Mechanism of Water-Splitting Reaction in Photosynthesis

The water-splitting and oxygen-evolving reaction in photosynthesis is extremely important as it supports the lives of oxygen-breathing organisms on earth by converting light energy into chemical energy used by these organisms and supplies oxygen to the atmosphere. This reaction is carried out by a membrane protein complex called photosystem II. The cyanobacterial photosystem II consists of 17 transmembrane proteins and three peripheral membrane proteins, and forms a dimer with a molecular weight of 350 kDa for each of the monomers. The crystal structure of this dimer was analyzed at a resolution of 1.9 Å using the synchrotron radiation at SPring-8 (Fig. 1, left). As a result, not only the structure of each subunit and the organization of components contributing to the electron transfer are revealed, but also the detailed structure of the catalyst for the water-splitting and oxygen-evolving reaction contained in the dimer is clarified. This catalyst has a composition of Mn_4CaO_5 and resembles the shape of a "distorted chair", with Mn_3CaO_4 forming a cubane-shaped "seat" and the fourth Mn ion connecting to the outside of the cubane by an oxo-oxygen (Fig. 1, right). One of the reasons for the distortion of the cubane shape is the long bond distance between one of the five oxygen atoms, called O5, and the surrounding Mn ions. This observation suggests the possibility that O5 is located in a special position and is cut out during reaction, serving as one of the substrate oxygen atoms for the formation of the oxygen molecule. To demonstrate this, the intermediate states of the water-splitting reaction (S_2 and S_3 states) were generated by flash irradiation and observed in a pump-probe experiment conducted using the X-ray free electron laser at SACLA. The results showed that a new water molecule (O6) is inserted not in the S_2 state but in the S_3 state in a position close to O5, suggesting that an oxygen–oxygen bond is formed between O5 and O6, releasing molecular oxygen (Fig. 2).

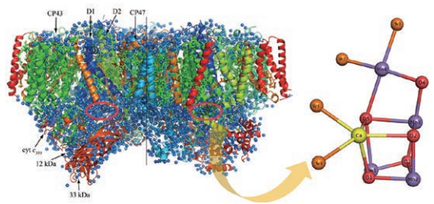

Fig. 1 Overall structure of photosystem II dimer (left) and structure of the Mn_4CaO_5 cluster (right).

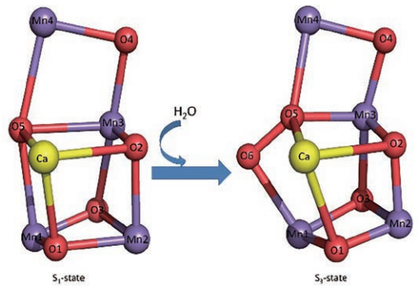

Fig. 2 Change in structure of Mn_4CaO_5 cluster during water-splitting reaction in photosystem II. The figure shows the structure in the S_1 state in the dark and that in the S_3 state generated by two-flash irradiation.

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Protein density measurement in eye lens by phase-contrast CT

The eyeballs of vertebrates consist of soft tissues such as cornea, lens, retina. Since soft tissues do not absorb X-rays sufficiently, they are barely visible by X-ray absorption imaging methods such as radiography. However, by using the phase-contrast X-ray CT method that uses phase change of X-rays, each tissue can be clearly visualized (Fig. 1). In particular, the shape of the lens and the density distribution of major structural proteins (crystallins) within the lens can be measured with high precision. The protein density distribution can be converted via a linear equation to the refractive index which is distributed as a gradient and this determines lens refraction and provides high image quality needed by the eye. At SPring-8, BL20B2 is equipped with a device that can easily perform phase-contrast imaging using diffraction gratings. This method allows the most accurate measurements of refractive index of any methods available and is the only means of obtaining shape and refractive index profiles in 3-dimensions from intact lenses (Fig. 1). For example, in the human crystalline lens, the shape of the lens changes with age without much change in the maximum value and distribution of the refractive index (Fig. 2). Water channel proteins in the lens, the aquaporins, have been shown to be important for the formation of the shape of the normal lens in zebrafish.
The technique is also the first to provide accurate measurements from lenses with opacifications. It has therefore made it possible for measurements of refractive index to be made in lenses with cataracts and has indicated how mutations in zebrafish lenses manifest as disturbances to the refractive index and how this can affect lens optics. In addition, it was found that the density distributions in the lenses of cataract model mice are different from those in wild type mouse lenses, and oxysterols, which have recently attracted attention as a compound that eliminates the opacity of the lens due to cataract, can restore normal density distribution. In this way, the high spatial and density resolution of phase-contrast X-ray CT is useful for studying the detailed structure and function of biological tissues.

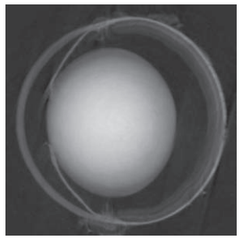

Fig. 1 Phase contrast image of a mouse eye showing eye lens

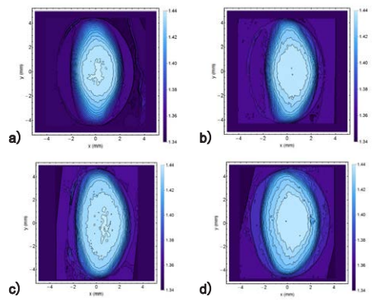

Fig. 2 Isoindicial contours of refractive index in human lenses aged a) 35 years, b) 48 years, c) 68 years and d) 86 years

BL20B2 Barbara Pierscionek (Anglia Ruskin University, UK)

