SUPPLEMENTAL TEXT

Cloning of Wheat Glucosidase cDNAs and the Primary Structrues of the Deduced TaGlu1s

To obtain the cDNA encoding wheat Hx-Glc glucosidase we used 3'-RACE. Total RNA was isolated from 48-hr-old wheat shoots (cv. Asakazekomugi) using RNeasy Plant mini kit (QIAGEN, Valencia, CA) and reverse transcription was performed with a 3'-RACE System (Invitrogen, Carlsbad, CA) by priming the oligo(dT)-containing adapter supplied in the kit. Two gene-specific degenerate primers (GSP1 and GSP2) were designed based on the amino acid sequences of a highly conserved region among the maize, rye, sorghum and oat glucosidases. The sequences of the degenerate primers were: 5'-GGATGGTWCYTRGAGCCHRTGGT-3' (GSP1) and 5'-CCHRTGGTKCGTGGTGAYTAYCC-3' (GSP2). The estimated size of the amplified DNA using GSP1 and the adapter primer was 900 bp. After size fractionation by agarose gel electrophoresis, DNA corresponding to about 900 bp was recovered. Because the nested-PCR with GSP2 and the adapter primer gave a 900 bp product, the DNA was purified from a gel and then cloned into the *Eco*RV site of pBluescript SK(-) (Stratagene, La Jolla, CA). The resulting plasmid was amplified in an *E. coli* strain, DH5α, and the sequence analysis of the inserted fragment revealed that the cloned DNA shared >90% identity with the *Scglu* gene.

For isolation of full length cDNA, the λZAP II-cDNA library constructed from shoots of 48-hr-old wheat, cv. Chinese Spring, was screened by the DNA fragment obtained from 3'-RACE as a probe. Labeling of the probe and signal detection was performed using AlkPhos Direct Labeling kit with CDP-Star and Hyperfilm (Amersham Biosciences, Piscataway, NJ) according to the manufacture's instructions. This procedure resulted in the isolation of the wheat glucosidase cDNA containing a 1710-bp open reading frame encoding a 569-amino acid glucosidase (TaGlu1a) (Supplemental Figure). The DNA sequence was analyzed on both strands.

The predictive programs ChloroP v.1.1 and TargetP v.1.01 indicate that TaGlu1a may possess a signal peptide for a plastid (Supplemental Figure). The cleavage site of the signal peptide predicted by both programs was between amino acids 49 and 50 (arginine and alanine, respectively). Our previous study revealed the N-terminus of the mature protein to be the glycine residue adjacent to alanine 50. When we performed N-terminal amino acid sequencing of the natural wheat and rye glucosidases, glycine was clearly detected as the major component

of the first reaction cycle together with significant amounts of alanine (about half the level of glycine) (data not shown). The second strongest peak observed in each successive reaction cycle corresponded to the primary peak of the preceding cycle. These results suggest that the purified enzyme is in fact a mixture of two polypeptides; the major component having an N-terminal sequence of GTPSLPAE and a minor component with the sequence AGTPSLPAE. For clarity, we will assign the alanine residue as the N-terminal amino acid of mature TaGlu1a and ScGlu.

The predicted molecular mass of the mature protein is 59,155 Da with a p*I* of 5.77, which correlates with the previous results for wheat glucosidase (Sue M, Ishihara A., and Iwamura H. (2000) Plant Sci. **155**: 67-74). However, it was impossible to determine from this value whether TaGlu1a represents the 60-kDa or 58-kDa band observed in SDS-PAGE analysis of purified wheat glucosidase (Sue M, Ishihara A., and Iwamura H. (2000) Planta **210**: 432-438). To examine whether wheat possesses other glucosidases of a different molecular size to TaGlu1a, we performed further cDNA library screening using *Taglu1a* as a probe.

The ORF of *Taglu1a* was used as a probe for the further screening of the cDNA library. Two more cDNAs encoding β-glucosidases were isolated by library screening and designated *Taglu1b* and *Taglu1c*. *Taglu1b* and *Taglu1c* comprised open reading frames of 1710- and 1713-bp encoding polypeptides of 569- and 570-amino acids, respectively (Supplemental Figure). The deduced molecular mass of mature TaGlu1b and TaGlu1c were 59,099 Da and 59,245 Da, respectively. The predicted p*I* values for TaGlu1b and TaGlu1c were 5.15, and 5.81, respectively.