The life science which the 5MW spallation neutron source provides.

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Abstract

Structural biology is one of the most important fields in the life sciences that will interest human beings in the 21st century. Neutrons can provide not only the positions of hydrogen atoms in biological macromolecules but also information on the dynamic molecular motions of hydrogen atoms and water molecules. The next generation neutron sources, the performance of which is expected to be 100 times better than that of the existing reactor JRR-3M, will be expected to provide a breakthrough in the life sciences of the 21st century.

1. INTRODUCTION

It is said that the universe began with the so-called big bang about 15 billion years ago and the first living cell was born about 3.5 billion years ago. As a consequence of evolutionary processes, a tremendous number of diverse living cells from different organisms exist on the earth. Human beings, for example, are constructed from many living cells with a total number of proteins of about 100 thousand. In the latter half of 20th century, X-ray single crystal structure analysis has been used to determine the three dimensional structures of biological macromolecules such as proteins and DNA, to bring many of the mysteries of life sciences to light. This structural information contributes to the understanding of the physiological function of proteins and DNA. This field is called structural biology.

Structural biology is one of the most important fields in the life sciences that will interest human beings in the 21st century. There is already clear evidence that hydrogen atoms and water molecules around proteins and DNA play a very important role in many physiological functions. However, since it is very hard for X-rays to determine the positions of hydrogen atoms in protein molecules, detailed discussion of protonation and hydration can be only speculative in many cases.

On the other hand, neutrons can provide not only the positions of hydrogen atoms in biological macromolecules but also information on the dynamic molecular motions of hydrogen atoms and water molecules. Since physiological functions accompany microscopic motion of atoms at active sites, neutron inelastic scattering is expected to provide experimental clues to clarify physiological functions on a microscopic scale. In principle, these contributions have been well identified, but there are only a few examples experimentally determined at present. Nevertheless, the results obtained have contributed to the field of the structural biology.

In the last section we show how the next generation neutron sources, the performance of which is expected to be 100 times better than that of the existing reactor JRR-3M (20 MW) in Japan Atomic Energy Research Institute (JAERI), will be expected to provide a breakthrough in the life sciences of the 21st century.

2. NEUTRON SCATTERING FOR STRUCTURAL BIOLOGY

A neutron has the following distinctive features of value to investigations in structural biology:

1) a neutron can identify hydrogen in a protein,

2) a neutron can distinguish deuterium from hydrogen,

3) a neutron can observe dynamics of atoms and molecules, and

4) a neutron does not cause radiation damage to a protein.

In order to apply these features to structural biology, single crystal neutron diffractometry, small angle neutron scattering and inelastic neutron scattering experiments are carried out.

3. HYDROGEN AND HYDRATES OF BIOLOGICAL MACROMOLECULES

The three dimensional structure determinations of biological macromolecules such as proteins and DNA by X-ray single crystal structure analyses has helped shed some light on many of the mysteries involved in life processes. At the same time, these results have clearly suggested that hydrogen atoms and water molecules around proteins and DNA play a very important role in many physiological functions. However, since it is very hard to determine the positions of hydrogen atoms in protein molecules using X-rays alone, a detailed discussion of protonation and hydration sites can only be speculated upon so far. In contrast, it is very well-known that neutron diffraction provides an experimental method of directly locating hydrogen atoms. In order to carry out this research in Japan Atomic Energy Research Institute (JAERI), two kinds of diffractometers

dedicated for protein crystallography have been constructed where technical innovations such as a neutron imaging plate [1] and a monochromator [2] had to be developed. By using these diffractometer, neutron diffraction experiments from rubredoxin, myoglobin and hen egg-white lysozyme have been carried out and the several results of these proteins are reported in the paper.

BIX-3

Laue diffractometry [3] is useful for quick data collection, but suffer from an upper limit of 2Å in resolution because of background problems. On the other hand, the fixed crystal method (using a monochromatized neutron beam and an area detector) provides the best S/N ratio, although this technique needs a large amount of computer storage since the sample crystal rotates at a small step angle and many frames of data collected at various positions need to be stored in memory. More importantly, this method is more time-consuming than the Laue diffractometry. However, especially in neutron protein crystallography, the most important thing is to collect a data set under the best S/N conditions since the neutron diffraction technique virtually guarantees the detection of all the hydrogen atom positions in proteins once data have been collected. Thus, the monochromatized beam utilization technique is expected to be the method of choice for collecting high resolution data (1.5Å or better) although it takes much machine time.

