Insight into the mechanism of active transport by calcium pump

Structural change of calmodulin molecule caused by calcium binding

Calmodulin is a small protein with a molecular weight of 17,000 that is expressed in almost all eukaryotic cells and plays a role of transporting intracellular information. When the calcium concentration in the cell is increased, an external stimulation stimulates the binding of calcium ions and then binds to other proteins such as enzymes, and causes various changes in the cell. Upon binding calcium, the structure of the calmodulin molecule changes, allowing it to bind to other proteins. X-ray crystallography revealed that the calmodulin molecule is extended in the absence of calcium, whereas it becomes globular upon binding calcium ions. However, this change occurs in a short time (milliseconds) and its details remained unclear. In this study, the structural change of the calmodulin molecule was clarified by the small-angle scattering technique using intense X-rays from the undulator beamline BL40XU. When calmodulin molecules were dispersed in solution and the calcium concentration was rapidly increased using a chelating agent that released calcium upon laser irradiation, the radius of gyration (an indicator of molecular size) of the calmodulin molecules decreased by ~25% in ~10 ms (Fig. 1).

BL40XU Yoshiteru Yamada, Hiroyuki Iwamoto, Naoto Yagi (JASRI, Osaka University), were essential to these structure determinations. These studies have revealed that the binding of calcium alone accompanies a surprisingly large-scale rearrangement of both transmembrane and cytoplasmic domains, and that the ion pumps work like mechanical pumps at an atomic scale. Also, the structure of a very strong inhibitor, thapsigargin (TG), bound to this pump was determined and may serve as a template for drugs targeted for membrane proteins. Calcium is a fundamental and ubiquitous factor in the regulation of intracellular processes. Therefore, the atomic structures of the calcium pump in different states have a tremendous impact on many fields, including medical treatment for myocardial diseases and cancer.

Life Science

Size effect of polymer micelles: treatment of intractable pancreatic cancer based on precise control of particle diameter

Recently, a drug delivery system (DDS) using nanoscale carriers, such as polymer micelles, has attracted attention as a means of increasing the efficacy and safety of drugs, including anticancer drugs. In the DDS, a drug is selectively delivered to cancer tissue by encapsulating a Pt-complex anticancer drug with the aim of realizing a DDS effective for treating pancreatic cancer. The mapping of Pt atoms by X-ray fluorescence analysis using BL37XU (Fig. 1) revealed that the Pt complex anticancer drug reached deep into the cancer tissue when encapsulated in polymer micelles with a diameter of ≤30 nm (Fig. 2). The evaluation of anticancer activity also indicated that polymer micelles with a diameter of ≤30 nm showed a marked anticancer activity. Thus, it was clarified that polymer micelles with a diameter of ≤30 nm could be effective for treating pancreatic cancer. Although the efficacy of cancer-targeting treatment using nanoscale carriers has been widely recognized, their size effect was still unclear. We clarified, for the first time in the world, that polymer micelles with a diameter of ≤30 nm could be effective for treating pancreatic cancer, for which no effective treatment method has yet been established. Our research results are expected to lead to the development of an innovative treatment method for pancreatic cancer, the so-called most intractable cancer.

Life Science

Medical Science

Elucidation of “How Newborns Start Breathing?”

A fetus does not require oxygen exchange via the pulmonary system; consequently, the lungs are filled with “lung water” until birth. Immediately after birth, however, the lungs must be inflated by sucking air into the lungs, thereby the newborn can begin breathing in a normal way. If this process does not go well, a newborn cannot breathe, and it will be necessary to use resuscitation measures by which a newborn’s lungs begin breathing. However, there have heretofore been no practical methods for observing the process of lung water being replaced with air, the details of which remain unclear. In this study, a new technique of refraction contrast imaging was developed in which X-rays are superimposed at the edge of an object according to the slight differences in the refractive index of the objects to be observed (Fig. 1). The real-time imaging experiment in a rabbit fetus was carried out at BL30BB (Fig. 2). It was found that air enters the lungs upon breathing and compresses the water from the lungs into tissues. This unexpected phenomenon suggests that inspiration plays an important role in removing lung water. If lung water remains in the lungs after birth, the newborn will go into respiratory distress. To avoid this, mechanical ventilation is required for premature infants or newborns with respiratory disorder. Because of this discovery, processes for mechanical ventilation of newborns must be significantly revised.

