

Title Cloning, biochemical characterization and expression of a sunflower (*Helianthus annuus* L.) hexokinase associated with seed storage compounds accumulation

Author M.A. Troncoso-Ponce, J. Rivoal, S. Dorion, M.-C. Moisan, R. Garcés and E. Martínez-Force

Citation Journal of Plant Physiology, Volume 168, Issue 4, 1 March 2011, Pages 299-308

Keywords Hexokinase; Sunflower; Biochemical characterization; Hexoses metabolism; Expression levels

Abstract

A full-length hexokinase cDNA, *HaHXK1*, was cloned and characterized from *Helianthus annuus* L. developing seeds. Based on its sequence and phylogenetic relationships, *HaHXK1* is a membrane-associated (type-B) hexokinase. The predicted structural model resembles known hexokinase structures, folding into two domains of unequal size: a large and a small one separated by a deep cleft containing the residues involved in the enzyme active site. A truncated version, without the 24 N-terminal residues, was heterologously expressed in *Escherichia coli*, purified to electrophoretic homogeneity using immobilized metal ion affinity chromatography and biochemically characterized. The purified enzyme behaved as a monomer on size exclusion chromatography and had a specific activity of 19.3 $\mu\text{mol}/\text{min}/\text{mg}$ protein, the highest specific activity ever reported for a plant hexokinase. The enzyme had higher affinity for glucose and mannose relative to fructose, but the enzymatic efficiency was higher with glucose. Recombinant *HaHXK1* was inhibited by ADP and was insensitive either to glucose-6-phosphate or to trehalose-6-phosphate. Its expression profile showed higher levels in heterotrophic tissues, developing seeds and roots, than in photosynthetic ones. A time course of HXK activity and expression in seeds showed that the highest HXK levels are found at the early stages of reserve compounds, lipids and proteins accumulation.