s8a.m4.p11 The Crystallisation and structure analysis of the dI Component of Transhydrogenase, a Proton-Translocating Membrane Protein. P.A. Buckley, J.B. Jackson, D.W. Rice, S.E. Sedelnikova, J.Burke, T. Shneider, M. Roth and P.J. Baker
Keywords: membrane proteins, receptors.

Membrane bound ion pumps are involved in metabolic regulation, osmoregulation, cell signalling, nerve transmission and energy transduction. Transhydrogenase is a conformationally-coupled, proton pump that links a proton gradient to the redox reaction between NAD(H) and NADP(H). It has three components. dI binds NAD(H), dII spans the membrane and dIII binds NADP(H).

This poster describes the first crystal structure determination of the dI NAD^+ complex component of *R. rubrum* transhydrogenase at 2Å resolution using the selenomethionine MAD technique [1, 2]. The monomer comprises two domains (α/β), one is a Rossmann fold that binds NAD^+ in a novel mode and the other is involved in dimer formation. The two domains can adopt different conformations with respect to each other, and in the most closed orientation the nicotinamide ring is expelled from the cleft between the two domains and is exposed on the outside of the protein. With the protein in this conformation it is possible to dock the structures of the dI/NAD^+ and the previously determined dIII/$NADP^+$ complexes [3, 4] together to provide the first insights into the molecular basis of the hydride transfer step.

The proposed model of the dI/dIII complex shows domain motion in dI with attendant changes in hydrogen-bonding, resulting in a shift in position of the nicotinamide ring of NAD^+. We propose that this movement is responsible for switching between the forbidden and allowed states for hydride transfer during proton pumping.

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s8a.m4.p12 Structure of a legume lectin from the bark of *Robinia pseudoacacia*. A. Rabijns[a], C. Verboven[a], E.J.M. Van Damme[b], W.J. Peumans[b], C.J. De Ranter[a], [a]*Laboratorium voor Analytische Chemie en Medicinale Fysicochemie, Fac. Farmaceutische Wetenschappen, K.U.Leuven, Van Evenstraat 4, B-3000 Leuven, Belgium.* [b]*Laboratorium voor Fytopathologie en Plantenbescherming, Fac. Landbouwkundige en Toegepaste Biologische Wetenschappen, K.U.Leuven, W. de Croylaan 42, 3001 Leuven, Belgium.*
Keywords: membrane proteins, legume lectin.

The legume lectins are a family of sugar binding proteins found in the seeds, roots, stems, leaves and bark of plants belonging to the *Leguminosae* family[1]. Despite the strong similarity in amino acid sequences and tertiary structures, the carbohydrate specificities and quaternary structures of legume lectins vary widely. Two different legume lectins occur in the bark of *Robinia pseudoacacia*: RPbAI and RPbAII. RPbAI is composed of two homologous 31.5- and 29-kDa lectin polypeptides (referred to as polypeptides A and B, respectively) that associate in all possible combinations into five different tetrameric isolectins (A_4, A_3B, A_2B_2, AB_3 and B_4)[2]. In this respect, the isolectin composition of RPbAI is reminiscent of that of the *Phaseolus vulgaris* agglutinin, which is also a mixture of five isolectins that originate from the association of E and L subunits in tetramers[3]. In this crystallographic study we focus on the RPbAI tetramer consisting of four 31.5-kDa subunits (A_4), which could be crystallised in two different crystal forms. Crystal form I grows in a condition containing 20% polyethylene glycol 8000, 0.1 M sodium cacodylate buffer pH 6.5 and 0.2 M magnesium acetate whereas crystal form II grows from 30% polyethylene glycol 4000 and 0.2 M ammonium sulphate. Synchrotron radiation data were collected on both crystal forms and molecular replacement solutions were found using the structure of phytohemagglutinin-L legume lectin (1FAT)[4]. Because of better data quality (1.8 Å), crystal form II was chosen for further refinement. The asymmetric unit of this crystal form comprises 1 monomer with the typical legume lectin fold. However, due to I222 symmetry a tetramer is formed which is very similar to the phytohemagglutinin-L legume lectin tetramer[4]. Differences between the structures of phytohemagglutinin-L and RpbAI A_4 will be discussed in detail.

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