Beta-Fructosidase from the Midgut of *Spodoptera frugiperda* (Lepidoptera) larvae.

Ferreira C., Faria G. , Terra W.R.

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo

Beta-fructosidases are present in bacteria, fungi, plants and in the midgut of Lepidoptera, where they are supposed to originate by horizontal transfer from bacteria. Their kinetical properties are not fully known and we describe the first attempts to characterize the enzyme from *S.frugiperda*. A transcriptome (454 Roche platform) of the insect midgut disclosed a single cDNA that codes for a beta-fructosidase with 486 amino acids containing a 18 amino acid signal peptide and the residues important to activity. The enzyme was purified from the midgut using hydrophobic and ionic exchange chromatographies. A recombinant enzyme (SfFru) was expressed in BL21 (DE3) cells and purified by Ni-NTA Agarose resin. Purified and recombinant enzymes have similar Km values using sucrose as substrate (4.4±0.3mM and 3.5±0.2mM respectively), indicating that the recombinant enzyme can be used to study enzyme properties and that the sequence found in the transcriptome corresponds to the enzyme purified from the insect. Moreover, molecular weight (theoretical) found for the sequence is 53,993.8 and for the purified and recombinant enzyme 55,600 and 56,000, respectively. The recombinant enzyme can hydrolyse raffinose (Km 35.96±6.66), has an optimum pH of 6.5 and is stable from pH 5.0 to 9.0 when incubated at 30^0C for 1 h. Previous studies with bacterial and plant beta-fructosidases hypothesized that the change in pKa of the proton donor residue (SfFru E214) that occurs after substrate binding is due to an Arg or Tyr residue. Phenyl glyoxal modification leads to an inactive enzyme whereas Tetranitro metane (TNM) modification decreases 70% of the activity, indicating that an Arg and a Tyr residue are important for activity. The unmodified and TNM modified enzyme presented the same activity pH profile, indicating that Tyr is not responsible for the E214 pKa change. Further studies with site directed mutants may shed light on the subject.

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