X-ray Diffraction Recordings from Single Sarcomere within Isolated Myofibril

The third-generation synchrotron radiation sources can focus their intense X-ray beams (10^{13}~10^{15} photons/sec) in a small area of ~0.01 mm^2, and a practical level of X-ray intensity remains even after the beam diameter is decreased to a micrometer-size. Such very thin X-ray beams, or X-ray microbeams, now have a wide range of applications including diffraction, element analysis and X-ray microscopy.

X-ray diffraction using microbeams (microdiffraction) has been used for analyzing local structures of synthetic and biopolymers, and hard dried tissues such as hair and feather. This technique is also potentially applicable to hydrated biological specimens, and is expected to be particularly effective for analyzing minute structures inside living cells (organelles). The first example of microdiffraction recording from hydrated biological specimens is that from single myofibrils of insect flight muscle, revealing that the entire myofibril (diameter, ~3 μm; length, ~3 mm) has common crystal lattice planes and therefore can be regarded as a single giant protein crystal [1]. The estimated volume of the specimen in the beam was ~10,000 μm^3.

However, most organelles of interest are usually much smaller than this, requiring longer exposure times with increased risks of radiation damage; hydrated biological specimens are much more vulnerable to radiation damage than dry biopolymers. To overcome this problem, we have developed a technique of quick-freezing hydrated biological specimens and recording microdiffraction patterns while the specimens are kept frozen [2]. It is known that the radiation damage can be greatly reduced by lowering the temperature, and the fine structure of the specimens can be preserved if they are frozen properly.

Figure 2 shows a series of microdiffraction patterns recorded, at **BL40XU** beamline, from quick-frozen single myofibrils isolated from an insect flight muscle. The orientation of the incident microbeam was made perpendicular to the myofibrillar axis, and this configuration generates a number of “equatorial” reflections arising from the hexagonal array of myofilaments (Fig. 1). In Fig. 2, the strongest equatorial reflections (1,0 and 2,0 reflections) are observed.

The series of diffraction patterns shown in Fig. 2 were obtained by scanning the specimen with respect to the microbeam. From the change in the intensity of reflection, one can estimate the size of the diffracting object. Figure 3(a) shows the change in 1,0 reflection intensity as the specimen is scanned across its long axis. The analysis indicates that there are two objects next to each other, one diffracting more strongly than the other. After corrections for microbeam diameter, the diameter of both objects was estimated to be ~3 μm, i.e., the diameter of a myofibril.

Generally, only a limited number of reflections are observed at the same time in a diffraction pattern from a crystal. This is because of the “Bragg condition”, which requires that the incident beam make a specific angle with respect to a lattice plane to generate reflections. For example, when the condition is met for the 1,0 and 2,0 reflections, the 1,1 reflection should

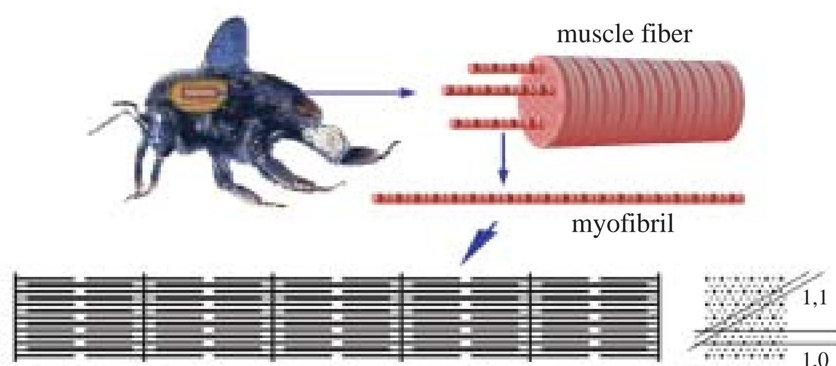

Fig. 1. Structure of myofibril from insect flight muscle, consisting of sarcomeres (minimal functional unit of muscle, ~3 mm in length) connected in series. In each sarcomere, the myofilaments are packed into a hexagonal array (lower right). Two lattice planes (1,0 and 1,1) are indicated.

not be observed. In the textbook diffraction patterns from muscle, however, the three reflections are always observed at the same time (dashed pale-green curve in Fig. 3(b)). This is because the specimen in usual settings (muscle cell or whole muscle) contains a large number of myofibrils with random lattice orientations. On the other hand, the 1,1 reflection is completely missing from the diffraction pattern from a myofibril (solid dark-green curve in Fig. 3(b)). This is what is expected for a single crystal, and therefore, another piece of evidence that the specimens were indeed single myofibrils.

