Tomato Phosphate Transporter Genes Are Differentially Regulated in Plant Tissues by Phosphorus

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Phosphorus is a major nutrient acquired by roots via high-affinity inorganic phosphate (Pi) transporters. In this paper, we describe the tissue-specific regulation of tomato (Lycopersicon esculentum L.) Pi-transporter genes by Pi. The encoded peptides of the LePT1 and LePT2 genes belong to a family of 12 membrane-spanning domain proteins and show a high degree of sequence identity to known high-affinity Pi transporters. Both genes are highly expressed in roots, although there is some expression of LePT1 in leaves. Their expression is markedly induced by Pi starvation but not by starvation of nitrogen, potassium, or iron. The transcripts are primarily localized in root epidermis under Pi starvation. Accumulation of LePT1 message was also observed in palisade parenchyma cells of Pi-starved leaves. Our data suggest that the epidermally localized Pi transporters may play a significant role in acquiring the nutrient under natural conditions. Divided root-system studies support the hypothesis that signal(s) for the Pi-starvation response may arise internally because of the changes in cellular concentration of phosphorus.

Phosphorus availability is considered one of the major growth-limiting factors for plants in many natural ecosystems (Barber et al., 1963). Plants have developed several adaptive mechanisms to overcome Pi stress (Marschner, 1995). Changes in the root growth and architecture (Anghinoni and Barber, 1980; Lynch, 1995), increased production of phosphatases and RNases (Duff et al., 1989; Goldstein, 1992; Loffler et al., 1992; Green, 1994; Bariola et al., 1995), and altered activity of several enzymes of the glycolytic pathway (Duff et al., 1989) are among the well-characterized responses to Pi deficiency in plants. In addition, an increase in the phosphate uptake rate of roots and cell cultures following phosphate starvation has been observed in several plant species (Clarkson and Scattengood, 1982; Drew et al., 1984; Katz et al., 1986).

Phosphate is acquired by plants in an energy-mediated co-transport process driven by a proton gradient generated by plasma membrane H⁺-ATPases (Epstein, 1976; Ullrich-Eberius et al., 1981, 1984; Sakano, 1990). The kinetic characterization of the Pi-uptake system by whole plants (Ullrich-Eberius et al., 1984) and cultured cells (Furihata et al., 1992) indicates a high-affinity transport activity operating at low concentrations (micromolar range) and a low-affinity activity operating at higher concentrations (millimolar range). In cultured cells of Catharanthus roseus the low-affinity system is expressed constitutively, whereas the high-affinity system is regulated by the availability of phosphorus (Furihata et al., 1992). When cells grown in Pi-rich medium were transferred to Pi-depleted medium, the high-affinity uptake activity increased significantly within 2 d. The enhanced uptake appears to be in part due to the increased synthesis of a carrier system in response to Pi starvation (Drew and Saker, 1984; Shimogawara and Usuda, 1995). Phosphorus stress in microorganisms is known to result in transcriptional activation of high-affinity Pi transporters and phosphatases (Torriani-Gorini et al., 1994). High-affinity phosphate transporter genes have been cloned and characterized from fungi (Bun-ya et al., 1991; Harrison and van Buuren, 1995; Versaw, 1995) and recently from plants (Muchhal et al., 1996; Kai et al., 1997; Leggewie et al., 1997; Smith et al., 1997). All of the characterized Pi transporter proteins are predicted to have a common structure containing 12 membrane-spanning domains, which are separated into two groups of 6 by a charged hydrophilic region.

In this paper we report the characterization of two tomato (Lycopersicon esculentum L.) phosphate transporters and regulation of their expression by phosphorus. To our knowledge, this is the first report showing an enhanced accumulation of phosphate transporter transcripts in root epidermis under Pi starvation. We also provide evidence for the regulation of the gene expression by internal signals during phosphate starvation.

MATERIALS AND METHODS

Tomato (Lycopersicon esculentum L.) plants were grown in an aeroponic growth facility similar to the one described by Liu et al. (1997). Tomato seeds of the variety OS4 were germinated in seedling trays filled with Ready Earth plug mixture (Scotts, Marysville, OH). When plants reached the four-leaf stage (20 d after sowing), they were removed from
the growing medium, and roots were washed free of medium and transferred to aeroponics. In aeroponic culture roots were sprayed with a fine mist that consisted of one-half-strength Hoagland solution (Jones, 1982) for 3 s every 10 min. Phosphorus-starvation treatments were initiated 1 week after the plants were transferred to aeroponics. For divided root-system studies, tomato plants were grown in aeroponics for 1 week as described above. Three aeroponic plants were transferred to an aerated hydroponic solution containing either 250 or 0 μM Pi. The roots of three more plants were separated into two sections, and each section of the roots was placed in an adjacent container with aerated nutrient solution containing either 250 or 0 μM Pi. Leaves and roots from P+ (250 μM Pi) and P− (0 μM Pi) and divided-root-system plants were harvested separately, frozen in liquid nitrogen, and stored at -70°C.

