

THERMODYNAMIC CHARACTERIZATION OF THE *MACROTYLOMA*
AXILLARE SEED LECTINS BY ITC AND DSC

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Lectins are carbohydrate-binding proteins that specifically recognize diverse sugar structures. It is important to obtain the complete thermodynamic characterization of such binding properties in order to understand their biological functions. Leguminous plants are recognized good lectins fonts. In this work, active fractions of a N-acetil- α -D-galactosamine (NacGal) specific lectin isolated from *Macrotyloma axillare* seeds (LMA) are studied by ITC and DSC methodologies. The lectin fractions were isolated from *M. axillare* seeds by an alternative purification methodology (required patent at INPI, Brazil, 2004). The DSC experiments were accomplished at a scanning speed of 60°C/Hour and the system was maintained at constant pressure of 30.0 psi. The protein concentration used was 0.020-0.041mM. The ITC experiments were carried out at 27°C and pH 7.2 and 6.2, protein concentration in the range of 0.16-0.27mM and ligand concentrations (NacGal, 2-NO₂-Phenyl- α -D-NacGal, 4-NO₂-Phenyl- α -D-NacGal and 4-NO₂-Phenyl- β -D-NacGal) about 5-27mM. Preliminary ITC results have shown that LMA have dissociation constants values in the range of 1.0-2.4 mM for the ligands tested. The DSC results have shown that the LMA showed irreversible denaturation (thermodynamically driven) and T_m about 98°C at pH 7.2. These results show that the LMA affinity by these ligands is weak, and the lectin thermal stability is large.

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