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Neutron Fiber Diffraction of Frog Muscle with the Contrast Variation Measurements

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Neutron scattering and diffraction has a unique ability that information on variation of scattering length density of a particle can be obtained by applying the "contrast variation" method. Here we applied this method to fiber diffraction. Neutron fiber diffraction patterns of "live" frog muscle, perfused with Ringer's solution containing a various amount of D_2O, were measured with the small-angle scattering instrument, SANS-J, of JAERI. Analysis of the contrast dependence of the amplitude of each reflection showed that there is definite variation of the scattering length density within the fibrous protein complexes in muscle.

KEYWORDS: neutron diffraction, fiber diffraction, skeletal muscle, contrast variation

§1. Introduction

Neutron scattering and diffraction have a specific feature that X-ray scattering and diffraction cannot have. Because of the large difference in the neutron scattering length of hydrogen and deuterium, the contrast of molecules against the solvent can be varied over a very wide range by changing the amount of D_2O in the solvent so that any component of biological macromolecules, proteins, nucleic acids, lipids, etc., can be matched by the solvent of particular H_2O/D_2O ratio. This "contrast variation" method is widely used in the area of solution scattering to extract the variation of the scattering length density within the molecule or the macromolecular complex.[1,2] This method is, however, not restricted to solution scattering but applicable to all types of low-resolution measurements. Low-resolution neutron crystallography, for example, has been successfully applied to extract information on the disordered region in various macromolecular complexes such as membrane protein-detergent complexes, viruses, and lipoproteins.[3–5] Here we applied this method to neutron fiber diffraction. We performed neutron diffraction measurements of skeletal muscle in living state.

In a muscle cell, two kinds of basic muscle proteins, myosin and actin, form two kinds of helical structures, the thick and the thin filaments respectively, complexed with other muscle proteins, and these two filaments are packed into a hexagonal lattice.[6] Understanding these structures in detail is important in elucidating the molecular mechanism of the muscle contraction. Neutron fiber diffraction studies of muscle were done in 1970s,[7,8] in which it was suggested that the variations of the scattering length density within the helical structures in the muscle are possible. Following these pioneering studies, we did more detailed measurements.

§2. Materials and Methods

Sartorius muscle was dissected from a live bullfrog (*Rana catesbeiana*), and mounted on a specimen chamber for neutron scattering experiments. Ringer's solutions containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 3 mM HEPES/NaOH (pH 7.2) in various D_2O concentrations (0%, 18.7%, 57.7%, 72.3%, and 92.9% D_2O) were prepared, and the oxygenated Ringer's solution was circulated through the chamber at about 10°C until the D_2O concentration in the muscle was reached the equilibrium. This process was monitored by measuring transmission of neutrons through the sample. It took about an hour to reach the equilibrium.

The neutron diffraction measurement was then carried out, during which the oxygenated Ringer's solution was circulated through the chamber at about 10°C. The muscle was kept under the "rest" state where no force was produced under this experimental condition. The neutron fiber diffraction patterns were measured with the Small-Angle Neutron Scattering Instrument (SANS-J) at the guide hall of the reactor JRR-3M in Japan Atomic Energy Research Institute. Neutrons with the wavelength of 0.653 nm ($\Delta\lambda/\lambda = 13.8\%$) were employed. Data were collected with a two-dimensional position-sensitive detector at a specimen-to-detector distance of 3.5 m. The exposure time was between 10 hours (for the muscle in 92.9% D_2O) and 36 hours (for the muscle in 0% D_2O (H_2O)).

The obtained patterns were scaled by the intensity of the incident neutrons and the sample transmissions. Subsequent data reduction was done with the CCP13 software suite XFIX[9] and XFIT.[10] Background intensity of the pattern was subtracted by the method of iterative low-pass filtering.[11] Integrated intensity of each reflection in the pattern was then calculated as follows. Rectangular regions containing the reflections of interest were selected, and integrated along the direction perpen-

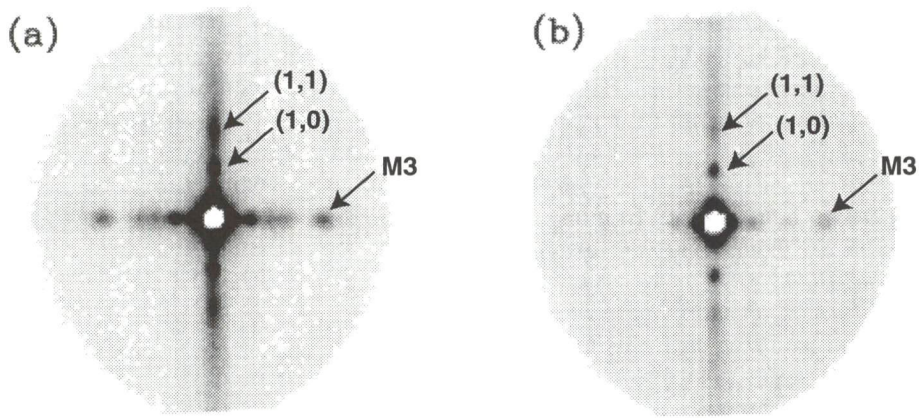

Fig. 1. Examples of neutron fiber diffraction patterns of frog sartorius muscle. Background was subtracted by the method described in §2. D_2O concentrations in the solvents were (a) 92.9% and (b) 0% (H_2O), respectively. The fiber axis of the muscle is set horizontally. The most prominent reflections, equatorial (1,0) and (1,1) reflections and the meridional reflection at 1/14.3 nm (M3), are indicated by arrows. Grey level in the patterns are liniear with the intensity.

dicular to the equator. The obtained profiles along the equator were fit with Gaussian peaks on the polynomial background. The area of the Gaussian peak was calculated to be the integrated intensity of the reflection.