Because the beam diameter was ~2 μm, i.e., shorter than a single sarcomere, it is considered that the diffraction patterns shown in Fig. 2 came from a single sarcomere within a single myofibril. The volume of specimen in the beam is estimated to be ~10 μm^3, i.e., 1/1,000 of the previous record [1]. This makes the similarly sized organelles realistic targets for structural analyses by X-ray microdiffraction.

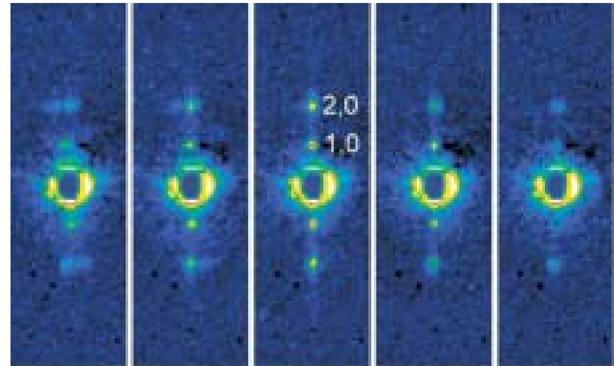

Fig. 2. Series of diffraction patterns from two quick-frozen single myofibrils (or single split myofibril) recorded by scanning the specimen by 0.87 μm steps.

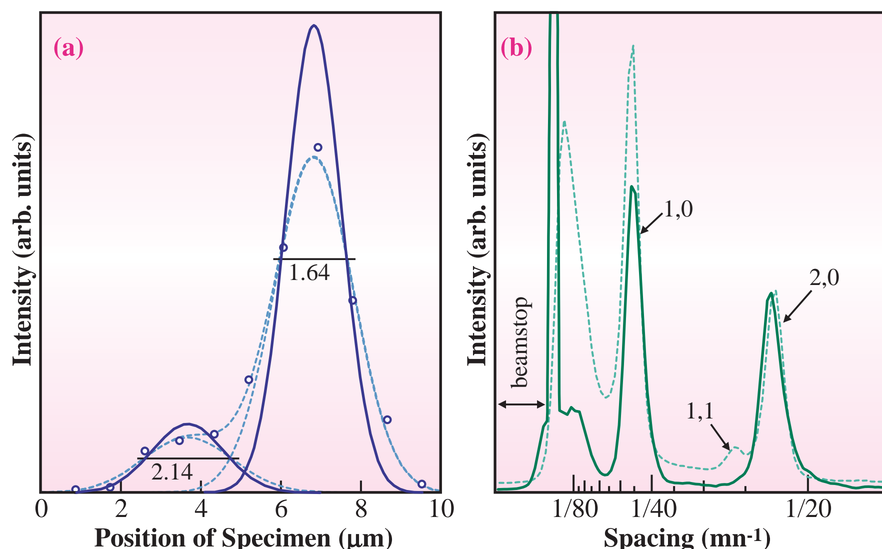

Fig. 3. **(a)** Intensity of 1,0 reflection as a function of the specimen position with respect to the beam. This provides information about the size of the specimen. The numbers indicate the full widths at half maximum of the two myofibrils. Dashed and solid curves represent the best-fit Gaussian distributions before and after correction for beam size, respectively. **(b)** Intensity profile of one of the diffraction patterns in Fig. 2 (solid dark-green curve), which lacks 1,1 reflection observed in the diffraction from bulkier muscle cell (dashed pale-green curve). All the figures except for the upper drawings shown in Fig. 1 are reproduced from [2] with permission from IUCR.

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References

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