RNA Isolation

Total RNA was isolated from roots and leaves of tomato plants by hot-pheno extraction and lithium chloride precipitation (Pawlowski et al., 1994). Poly(A+) RNA was isolated by the oligo(dT)-cellulose batch-binding method (Sambrook et al., 1989).

cDNA Library Construction and Screening

A cDNA library representing the mRNA isolated from tomato roots starved for phosphate for 5 d was constructed in the XhoI-EcoRI site of the Uni-ZAP-XR vector (Stratagene) according to the manufacturer’s instructions. The two Arabidopsis thaliana cDNA clones (AtPT1 and AtPT2) encoding the phosphate transporters (Muchhal et al., 1996) were radiolabeled by random priming (DECAprimeII, Ambion, Austin, TX) and used for screening the tomato cDNA library according to standard procedures (Sambrook et al., 1989). Hybridizations for screening were carried out in a solution containing 50% (v/v) formamide at 38°C. Final washing of the filters was done with 1× SSC and 0.2% (w/v) SDS at 60°C for 30 min. Two sets of cDNA clones were obtained from this screening. Based on the insert size and restriction mapping, one full-length representative from each of these sets was used for further analysis. The sequence of these two clones was determined on both strands by the dideoxy method using Sequenase (United States Biochemical). The Genetics Computer Group (University of Wisconsin, Madison) software package was used for sequence analysis and database searches.

Northern Blots

Ten micrograms of total RNA was electrophoretically separated on 1% denaturing formaldehyde agarose gels and blotted onto a BA-S nitrocellulose membrane (Sambrook et al., 1989). The nitrocellulose filters were hybridized overnight with a 32P-labeled probe (106 cpm/mL) in a solution containing 50% formamide, 5× Denhart’s solution, 0.1% (w/v) SDS, 6× SSPE, and 100 μg/mL denatured salmon-sperm DNA at 42°C. Filters were washed twice in 2× SSC and 0.2% SDS at room temperature for 10 min, twice in 1× SSC and 0.2% SDS at 50°C for 15 min, and twice in 0.1× SSC and 0.2% SDS at 62°C for 20 min before autoradiography.

Southern Blots

High-molecular-weight genomic DNA was isolated from young leaves of tomato, as described by Dellaporta et al. (1983). Ten micrograms of genomic DNA was digested with restriction enzymes, electrophoretically separated through 0.8% agarose gels, denatured, and transferred to a supported nitrocellulose membrane (Sambrook et al., 1989). The hybridization and washing conditions were the same as those described above for northern blots.

In Situ Localization of Tomato Phosphate Transporter Transcripts

Roots of tomato plants grown in aeroponics were sprayed with nutrient solutions with Pi (250 μM) or without Pi for 5 d. Root and leaf samples were harvested and fixed in a solution containing 3.7% (v/v) formaldehyde, 5% (v/v) acetic acid, and 50% (v/v) ethanol (Niu et al., 1996). Fixed tissue samples were dehydrated in an ethanol dilution series and embedded in wax (Paraplast, Fisher Scientific). Ten-micrometer sections cut with a microtome were transferred to Super-Frost Plus slides (Fisher Scientific) and incubated at 42°C overnight. Sense and antisense probes representing LePT1 and LePT2 were transcribed by T3 or T7 RNA polymerase (Ambion) from linearized pBluescript-SK containing the cDNA. The probes were labeled with digoxigenin following the procedure described by the manufacturer (Boehringer Mannheim). Tissue-section pretreatment and in situ hybridization were performed as described by Niu et al. (1996). Successive sections from roots obtained from three plants were used for hybridizing with sense and antisense probes. After color development for 16 to 24 h, sections were photographed using an Optiphot microscope (Nikon).

RESULTS

Structure and Organization of the Tomato Pi Transporters

Two full-length cDNA clones, LePT1 and LePT2, encoding the tomato phosphate transporters were isolated from a phosphate-starved root library using a mixture of Arabidopsis AtPT1 and AtPT2 cDNA clones (Muchhal et al., 1996; Mukatira et al., 1996) as heterologous probes. LePT1 is 2023 bp long and contains an open reading frame encoding a 538-amino acid polypeptide (58.7 KD), whereas LePT2 is 1826 bp long and encodes a 528-amino acid polypeptide (58.7 KD). The open reading frames of LePT1 and LePT2 are flanked by 151 and 37 bp of untranslated sequence at the 5’ end and by 258 and 205 bp of untranslated sequence, including the poly(A) tail, at the 3’ end. The LePT1 and LePT2 polypeptides are 80% identical in their amino acid sequence. The two polypeptides share the greatest degree of similarity with the recently characterized phosphate transporters (Fig. 1) from potato (Solanum tuberosum L.;
Legewie et al., 1997), Arabidopsis (Muchhal et al., 1996; Smith et al., 1997), and Catharanthus roseus (Kai et al., 1997).