Medical Science

X-ray fluorescence imaging

Fig. 1 Calibration of Pt X-ray fluorescence. The Pt content in the sample can be determined from the peak height of Pt X-ray fluorescence.

B) 70 nm micelles

Fig. 2 X-ray fluorescence technique used in radiographs

Fig. 3 Absorption contrast imaging
Two-Layer Convection in Liquid Outer Core of Earth

At the center of the earth, there is a core mainly composed of iron with a diameter of 3,500 km. The metallic core is divided into two regions at a diameter of 6,160 km: a liquid outer core and a solid inner core (Fig. 1). The magnetic field of the earth is induced by the convection of the liquid metal in the outer core. Conventionally, it has been considered from the observation of seismological waves that the convection in the outer core occurs in a single layer. However, the mechanism has not been clarified in detail. In this study, the change in the crystal structure of FeO in the outer core was examined to investigate the convection state in the outer core. Using the high-pressure research beamline (BL10XU) at SPring-8, the crystal structure of FeO, a component of the outer core, was studied at pressures and temperatures similar to those in the outer core. It was found that the sodium chloride (NaCl-type) (B1) structure transformed to a cesium chloride (CsCl-type) (B2) structure at 240 GHz at 4,000 K, similar to the conditions of the middle of the outer core. This is the world’s first demonstration of the existence of a CsCl-type FeO structure. This change in the crystal structure suppresses the convection and may change the convection state in the outer core. A numerical simulation showed that a two-layer convection, instead of single-layer convection as conventionally thought, occurs in the outer core because the convection is suppressed at the depth where the FeO transforms to a CsCl-type structure (Fig. 2).

Origin and evolution of Itokawa regolith

The Hayabusa spacecraft of Japan Aerospace Exploration Agency (JAXA) arrived at the asteroid Itokawa in September 2005 and collected samples of the microparticles (fine sand particles formed upon the impact of meteorites) from its surface. The spacecraft returned to Earth in June 2011, and the analysis of the samples brought back by Hayabusa began. In this study, the three-dimensional structure of Itokawa microparticles was nondestructively examined by X-ray microcomputed tomography (microCT) at SPring-8. Using the projection-type CT system installed at the beamline BL10XU, the three-dimensional external and internal structures of 46 microparticles (50–180 μm in diameter) were determined with a spatial resolution of 0.02 or 0.05 μm. In addition, CT imaging with two different X-ray energies (7 and 8 keV) enabled the estimation of the distribution of elements in the microparticles. The three-dimensional spatial distribution of minerals that constitute the microparticles was nondestructively determined. The obtained abundances of the minerals in all 46 microparticles were similar to that of LL chondrites, which are often found in meteorites. Moreover, textures that might have undergone thermal metamorphism were found. The 3D-axis ratios of the three-dimensional shapes of the microparticles obtained by void approximation were similar to that of pieces obtained in a laboratory impact experiment, suggesting that the microparticles were formed upon impact. Any structures that indicate large-scale melting were not observed in the Itokawa microparticles. This is consistent with relatively low impact velocities (~6 km/s).

The analysis of the Itokawa microparticles in this study revealed the appearance of an active asteroid surface that had never been clarified by conventional observational and meteoritical studies. This is a scientifically significant finding that will contribute to the clarification of the formation process of the solar system.
**Materials Science**

**Visualization of large cage-like network structure of glass and solvated electrons**

Generally, glass is formed by melting a raw material to a melt (liquid) state at high temperature followed by cooling. Not all materials can be made into glass; many materials form crystals instead of glass when a melt is cooled.

To understand the glass formation of materials, the research group focused on CaO-Al₂O₃ systems that form or do not form glass depending on slight differences in the components. The group then rapidly cooled to form glass (Fig. 1). The atomic structures of the glasses were examined by high-energy X-ray scattering and X-ray absorption fine structure (XAFS) at SPring-8 (Fig. 2).

The group utilized large-scale theoretical calculations using a supercomputer to determine the atomic and electronic structures of the glasses. The results indicate that a large “cage-like network structure” (Fig. 3) forms in the materials with a high glass-forming ability. In contrast, such a structure does not exist in the materials with a low glass-forming ability.

Researchers have recently reported that electrode glass can be formed by solvated electrons through the removal of oxygen in a reducing atmosphere. The solvated electrons are located in the cage-like network structure and were found to lead to an energetically stable a glassy structure by the large-scale theoretical calculation.