Monochromatized neutron beams represent the oldest and most classical sources of neutrons, and most of the previous neutron protein crystallographic data sets have been collected using this type of radiation. Monochromatized neutron utilization is still charming in its way as discussed in comparison with Laue methods. In this method, a monochromator is indispensable in order to obtain a neutron beam of a specific wavelength from a 'white radiation' source. At JAERI, a new type of elastically-bent perfect-Si crystal (EBP-Si) monochromator for a neutron diffractometer, dedicated to protein crystallography, has been developed [2]. The EBP-Si monochromator has been successfully applied to a diffractometer named as BIX-3 [4] at JAERI.

Rubredoxin

With this BIX-3 a single-crystal neutron diffraction analysis of the structure of small protein rubredoxin from the hyperthermophile Pyrococcus furiosus is currently under way. [5] Rubredoxins are small protein containing an iron atom coordinated by sulfur atoms of four cysteine side chains. Although the physiological role for rubredoxins have not been definitively established, it is likely that they function as electron transfer proteins. Despite the uncertainty of its function in most species, rubredoxins from different organisms have been extensively studied by structural and spectroscopic methodologies since rubredoxin from the hyperthermophile Pyrococcus furiosus is extraordinary stable in heat. In order to understand the stability, very high resolution X-ray crystallography study has been carried out by comparing the structure between wild and the mutant which is not so stable in heat, but the special differences of backbone structure between them could not been found. [6] We assume that there might be differences in hydrogen bonding and/or hydration structures and as a first step a neutron diffraction study of the wild type of rubredoxin with BIX-3. Data were collected at room temperature up to a resolution of 1.5 Å (so far the highest resolution neutron data set), using wavelength λ=2.35 Å. A single crystal of dimension 2 x 2 x 1 mm, grown via vapor diffusion from 3.6 M NaK phosphate, is being used in this project. Two sets of data from the same crystal, roughly corresponding to the crystal being mounted along the a and c axes, are being collected and merged. Data collection is by the step-scan method, with 0.3° intervals in ϕ and exposure times ranging from 60 to 75 minutes per frame. The completeness factor of the 1.5 Å resolution data set is currently at 78.2%. 301 hydrogen atoms and 49 deuterium atoms are included in the refinement of the structure of rubredoxin. 26 water molecules are also identified. In the present model R factor and R-free are 0.254 and 0.279, respectively.

Fig.1 shows the $|2|F_o| - |F_c||$ map of Tyr 10. Because of the high resolution, the clear hole is seen in the center of the 6 members ring and hydrogen atoms and replaced deuterium atom are clearly seen. The replacement ratio of hydrogen atoms by the deuterium atoms bound to nitrogen, oxygen and sulfur atoms has been determined and it is strongly correlated on ASA and the temperature factors. The results are shown in Fig.2.

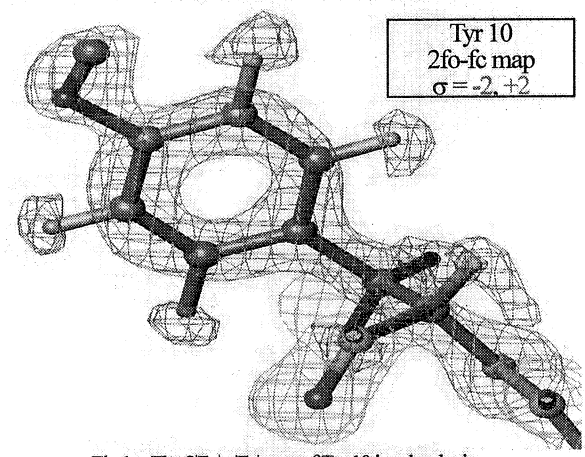

Fig.1 The $2|F_o| - |F_c|$ map of Tyr 10 in rubredoxin.

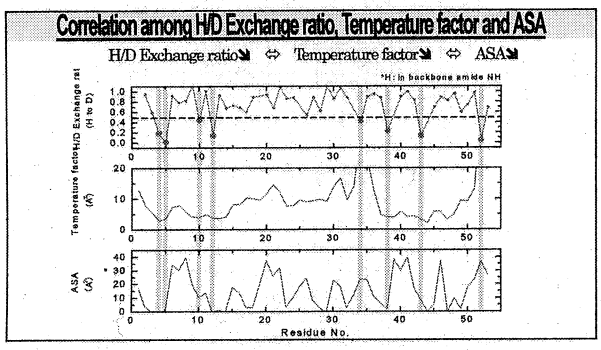

Fig.2 The replacement ratio, ASA and the temperature factor of hydrogen atoms by the deuterium atoms bound to nitrogen, oxygen and sulfur atoms in rubredoxin.

