Expression of Active *Cratylia mollis* Seed Lectin (CRAMOLL 1) in *Escherichia coli*

Varejão, N.[1,2]; Almeida, M.S.[1]; Coelho, L.C.B.B.[2]; Correia, M.T.S.[2]; Foguel, D.[1]

[1]IBqM, CCS, UFRJ; [2]DBioq, CCB, UFPE

varejao@bioqmed.ufrj.br

CRAMOLL 1 is a glc/man isolectin isolated from *Cratylia mollis* seeds. CRAMOLL 1 has 82% sequence identity with Con A. As Con A, CRAMOLL 1 undergoes a posttranslational processing that has raised objections to traditionally molecular cloning and heterologous expression. We now report the expression and recovery of functional recombinant CRAMOLL 1 (rCRAMOLL 1) in *Escherichia coli*. This was accomplished by using a chemically synthesized DNA, which was based on the mature CRAMOLL 1 primary amino acid sequence. The proper folding of the rCRAMOLL 1 and its biological activity have been checked and compared with its plant lectin counterpart. SDS-PAGE revealed that purified rCRAMOLL 1 is a homogeneous protein stained as a single band on gel, since no naturally occurring fragments were detected. Native PAGE confirmed that the global charge of rCRAMOLL 1 is similar to nCRAMOLL 1. Mass spectroscopy detected rCRAMOLL 1 with its expected molecular mass of 25,336 Da. Size-exclusion chromatography shows that rCRAMOLL 1 is a tetrameric protein at pH 7.0. Circular dichroism and intrinsic fluorescence emission spectra, pointed that rCRAMOLL 1 maintains the same folding of its native plant lectin; presenting the all-β secondary structure (maximum negative at 222 nm) and its tryptophan residues deep buried in a hydrophobic core (center of mass = 332 nm). Moreover, rCRAMOLL 1 sample yielded a 1D ^{1}H-NMR spectrum with good chemical shift dispersion in the amide region, reinforcing the indication of well-defined structure. Finally, we have showed that rCRAMOLL 1 is also able to agglutinate rabbit erythrocytes. The availability of this expression system will enable us to perform structure/function relationship studies of this lectin.

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