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Purification, properties and specificity of a NDP kinase from *Alyssum murale* grown under Ni^{2+} toxicity

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Summary

During the growth of *Alyssum murale*, a nickel accumulator plant, three root peptides chains of 55, 18 and 16 kDa undergo phosphorylation. The intensity of the phosphorylated bands decreased in the course of growth in nutrient solution supplied with 0.5 mM Ni^{2+}. In the shoot only two phosphorylated peptide chains with a size of 18 and 16 kDa were detected. These two shoot peptides disappeared on the 19th day of growth in Ni^{2+}-exposed plants, while the root peptide of 16 kDa continued to be present in less intensity. This peptide was identified as the catalytic subunit of nucleoside diphosphate kinase (NDP kinase: E.C. 2.7.4.6) and was named NDPK-B. The enzyme was purified by means of ammonium sulphate precipitation, DEAE-sepharose and hydroxyapatite column chromatography. NDPK-B was thermostable, displayed a molecular mass of 103,000 and was comprised of six catalytic subunits. The autophosphorylated enzyme displayed an isoelectric point (pI) of 6.5. The NDPK-B autophosphorylation activity was metal-dependent. With regard to the transfer reaction, NDPK-B exhibited the following properties: (a) the enzyme had an optimum pH of 7.6; (b) it was capable of using both (γ-^{32}P) ATP and (γ-^{32}P) GTP as phosphate donors and of using all the available NDPs except dCDP as phosphate acceptors; (c) its activity using NDPs as substrates was metal dependent; (d) in the presence of (γ-^{32}P) GTP as the phosphate donor, it phosphorylated exclusively ADP when a mixture of NDPs was added in the reaction mixture; and, (e) ADP had a very low K_m value towards 8.4 nM. This high affinity towards ADP suggests that the enzyme may

Abbreviations: ATP-PRT, ATP-phosphoribosyltransferase; EDTA, ethylene-diamine-tetra-acetic acid; IEF, isoelectric focusing; NDP, purino- or pyrimido- diphosphonucleoside; NDPK, kinase of diphosphonucleoside

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play a crucial function in the formation of the amount of ATP necessary for *Alyssum murale* to survive Ni^{2+} stress.

Introduction

Alyssum murale is a nickel hyperaccumulator plant. Hyperaccumulators are plants native to metalliferous soils that take up large quantities of metallic elements compared to coexisting species. Nickel is the most frequently hyperaccumulated metal. In the Brassicaceae family 48 *Alyssum* species can accumulate 1280–29,400 $\mu g\,g^{-1}$ Ni^{2+} in their leaves (dry weight), while Ni^{2+} concentrations in the leaves of plants normally range between 0.5 and 10 $\mu g\,g^{-1}$. Nickel was established as an essential micronutrient for the growth of higher plants (Brown et al., 1987).

Data regarding the tolerance range and the toxic effect of metals on serpentine species are scarce. It has been observed in two serpentine plants, namely *Festuca rubra* and *Alyssum bertolonii*, that phosphatase activity is increased by high Ni^{2+} concentrations (Johnston and Proctor, 1984; Gabbrielli et al., 1989), while no significant response is found in peroxidase activity of *Alyssum bertolonii* (Gabbrielli et al., 1990). The response of antioxidative enzymes (superoxide dismutase, ascorbate peroxidase and glutathione reductase) was also examined in plants of the genus *Alyssum* under Ni^{2+} and Cd^{2+} stress (Schickler and Caspi, 1999). Recently the response of a new DNase in relation to nickel and manganese accumulation in *Alyssum murale* has been reported (Abou Auda et al., 2002). Furthermore, Ingle et al. (2005) have suggested that ATP-phosphoribosyltransferase [ATP-PRT, the first enzyme involved in histidine (His) biosynthesis] expression is very important in regulating the pool of free His and that it contributes to the exceptional Ni tolerance of hyperaccumulator *Alyssum* species. The association of His with Ni tolerance of hyperaccumulator plants is attributed to detoxification of the metal anion by chelation and vacuolar compartmentalization, as well as to the effective translocation of Ni from root to shoot in the xylem (Krämer et al., 1996, 2000). In *Alyssum murale* Ni concentrates in stem and leaf dermal (epidermal vacuoles) tissues (McNear et al., 2005).

Diphosphonucleoside kinases (NDP kinases) (E.C.2.7.4.6) are classic metabolic enzymes that catalyse the transfer of γ-phoshate group of 5′-triphosphonucleosides (NTPs) to 5′-diphosphonucleosides (NDPs) through a ping–pong mechanism according to the following reactions:

$$E + N_1TP \rightleftharpoons E{\sim}P + N_1DP$$
$$E{\sim}P + N_2DP \rightleftharpoons N_2TP + E$$
$$N_1TP + N_2DP \rightleftharpoons N_2TP + N_1DP$$

N_1 and N_2 represent any type of purino- or pyrimidine-nucleoside. The enzymes are also unspecific regarding the nature of the nucleoside sugar (ribo- or deoxyribo-). The intermediate autophosphorylated enzyme (E∼P) is stable and can be separated in the absence of phosphate acceptor (NDP). NDP kinases are the bridge between oxidative phosphorylation and other cell processes, such as the synthesis of nucleic acids (Creanor and Mitchison, 1989) sugars and lipids (Bovet and Siegenthaler, 1997; Marechal et al., 1997).

NDP kinases also participate in the regulation of growth, development and in the transmission and transduction of signals into cells (Sommer and Song, 1994; Zhang et al., 1995; Pan et al., 2000). Plant NDP kinases have been studied among and within species such as pea seeds (Edlund, 1974) and mitochondria (Struglies and Hakansson, 1999), spinach chloroplast (Bovet and Siegenthaler, 1997), imbibing soybean (Krishnan et al., 1999), *Acer pseudoplatanus* L. (Perata et al., 1992), oat (Sommer and Song, 1994) and sugar cane (Moisyadi et al., 1994).

At the molecular level genes that express plant NDP kinases have been identified and cloned in, for example, pea (Finan et al., 1994), rice (Yano et al., 1995), oat (Sommer and Song, 1994), spinach (Nomura et al., 1992) and ryegrass (Larsen, 2001). Furthermore, a transgenic plant has been created (Pan et al., 2000).

The aim of the present work is to study the effect of nickel accumulation on the endogenous protein phosphorylation of *Alyssum murale* soluble protein extract. Furthermore, a Ni^{2+}-tolerant root isoform of NDP kinase isoform (named NDPK-B) with high affinity to ATP was purified and characterized.

Materials and methods

Plant material

Seeds of *Alyssum murale* Waldstein and Kitaibel (Brassicaceae) were collected from plants grown in a metalliferous region of North Greece (Vavdos, Chalkidiki,