



Application for beam time at ESRF – Experimental Method

This document should consist of a maximum of two A4 pages with a minimal font size of 12 pt.

Aims of the experiment and scientific background

Lectins isolated from seeds of *Diocleinae* (*Phaseolae*, *Leguminosae*) have highly related amino acid sequences but exhibit distinct pH-dependent dimer-tetramer equilibria, and show different biological activities. Since only the tetravalent form is able to cause cross-linking of receptors on the cell surface, the different ratio between divalent and tetravalent lectin species at a given pH may contribute to the variability of biological functions. We have reported the crystal structure of *D. guianensis* (Dguia) seed lectin at 2.0 Å resolution. Comparison of the structures of Dguia and *D. grandiflora* lectins (1DGL), a lectin which does not display dimer-tetramer equilibrium, indicated that substitution of His 131 for Asn drastically reduces interdimer contacts along with disordering of the loop comprising residues 117-123 which, in its ordered conformation, stabilises the pH-independent tetrameric association of the Dgran lectin. In order to test the hypothesis that replacement of His for Asn at position 131 may explain the existence of pH-dependent dimer-tetramer equilibrium, we searched for other lectins displaying pH-dependent tetrameric association. We have crystals from *Dioclea violacea* and *Cratylia floribunda* seed lectins. The aim of the experiment is to resolve by molecular replacement the structure of this lectins (native and in complex with mannose), looking for a common mechanism for the regulation of the dimer-tetramer association.

Parkia platycephala seed lectin structure can't be resolved by molecular replacement. We expected to resolve the crystal structure using MAD.

Experimental method

*Crystals from *Dioclea violacea* seed lectin (DVL) and *Cratylia floribunda* seed lectin (CFL) in complex with mannose: Cryocooling, fixed energy.

* Crystals from *Parkia platycephala* seed lectin (PPL): Cryocooling, tunable energy.

Results expected

* Resolve the structure of DVL by molecular replacement. Resolved the structure of CFL in complex with mannose.

References

Wah DA, Romero A, Gallego del Sol F, Cavada BS, Ramos MV, Grangeiro TB, Sampaio AH, Calvete JJ. Crystal structure of native and Cd/Cd-substituted *Dioclea guianensis* seed lectin. A

novel manganese-binding site and structural basis of dimer-tetramer association J Mol Biol. 2001 Jul 20;310(4):885-94. PMID: 11453695