Title A cryoprotective and cold-adapted 1,3-β-endoglucanase from cherimoya (*Annona cherimola*) fruit

Author Oscar Goñi, María T. Sanchez-Ballesta, Carmen Merodio and María I. Escribano

Citation Phytochemistry, Volume 72, Issue 9, June 2011, Pages 844-854

Keywords *Annona cherimola*; *Annonaceae*; Protein purification; 1,3-β-Glucanase activity; Kinetic and thermodynamic characterization; Acidic endo-1,3-β-glucanase; Cryoprotective and cold-adapted 1,3-β-glucanase protein; Cryoprotection; Glycine-betaine

Abstract

A 1,3-β-glucanase with potent cryoprotective activity was purified to homogeneity from the mesocarp of CO_2-treated cherimoyafruit (*Annona cherimola* Mill.) stored at low temperature using anion exchange and chromatofocusing chromatography. This protein was characterized as a glycosylated endo-1,3-β-glucanase with a M_r of 22.07 kDa and a p*I* of 5.25. The hydrolase was active and stable in a broad acidic pH range and it exhibited maximum activity at pH 5.0. It had a low optimum temperature of 35 °C and it retained 40% maximum activity at 5 °C. The purified 1,3-β-glucanase was relatively heat unstable and its activity declined progressively at temperatures above 50 °C. Kinetic studies revealed low k_{cat} (3.10 ± 0.04 s^{-1}) and K_m (0.32 ± 0.03 mg ml^{-1}) values, reflecting the intermediate efficiency of the protein in hydrolyzing laminarin. Moreover, a thermodynamic characterization revealed that the purified enzyme displayed a high k_{cat} at both 37 and 5 °C, and a low E_a (6.99 kJ mol^{-1}) within this range of temperatures. *In vitro* functional studies indicated that the purified 1,3-β-glucanase had no inhibitory effects on *Botrytis cinerea*hyphal growth and no antifreeze activity, as determined by thermal hysteresis analysis using differential scanning calorimetry. However, a strong cryoprotective activity was observed against freeze–thaw inactivation of lactate dehydrogenase. Indeed, the PD_{50} was 8.7 μg ml^{-1} (394 nM), 9.2-fold higher (3.1 on a molar basis) than that of the cryoprotective protein BSA. Together with the observed accumulation of glycine-betaine in CO_2-treated cherimoya tissues, these results suggest that 1,3-β-glucanase could be functionally implicated in low temperature-defense mechanism activated by CO_2.