**Chemical Science**

**Development of high-energy-density, high-safety, and low-cost magnesium rechargeable batteries**

The practical use of rechargeable magnesium batteries, which are superior to lithium ion rechargeable batteries, has been a high-priority research theme. However, high theoretical energy density, magnesium resources are abundant, and rechargeable magnesium batteries are safe. However, divalent magnesium ions have the drawbacks of a strong ionic interaction, difficulty of diffusion in the solid phase, and extremely slow electrode reaction compared with monovalent lithium ions. In addition, no electrolyte for safe and stable operation of magnesium batteries that enables magnesium deposition/dissolution has been found. Therefore, issues related to the cathode and electrolyte should be resolved before the fabrication of rechargeable magnesium batteries. In this study, the research group succeeded in the synthesis of a cathode material (ion-exchanged MgFeSiO₄) in which the diffusion path of magnesium ions is secured by precisely controlling the crystal structure of the cathode material. With the use of MgFeSiO₄, the insertion and extraction of double the number of magnesium ions compared with conventional cathode materials becomes possible. The reason behind the stable and high-capacity reaction of MgFeSiO₄ was examined in detail by powder X-ray diffraction and X-ray absorption spectroscopy using high-brilliance synchrotron radiation X-rays. The results indicate that the three-dimensional structure of Si-O and Fe-O forms a skeleton structure and that the reaction proceeds by the single-phase reaction of the insertion and extraction of magnesium ions while maintaining the three-dimensional crystal structure (Fig. 1(a)). Also, the mechanism of change compensation through the change in the iron valence along with the insertion and extraction of magnesium ions was clarified (Fig. 1(b)). Rechargeable magnesium batteries with the highest performance in the world were realized by combining the MgFeSiO₄ cathode, a newly developed electrolyte solution, and a magnesium metal anode (Fig. 2).

**Materials Science**

**Clariﬁcation of thin-ﬁlm transistor properties using the transparent oxide semiconductor, indium gallium zinc oxide (IGZO)**

A transparent oxide semiconductor, indium gallium zinc oxide (IGaZnO), generally abbreviated as ISZO, is optically transparent because of its wide bandgap of 3.0 eV. In addition, IGZO thin-ﬁlm transistors (TFTs) with electron mobility one order of magnitude higher than that of amorphous silicon TFTs can be easily fabricated. Furthermore, IGZO has a high switching performance. Therefore, ISZO has been widely recognized as a material for next-generation electronics device, such as high-performance transparent transistors, that have been difﬁcult to realize using conventional semiconductors (Fig. 1). In recent years, research and development for the improvement of the performance of devices and for the search of new materials has been active. The analysis of electronic states, such as the tail level, which signiﬁcantly affects TFT performance and stability, has been high-priority research themes. In general, X-ray photoelectron spectroscopy (XPS) is known to be a powerful experimental tool for directly observing electronic states. However, the data obtained by general XPS using soft X-rays are affected by the surface conditions and it is extremely difﬁcult to identify the original electronic states of the sample used as the material of an actual device. In order to overcome this problem, hard X-ray photoemission spectroscopy (HAXPES) using high-energy X-rays (5-10 keV) as excitation light source was developed at SPring-8 for the purpose of analyzing the electronic states, such as the tail level, which signiﬁcantly affects TFT performance and stability, by precisely controlling the crystal structure of the cathode material. With the use of MgFeSiO₄, the insertion and extraction of double the number of magnesium ions compared with conventional cathode materials becomes possible. The reason behind the stable and high-capacity reaction of MgFeSiO₄ was examined in detail by powder X-ray diffraction and X-ray absorption spectroscopy using high-brilliance synchrotron radiation X-rays. The results indicate that the three-dimensional structure of Si-O and Fe-O forms a skeleton structure and that the reaction proceeds by the single-phase reaction of the insertion and extraction of magnesium ions while maintaining the three-dimensional crystal structure (Fig. 1(a)). Also, the mechanism of change compensation through the change in the iron valence along with the insertion and extraction of magnesium ions was clarified (Fig. 1(b)). Rechargeable magnesium batteries with the highest performance in the world were realized by combining the MgFeSiO₄ cathode, a newly developed electrolyte solution, and a magnesium metal anode (Fig. 2).

