Poster Presentation

MS77.P13

Agarose Hydrogel Protects Protein Crystals From Osmotic Shock

S. Sugiyama[1], T. Terai[2], H. Matsumura[3], M. Maruyama[3], Y. Takahashi[3], H. Yoshikawa[4], M. Yoshimura[3], H. Adachi[5], K. Takano[6], S. Murakami[7], T. Inoue[3], T. Nagano[2], Y. Mori[3]

[1]Osaka University, Graduate School of Science, Osaka, Japan, [2]The University of Tokyo, Graduate School of Pharmaceutical Sciences, Tokyo, Japan, [3]Osaka University, Graduate School of Engineering, Osaka, Japan, [4]Saitama University, Graduate School of Chemistry, Saitama, Japan, [5]SOSHO Inc., Osaka, Japan, [6]Kyoto Prefectural University, Graduate School of Life and Environmental Sciences, Kyoto, Japan, [7]Tokyo Institute of Technology, Graduate School of Bioscience and Biotechnology, Yokohama, Japan, [8]JST, ERATO, Lipid Active Structure Project, Osaka, Japan

High-throughput protein X-ray crystallography offers an unprecedented opportunity to facilitate drug discovery. The most reliable approach is to determine the three-dimensional (3D) structure of the protein-ligand complex by soaking the ligand in apo-crystals, but many lead compounds are not readily water-soluble. Such lead compounds must be dissolved in concentrated organic solvents such as DMSO. Therefore, to date, it has been impossible to produce crystals of protein-ligand complexes by soaking in apo-crystals, because protein crystals dissolve immediately upon soaking in concentrated organic solvents containing lead compounds. The problem arises from the influence of osmotic shock on crystal packing during soaking. We propose an approach to avoid the damage by growing protein crystals in a high-strength hydrogel(1-3). Interestingly, the hydrogel-grown crystals did not dissolve at all for more than thirty minutes in concentrated organic solvents and ionic-strength solutions such as 60% DMSO, and 5.0M lithium acetate. Their X-ray diffraction data were suitable for structure analysis. Surprisingly, some of the crystals diffracted to the highest resolution reported in the Protein Data Bank. Furthermore, the 3D structure determined from hydrogel-grown apo-avidin crystals which were transferred to a solution containing the ligand revealed a clear electron-density map of the ligand bound to the active site. This result indicates that it is possible to bind ligand compounds into hydrogel-grown apo-crystals.

[1] *Sugiyama et al. (2012) Jpn. J. Appl. Phys., 48, 075502,* ***[2]*** *Sugiyama et al. (2012) J. Am. Chem. Soc., 134, 5786-5789,* ***[3]*** *Sugiyama et al. (2013) Cryst. Growth Des., 13, 1899-1904*

Keywords: protein crystallization, hydrogel, drug discovery