molecule assay for BGL activity in a microchamber array chip.

The microchamber array chips were fabricated by micromolding processes with polydimethylsiloxane polymer. BGL1B from the basidiomycete *Phanerochaete chrysosporium* was confined with fluorogenic substrate [Fluorescein di- β -D-glucopyranoside (FD-Glu), TokyoGreen- β Glu (TG-Glu) or Resorufin β -D-glucopyranoside (R-Glu)] in microchambers with a volume of 50 fL. The enzyme produced a large number of fluorescent molecules by hydrolyzing fluorogenic substrates, leading to fluorescence increase in microchambers. However, in the absence of BGL1B, TG-Glu was found to be hydrolyzed spontaneously in microchambers, whereas the others not. We also proved that R-Glu is more adequate for the enzymatic assay than FD-Glu. Unlike FD-Glu that requires a two-step hydrolysis to generate maximum fluorescence, R-Glu requires only a single-step hydrolysis reaction to attain full fluorescence, thereby affording a higher rate of fluorescence increase and ensuring the linear relationship between the fluorescence intensity and the BGL activity. Hence, using R-Glu, we are trying to monitor the single-molecule enzymatic activity of BGL1B in microchambers.

1PT237 フェリチン空洞内でのレアアース(Eu、Tb、Tm、Y)ナノ粒子合成 Synthesis of rear earth (Eu, Tb, Tm and Y) nanoparticles in apoferritin cavity

Tomoaki Harada, Hideyuki Yoshimura (*Meiji Univ.*)

Eu, Tb, Tm and Y nanoparticles were fabricated in the cavity of a cage shaped protein, recombinant apoferritin (fer0). Apoferritin is a spherical protein of 13 nm diameter with a cavity of 7 nm diameter. It is known to mineralize several metal ions in the cavity. Eu nanoparticles were synthesized by mixing 0.1 mg/ml apoferritin with 100 mM MOPS (pH 6.8) and 1 mM $\text{Eu}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ and kept standing for 24 hours at room temperature. This solution was centrifuged for 3 minute at 12000 rpm to remove small amount of precipitation. The supernatant was dialyzed with 20 mM NH_3 acetate, whose pH was adjusted to 8.0 by adding NH_3 , to stabilize core in the ferritin cavity. Tb, Tm and Y nanoparticles were prepared as the same method, except 100 mM MOPS (pH 7.0) was used for Y nanoparticles. The nanoparticles formation was confirmed with transmission electron microscopy (TEM) with and without aurothioglucose negative staining. The lattice structure of the cores was observed by high resolution TEM (HRTEM). Some of cores were showed lattice structure, however most of cores were amorphous. These nanoparticles were analyzed with an EDX and the specific peaks of expected elements were observed. The solution of Eu nanoparticles was applied to gel-filtration (Sephadex G-25) with elution buffer of 5 mM NH_3 acetate (pH 8.0) and was dried at 60°C. Subsequently, it was sintered at 800°C for 4 hours in the air. The sintered specimen showed electronic diffraction pattern corresponding to the lattice spacing of Eu_2O_3 .

1PT238 カゴ状蛋白質アポフェリチンを使用したガドリニウムナノ粒子の作成 Synthesis of gadolinium-based nanoparticles using the protein cage of apoferritin

Hiroko Fukano, Hideyuki Yoshimura (*Physics. Univ. Meiji*)

Gadolinium-based nanoparticles are expected as a better contrast agent for magnetic resonance imaging (MRI), since their effect for the relaxation rate is much higher than those of chelated gadolinium (III) complexes used in the medical field. Here we report the synthesis of gadolinium oxide nanoparticles in the apoferritin cavity which is known to reserve metals as oxide or oxy-hydroxide state. The apoferritin coated-gadolinium nanoparticles were synthesized by the following simple procedure; the mixture solution of 0.1 mg/mL recombinant apoferritin, 1 mM $\text{Gd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, and 0.1 M MOPS (pH 7.0) was just incubated for overnight at 4 °C. The synthesized nanoparticles were characterized by TEM and EDS. Homogeneous and spherical nanoparticles coated with protein shell were confirmed by TEM. The average diameter of them was determined to be 5.8 ± 0.5 nm by evaluating 900 particles. According to the high-resolution TEM images, the nanoparticles showed single-crystal, multi-crystal, or amorphous state. With EDS analysis, the peaks of gadolinium ($L\alpha$; 6.06 keV, $L\beta$; 6.71 keV, $M\alpha$; 1.19 keV) were detected only at the synthesized nanoparticles. The electron diffraction pattern suggested that the nanoparticles were gadolinium oxide. However further investigation of crystal identification is needed because the electron diffraction image was broad and weak.