**Chemical Science**

**Shedding New Light on Efforts toward Elucidation of the Degradation Mechanism in Membrane-electrode Assembly**

The fuel cell is coming into widespread use both in households (e.g., “Eine Farm” residential fuel cell) and industry (typically, commercialization for automobiles) as a next-generation energy source. Still, it faces challenges, including the upgrade of power generation efﬁciency and reduction of power generation cost, to become competitive with conventional energy supply systems. For example, the use of magnesium batteries, which are superior to lithium batteries, has been high-priority research themes. In general, X-ray photoelectron spectroscopy (XPS) is known to be a powerful experimental tool for directly observing electronic states. However, the data obtained by general XPS using soft X-rays are affected by the surface conditions and it is extremely difﬁcult to identify the original electronic states of the sample used as the material of an actual device. In order to overcome this problem, hard X-ray photoemission spectroscopy (HAXPES) using high-energy X-rays (5-10 keV) as excitation light source was developed at SPring-8 for the purpose of analyzing the electronic states, such as the tail level, which signiﬁcantly affects TFT performance and stability, by precisely controlling the crystal structure of the cathode material. With the use of MgFeSiO₄, the insertion and extraction of double the number of magnesium ions compared with conventional cathode materials becomes possible. The reason behind the stable and high-capacity reaction of MgFeSiO₄ was examined in detail by powder X-ray diffraction and X-ray absorption spectroscopy using high-brilliance synchrotron radiation X-rays. The results indicate that the three-dimensional structure of Si-O and Fe-O forms a skeleton structure and that the reaction proceeds by the single-phase reaction of the insertion and extraction of magnesium ions while maintaining the three-dimensional crystal structure (Fig. 1(a)). Also, the mechanism of change compensation through the change in the iron valence along with the insertion and extraction of magnesium ions was clarified (Fig. 1(b)). Rechargeable magnesium batteries with the highest performance in the world were realized by combining the MgFeSiO₄ cathode, a newly developed electrolyte solution, and a magnesium metal anode (Fig. 2).
Magnetism

Visualization of antiferromagnetic spins in spintronic devices

Spintronics is a research field dealing with both charges and spins of electrons and is used in fields such as magnetic recording. Spin valve films are spintronic devices and have been used in the magnetic reading head of hard disk drives (Fig. 1). The spin valve film basically has the ferromagnetic/antiferromagnetic magnetic sandwich layer structure. In the spin valve film, the electric resistance changes in accordance with the relative direction of magnetization of the two ferromagnetic layers. To change the relative direction of magnetization, the direction of the magnetization of one of the ferromagnetic layers must be fixed (exchange bias). It is considered that the exchange bias is induced by the antiferromagnetic spins with respect to the magnetic spins; however, the detailed mechanism behind this phenomenon has not yet been clarified.

It is difficult to detect antiferromagnetic spins by general magnetization measurement methods because spins are oriented in an antiferromagnetic material. When a circularly polarized X-ray is incident on a spin-polarized material, X-ray absorption intensity differs between left and right circularly polarized lights. This is used to X-ray magnetic circular dichroism (XMCD). By adjusting the energy of the incident X-ray with the absorption edge of the target element, the spin-polarized information can be obtained. In this study, XMCD measurement was carried out using BL23SU to detect the unoccupied antiferromagnetic spins induced by exchange coupling between antiferromagnetic and ferromagnetic spins by a technique that is interface sensitive. In the element-specific magnetization curves of O1s and O2p in the antiferromagnetic chromium oxide (Cr2O3) layer, theXMCD spectra with respect to positive and negative magnetic fields were confirmed (Fig. 2A). From these results, it was confirmed that O2p spins are pinned with respect to the magnetic field, and the XMCD signal is caused by the pinned spins in the interface layer (Fig. 2B). These findings are expected to be basic information in the clarification of the origin of exchange bias and the development of spintronic devices.

Magnetism

Photoemission electron microscopy (PEEM)

Visualization of magnetic domain structure at the interface of magnetic thin films

In modern society, we receive great benefit from magnetic recording devices such as memories and hard disks. These magnetic recording devices make use of the phenomenon called exchange bias, and its detailed observation is necessary to use the phenomenon in a wider range of applications. For example, the observation of the exchange coupling of spins between an antiferromagnetic substrate and a ferromagnetic thin film formed on it is required. The element-specific observation of magnetic domains of various magnetic materials is realized by combining photoemission electron microscopy (PEEM), X-ray magnetic circular and linear dichroism (XMCD) in BL17SU and BL23SU. The figure shows the schematic of this experimental method. Depending on the direction of magnetization in the magnetic domain of a sample and the direction of the polarization vector of the incident X-rays, the number of electrons emitted from the surface differs. The image of the region where electrons are emitted is bright whereas the region with fewer electrons being emitted is dark, when the images are magnified using an electron microscope.

