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Nucleoside Diphosphate Kinase Is a Possible Component of the Ethylene Signal Transduction Pathway

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Abstract—In etiolated seedlings of *Pisum sativum* and leaves of *Arabidopsis thaliana*, *in vivo* ethylene treatment resulted in an increase in *in vitro* phosphorylation of 17 kD (*P. sativum*) or 16 and 17 kD (*A. thaliana*) polypeptides. These polypeptides were identified as nucleoside diphosphate kinase (NDPK) based on both biochemical properties and interaction with antibodies against NDPK from *P. sativum*. Using the receptor-directed antagonist of ethylene action 2,5-norbornadiene and the ethylene-insensitive mutants of *A. thaliana etr1-1* and *eti5*, ethylene specificity and receptor dependence of NDPK phosphorylation have been demonstrated. In pea epicotyls, ethylene treatment also led to increase in nucleoside transferase activity unlike in *A. thaliana* leaves. The increases in nucleoside transferase activity and NDPK phosphorylation were very rapid and transient. The results suggest a role for NDPK as a possible component of the ethylene signal transduction chain.

Key words: *Arabidopsis thaliana*, *Pisum sativum*, nucleoside diphosphate kinase, signal transduction, phosphorylation, ethylene

Nucleoside diphosphate kinase (NDPK; ATP:NDP phosphotransferase; EC 2.7.4.6) catalyzes the transfer of the γ-phosphate of 5′-nucleoside triphosphates to 5′-nucleoside diphosphates with low substrate specificity [1]. The enzyme appears to be ubiquitously distributed in all living organisms from bacteria to animals and plants. It is a 70- to 100-kD protein composed of several copies of polypeptides of 16-20 kD. In most eukaryotic species, NDPK exists as a hexamer, while in prokaryotic species it is a tetramer [2]. Both cytosolic and membrane-associated isoforms have been isolated from bacteria [3], animals [4], and plants [5]. Initially, the functions of NDPK have been thought to be the maintenance of nucleoside triphosphate pool in the cells [1]. Recent reports have revealed a number of distinct biochemical activities for NDPK other than transfer of the phosphate group between nucleotides [6, 7]. Thus, in animal cells NDPK is implicated in cell proliferation [8] and transcription regulation [4, 9]. On the other hand, the enzyme can function as a protein kinase [10, 11]. In plants, UV-B signaling [12] and phytochrome B response [13] are the processes in which NDPK has been shown to be involved. In relation to ethylene responses, of major significance is the observation that the expression of the gene *TAB2* encoding NDPK is upregulated rapidly in response to wounding in tomato leaf and stem tissues [14]. There is also evidence that NDPK is involved in response to heat shock in cultured sugarcane cells (*Saccharum officinarum* L.) [5] and in pea plants (*Pisum sativum* L.) [15].

The objective of this work was to study ethylene effects on NDPK activity and NDPK phosphorylation in relation to a possible involvement of this enzyme in ethylene signaling.

MATERIALS AND METHODS

Plant material and treatments. Etiolated pea seedlings (*Pisum sativum* L., cv. Alaska) were grown in vermiculite in the dark for 5 days at 23 ± 2°C. *Arabidopsis thaliana* (L.) Heynh., ecotype Columbia (wild-type plants and ethylene-insensitive *etr1-1* and *eti5* mutants)

Abbreviations: DTT) dithiothreitol; NBD) 2,5-norbornadiene; NDPK) nucleoside diphosphate kinase; PMSF) phenylmethylsulfonyl fluoride; PVPP) polyvinylpolypyrrolidone; TBST) 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20.

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were grown in compost in growth cabinets at 21 ± 1°C, 16-h photoperiod, and photosynthetically active radiation of 90 μmol·m^{-2}·sec^{-1}. Plants were collected at the early flowering stage (five weeks old).

For treatments, plant tissue was placed in a 1-liter Kilner jar sealed with a Perspex lid fitted with a SubaSeal injection port. Since at room temperature NBD is an easily evaporating liquid, it was introduced into jars by spotting appropriate volumes onto filter paper strips to facilitate evaporation. NBD concentrations are given in the gas phase.

All manipulations with etiolated pea seedlings were performed under dim green light. Intact pea seedlings (15 seedlings per jar) were treated at 23 ± 2°C in the dark as follows: with 1 μl/liter ethylene for various time intervals as indicated, with 2000 μl/liter NBD for 2 h, or with 2000 μl/liter NBD for 2 h followed by 1 μl/liter ethylene. After treatment, the apical 1.5-2 cm of epicotyls were excised and immediately frozen in liquid nitrogen, and stored at −70°C.

