Structural changes upon de- and re-metallization of *Pterocapus angolensis* seed lectin

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Lectins are a class of proteins of non-immune origin that specifically recognize carbohydrates without having any enzymatic activity on them. Many structurally and functionally diverse families of lectins have been identified in animals, plants and micro-organisms [1]. The legume lectin family is extremely well studied and serves as a very useful model system [2]. In the past few years, our lab has focused on the mannose/glucose-specific lectin present in the seeds of the African bloodwood tree (*Pterocarpus angolensis* - PAL). We have determined the structure of this protein in its free state as well as in complex with 11 different mono- and oligosaccharides [3-5]. In addition, the thermodynamic parameters for mono-and oligosaccharide binding have been measured using isothermal titration calorimetry, allowing a detailed analysis of the structure-function relationship for this protein.

Like other members of the legume lectin family, the PAL requires stoichiometric binding of calcium and a transition metal for its activity. The structural effects of demetallization and the pathway of de- and remetallization have been studied extensively for the model lectin concanavalin A [6-9]. Anecdotal evidence however suggests that the results obtained for concanavalin A are not universal within the legume lectin family. Therefore we set out to study the structural changes upon de- and remetallization of PAL by X-ray crystallography.

Demetallization of PAL in the crystal is a slow process that takes about 2 weeks to complete. PAL crystals withstand treatment with 100 mM EDTA for 2 weeks and still diffract to 2.3 Å resolution (Figure 1). The resulting structure shows significant conformational changes in the metal binding loop and an adjacent loop that contains a conserved cis-peptide bond. Both loops are involved in carbohydrate binding as well, thus linking carbohydrate binding with the presence of the two metal ions. In contrast to concanavalin A, the crucial cis-peptide bond preceding Asp86 itself does not convert to the trans conformation upon removal of the metal ions.

Figure 1: Crystals of metal-free PAL and their diffraction pattern. (A) PAL crystal treated for about 2 weeks with 100 mM EDTA shows visible cracks at its surface. (B) Diffraction pattern of the crystal shown in panel A. Despite the cracking, the diffraction pattern is still of high quality and extends to 2.3 Å resolution.
We studied the time course of the demetallization reaction by collecting data sets after 1 hour, 1 day, 1 week and 2 weeks of incubation with 100 mM EDTA. The structures of the crystals that were treated for 1 hour or 1 day were identical to the fully metallized structure. After 1 week, partial demetallization is observed, resulting in electron density that indicates the superposition of 2 distinct conformations: the fully metallized conformation and the fully demetallized conformation. No additional intermediate conformation is observed, suggesting that the reaction is a 2-state process.

Figure 2: Metal-dependent conformational states of PAL. (A) Fully metallized lectin containing one calcium (green) and one manganese (orange) ion which are necessary for an intact and functional carbohydrate binding site. (B, C) Upon demetallization, part of the metal binding loops (yellow) becomes disordered or adopts a different conformation. Also a neighboring loop (blue) containing a conserved cis-peptide bond can change conformation, although the cis-peptide is retained. Panels B and C correspond to molecules A and B in the asymmetric unit of the crystals respectively.

The EDTA-treated metal-free protein can be remetallized in the crystal indicating full reversibility. Remetallization in the crystal is a much faster process, as even after 1 hour full occupancy of the metal ions and a native conformation are observed.

References