SCREENING, CHARACTERIZATION AND *DE NOVO* SEQUENCING OF BROCCOLI PLASMA MEMBRANE AQUAPORINS BY HIGH RESOLUTION MASS SPECTROMETRY

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Plasma membrane Intrinsic Proteins (PIPs), a subclass of aquaporins, are ubiquitous membrane channel proteins that play a crucial role in water relations and other cell functions.

Bottom-up protein identification is carried out by cleaving proteins into their constituent peptides by endoprotease digestion, followed by separation and analysis by reverse phase HPLC-MS/MS. The recent introduction of mass spectrometers able to measure with high mass accuracy (≤ 5 ppm), together with new alternative ways to fragment peptides such as HCD (higher-energy C-trap dissociation) allow the screening of proteins from not sequenced organisms and the characterization of post-translational modifications (PTMs). In this study we took advantage of the high mass accuracy of LTQ Orbitrap mass spectrometer combining collision induced dissociation (CID) and HCD fragmentation to identify a total of 21 peptides belonging to several isoforms of PIPs in Broccoli (*Brassica oleracea* L. var italica) roots. In addition, 7 of them present PTMs. The phosphorylation on Ser^{280} and Ser^{283} on the C-terminal tail of *At*PIP2 has been described (Prak et al., 2008). Our results with broccoli PIPs only showed the presence of phosphorylated Ser^{283} on two different peptides (SLGSFRSAANV and SLGSFRSAA), both containing three Ser residues. We have also evidenced the presence of methylation (+14.0157 amu) on the tryptic doubly charged peptide with sequence DYEDPPPTPFFDADEmeLTK. Methylated Glu residues on PIP proteins have been described, although it is quite uncommon in eukaryotic proteins (Maurel, 2007).

Finally, 8 peptides from PIP proteins were *de novo* sequenced and validated searching homologue peptides using BLASTp. Sequence analysis by LTQ Orbitrap is a useful tool to provide reliable information on peptide sequences from a complex peptide-protein mixture, even when dealing with organism under-represented in databases. The results described here will lead to a better understanding of aquaporin regulation and the role of different PIP isoforms in specific subcellular compartments.

Maurel C. 2007. FEBS Letter 581: 2227-2236.
Prak S, Hem S, Boudet J, Viennois G, Sommerer N, Rossignol M, Maurel C, Santoni V. 2008. Molecular and Cellular Proteomics 7: 1119-1030.