angle X-ray diffraction methods. The biggest single need at these 3rd generation sources is to have very fast readout detectors such as RAPID (Lewis *et al.*, 1996) which can cope with high fluxes, have a high dynamic range and can be read out on a timescale, milliseconds or better, at which interesting biological events occur. Without such detectors the high brilliance of the new low-angle beamlines, which potentially renders them ideal for time-resolved X-ray diffraction studies of muscle such as those described here, will never be fully exploited.

Acknowledgements

The work described here was supported by project grants from the BBSRC and the Wellcome Trust (#061729) and the data analysis made use of CCP13 programs (BBSRC/ EPSRC grant #25/B15281). We acknowledge the help of Ngai-Shing Mok in preparing Figure 3 and of Dr. Liam Hudson in preparing Figure 4.

REFERENCES

- [1] AL-Khayat, H.A., Yagi, N. & Squire, J.M. (1995) Structural changes in actin-tropomyosin during muscle regulation: computer modelling of low-angle X-ray diffraction data. *J. Mol. Biol.* **252**, 611-632.
- [2] Bliss, N., Bordas, J., Fell, B.D., Harris, N.W., Hellsby, W.I., Mant, G.R., Smith, W. & Towns-Andrews, E. (1995) W16.1: A new fixed wavelength diffraction station at the SRS Daresbury. *Rev. Sci. Instrum.* **66**, 1311-1313.
- [3] Duke, E.M., Hadfield, A., Martin, J.L., Clifton, I.J., Hajdu, J., Johnson, L.N., Reid, G.P., Tretham, D.R., Bruce, I. & Fleet, G.W. (1991) Towards time-resolved diffraction studies

