

Abstract

Two cDNA clones, OsFKI and OsFKII, encoding fructokinase (EC 2.7.1.4) were isolated from immature seeds of rice (*Oryza sativa* L.) by PCR. OsFKI cDNA encoded a deduced protein of 323 amino acids that was 59–71% identical to previously characterized plant fructokinases. In contrast, OsFKII cDNA encoded a deduced protein of 336 amino acids that shared only 64% amino acid identity with OsFKI. The deduced proteins both possessed an ATP-binding motif and putative substrate recognition site sequences that were previously identified in bacterial fructokinases. Genomic DNA blot analysis also revealed that each fructokinase gene exists as a single copy in the rice genome. The identity of OsFKI and OsFKII as fructokinases was confirmed by the expression of enzyme activity in *E. coli*. Although both OsFKI and OsFKII utilized fructose as substrate, only OsFKII activity was strongly inhibited at a high fructose concentration. The mRNA corresponding to OsFKII accumulated at high levels in developing rice grains, whereas there were only low levels of OsFKI transcripts in immature seeds. These results indicate that fructokinase in rice endosperm is encoded by two divergent genes, which play different roles in rice grains for starch storage based on their sensitivity to substrate inhibition and level of transcripts in endosperm.