Rosettes of 5-week-old *A. thaliana* plants were separated from roots and flower stalks and placed into Kilner jars (approximately 10 g fresh weight per jar) to which 1 μl/liter ethylene was injected. Treatments were conducted in the light at room temperature. After treatment, rosettes were used immediately for membrane isolation or, where it was appropriate, frozen in liquid nitrogen and stored at −70°C.

Isolation of membrane-enriched fractions. All procedures were performed at 4°C. Pea epicotyl tips were homogenized in buffer (1 : 2, w/v) containing 50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 2 mM Na-EDTA, 1 mM DTT, 1 mM PMSF, 1 mM diethyldithiocarbamic acid Na-salt, and 250 mM sucrose. PVPP was added into the buffer at ratio 1 : 20 (w/w) of plant tissue. The homogenate was filtered through 250-μm nylon mesh and centrifuged for 20 min at 13,000*g*. The pellet was discarded, and the supernatant was centrifuged for 4 h at 130,000*g*. The resulting supernatant was discarded and the pellet was resuspended in the homogenization buffer without diethyldithiocarbamic acid Na-salt but supplemented with 20% (w/v) glycerol to a protein concentration of about 10 mg/ml. Then the membrane suspension was divided into aliquots (100-1000 μl), frozen in liquid nitrogen, and stored at −70°C.

A. thaliana leaves were homogenized in buffer (1 : 1.5, w/v) composed of 50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 2 mM Na-EDTA, 1 mM DTT, 1 mM PMSF, 1 mM diethyldithiocarbamic acid Na-salt, 5 mM L-ascorbic acid, 3.6 mM L-Cys, and 250 mM sucrose. PVPP (1 : 10, w/w) was added into the buffer prior to homogenization. The homogenate was filtered through 250-μm nylon mesh and centrifuged for 20 min at 13,000*g*. The pellet was discarded, and the supernatant was centrifuged for 1 h at 50,000*g*. The 50,000*g* supernatant was collected and centrifuged for 4 h at 130,000*g*. The resulting membrane pellet was resuspended in the homogenization buffer supplemented with 20% (w/v) glycerol but without diethyldithiocarbamic acid Na-salt, L-ascorbic acid, and L-Cys to a final protein concentration of 5-6 mg/ml. The membrane suspension was divided into aliquots (100-1000 μl), frozen in liquid nitrogen, and stored at −70°C.

Membrane protein phosphorylation *in vitro* was performed in a mixture (50 μl) containing 50 mM Tris-HCl, pH 7.6, 15 mM $MgCl_2$, 0.1% (w/v) Triton X-100, 10 mM NaF, 20 nM ATP, and 37 kBq [γ-^{32}P]ATP (specific activity 110 TBq/mmol, Amersham Pharmacia Biotech, UK-Sweden). The reaction was initiated by addition of 10-20 μg protein and allowed to proceed for 10 min at 30°C. Then the reaction was terminated with 50 μl double SDS-PAGE sample buffer followed by boiling for 5 min. Polypeptides were separated by 15% SDS-PAGE, stained with 0.2% Coomassie Brilliant Blue in 40% (v/v) methanol with 10% (v/v) acetic acid, dried, and autoradiographed with Kodak Biomax MR-1 film (Kodak, USA).

We previously found that there was no specific ethylene effect upon phosphorylation in 50,000*g* membrane fraction isolated from *A. thaliana* leaves [16]. Therefore, only the 50,000-130,000*g* membrane fraction was examined in this study.

Assay for GTP formation by NDPK. For assaying NDPK phosphotransferase activity, membrane proteins were solubilized. For this, the membrane suspension (50,000-130,000*g*) was mixed with 25 mM Tricine-NaOH, pH 8.0, 1 mM Na-EDTA, 1 mM DTT, 5 mM Mg-acetate, 0.1 mM PMSF, and 0.1% CHAPS (Sigma, USA) and stirred for 30 min at 4°C. Then the suspension was centrifuged for 1 h at 130,000*g* and the supernatant was collected and used for the assay. In preliminary experiments several detergents—0.1% (w/v) CHAPS, 1% (w/v) CHAPS, 1% (w/v) MEGA-9 (Sigma), 1% (w/v) Triton X-100, 1% (w/v) deoxycholic acid, 1% (w/v) cholic acid, and 1% (w/v) Empigen (Calbiochem, USA)—were tested for their ability to solubilize NDPK activity. The maximum NDPK activity was found when proteins were solubilized with 0.1% CHAPS, which was used in all subsequent experiments. The enzyme activity was measured in an assay mixture (10 μl) containing 50 mM Na-Hepes, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 5 μg BSA, 2 mM GDP, 2 mM ATP, and 74 kBq [γ-^{32}P]ATP (specific activity 110 TBq/mmol; Amersham Pharmacia Biotech) [3]. Solubilized membrane protein (50-200 ng) was added to start the reaction. The mixture was incubated for 10 min at 30°C and the reaction was terminated with 50 μl 10 mM Na-EDTA, pH 3.65. Aliquots of 1 μl were spotted onto a polyethyleneimine-cellulose TLC plate and reaction products were resolved by ascending chromatography with 0.75 M KH_2PO_4, pH 3.65. The plate was dried and exposed to Kodak Biomax MR-1 film.