Based on the amino acid sequence identity, LePT1 is more similar to potato STPT1 and Arabidopsis to AtPT2, whereas LePT2 is more similar to STPT2 and AtPT1. Phylogenetically, the phosphate transporters from plants and fungi belong to a closely related family, even though the similarity between the plant transporters is significantly higher than that between plants and fungal transporters (Muchhal et al., 1996).

Hydropathy plots of the deduced polypeptides suggest that both tomato transporters are integral membrane proteins that consist of 12 membrane-spanning regions, a common feature shared by proteins responsible for transport of substrates as diverse as sugars, ions, antibiotics, and amino acids (Griffith et al., 1992; Henderson, 1993; Marger et al., 1994).

**Figure 1.** A, Alignment of the deduced amino acid sequence of LePT1 and LePT2 with that of A. thaliana (AtPT1 and AtPT2), potato (STPT1 and STPT2), and C. roseus (PT1) phosphate transporters. Identical amino acids are indicated by asterisks and conserved substitutions are indicated by dots. The membrane-spanning domains of LePT1 and LePT2 as predicted by TopPred (Claros and von Heijne, 1994) are underlined and their numbering is indicated by roman numerals (I–XII). The open and boxed sequences are consensus sites for phosphorylation by casein kinase II, and boxed and shaded sequences are consensus sites for phosphorylation by protein kinase C. B, Summary of the percentage of amino acid identity between tomato and other plant phosphate transporters.
Saier, 1993). The position and spacing of these membrane-spanning regions in tomato transporters are very similar to those in other Pi transporters (Fig. 1). Based on secondary structure analyses, both the N and C termini of the polypeptides are predicted to be on the cytoplasmic side of the plasma membrane. The amino acid domains for protein kinase C- and casein kinase II-mediated phosphorylation are present in similar conserved regions, as seen with Arabidopsis Pi transporters (Muchhal et al., 1996).

Full-length LePT1 and LePT2 cDNA probes hybridized with two or three distinct bands on Southern blots (Fig. 2) of tomato genomic DNA digested with different restriction enzymes, suggesting the presence of a small gene family.

LePT1 and LePT2 Transcripts Are Induced by Phosphate Starvation in Roots

The expression of LePT1 and LePT2 in tomato plants grown either in the presence of 250 μM phosphate or no phosphate was compared by northern-blot analysis of total RNA isolated from different tissues. Both probes hybridized to approximately 2-kb transcripts (Fig. 3). Their expression was markedly increased in plants grown under Pi-limiting conditions. LePT1 is primarily expressed in roots, with a small amount of the message also detectable in leaves (Fig. 3), stems, and petioles (data not shown) of tomato plants subjected to Pi starvation. LePT2 is expressed only in the roots. The relative abundance of both of the messages was similar in the Pi-starved roots. An increase in the transcript level of both of the genes was detected within 24 h of Pi starvation in roots (Fig. 4). The transcript levels continued to increase with increased duration of Pi starvation, and reached a maximum after 5 d. These results indicate that expression of LePT1 and LePT2 responds to changing nutritional conditions.

To investigate the expression of LePT1 and LePT2 in response to deficiency of other nutrients, tomato plants grown in aeroponics were subjected to starvation of three other nutrients, nitrogen, potassium, and iron. After 5 d of visible retardation in growth was noticed in the Pi- and nitrogen-starved plants, but no visible difference in growth was observed in the plants starved of potassium and iron. The LePT1 and LePT2 transcript levels increased greatly in Pi-starved plants but remained low in the roots of plants subjected to the other three nutrient-starvation treatments (Fig. 5). Although we have not tested the effect of starvation of other essential nutrients, the data suggest a strong correlation between LePT1 and LePT2 expression and Pi starvation.

