

PURIFICATION, CHARACTERIZATION AND PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF A LACTOSE-SPECIFIC LECTIN FROM *CYMBOSEMA ROSEUM* SEEDS

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The unique carbohydrate binding property of lectins has rendered them invaluable as tools in biomedical research. Here we report the purification, partial primary structure, carbohydrate affinity characterization, crystallization and preliminary X-ray diffraction analysis of a lactose-specific lectin from *Cymbosema roseum* seeds (CRLII). Isolation and purification of CRLII was performed by a single step using a Sepharose-4B-lactose affinity chromatography column. The affinity characterization was carried using haemagglutinating activity and haemagglutinating-inhibition assays. Protein sequencing by mass spectrometry was obtained by the digestion of CRLII with trypsin, Glu-C and AspN. CRLII showed haemagglutinating activity toward rabbit erythrocytes. Desialylated bovine lactotransferrin inhibited CRLII at 2.4 $\mu\text{g}\cdot\text{mL}^{-1}$ and the O-glycoproteins from mucine origin showed the most potent inhibition capacity at a minimum concentration of 1.2 $\mu\text{g}\cdot\text{mL}^{-1}$. CRLII partial protein sequence exhibits 46 % similarity with the ConA-like α chain precursor. Suitable protein crystals were obtained from drops containing 8% of etilene glicol, Tris-HCL 0.1M pH 8.5 and PEG 8,000 11%. The crystals are monoclinics, belonging to the P2₁ space group with unit cell parameters of a=49.4, b=89.6 and c=100.8 Å.

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