Immunodetection of proteins was performed using the western blot technique. After SDS-PAGE in 12.5% gel, polypeptides were transferred onto Hybond-C Pure nitrocellulose membrane (Amersham Pharmacia Biotech) in a Trans-Blot SD Electrophoretic Semi-Dry Transfer Cell (Bio-Rad, USA). Transfer was conducted in buffer consisting of 25 mM Tris-HCl, pH 8.4, 191 mM Gly, 20% (v/v) methanol, and 0.1% (w/v) SDS at 0.8 mA/cm^2 and 25 V for 2 h. Then membranes were washed with TBST and blocked in 2% (w/v) gelatin (Sigma) in TBST. Blots were challenged with rabbit polyclonal antibodies raised to NDPK isolated from pea epicotyls (a generous gift from Dr. P. A. Millner, Biochemistry Department, Leeds University, UK). Primary antibodies were diluted with 2% gelatin and normal rabbit antiserum (1 : 500 dilution; Sigma) and incubation was carried out overnight at 4°C. Alkaline phosphatase-conjugated goat anti-rabbit IgGs (Sigma) were used as secondary antibodies. The reaction was visualized by incubating blots with 2 μl/ml 5-bromo-4-chloro-3-indolyl-1-phosphate and 4 μl/ml nitro blue tetrazolium (both Pierce Biotechnology, USA) in 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, and 5 mM $MgCl_2$.

Protein concentration was determined with a BCA Protein Assay Kit (Pierce) according to manufacturer's instruction using BSA as a standard protein. Bicinchoninic acid- (BCA) based reagent does not interfere with ionic and nonionic detergents (Triton X-100, CHAPS, SDS), high salt contents, NaOH, and Na-EDTA within a range of concentrations used in this study.

Quantification of autoradiographs and immunoblots was performed after scanning in a GS-690 Imaging Densitometer (Bio-Rad) using Molecular Analyst Software (Bio-Rad) or Phoretix 1D software (Nonlinear Dynamics, USA).

All experiments were performed 3-5 times with different batches of membrane preparations. The results were fully reproducible and typical data are presented.

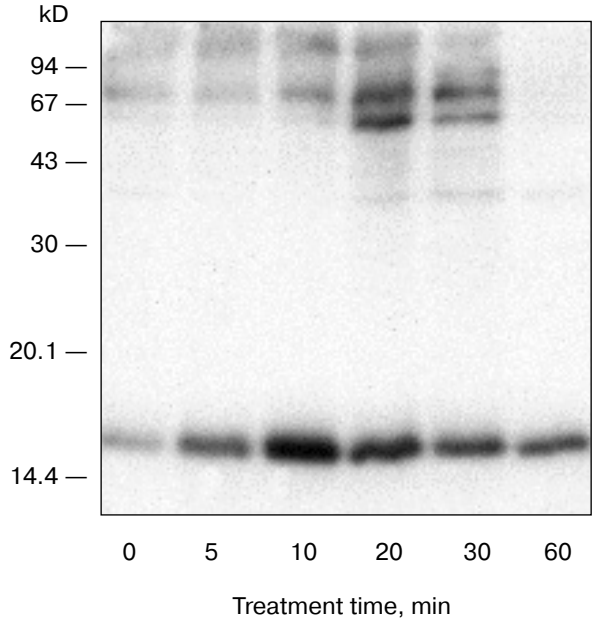

Fig. 1. Time course of 17-kD polypeptide phosphorylation as affected by ethylene (1 μl/liter).

RESULTS

Phosphorylation of 17-kD membrane protein from etiolated pea epicotyls in the absence of Mg^{2+}. Figure 1 shows the effect of ethylene on protein phosphorylation in pea membrane preparations. Ethylene treatment led to a marked increase in phosphorylation of a 17-kD polypeptide. Time-course studies on *in vitro* phosphorylation revealed that the phosphorylation level of the 17-kD polypeptide increased within 5 min of ethylene treatment and peaked after 10 min followed by a slow decrease over the next 50 min (Fig. 1).

