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Physiological implications of trehalase from *Phaseolus vulgaris* root nodules: partial purification and characterization

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Abstract

The purification and characterization of trehalase from common bean nodules as well as the role of this enzyme on growth, nodulation nitrogen fixation by examining the effects of the trehalase inhibitor validamycin A, was studied. Validamycin A did not affect plant and nodule mass, neither root trehalase and nitrogenase activity; however this treatment applied at the time of sowing increased nodule number about 16% and decreased nodule trehalase activity (16-fold) and the size of nodules. These results suggest that nodule trehalase activity of *Phaseolus vulgaris* could be involved in nodule formation and development. In addition, acid trehalase (EC 3.2.1.28) was purified from root nodules by fractionating ammonium sulfate, column chromatography on DEAE-sepharose and sephacryl S-300, and finally on native polyacrylamide gel electrophoresis. The purified homogeneous preparation of native acid trehalase exhibited a molecular mass of 42 and 45 kDa on SDS-PAGE. The enzyme has the optimum pH 3.9, K_m of 0.109 mM, V_{max} of 3630 nkat mg^{-1} protein and is relatively heat stable. Besides trehalose, it shows maximal activity with sucrose and maltose and, to a lesser degree melibiose, cellobiose and raffinose, and it does not hydrolyze on lactose and turanose. Acid trehalase was activated by Na^+, Mn^{2+}, Mg^{2+}, Li^+, Co^{2+}, K^+ and inhibited by Fe^{3+}, Hg^+ and EDTA.

Keywords: Carbon metabolism; Nodules; *Phaseolus vulgaris*; Trehalase

1. Introduction

Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside), a non-reducing disaccharide, has been found in a wide variety of organisms such as yeast, fungi, bacteria, plants, insects, and other invertebrates. In invertebrates and fungi trehalose serves as an endogenous energy reserve, for a variety of physiological activities, and as an intracellular stress manager [37,38]. In addition, in *Saccharomyces cereviceae*, trehalose is one of the major storage carbohydrate accounting for 1% to more 23% of dry weight of the cells, depending on the growth conditions and stage of the life cycle [18], where it plays the role of a protector of plasma membranes and cytoplasmic enzymes [11,41]. Trehalose also plays an important role as an abiotic stress protectant in a large number of organisms, including bacteria, yeast and invertebrate [2]. Trehalose has been shown to stabilize dehydrated enzymes and membranes as well as to protect biological structures from desiccation damage.

In vascular plants, trehalase occurs in certain pteridophytes (Clubmosses) and several eusporangiate ferns, where it often exceeds sucrose in concentration and indeed appears to replace sucrose as a translocated and stored disaccharide [16]. However, in higher vascular plants, trehalose is a rare sugar and it may be even deleterious [9,40]. It was found that trehalose may occur in the plants with diseases [12] or colonized by microorganisms, for example in mycorrhizal roots [7], nitrogen-fixing nodules [33] and actinorhizal nodules [20]. Large amounts of trehalose were detected in the soybean root nodule [24,26]. After the cell fractionation it was found that about 70% of the trehalose was localized in the bacteroid and 30% was present in the soluble plant material [34].

Abbreviations: DAS, days after sowing; DW, dry weight; EDTA, ethylenediamine tetraacetic acid; MES, 2-(*N*-morpholino)-ethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; Tris–HCl, 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochlorid.

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