LUFFA ACUTANGULA AGGLUTININ (LAA): PRIMARY SEQUENCE DETERMINATION AND ANALYSIS OF ROLE OF TRYPtokPHAN IN SUGAR BINDING ACTIVITY

Avadhesha Surolia¹

¹Molecular Biophysics Unit, Indian Institute of Science, Bangalore, Karnataka, India

Introduction and Objectives:
Phloem lectins constitute a specialized group of chitin binding proteins. They were initially discovered in the fruits of plants belonging to Cucurbitaceae family and have been most widely studied members of this group. This class of lectins are underrepresented in terms of structural studies as well as in understanding their physiological roles in plants. In the present study, a lectin from phloem exudates of Luffa acutangula (ridge gourd) was characterised for its primary sequence and studying the role of tryptophan in carbohydrate binding.

Materials and Methods:
Lectin was purified on chitin affinity matrix to near homogeneity and purity and molecular mass was determined using SDS-PAGE. The gel separated protein was subjected to various proteolytic digestion and the resulting peptides were analysed on LC coupled ESI ion trap mass spectrometer. The peptide precursor ions were fragmented by both CID or ETD experiments and manual interpretation of MS2 was performed to deduce amino acid sequence. Chemical modification of tryptophan was performed using N-bromosuccinimide and 2-hydroxy-5-nitrobenzyl bromide.

Results and Discussion:
On SDS-PAGE the lectin showed an Mr 24000 ± 1000 Da. The de novo sequencing gave rise to 95% sequence coverage of lectin and showed high sequence similarity with deduced sequences of phloem lectins present in the database. The lectin after tryptophan modification showed loss of both the hemagglutinating and saccharide binding activities. However, the retention of the lectin activity was seen when the modifications were performed in presence of chitooligosaccharides.

Conclusions:
This study identify a particular tryptophan residue in the activity of the lectin.