Upregulation of the 17-kD polypeptide phosphorylation by ethylene was specific for this phytohormone since it was significantly reduced when the epicotyls were pretreated with the receptor-directed inhibitor of ethylene action NBD. NBD alone did not elicit ethylene response but its binding to ethylene receptors antagonizes the "triple response" in pea seedlings [17]. We found that ethylene treatment (1 μl/liter) of pea seedlings resulted in 3.8-fold increase in 17-kD polypeptide phosphorylation while in the presence of NBD (2000 μl/liter) this increase was 2-fold. NBD applied alone consistently increased 17-kD polypeptide phosphorylation up to 1.9-fold but always less than shown by ethylene in the same experiment. Since NBD is capable of occupying ethylene receptors, this binding possibly triggers alteration in protein phosphorylation, but at a level that does not elicit growth responses.

In the presence of Na-EDTA (10-15 mM), phosphorylation of most pea membrane proteins was inhibited (Fig. 2, lane *2*) as compared to phosphorylation in the presence of free Mg^{2+} (Fig. 2, lane *1*). In the absence of free Mg^{2+} the only phosphorylated protein was the 17-kD polypeptide. This observation is consistent with the properties of NDPK, since unlike most protein kinases NDPK shows autophosphorylation irrespectively of free Mg^{2+} [7, 18].

Earlier, it has been demonstrated that NDPK can utilize both ATP and GTP as phosphate donors for autophosphorylation [19, 20]. To determine the preferred phosphate donor, pea membrane proteins were phosphorylated in the presence of various concentrations of ATP

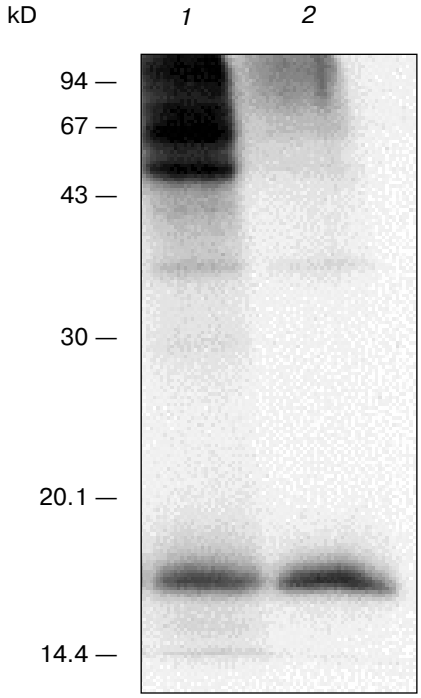

Fig. 2. Effect of Mg^{2+} on phosphorylation of membrane proteins from etiolated pea epicotyls. Phosphorylation *in vitro* was performed in the absence (lane *1*) or in the presence (lane *2*) of 10 mM Na-EDTA.

or GTP. As Fig. 3 shows, GTP is more effective as a donor of phosphate for an acid-stable phosphorylation (Ser/Thr) since it required an order of magnitude lower concentration of unlabeled GTP than ATP to inhibit phosphorylation of 17-kD polypeptide by 50%. Similar results were obtained for NDPK from intact pea mitochondria [18]. Human NDPK isoforms have also been reported to be more readily phosphorylated with GTP than any other NTP [19].

Immunological study of 17-kD polypeptide from pea epicotyl membranes. In order to support our assumption that 17-kD polypeptide is likely to be NDPK, antibodies to NDPK from pea epicotyls were employed. Western analysis demonstrated that the only protein recognized by the antibodies was the 17-kD polypeptide (Fig. 4, panel *2*). Although no difference in 17-kD polypeptide content was detected after ethylene treatment (Fig. 4, panel *2*), the enhancing effect of ethylene on phosphorylation of the polypeptide was clearly observed (Fig. 4, panel *3*).

NDPK activity in membrane preparations from etiolated pea epicotyls. NDPK activity in preparations of solubilized membrane proteins was evaluated by the capacity to convert GDP into [γ-^{32}P]GTP in the presence of [γ-^{32}P]ATP. Figure 5 shows that ethylene promoted formation of [γ-^{32}P]GTP. The activation was transient, reaching a maximum at 5-10-min treatment and thereafter decreasing to the level detected in samples from untreated pea epicotyls.