§3. Results and Discussion

Figure 1 shows examples of the neutron diffraction patterns of muscle in 92.9% D_2O and in 0% D_2O (H_2O), recorded with the fiber axis set horizontally. Resolution of these measurements was about 14 nm. Prominent equatorial reflections, which can be indexed as 100 and 110 reflections (referred to as (1,0) and (1,1) below) from the hexagonal lattice in the muscle structure, and the meridional reflection at 1/14.3 nm (M3) coming from the myosin head axial repeat in the thick filaments, were clearly observed. Less prominent other reflections ((2,0), (2,1), and (3,0)) on the equator, the meridional reflections in the lower q-region than M3, and the off-meridional myosin first layer line, were also observed. These reflections were observed in all patterns measured in different D_2O concentrations, although the intensity of each reflection and the background levels were different. It was thus possible to analyze the contrast dependence of the amplitudes of these reflections.

The scattering amplitude, i.e., a square root of the integrated intensity calculated as described in 2, of each reflection was plotted against D_2O concentration in the sample. Figure 2 shows the contrast dependence of the scattering amplitudes of some of the observed reflections. Fitting of the contrast dependence with a straight line is also shown. At this resolution, phases of the equatorial reflections can be assumed to be either 0 or π.[12] The meridional reflections due to the thick filaments are also assumed to have the phases of 0 or π.[7] Intercepts of these lines with the abscissa indicate the contrast-matching points of the reflections. It is clearly seen

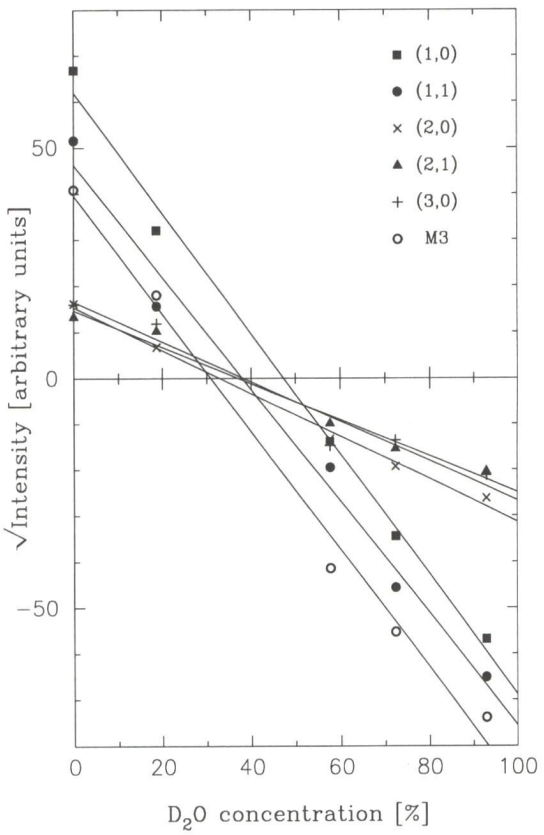

Fig. 2. Contrast dependence of the scattering amplitudes of some of the reflections on the equator and the meridian. Fitting with straight lines is also shown.

that there are reflections having the different contrast-matching points. Particularly notable is that the matching point of (1,0) was about 47% D_2O while that of (1,1) was about 40% D_2O. This is concomitant with the result that the ratio of the integrated intensity of (1,0) and that

of (1,1) was changed from the values larger than unity to those smaller than unity as the D_2O concentration in the sample was increased. This is reminiscent of the fact that when the physiological state of the muscle is changed from the relaxing state to the rigor state, this ratio changes from the value larger than unity to that smaller than unity, reflecting the structural change of the muscle.[12] Thus, there are two possibilities for the differences in the matching point: the one is attributed to the true contrast effects, the other is to the structural change similar to that occuring in the transition from the relaxing state to the rigor state when the solvent around the muscle is changed from H_2O to D_2O. To clearify these possibilities, X-ray diffraction measurements were done.

X-ray diffraction patterns of frog sartorius muscles, in equilibrium with Ringer's solution either in H_2O or in D_2O, were recorded with the imaging plates. The patterns were virtually the same in H_2O and in D_2O (data not shown). It is therefore concluded that the difference in the integrated intensity of the reflections in different D_2O concentration was due actually to the effects of the contrast.

Such differences in the matching points of the reflections indicate the definite variation of the scattering length density in the unit cell of the muscle structure. Detailed analysis using, for example, Fourier synthesis should provide information on the location of the density variation within the structures of the muscle. The analysis by the density maps computed by Fourier synthesis is currently underway, and the results will be described elsewhere.

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