- with glycogen phosphorylase. *CIBA Foundation Symp.* **161**, 75-86 (& 86-90).
- [4] Harford, J.J. & Squire, J.M. (1986) "Crystalline" myosin cross-bridge array in relaxed bony fish muscle: low-angle X-ray diffraction from plaice fin muscle and its interpretation. *Biophys. J.*, **50**, 145-155.
- [5] Harford, J.J. & Squire, J.M. (1992) Evidence for structurally different attached states of myosin cross-bridges on actin during contraction of fish muscle. *Biophys. J.*, **63**, 387-396.
- [6] Harford, J.J., Denny, R.C., Hudson, L., Mendelson, R., Morris, E.P. & Squire, J.M. (1998) Myosin head configurations in relaxed, active, rigor and S1-labelled fish muscle: Evidence for characteristically distinct states. *Fibre Diffraction Review* **7**, 45-50.
- [7] Hajdu, J. & Johnson, L.N. (1990) Progress with Laue diffraction studies on protein and virus crystals. *Biochemistry* **29**, 1669-1678.
- [8] Helliwell, J.R. (2002) New opportunities in biological and chemical crystallography. *J. Synchrotron Radiation* **9**, 1-8.
- Helliwell, J.R. & Rentzepis, P.M. (1997) *Time-Resolved Diffraction*. (Pp. 454). Clarendon Press.
- [9] Holmes, K.C. (1994) Solving the structures of macromolecular complexes. *Structure* **2**, 589-593.
- [10] Holmes, K.C., Popp, D., Gebhard, W. and Kabsch, W. (1990) Atomic model of the actin filament. *Nature* **347**, 44-49.
- [11] Hudson, L., Harford, J.J., Denny, R.J. & Squire, J.M. (1997) Myosin head configurations in relaxed fish muscle: resting state myosin heads swing axially by 150Å or turn upside down to reach rigor. *J. Mol. Biol.* **273**, 440-455.
- [12] Irving, M., Lombardi, V., Piazzesi, G. & Ferenczi, M.A. (1992) Myosin head movements are synchronous with the elementary force-generating process in muscle. *Nature* **357**, 156-158.
- [13] Kabsch, W., Mannherz, H.G., Suck, D., Pai, E.F. & Holmes, K.C. (1990) Atomic structure of the actin: DNaseI complex. *Nature* **347**, 37-44.
- [14] Kaplan, J.H., Forbush, B. III & Hoffman, J.F. (1978) Rapid photolytic release of adenosine 5'-triphosphate from a protected analogue: utilization by the Na:K pump of human red blood cell ghosts. *Biochemistry* **17**, 1929-1935.
- [15] Lewis, R.A., Hall, C., Parker, B. Jones, A., Helsby, W., Sheldon, J., Clifford, P., Hillen, M. & Fore, N. (1996) The "RAPID" high rate area X-ray Detector System. *Fibre Diffraction Review* **5**, 30-34.
- [16] Lombardi, V., Piazzesi, G., Ferenczi, M.A., Thirlwell, H., Dobbie, I. & Irving, M. (1995) Elastic distortion of myosin heads and repriming of the working stroke in muscle. *Nature* **374**, 553-555.
- [17] Lorenz, M., Popp, D. & Holmes, K.C. (1993) Refinement of the F-actin model against X-ray fiber diffraction data by the use of a directed mutation algorithm. *J. Mol. Biol.*, **234**, 826-836.
- [18] Martin-Fernandez, M.L., Bordas, J., Diakun, G., Harries, J., Lowy, J., Mant, G.R., Svernnson, A. & Townes-Andrews, E. (1994) Time-resolved X-ray diffraction studies of myosin head movements in live frog sartorius muscle during isometric and isotonic contractions. *J. Mus. Res. Cell Motil.* **15**, 319-348.
- [19] Maruta, S., Aihara, T., Uyehara, Y., Homma, K., Sugimoto, Y. & Wakabayashi K. (2000) Solution structure of myosin-ADP-MgFn ternary complex by fluorescent probes and small-angle synchrotron X-ray scattering. *J. Biochem.* **128**, 687-94.
- [20] Perkins, S.J., Gilbert, H.E., Aslam, M., Hannan, J., Holers, V.M. & Goodship, T.H. (2001) Solution structures of complement components by X-ray and neutron scattering and analytical ultracentrifugation. *Biochem Soc Trans.* **30**, 996-1006.
- [21] Perkins, S.J., Smith, K.F. & Sim, R.B. (1993) Molecular modelling of the domain structure of Factor I of human complement by X-ray and neutron solution scattering. *Biochem. J.* **295**, 101-108.
- [22] Squire, J.M. (1997) Architecture and function in the muscle sarcomere. *Curr. Opin. Struct. Biol.* **7**, (1997) 247-257.
- [23] Squire, J.M. & Morris, E.P. (1998) A new look at thin filament regulation in vertebrate skeletal muscle. *FASEB J.* **12**, 761-771.
- [24] Squire, J.M., Harford, J.J. & AL-Khayat, H.A. (1994) Molecular movements in contracting muscle: towards Muscle - The Movie. *Biophys. Chem.*, **50**, 87-96.
- [25] Squire, J.M., Cantino, M., Chew, M., Denny, R., Harford, J.J., Hudson, L. & Luther, P.K. (1998) Myosin rod packing schemes in vertebrate muscle thick filaments. *J. Struct. Biol.* **122**, 128-138.
- [26] Sugimoto, Y., Tokunaga, M., Takezawa, Y., Ikebe, M. & Wakabayashi, K. (1995) Conformational changes of the myosin heads during hydrolysis of ATP as analyzed by X-ray solution scattering. *Biophys. J.* **68** (4 suppl) 29s-34s.
- [27] Svergun, D.I. & Koch, M.H.J. (2002) Advances in structure analysis using small-angle scattering in solution. *Curr. Op. Struct. Biol.* **12**, 654-660.
- [28] Taubes, G. (1994) X-ray movies start to capture enzyme molecules in action. *Science* **266**, 364-365.
- [29] Wriggers, W., Milligan, R.A. & McCammon, J.A. (1999) Situs: A package for docking crystal structures into low-resolution maps from electron microscopy. *J. Struct. Biol.* **125**, 185-195.