NDPK in *A. thaliana* leaves. Previously, we demonstrated that ethylene at physiological concentrations stimulated phosphorylation of 14-20-kD membrane proteins from wild-type *A. thaliana* leaves [16]. In contrast to pea

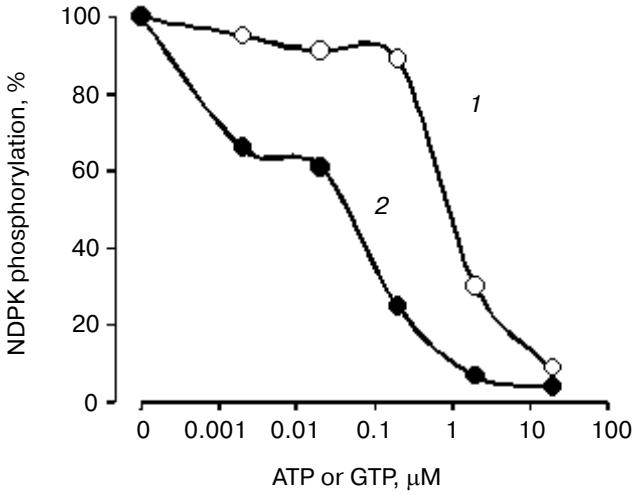

Fig. 3. Phosphorylation of membrane-associated NDPK from etiolated pea epicotyls versus concentration of ATP (*1*) or GTP (*2*). After phosphorylation, proteins were subjected to SDS-PAGE followed by autoradiography; autoradiographs were scanned and 17-kD band phosphorylation was estimated from the optical density. Phosphorylation of the 17-kD band in the absence of unlabeled ATP or GTP was taken as 100%.

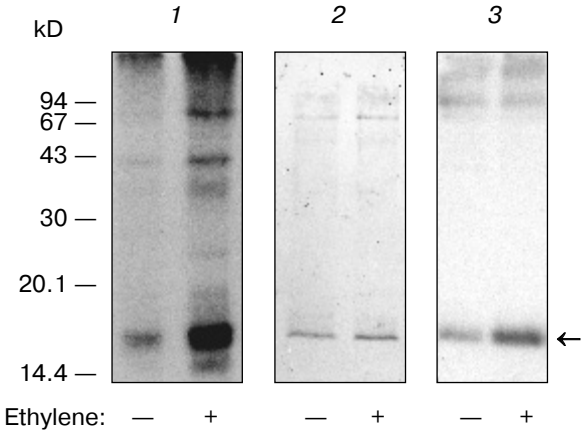

Fig. 4. Western analysis of membrane proteins from pea epicotyls with anti-NDPK antibodies. Panels: *1*) autoradiograph of *in vitro* phosphorylated membrane proteins; *2*) immunoblot of membrane proteins; *3*) autoradiograph of the immunoblot.

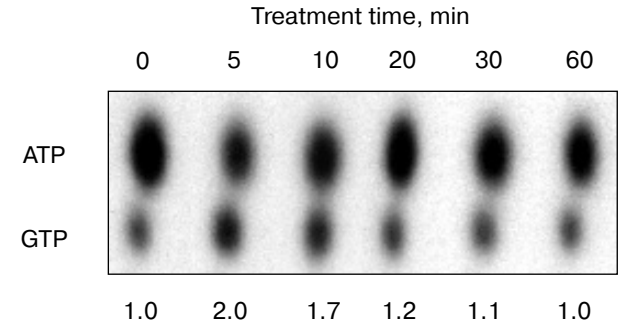

Fig. 5. Effect of ethylene (1 μl/liter) on membrane-associated NDPK activity from pea epicotyls. Autoradiographs of TLC plates were normalized against GTP level formed at zero time (untreated control).

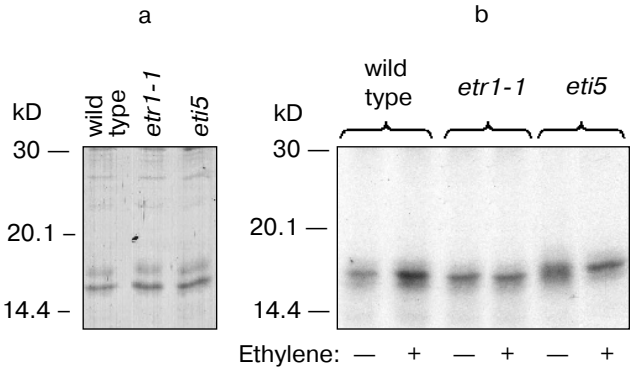

Fig. 6. Western blot of membrane proteins from *A. thaliana* leaves probed with anti-NDPK antibodies (panel (a)). Panel (b) is an autoradiograph of (a).

epicotyls, two bands at 16 and 17 kD interacted with antibodies to pea NDPK, the content of 16-kD polypeptide being higher (Fig. 6a). Radioactive bands corresponding to both polypeptides were observed (Fig. 6b) but phosphorylation of 17-kD polypeptide was greater. Ethylene treatment led to an increase in phosphorylation of both polypeptides. Thus, it appears that NDPK in *A. thaliana* leaves is composed of 16- and 17-kD polypeptides.

