X-ray structure analysis of antibodies

Yoshihiro Mezaki* (0003397), Yoshio Katsuya (0003395), Nobuyuki Mouri (0004216), Taira Fujimura (0004792), Yoshiki Matsuura+ (0003798)

Hyogo Prefectural Institute of Industrial Research, 3-1-1, Yukihira-cho, Suma-ku, Kobe 654-0037, Japan

+Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan

Antibodies play a crucial role to humoral immunity of higher animals: they distinguish unpreferable alien cells or molecules from the self, followed by their degradation and removal. Such a specific recognition of antigens is very useful in pharmaceutical or bioengineering fields and therefore, a large amount of monoclonal antibodies against various antigens have been produced so far. Of these, monoclonal antibodies against (4-hydroxy-3-nitrophenyl)acetyl (NP) are one of the most widely known and some crystal structures of these anti-NP antibodies have been reported. Comparison of tertiary structures of anti-NP antibodies in combination with their affinity to NP would reveal how different amino acids in the complementarity determining regions of antibodies recognize NP and to that end, lead to antibody design of any desired specificity. In this project, we crystallized an anti-NP antibody Fab fragment, originated from the clone G6 antibody, and subjected to the measurement by using brilliant X-rays at BL24XU of SPring-8.

G6 Fab was crystallized by hanging drop vapor diffusion method using 1.2 to 1.8 M ammonium sulfate as a precipitant. Though microcrystals were observed within a week and grew gradually, these crystals soon began to collapse from the core. Moreover, slight change of temperature led to a cloudiness of hanging drops and this precipitation seemed to affect the quality of protein crystals. So crystals grown properly (about 0.1 mm) were immediately dipped into liquid nitrogen and then transferred into the cold stream above the goniometer in the experimental hutch. About 4:1 mixture of precipitant and glycerol was successfully used as a cryo-protectant.

X-ray was monochromatized by diamond crystals to a wavelength of 0.834 angstrom, collimated to the size of 0.1mm x 0.1 mm and irradiated onto protein crystals. Diffraction images were recorded on imaging plates of Rigaku R-AXIS IV. Unfortunately, G6 crystals gave only low-resolution diffraction up to at most five angstrom. Analysis of low-resolution data suggested that these G6 crystals belong to space group *R*32 with cell dimensions of a = b = 161 angstrom and c = 306 angstrom. Further studies for improvement of protein crystal quality are needed in order to reveal three-dimensional structure of G6-Fab by the molecular replacement method.