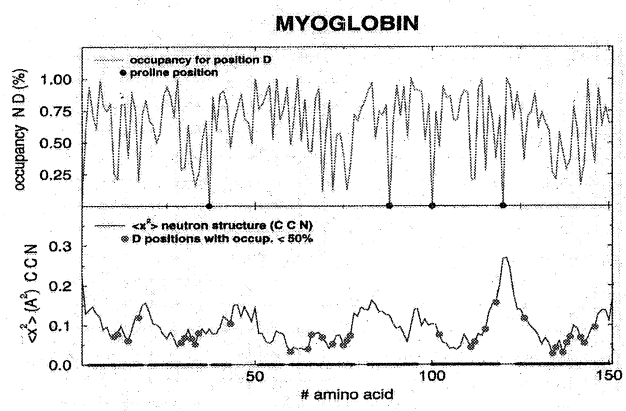

Fig.3 The replacement ratio and mean displacement of hydrogen atoms by the deuterium atoms bound to nitrogen in myoglobin.

Myoglobin

Myoglobin serves as a „model-protein" in biophysics. It is investigated by many research groups with a wide range of physical and biochemical methods. The respiratory heme protein myoglobin from sperm whale which we have used for the neutron diffraction experiments consists of 153 amino acids which form 8 α-helices. .We determined the neutron structure of met-myoglobin up to 1.5Å resolution. The myoglobin crystal (2.5 x 2.5 x 1.0 mm^3) used in this study was soaked in deuterated mother liquor for years. [7] The space group is $P2_1$ with the lattice constants a = 64.8Å, b = 31.1Å, c = 35.0Å and β = 105.8°. Neutron diffraction data were collected at room temperature at the neutron single crystal diffractometer BIX3. This high resolution data set provides the possibility to analyze hydrogen bridges as well as the extent of exchange of hydrogen atoms by deuterium within the protein.

The occupancy of deuterium atoms which corresponds to replacement ratio of hydrogen atoms by the deuterium atoms bound to amide nitrogen atoms has been determined and it is strongly correlated on the local position of amide atoms and the temperature factors as shown in Fig.3 and 4. Fig. 3 shows the occupancy and mean displacement of atoms of deuterium atoms of amide, and Fig.4 shows the location of deuterium atoms with the occupancy less than 50%, and it is clearly localized in the hydrophobic region.

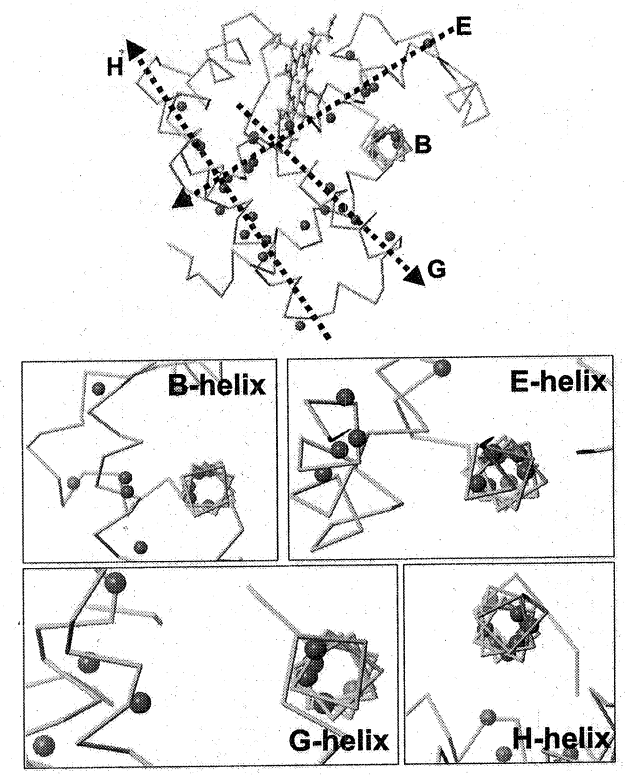

Fig.4 The location of deuterium atoms which has the replacement ratio less than 50% in myoglobin.