Novel Drug Delivery System Using Cancer-Cell-Targeted Protein Nanocapsules

Small-angle X-ray scattering (SAXS) is an effective technique for obtaining submicron- to nanometer-scale structural information. SAXS can be measured in air with little limitation of the sample shape and measurement environment. A drug delivery system (DDS) is a system that encapsulates poorly water-soluble or easily degradable drugs into particles (nanocapsules) to solubilize or preserve the drugs, transports them to target cells through, for example, blood vessels, and releases them in the target cells. SAXS is suitable for studying the structure of DDS particles in solution. The objective of this study is to develop the DDS using lipocalin-type prostaglandin D synthase (L-PGDS), a biocompatible transport protein, as the particles. Although 7-ethyl-10-hydroxycamptothecin (SN-38) has high anti-tumor activity, it is not used in clinical practice because of its poor water solubility. We therefore tried to solubilize SN-38 by encapsulating it into L-PGDS and clarified the changes in the structure of L-PGDS with encapsulated drugs by SAXS performed at BL40B2. The scattering curves obtained by SAXS showed that the overall structure of L-PGDS is spherical and that L-PGDS does not coagulate but is monodispersed even in solution (Fig. 1). Also, the radius of gyration (R_g) was obtained from the Guinier plot for the small-angle region in the scattering curves. The R_g of L-PGDS decreased when SN-38 was encapsulated into it (Fig. 2). These results showed that L-PGDS is a very flexible protein that becomes compact when SN-38 is encapsulated into it.

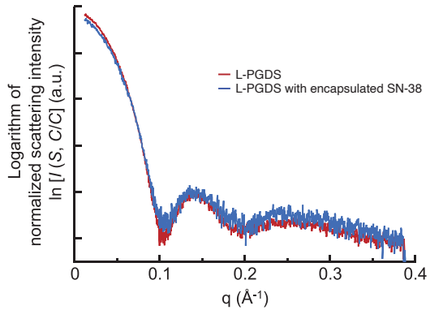

Fig. 1 Scattering curves of L-PGDS and L-PGDS with encapsulated SN-38

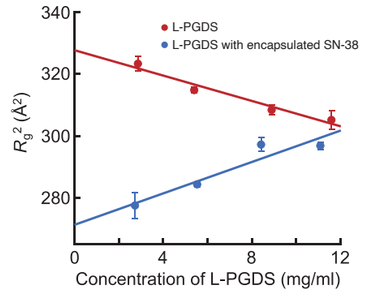

Fig. 2 L-PGDS concentration dependence of R_g^2 obtained by Guinier plot for L-PGDS with encapsulated SN-38

BL40B2 Masatoshi Nakatsuji, Takashi Inui (Osaka Metropolitan University)

Intratumoral Distribution of Very Small Amounts of Anticancer Drug Visualized via High-Intensity Nanobeam at SPring-8

Oxaliplatin (L-OHP), a third-generation platinum complex antitumor drug, is widely used in the treatment of colorectal cancer. However, L-OHP is effective in ~50% of the patients with advanced/recurrent colorectal cancer. Moreover, the use of L-OHP causes adverse effects such as neutropenia and peripheral sensory neuropathy. Establishing techniques to predict and evaluate the therapeutic effects of this drug is desired for achieving an effective and safe chemotherapy regimen. We therefore quantitatively analyzed and visualized the distribution of the platinum contained in L-OHP and essential metals by synchrotron radiation X-ray fluorescent spectrometry (SR-XRF) analysis to human cancer tissues and examined how this distribution is related to the effectiveness of chemotherapy and clinicopathological factors.
In the SR-XRF analysis of 30 rectal cancer specimens resected from patients who received L-OHP-based preoperative chemotherapy, the platinum concentration in the cancer tissue was 2.85–11.44 ppm (lower detection limit: 1.848 ppm). In the tumor epithelium, the concentration of accumulated platinum was significantly higher in the areas showing marked improvement by chemotherapy than in other areas. Conversely, in the tumor stroma, the concentration of accumulated platinum was higher in patients with limited effects of chemotherapy. The results of multivariate analysis showed that the concentration of accumulated platinum in the tumor stroma is an independent predictive factor of therapeutic effects. The results of principal component analysis suggested that copper transporters contribute to drug resistance.

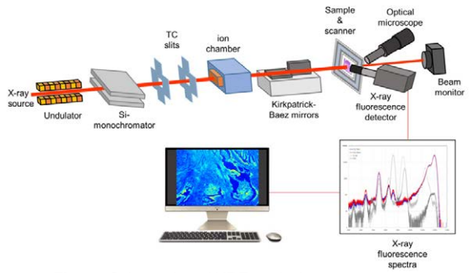

Fig. 1 Schematics of XRF analysis apparatus.

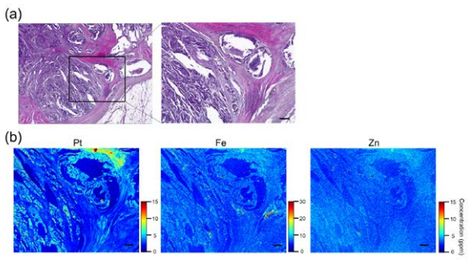

Fig. 2 (a) Representative histopathologic image of rectal cancer section. The image on the right is an enlarged view of the square in the image on the left. (b) XRF mapping of platinum, iron, and zinc in the same sample.

BL37XU Maiko Nishibori, Hayato Fujita, Ryo Koba (Kyushu University)

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