Neutron structure of type-III Antifreeze Protein allows the reconstruction of AFP-ice interface

Eduardo I. Howard[a*], Matthew P. Blakeley[b], Michael Haertlein[b,c], Isabelle Petit-Haertlein[b,c], Gabriel Koremblit[d], Andre Mitschler[e], Stuart J. Fisher[b,e], Alexandra Cousido-Siah[e], Andrés G. Salvay[a,f], Alexandre Popov[g], Christoph Muller-Dieckmann[g], Tatiana Petrova[h], Alberto Podjarny[e*]

[a]IFLYSIB, UNLP-CONICET, Calle 59, 789, B1900BTE, La Plata, Argentina
[b]Institut Laue-Langevin, 6 rue Jules Horowitz, 38042, Grenoble, France,
[c]ILL–EMBL Deuteration Laboratory, Partnership for Structural Biology, 6 rue Jules Horowitz, 38042 Grenoble, France,
[e]UTN–URRG, Islas Malvinas N° 1650, Ushuaia, Tierra del Fuego, Argentina
[e]IGBMC, CNRS, INSERM, Université de Strasbourg, 1 rue Laurent Fries, Illkirch, France
[f]Department of Molecular Biology, Faculty of Natural Sciences, University of Salzburg, Salzburg, Austria
[g]Universidad Nacional de Quilmes, Roque Sáenz Peña 352, B1876BXD Bernal, Argentina
[h]ESRF, 6 rue Jules Horowitz, 38043, Grenoble, France
[i] Institute of Mathematical Problems of Biology, Russian Academy of Sciences, Pushchino, 142290 Russia

The prototypical type-III AFPs have been extensively studied, notably by X-ray crystallography, solid-state and solution NMR, and mutagenesis, leading to the identification of a compound ice-binding surface (IBS) composed of two adjacent ice-binding sections, each which binds to particular lattice planes of ice crystals, poisoning their growth. This surface, including many hydrophobic and some hydrophilic residues, has been extensively used to model the interaction of AFP with ice. Experimentally observed water molecules facing the IBS have been used in an attempt to validate these models. However, these trials have been hindered by the limited capability of X-ray crystallography to reliably identify all water molecules of the hydration layer. Due to the strong diffraction signal from both the oxygen and deuterium atoms, neutron diffraction provides a more effective way to determine the water molecule positions (as D_2O). Here we report the successful structure determination at 293K of fully perdeuterated type-III AFP by joint X-ray and neutron diffraction providing a very detailed description of the protein and its solvent structure. X-ray data were collected to a resolution of 1.05 Å, and neutron Laue data to a resolution of 1.85 Å with a “radically small” crystal volume of 0.13 mm^3. The identification of a tetrahedral water cluster in nuclear scattering density maps has allowed the reconstruction of the IBS-bound ice crystal primary prismatic face. Analysis of the interactions between the IBS and the bound ice crystal primary prismatic face indicates the role of the hydrophobic residues, which are found to bind inside the holes of the ice surface, thus explaining the specificity of AFPs for ice versus water.

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