Hydrogen bonds in α helix

The α-helix in proteins is one of the fundamental secondary structure. It is famous that the existence of the helix structure model proposed by Pauling was firstly verified by the X-ray crystal structure analysis of myoglobin. The helix structure has been stabilized by the formation of straight N-H··· O hydrogen bonds between N-H group in the amino acid residue (residue) n and C-O group in residue n+4 as originally proposed by Pauling. However, the formation of hydrogen bonds has been characterized by the discussion of the distance between proton donor and acceptor and precise structure of hydrogen bonds

including hydrogen atoms has remained in dispute, simply because it is difficult for X-ray crystallography to identify all the hydrogen atoms in proteins.

On the other hand, neutron diffraction provides an experimental method of directly locating hydrogen atoms and the nature of hydrogen bonds. Neutron diffraction data from tetragonal hen egg-white lysozyme (HEWL) [8] and myoglobin [7] were analyzed and the position of all the hydrogen atoms in both of the protein were identified.

The identification of hydrogen atoms provides us the accurate information of hydrogen bonds in proteins. There are 6 α- helices in a HEWL molecule and 8 α- helices in a myoglobin. The "N-D—O" type intramolecular hydrogen bonds in the α- helices, which play an important role in the stabilization of the α- helices of proteins, were surveyed.

4. DYNAMICS OF BIOLOGICAL MACROMOLECULES

It is expected that most physiological functions correlate strongly with the dynamics of biological macromolecules. The temperature dependence of isotropically-averaged mean-square displacements of atoms of myoglobin from neutron experiments was observed. For temperatures below 200 K, the mean-square displacement increases linearly with temperature, in accord with harmonic models for internal dynamics. Above 200 K there is a transition above which the mean-square displacement increases more rapidly with temperature. This nonlinear behaviour implies an anharmonicity in the potential-energy surface. It should be noted that most physiological functions (such as the processes mentioned earlier: hydrolysis, dehydrogenation, etc.) occur most efficiently at temperatures above 200 K, and not many of them take place below 200 K.

This predicts that protein dynamics are strongly correlated with physiological functions and INS experiments from proteins can be expected to produce useful results.

Dynamics of biological macromolecules are theoretically treated by normal mode analysis or molecular dynamics that can provide the dynamic structure factor observed by INS experiments. The vibrational frequency distribution from the molecular dynamics of BPTI (bovine pancreatic trypsin inhibitor) in solution has been calculated and compared with the neutron-derived frequency distribution (coming from incoherent scattering) from the BPTI in solution experiment and the good coincidence between them has been reported. How neutron scattering can provide unique information on the configurations and dynamics of native and unfolded protein has been already reported. However, there is one mystery in the INS data. No big differences in time-of-flight spectra from BPTI and HEWL are evident except at a large energy transfer region, which is not so relevant for protein dynamics, although the molecular structures as well as physiological functions of the two proteins are different. Why? Is INS useless for studying protein dynamics? In the INS spectra of proteins, the incoherent scattering coming from the nuclear spins of hydrogen atoms in the sample is dominant. Generally speaking, INS observes the quantity $\langle \mathbf{R}_i(0)\mathbf{R}_j(t)\rangle$, where $\mathbf{R}_i(0)$ and $\mathbf{R}_j(t)$ are the position vectors of the i-th atom at t=0 and the j-th atom at t=t, respectively. When the i-th and j-th atoms are different from each other, it is coherent, and when they are the same, it is incoherent. Coherent or incoherent scattering provides the dynamical amplitude between different atoms or of an atom itself, respectively. Big differences among proteins might not appear in the dynamical amplitudes of the atoms themselves. If so, coherent scattering must be separated from incoherent scattering by measuring neutron spin flip and non-spin flip processes.

5. THE NEXT GENERATION NEUTRON SOURCES AND THE FUTURE OF NEUTRON STRUCTURAL BIOLOGY

At present, because of the low level of current neutron fluxes, it takes a prohibitively long amount of time to collect a full diffraction data set, and/or it is necessary to prepare a large specimen. An improvement in neutron flux would be beneficial not only to ease the above limitations but also to enable new experiments to be carried out.

The type of proteins that can be measured by neutron single crystal diffractometry in accordance with increments in incident neutron flux will be discussed. Note that in a diffraction experiment the neutron diffraction intensity is proportional to the crystal volume, and to the inverse square of the cell volume. If the next generation neutron sources project (for example, a several-megawatt spallation neutron source) is realised, the neutron source resulting from the next generation neutron sources project will cover most of the important proteins to be investigated.

To date, neutron structural biology was expected to be entirely complementary to X-ray structural biology, but it has thus far failed to meet this expectation. The next generation neutron sources project will surely initiate a new generation in

neutron structural biology and consequently make a major contribution to the life sciences.

Acknowledgement

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