OLIGOMERIZATION AND PHOSPHORYLATION OF LENTIL SEED AQUAPORINS

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Aquaporins belong to the MIP superfamily of transmembrane channel proteins. In plants, aquaporins are thought to regulate the water flow through membranes during growth, development and stress responses. Two homologous cDNA have been cloned from bean seeds and from *Arabidopsis* seeds. They were respectively called α- and β-TIP. This suggests that two putative aquaporins could be expressed simultaneously in plant seeds.

We have purified two TIP isoforms from the protein storage vacuoles (PSV) membrane of *Lens* seeds. The amino-terminal sequence of the 25 kDa protein showed 87 % identity with the β-TIP of *Arabidopsis thaliana* and 60 % identity with α-TIP protein of *Phaseolus vulgaris*. The amino-terminal end of the 26 kDa polypeptide was blocked. To overcome this problem, an internal sequence was determined after trypsinolysis. Its sequence also shared a high homology with both *Arabidopsis* β-TIP (73 %) and *Phaseolus* α-TIP (78 %). To identify the 25 kDa and the 26 kDa bands more precisely, they were blotted on a PVDF membrane and cleaved with CNBr. For each protein, comparison of the sequences of five internal peptides were done.

Cross-linking experiments were performed in order to show that close physical association between different TIP molecules occurs within the PSV membranes. SDS-PAGE revealed that as the incubation time increased, both 25 and 26 kDa bands disappeared and concommitently high molecular weight bands became visible. Oligomers larger than tetramers did not migrate on a 12% SDS-PAGE gel. The presence of a dimer is observed after only ten seconds incubation. To minimize intermolecular coupling, membrane proteins were diluted in their lipidic environment. In this aim, liposomes were fused with the TIP-enriched membranes in order to increase the lipid to protein ratios. Increasing the lipid to protein ratio delayed the formation of trimers and tetramers but not that of the dimers. This is consistent with the presence of a dimer in the PSV membrane. Oligomers crosslinked with DTSP were dissociated in monomers in the presence of β-mercaptoethanol. Our results indicates that each oligomer was made of both 25 kDa and 26 kDa proteins.

Both proteins are phosphorylated by a 52 kDa magnesium-dependent calcium-regulated membrane-bound kinase (CDPK). For both proteins, the incorporation of ^{32}P was maximal at 30°C and at pH 6.5. The pH-dependence was slightly affected by the $MgCl_2$ concentration. Increasing the $MgCl_2$ concentration from 2 to 10 mM shifted the pH response curve to lower pH by about 0.5 unit. Addition of W-7, a calmodulin antagonist also known as a CDPK inhibitor to the reaction mixture resulted in a 30% loss in kinase activity.

In order to get the apparent molecular weight of the kinase we have used an in-gel assay. Calf histone H1 were used as substrates for the kinase and mixed with the acrylamide solution of a 12 % SDS-PAGE gel. Membrane proteins were then separated in the gel by electrophoresis.