In the two ethylene-insensitive mutants *eti5* and *etr1-1*, the same 16- and 17-kD polypeptides interacted with NDPK antibodies (Fig. 6a). While the amounts of polypeptides were similar to those in wild-type plants, the constitutive phosphorylation of the 17-kD band was higher in *eti5* than in wild type, whereas in *etr1-1* it was similar to the latter (Fig. 6b). The most important observation was that in both *etr1-1* and *eti5* mutants phosphorylation of the 17-kD polypeptide was unaffected by ethylene (Fig. 6b). This fact lends support to the receptor-dependent activation of NDPK phosphorylation because neither mutant responds to ethylene. Equally, ethylene-binding capacity in *etr1-1* and *eti5* is reduced by 80% [21] and 60% [22], respectively, as compared to wild type.

NDPK activity was assayed in all membrane preparations, but no ethylene effect was detected. In addition, there was no significant difference in the enzyme activity in wild type and *eti5* (Fig. 7).

DISCUSSION

The work presented here shows that ethylene rapidly and transiently and with high specificity promotes phosphorylation of a 17-kD membrane polypeptide from etiolated pea epicotyls (Fig. 1). Since autophosphorylation in the absence of free Mg^{2+} is the characteristic feature of NDPK [7, 18], the data shown in Fig. 2 suggest that the 17-kD polypeptide is a subunit of NDPK.

Western analysis revealed the interaction of the 17-kD band with antibodies raised to NDPK from pea epicotyls (Fig. 4), which supports the above suggestion. The finding of enzymatic activity catalyzing the conversion of GDP to GTP (Fig. 5) is further evidence for our suggestion.

In membranes from wild-type *A. thaliana*, two polypeptides with M_r of 16 and 17 kD were found whose phosphorylation was regulated by ethylene. These polypeptides correspond to the M_r values of NDPK subunits. Probing *A. thaliana* membranes with antibodies to pea NDPK showed that both polypeptides interacted with antibodies (Fig. 6a).

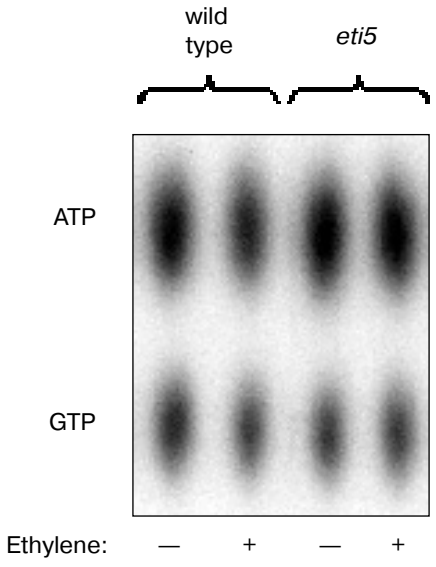

Fig. 7. Effect of ethylene (1 μl/liter) on NDPK activity in membranes from *A. thaliana* leaves.

Apparently, in *Arabidopsis* leaves NDPK consists of two subunits, which is typical for most eukaryotes [2].

It is well known that phosphorylation of enzymes can lead to changes in their activities. Indeed, in pea membranes we observed an increase in NDPK activity under ethylene treatment (Fig. 5) as well as phosphorylation of NDPK polypeptide of 17 kD (Figs. 1 and 4), whereas in *A. thaliana* leaves ethylene-activated phosphorylation of NDPK (Fig. 6b) did not affect the capacity to convert GDP to GTP (Fig. 7). It seems likely that ethylene treatment brought about a change in NDPK as a protein kinase as has been ascribed for NDPKs of different origin [10, 11, 20, 23]. It might be significant that ethylene-regulated acid-stable phosphorylation of NDPK, which is shown in this work, is autophosphorylation on Ser/Thr residues as found for human NDPK-B [6], sugarcane NDPK [5], and spinach chloroplast NDPKII [7]. Upregulation by ethylene of acid-stable NDPK phosphorylation in *Arabidopsis* leaves may relate to the regulatory role of NDPK as a protein kinase.

