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Original article

Partial purification and characterization of a non-specific acid phosphatase in leaves and root nodules of *Phaseolus vulgaris*

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Abstract

Acid phosphatase (ACP) activity in common bean grown with or without 1.5 mM of phosphate has been examined. Leaves and root nodules responded to the absence of an exogenous phosphate source with an increase in ACP activity. Increases in enzyme activity were not associated with the synthesis of new isoforms of the enzyme. We partially purified and characterized the ACPs, which consisted of three proteins, one of leaf and two of nodule. Proteins of leaf migrated at 72 and 51 kDa in SDS-PAGE, whereas that of nodule migrated at 72, 49, 41 and 34 kDa. Enzymes of both organs had a pH optimum of 5.6, and were relatively heat stable. The enzymes exhibit a broad substrate selectivity, with maximal activity obtained with α-naphthyl-phosphate, ribulose 1,5-bisphosphate and *p*-nitrophenyl-phosphate (*p*-NPP). Potent inhibition by Zn^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+}, Al^{3+} and $(MoO_4)^{2-}$ was observed.

Keywords: Acid phosphatase; *Phaseolus vulgaris*; Phosphate metabolism; Purification

1. Introduction

Phosphorus is essential for the growth and development of organisms and is one of the most important but least available mineral nutrients required by plant, in part because it is commonly bound to many soil constituents. For organic phosphorus sources to be used they must be first hydrolyzed by phosphatases [10]. Plant acid phosphatase (ACP) examined include the enzyme from seeds [8,12,17], leaves [28], bulbs [13], root nodules [23], coleoptiles [22], cotyledons [27], tubers [9], roots [18] and plumules [14].

ACPs (ortophosphoric-monoester phosphohydrolase, EC 3.1.3.2) are ubiquitous in a broad variety of animals, plants and microorganisms. ACPs are widely distributed in plants and exist as compartment and/or tissues-specific isoforms that can significantly differ in subunit molecular mass, substrate specificity, and their susceptibility to inhibition by various compounds [23,31,34]. ACPs catalyze the hydrolysis of phosphate esters, releasing inorganic phosphorus from a phosphorylated substrate in vitro, making their cellular role(s) difficult to define [23]. They tend to display little substrate specificity and appear to be important in the production, transport and recycling of inorganic phosphorus. Due to low substrate specificity, ACPs are presumed to be involved in non-specific hydrolysis of organic phosphorus resulting in elevation of the (Pi) phosphorus pool and appear to function in response to phosphate deficiency [6,7]. However, changes in specific isoforms of phosphatases under Pi starvation are commonly observed [4,11,29].

Related to this, it has been observed that common bean (*Phaseolus vulgaris*) root nodules export nitrogen in the form of ureides allantoin and allantoic acid, which are derived from de novo-synthesized purines [5,26]. Even though the first step in the conversion of purines to ureides is the removal of the 5′-phosphate group by a non-specific ACP [15] or by a 5′-nucleotidase [3,5,20], a comprehensive understanding of the metabolic function of ACPs in nodules and leaves from legumes is lacking partly because of the heterogeneity and large number of phosphatases.

In the present work we describe the partial purification and characterization of a soluble ACP from nodules and leaves of common bean. We also discuss the effect of P doses (–P and +P) supplied to the plants on ACP activity in both

Abbreviations: ACP, acid phosphatase; PAGE, polyacrylamide gel electrophoresis; PEP, phosphoenolpyruvate; *p*-NPP, *p*-nitrophenyl-phosphate; SDS, sodium dodecyl sulfate.

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