PURIFICATION OF A MEMBRANE-BOUND TREHALASE FROM SPODOPTERA FRUGIPERDA MIDGUT AND CLONING OF THE CORRESPONDING cDNA.

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Trehalose (glucose a-1,1-glucose) is a disaccharide occurring in invertebrates, plants and fungi. In insects this sugar is the reserve of metabolic energy, and is mobilized only by trehalase. The midgut of *Spodoptera frugiperda* larvae has two trehalases: one soluble and one membrane-bound. The soluble enzyme has already been characterized, sequenced, cloned and expressed. The membrane-bound trehalase apparently is solubilized by an endogenous serine proteinase during homogenate manipulation. Purification of the solubilized enzyme was achieved submitting the enzyme to the following sequential chromatographic steps: ion-exchange chromatography in HighQ column, hydrophobic chromatography in Methyl Sepharose column, ion-exchange chromatography in MonoQ column, hydrophobic chromatography in Isomethyl column and a gel filtration in Superose 12. After SDS-PAGE of the pure enzyme only one band with molecular weight of 67 kDa was seen. The enzyme is purified 10 times with a specific activity of 10 U/mg. Using different strategies we sequenced the cDNA that putatively code for this trehalase. The deduced protein contains 652 amino acid residues, has the predicted signal sequence cleavage site between Gly 18 and Met 19, six potential N-glycosylation sites (Asn-X-Thr/Ser) and a transmembrane region spanning from Gly 580 to Tyr 606. The membrane- bound trehalase is more similar (90%) to a midgut membrane-bound trehalase present in *Bombyx mori*. Supported by Fapesp and CNPq.