Characterization of high affinity Na^+ K^+ transporter gene from higher plants

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Summary

The ions concentration in plant cell is crucial for plant osmoregulation, which correlated to Na^+ tolerance and dehydration resistance. The molecular mechanism for plant Na^+ uptake however remains unknown. We have isolated the Na^+K^+ transporter homologue from *Arabidopsis thaliana*, AtHKT1 and have determined DNA sequence.

To confirm the amplified 5' cDNA sequence and to isolate the promoter region of AtHKT1, the 5' flanking DNA sequence of AHKT1 was isolated from genomic DNA from *Arabidopsis thaliana*. Isolation of the 5' region was performed according to thermal asymmetric interlaced (TAIL) PCR method. The longest genomic PCR products extends about 850 bp beyond the putative translation start codon. The DNA sequence was confirmed independently by isolating using PCR primers located at 5' end of the sequence and the primer inside AtHKT1 cDNA. The sequence of the genomic DNA matched RACE-extended cDNA sequence except for the one nucleotide corresponding to amino acid at the position 9. A putative TATA boxes (TATATA) that exhibit a high degree of similarity to the plant TATA consensus sequence The two putative CAAT boxes were identified at the promoter region.

The wheat HKT1 (wHKT1) confers the K^+ permeation ability but AtHKT1 did not possess the K^+ uptake property. To elucidate the pore region which determines the ion selectivity, we constructed chimera plasmids from wHKT1 and AtHKT1. The N-terminus region of wHKT1 is responsible for K^+ permeation. This study is now in progress.

A glutathione-S-transferase (GST)-AtHKT fusion protein was purified for antibody production. The amino acid sequences that shows highly hydrophilic region located at the middle part of AtHKT1 protein based on the hydrophobic profile was chosen as an epitope to produce AtHKT1-antibody. Immunoblot analysis of protein from Arabidopsis root and leaf showed the detection of a protein at predicted molecular mass of AtHKT1 of 56kD in membrane fraction not but in soluble fraction. This is consistent with the membrane protein of AtHKT1 The antibody also recognized a higher molecular weight band that is present in only membrane fraction. The leaf fraction exhibited a slightly higher in intensity of the signal of 56kD.