Structural basis for the control of antigen-antibody reaction

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The immune system has been shown to recognize and distinguish foreign antigens by generating a large and diverse repertoire of antibody molecules and amplifying those with the requisite binding affinity and specificity in an affinity-based selection. Now it becomes practicable to produce antibodies with sequences we expect. Therefore, generation of a genetically engineered antibody with improved specificity and binding ability has been a focus in biotechnology.

The monoclonal antibody TT1 binds tri-iodothyronine (T3) and thyroxine (T4), both of which are structurally related thyroid hormones. Recently, Taya *et al.*[*] reported that the designed amino acid substitution in the frame region of TT1 results in antibodies that have the different specificity to T3 and T4 compared to those of the parent TT1. Our final goal is to understand the structural basis for the control of the specificity of thyroid hormones-antibody interaction and to apply the understanding to the generation of a genetically engineered antibody with greater thyroid hormones-TT1 binding affinity.

In this experiment, our purpose was to collect the diffraction data of the Fab fragment crystal of the parent TT1. Crystals were obtained by the hanging drop method. Protein solution consisted of 5 mg/ml in phosphate buffer. The mother liquor was 2.0 M $(NH_4)_4SO_4$, 5% isopropanol. The octahedral crystals with dimensions 80 x 40 x 40 μm were grown after three to four months. A preliminary data collection using R-axis IIC on the Rigaku RU-300 rotating anode with Cu Kα showed that the resolution of the crystals was low and crystals were damaged by the radiation. So we planed to obtain higher resolution data at BL41XU of SPring-8 at 100 K. Since the number of crystals was limited, the screening of suitable cryoprotectant was carried out in our beam time period. The crystals in 20 % glycerol and 20 % PEG400 solutions were cracked but 30 % trehalose solution was available. The crystals soaked in the solution were flash-frozen in a stream of nitrogen gas at 100 K. However, we could not solve the several problems perfectly. First it was difficult to put the crystal on the cryoloop because of the small crystal and lower viscosity of the solution. Second it was hard to center the crystal in the X-ray beam because of invisible small crystal in glass solution on the cryoloop. Finally the frozen crystal was covered with frost during the measurements.

We are trying to make larger crystals and will collect the data except in the rainy season.

[*]Taya, T., Yasukawa, K., Miyata, K., Kidokoro, S. & Inouye, K., *Biotech. Lett.*, 18, 1325 (1996)