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High pressure crystallization and crystallography of glucose isomerase

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Crystallization of protein is still a “bottleneck” for the three-dimensional (3D) structure analysis of a protein molecule. Therefore, the study on the acceleration of the crystallization is important. Visuri et al. repoted, for the first time, that the crystallization of glucose isomerase (GI) crystals was significantly enhanced with increasing pressure [1], while they did not discuss further effects of pressure on the crystallization. We measured solubilities, growth rates of a crystal face, step velocities and two-dimensional (2D) nucleation rates, three-dimensional (3D) nucleation rates of the GI crystal under high pressure. From these results, we found the negative molar volume change of crystallization delta V, the decrease in a step ledge surface energy and the increase in a step kinetic coefficient with increasing pressure. Activation volume of crystallization delta V‡ was discussed and the absolute value of it was almost half of that of delta V [2]. X-ray crystallography is also very important to study how the three dimensional structure of protein is related to its function under high pressure at atomic level. Kundrot et al. analyzed the crystal structure of lysozyme at 100 MPa using a beryllium (Be) cell. The crystal was prepared under 0.1 MPa. Charron et al. reported that thaumatin crystals grown under 150 MPa were analyzed under 0.1 MPa. Although Kundrot et al. concluded that the protein structure under high pressure was compressed, they could not estimate the influence of the bond structure of the crystal at 0.1 MPa on the compressed structure. In Charron’s case, the thaumatin structures in crystals prepared under 0.1 MPa and 150 MPa were almost identical, when they did X-ray analyses of both crystals at 0.1 MPa. From these two results, we considered that compression of the crystals was reversible. We conducted both Kundrot’s and Charron’s methods for GI using a stand-alone type Be vessel [3]. We found that (1) GI molecule was reversibly compressed under high pressure, (2) the distributions of water sites around GI molecules were almost identical in the range of pressures from 0.1 to 100 MPa.

[1] *K. Visuri, et al, Bio/Technology, 1990, 8, 547-549,* ***[2]*** *Y. Suzuki, et al, High Press. Res., 2010, 30, 483-489,* ***[3]*** *Y. Suzuki, et al, Rev. Sci. Instrum., 2010, 81, 084302-1-3*

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