Mention should be made here of the differences in responses to ethylene in etiolated pea epicotyls and fully expanded *Arabidopsis* leaves at the biochemical level and, in particular, ethylene effects on NDPK activity. If NDPK is involved in ethylene signaling, its upstream partners and/or downstream effectors may vary depending on plant age or plant tissue.

Receptor-dependent regulation of NDPK phosphorylation also points to a possible involvement of the enzyme in ethylene signal transduction. Thus, in etiolated pea epicotyls and *Arabidopsis* wild-type leaves, ethylene treatment led to an increase in NDPK phosphorylation while in the ethylene-insensitive *Arabidopsis* receptor mutants *etr1-1* and *eti5*, which possess decreased levels of ethylene binding, the phytohormone had no effect on phosphorylation of the enzyme.

The rapid and transient increase in NDPK activity in pea membranes also suggests a link between NDPK and ethylene-regulated growth processes in etiolated pea seedlings. These results are consistent with the report that NDPK plays an important role in providing nucleoside triphosphates at early stages of rice seed germination [24]. Moreover, expression of an *NDPK* gene in antisense orientation in rice resulted in plant dwarfism [25] indicating that this gene is essential for the cell elongation. In this connection, perhaps, it is important to note that in animal cells NDPK is associated with microtubules [26, 27]. If in plants NDPK is also bound to microtubules (and ethylene has been shown to affect microtubule orientation and assembly [28]), one should expect an involvement of NDPK in controlling cell growth responses to ethylene.

Besides the key role in synthesis of nucleoside triphosphates, NDPK might be also engaged in signal transduction events via an interaction with G-proteins. Thus, in *Dictyostelium discoideum* a portion of the cellular NDPK has been shown to be plasma membrane-associated. Furthermore, *Dictyostelium* possesses surface cAMP receptors, which couple to G-proteins, but NDPK appears to be activated by the receptor followed by G-protein activation [29]. This activation could be eliminated by the addition of antibodies against NDPK and it was proposed that NDPK was supplying GTP for G-protein activation. One of the most compelling pieces of evidence comes from the work on bovine retinal rod-outer-segment membranes [30]. In bovine retina, NDPK is a key enzyme providing intracellular GTP for transducin and guanylate cyclase. Moreover, the stimulation of G_i-protein in HL-60 cells has been reported to occur via activation of NDPK [31]. In addition to a putative interaction between NDPK and heterotrimeric G-proteins, the enzyme seems to be implicated in an interaction with monomeric G-proteins. In *Pseudomonas aeruginosa*, the Pra (*Pseudomonas* Ras-like) protein can form a complex with one of the NDPK isoforms [32]. Recently we demonstrated that ethylene at physiological concentrations specifically and differentially affected GTP-binding activity of monomeric G-proteins of the Ras superfamily [33]. The kinetics of activation of GTP binding upon ethylene treatment was similar to those presented here for both NDPK phosphorylation and NDPK activity, thus suggesting that NDPK and monomeric G-proteins are involved in the same ethylene signaling pathway. Hence, the NDPK activity upregulation by ethylene in pea epicotyls demonstrated in this work could be associated with NDPK function as a GTP supplier for G-proteins.

In eukaryotic organisms, both heterotrimeric and monomeric G-proteins are prominent signaling molecules involved in transduction pathways for a wide variety of stimuli [34]. In this regard, the results described here may have greater importance than appears at first sight. Thus, the effects of ethylene on NDPK phosphorylation (in both pea epicotyls and *Arabidopsis* leaves) as well as on NDPK activity (in pea epicotyls) tend to support the view of a possible involvement of NDPK in ethylene signaling. In a broader context, this raises the point that G-proteins have a role to play in ethylene signaling.

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REFERENCES

1. Parks, R. E., Jr., and Agarwal, R. P. (1973) in *The Enzymes* (Boyer, P. D., ed.) Vol. 8, Academic Press, New York, pp. 307-333.
2. Dumas, C., Lascu, I., Morera, S., Glaser, P., Fourme, R., Wallet, V., Veron, M., and Janin, J. (1992) *EMBO J.*, **11**, 3203-3208.
3. Munoz-Dorado, J., Inouye, S., and Inouye, M. (1990) *J. Biol. Chem.*, **265**, 2707-2712.
4. Postel, E. H., Berberich, S. J., Flint, S. J., and Ferrone, C. A. (1993) *Science*, **261**, 478-480.
5. Moisyadi, S., Dharmasiri, S., Harrington, H. M., and Lukas, T. J. (1994) *Plant Physiol.*, **104**, 1401-1409.
6. MacDonald, N. J., De La Rosa, A., Benedict, M. A., Freije, J. M. P., Krutsch, H., and Steeg, P. S. (1993) *J. Biol. Chem.*, **268**, 25780-25789.