In this study, an iron film (thickness: ~0.9 nm) was formed on a nickel oxide (NiO) substrate, which is known as a typical antiferromagnetic material, to analyze the magnetic structures. Using various states of magnetic dichroism at the absorption edges of elements constituting the system, information on (i) the magnetic domain originating from the antiferromagnetic domain of the NiO substrate (yellow frame), (ii) the magnetic domain of the upper layer of the Fe thin film, and (iii) the magnetic domain of the interface in which Fe, Ni, and O are mixed is obtained. The sample was rotated with respect to the incident direction of the light. From the change in the contrast of the obtained images, the spin directions in each magnetic domain were determined. The exchange coupling of spins among the substrate, top layer, and interface was clarified.

The observation technique is considered to be applicable to the development of new materials for inductor circuits, in addition to magnetic recording. At Spring-8, this technique is combined with time-resolved measurement of the movements of the magnetic domain spin with magnetic field applied to an external field. BL17SU, BL23SU, Tohoku University (JASPR)

Industrial Application

Infrared microspectroscopy imaging

Success in visualization of localizations of proteins and lipids in hair

Around the age of 30 years, people’s hair concerns shift from hair damage to hair aging. Curliness, waviness, and dryness are included in the top common hair problems and cause people to feel a change in their hair quality. A well-known change in hair caused by aging is the increase in the number of hair waves. The localization of one hair in such curvy hair has been studied with various methods. However, the localization of lipids in the hair has remained unclear. In this study, the localizations of proteins and lipids were visualized using an infrared (IR) microspectroscopy system at Spring-8. This system combines a microscope and an IR spectrophotometer and can be used to determine the localization of components in a hair fiber. Detailed data on the cross-sectional localization of components in hair have not been available.

Cross-sectional imaging revealed that proteins (amide bands) and lipids (CH bond) were unevenly localized in hair fibers as X-ray magnetic circular dichroism experiment suggested that the inside of the hair had a uniform composition localization (Fig. 1). For curly hair, however, lipids were sparsely localized (the number of CH bonds was small) at sites where proteins were densely localized (the number of amide bands was large). In contrast, the lipids were densely localized at sites where proteins were sparsely localized (Fig. 2). That is, the localizations of proteins and lipids were in an inverse relationship.

In this study, an uneven localization of hair components, such as proteins and lipids, in curly hair was confirmed at the microscopic by visualizing a more detailed localization of hair components than before. It was found that the localization of proteins is different from that of lipids in curly hair; this is the so-called state of “hair distortion”. Therefore, the curviness of hair can be improved by correlating the uneven localizations of proteins and lipids throughout the curly hair.

Molecular Science

Momentum Imaging

Establishment of Four-Dimensional (4D) Nano Design, a Technology for the Development of New Materials Used for High-Performance and High-Quality Tires

The development of fuel-efficient tires is very important from the viewpoint of the global environment, and the development and promotion of technologies related to fuel-efficient tires are strongly desired. Grip performance is realized owing to the energy loss due to the deformation of a tire as a result of its contact with a road surface. Therefore, the two contradictory requirements, i.e., improving the energy efficiency and improving tire performance, should be balanced by advanced methods. In this study, the technique of two-dimensional ultrasmall-angle X-ray scattering (2D-USAXS) with a camera length of 160 m using the high-brightness X-ray from BL20SU was developed. The transmission X-ray scattering (TXS) imaging (2D-USAXS), together with the 2D small-angle X-ray scattering (2D-SAXS) (Fig. 1), were analyzed by the two-dimensional pattern reversal Monte Carlo method (2D-PARM) which was developed at the Earth Simulator supercomputer (Fig. 2).

With the results of the study, a booth-modified polymer was developed by Sumitomo Chemical Co., Ltd., which was used to develop fuel-efficient tires. These tires have approximately 19% (approximately 6% less fuel consumption in JC08 mode) and the wet grip performance was improved by approximately 9% compared with those of conventional tires manufactured by Sumitomo Rubber Industries, Ltd.