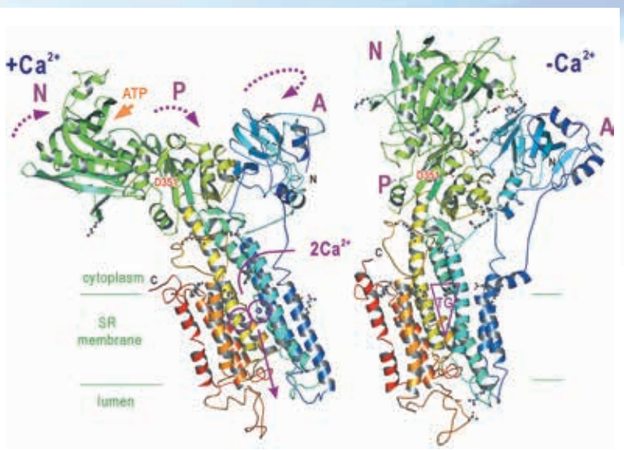
Life Science — Protein Crystallography

Insight into the mechanism of active transport by calcium pump

SPring-8 played a vital role in the recent structure determinations of the sarcoplasmic reticulum (SR) calcium pump in the calcium bound and unbound states. The ATP-driven calcium pump is an integral membrane protein (molecular weight of 110 k) that relaxes muscle cells by pumping calcium released during contraction back into the sarcoplasmic reticulum. The crystals were thin (<20 μm; Ca^{2+}-bound form) or had a very large unit cell dimension (nearly 600 Å; Ca^{2+}-unbound form). Hence, the use of very bright and highly parallel X-ray beam available in undulator beamlines, such as BL41XU (Structural biology I) and BL44XU (Protein Institute, 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.



BL41XU Chikashi Toyoshima (University of Tokyo)

Life Science — Protein solution scattering

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 by an external stimulation, calmodulin binds 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). This indicates that the calmodulin molecules bound calcium ions and became compact. When a peptide was present as a binding partner in the solution, the compact form was stabilized and maintained (Fig. 1, white). Without the binding partner, however, the calmodulin molecules returned to the original extended state in ~150 ms (Fig. 1, black).

The existence of the compact structure observed immediately after calcium binding (Fig. 2) was demonstrated for the first time. This structure corresponds to the crucial state in the transmission of the calcium signal by calmodulin molecules through binding to other proteins. This finding will lead to clarification of cellular functions and to development of agents that target these proteins.

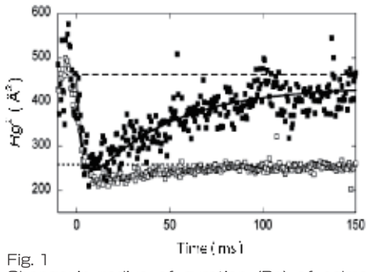


Fig. 1
Change in radius of gyration (Rg) of calmodulin molecules when calcium concentration was increased at time 0

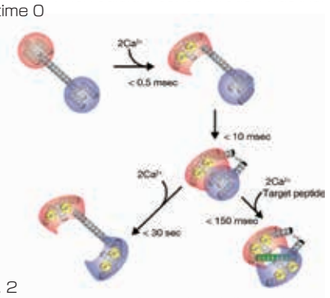


Fig. 2
Structural change of a calmodulin molecule upon calcium binding

BL40XU Yoshiteru Yamada, Hiroyuki Iwamoto, Naoto Yagi (JASRI)

Medical Science — Imaging

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 lung water must be removed so that air can enter 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 die. To avoid such an event, we need to understand the mechanisms by which a newborn's lungs begin breathing. However, because there have heretofore been no practical methods for observing the process of lung water being replaced with air, the details have remained unclear. In this study, the novel technique of refraction contrast imaging was used, in which X-rays are superimposed at the edge of an object according to the slight differences in the refractive index of the object's materials (Fig.1). The real-time imaging experiment on rabbit fetuses was carried out at BL20B2 (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, procedures for mechanical ventilation of newborns must be significantly revised.

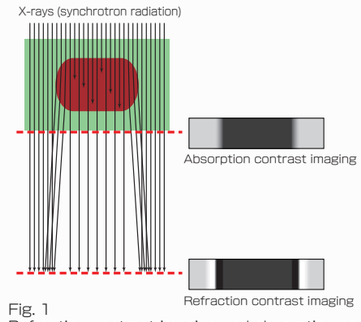


Fig. 1
Refraction contrast imaging and absorption contrast imaging. Absorption contrast imaging is the conventional X-ray imaging technique used in radiographs

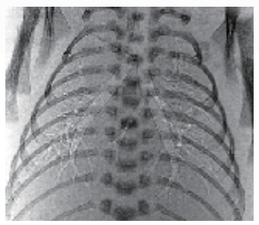


Fig. 2
A chest refraction contrast X-ray image of a rabbit newborn during air feeding

BL20B2 Rob Lewis (Monash University)

Medical Science — X-ray fluorescence imaging

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 the drug in nanoscale carriers. However, no DDS effective for pancreatic cancer has yet been developed because drugs and the DDS cannot reach pancreatic cancer cells. In this study, the research group clarified the size effect of polymer micelles with a precisely controlled particle diameter (≤100 nm) that encapsulated 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 ≤50 nm (Fig. 2). The evaluation of anticancer activity also indicated that the polymer micelles with a diameter of ≥50 nm were not effective in models of human pancreatic cancer, whereas those with a diameter of 30 nm showed a marked anticancer activity. Thus, it was clarified that polymer micelles with a diameter of ≤50 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 ≤50 nm are 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.

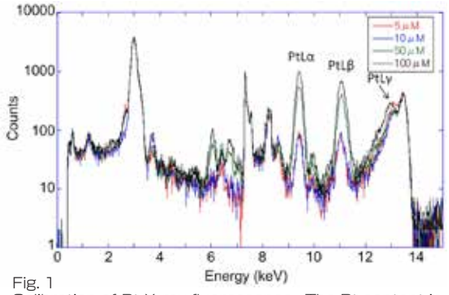


Fig. 1
Calibration of Pt X-ray fluorescence. The Pt content in the sample can be determined from the peak height of Pt X-ray fluorescence

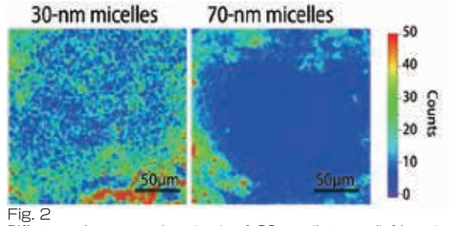


Fig. 2
Difference in penetration depth of 30-nm-diameter (left) and 70-nm-diameter (right) polymer micelles into a model of human pancreatic cancer, clarified by Pt elemental distribution mapping

BL37XU Horacio Cabral, Kazunori Kataoka (The University of Tokyo)