7. Bovet, L., and Siegenthaler, P.-A. (1997) *Plant Physiol. Biochem.*, **35**, 455-465.
8. Cipollini, G., Berti, A., Fiore, L., Rainaldi, G., Basolo, F., Merlo, G., Bevilacqua, G., and Caligo, M. (1997) *Int. J. Cancer*, **73**, 297-302.
9. Ji, L., Arcinas, M., and Boxer, L. M. (1995) *J. Biol. Chem.*, **270**, 13392-13398.
10. Engel, M., Seifert, M., Theisinger, B., Seyfert, U., and Welter, C. (1998) *J. Biol. Chem.*, **273**, 20058-20065.
11. Wagner, P. D., and Vu, N.-D. (2000) *Biochem. J.*, **346**, 623-630.
12. Zimmermann, S., Baumann, A., Jaekel, K., Marback, I., Engelberg, D., and Frohnmeyer, H. (1999) *J. Biol. Chem.*, **274**, 17017-17024.
13. Choi, G., Yi, H., Lee, J., Kwon, Y. K., Soh, M. S., Shin, B., Luka, Z., Hahr, T. R., and Song, P. S. (1999) *Nature*, **401**, 610-613.
14. Harris, N., Taylor, J. E., and Roberts, J. A. (1994) *Plant Mol. Biol.*, **25**, 739-742.
15. Galvis, M. L. E., Marttila, S., Hakansson, G., Forsberg, J., and Knorpp, C. (2001) *Plant Physiol.*, **126**, 69-77.
16. Novikova, G. V., Moshkov, I. E., Smith, A. R., Kulaeva, O. N., and Hall, M. A. (1999) *Planta*, **208**, 239-246.
17. Sisler, E. C., Blankenship, S. M., and Guest, M. (1990) *Plant Growth Regul.*, **9**, 157-164.
18. Struglics, A., and Hakansson, G. (1999) *Eur. J. Biochem.*, **262**, 765-773.
19. Schaertl, S., Konrad, M., and Geeves, M. A. (1998) *J. Biol. Chem.*, **273**, 5662-5669.
20. Ann, K.-S., and Nelson, D. L. (1996) *J. Eucaryot. Microbiol.*, **43**, 365-372.
21. Bleecker, A. B., Estelle, M. A., Somerville, C., and Kende, H. (1988) *Science*, **241**, 1086-1089.
22. Sanders, I. O., Harpham, N. V. J., Raskin, I., Smith, A. R., and Hall, M. A. (1991) *Ann. Bot.*, **68**, 97-103.
23. Lu, Q., Park, H., Egger, L. A., and Inouye, M. (1996) *J. Biol. Chem.*, **271**, 32886-32893.
24. Yano, A., Umeda, M., and Uchimiya, H. (1995) *Plant Mol. Biol.*, **27**, 1053-1058.
25. Pan, L., Kawai, M., Yano, A., and Uchimiya, H. (2000) *Plant Physiol.*, **122**, 447-452.
26. Biggs, J., Hersperger, E., Steeg, P. S., Liotta, L. A., and Shearn, A. (1990) *Cell*, **63**, 922-940.
27. Lombardi, D., Sacchi, A., D'Agostino, G., and Tibursi, G. (1995) *Exp. Cell Res.*, **217**, 267-271.
28. Shibaoka, H. (1991) in *The Cytoskeletal Basis of Plant Growth and Form* (Lloyd, C. W., ed.) Academic Press, New York, pp. 159-168.
29. Bominaar, A. A., Molijn, A. C., Pestel, M., Veron, M., and van Haastert, P. J. M. (1993) *EMBO J.*, **12**, 2275-2279.
30. Lagnado, L., and Baylor, D. (1992) *Neuron*, **8**, 995-1002.
31. Klinker, J. F., Hageluken, A., Grunbaum, L., Heilmann, I., Nurnberg, B., Harhammer, R., Offermann, S., Schwaner, I., Ervens, J., Wenzelseifert, K., Muller, T., and Seifert, R. (1994) *Biochem J.*, **304**, 377-383.
32. Chopade, B. A., Shankar, S., Sundin, G. W., Mukhopadhyay, S., and Chakrabarty, A. M. (1997) *J. Bacteriol.*, **179**, 2181-2188.
33. Moshkov, I. E., Novikova, G. V., Mur, L. A. J., Smith, A. R., and Hall, M. A. (2003) *Plant Physiol.*, **131**, 1718-1726.
34. Hall, A. (ed.) (2000) *GTPases*, Oxford University Press, Oxford.