Large protein crystal growth for neutron crystallography

Monika Budayova-Spano[1/2], François Dauvergne[1], Marie-Thérèse Dauvergne[1], Peter Timmins[2] and Stephen Cusack[1]

[1]Neutron Diffraction Group EMBL Grenoble Outstation / [2]LSS Group ILL Grenoble

6, rue Jules Horowitz, BP 181, 38042 Grenoble Cedex 9, France

To this date, the bottleneck in biomacromolecular crystallography still remains the growth of single crystals with good crystal quality (low mosaicity, long range crystal order, high resolution diffraction power) and with good crystal size.

Whereas development of third generation synchrotron sources has allowed X-ray protein structures to be solved from crystals of a few 10^{-4} mm^3, a major hurdle to neutron protein crystallography is that unusually large crystals (~1mm^3) are required to compensate for the weak flux of available neutron beams [1].

Deuteration is a special case of isotopic substitution, and H-D exchange can significantly alter the physico-chemical properties of proteins solutions. The fixed-point properties of D_2O are all significantly different from the corresponding values of H_2O. In the same way, protein solubility, intermolecular interactions and association between the macromolecules in solution are particularly affected by deuteration [2]. Systematic studies of the effects of D_2O and deuteration on physico-chemical properties of biological macromolecules, specifically on their crystallization, are of particular interest in the development of techniques that allow getting large high-quality crystals.

We have invented a novel method for the crystallization of proteins allowing alteration and optimisation of the conditions in order to grow crystals that are appropriate for neutron diffraction analysis.

We propose a rational physico-chemical approach of crystallization based on knowledge of the phase-diagram [2]. We have constructed a device, which enables the phase diagram to be investigated, the nucleation and crystal growth of biological macromolecules to be controlled, and the solubility of seeded H/D-labelled biological macromolecule crystals to be manipulated, as a function of the temperature. This semi-automated crystallization tool is also intended for *in situ* observation by optical microscopy and allows sequential image acquisition, processing and storage. We report here our first experimental results with perdeuterated protein systems.

References

[1] - D.A.A. Myles, C. Bon, P. Langan, F. Cipriani, J.C. Castagna, M.S. Lehmann, C. Wilkinson, Physica B **241-243**, 1122, (1998)

[2] - M. Budayova-Spano, S. Lafont, J.P. Astier, C. Ebel, S. Veesler, J. Cryst. Growth **217**, 311, (2000)