LePT1 and LePT2 Expression Is Dependent on the Availability of Pi in the Medium

To define the Pi concentration at which the phosphate-transporter genes are expressed, tomato plants were grown in the presence of different concentrations of phosphorus. After 5 d of treatment the roots were harvested for isolation of RNA. Increased expression of both LePT1 and LePT2 was detected in the roots of plants provided with 100 μM or less (Fig. 6), suggesting a correlation between the amount of phosphorus present in the medium and the level of LePT gene expression. It appears that expression of LePT1 is relatively more sensitive to Pi concentration changes than that of LePT2.
The regulation of LePT1 and LePT2 expression by phosphorus availability was further examined by resupplying Pi to the plants that were Pi deficient and strongly expressing the genes. When 250 μM Pi was resupplied to these plants, transcript levels of both genes decreased within 24 h (Fig. 7) and reached a significantly low level within 2 d. These observations suggest the existence of a fine coordination between gene expression, presumed to lead to the synthesis of more transporters and increased uptake, and the availability of phosphorus in the soil.

Phosphate Transporter Expression Is Likely Regulated by Internal Signals

The increase in transcript levels of tomato Pi transporters may be due to a combination of a lack of phosphate supply to roots and/or depletion of internal Pi reserves. To obtain further insight into the origin of the signals that regulate expression of the Pi transport system in plants, one-half of the roots was exposed to a Pi-deficient solution, and the other half was exposed to a solution with 250 μM Pi. Under these conditions the LePT1 and LePT2 transcript levels remained comparable in the roots exposed to either 250 μM or no Pi (Fig. 8). The accumulation of these transcripts in divided-root plants was similar to those sprayed with 250 μM Pi. Similarly, the transcript levels of LePT1 in leaves of divided-root plants and Pi+ control plants were also comparable. The expression of TPSI1, a gene specifically induced in response to phosphate starvation in tomato (Liu et al., 1997), was also similar to those of phosphate transporters. These data suggest that even if Pi is supplied to a portion of the root system, expression of Pi transporters in other portions of roots exposed to Pi-deficient conditions do not increase compared with Pi-deficient plants.

LePT1 and LePT2 Are Strongly Expressed in the Epidermis of Phosphate-Starved Roots

In situ localization of the LePT1 and LePT2 transcripts was done with digoxigenin-labeled probes to obtain information about tissue-specific expression of the phosphate transporter genes.
transporters. In tomato plants grown under phosphorus-deficient conditions, a significant amount of chromogenic product signal for LePT1 and LePT2 transcripts was observed in the root epidermis (Fig. 9). Low levels of LePT1 transcripts in other cell types, including the central cylinder, was also noticed in Pi-starved roots. In addition, accumulation of the LePT1 message was also detected in palisade parenchyma and phloem cells (data not shown) of leaves under phosphate starvation. The significance of the presence of higher transcript levels in palisade parenchyma cells in leaves is not clear. Higher demand for phosphorus by the actively photosynthesizing palisade parenchyma cells may be one of the reasons for increased expression of phosphate transporters. These transporters may be involved in active transport of Pi from neighboring tissues or in the release of Pi in the apoplastic space into the palisade parenchyma cells. Expression of LePT2 was not detected in leaf tissue even under phosphate starvation. In situ localization of the phosphate transporter message agrees with northern analysis of RNA.

DISCUSSION

The uptake system for phosphate and other ions in plants consists of high- and low-affinity components (Epstein, 1976). Under most natural conditions in which the concentration of available Pi in soil is very low (Barber et al., 1963), the transport of Pi by plant cells proceeds through the high-affinity, energy-dependent proton/phosphate symport mechanism. At the molecular level the main protein component of this system, the phosphate transporter, has been recently characterized from Arabidopsis (Muchhal et al., 1996; Smith et al., 1997), potato (Leggewie et al., 1997), and C. roseus (Kai et al., 1997). These proteins show significant structural similarity with known high-affinity phosphate transporters and were able to complement yeast mutants defective in high-affinity phosphate uptake activity (Muchhal et al., 1996; Kai et al., 1997; Leggewie et al., 1997). Furthermore, overexpression of AtPT1 (PHT1) in tobacco cell cultures enhanced cell growth and Pi uptake under phosphate-limited conditions (Mit-
sukawa et al., 1997). The deduced amino acid sequences of LePT1 and LePT2 polypeptides show a high degree of sequence similarity to other plant phosphate transporters. LePT1 and LePT2 are greater than 95% identical at the amino acid sequence level to STPT1 and STPT2 from potato, respectively. This degree of similarity at the primary sequence level among the members of the Solanaceae family is interesting, considering the fact that, despite similar functionality, the overall sequence similarity among phosphate transporters from plants and fungi is not more than 42% (Muchhal et al., 1996).

The results from expression studies show that both LePT1 and LePT2 transcripts accumulate primarily in roots, and their expression is highly induced under Pi-deficient conditions. Roots are the organs involved in nutrient acquisition, and the expression pattern of these two genes in roots correlates well with their function. A small amount of LePT1 message was also detectable in the leaves, stem, and petioles of tomato plants starved of Pi, suggesting a global role for this in plants. The differential expression of LePT1 and LePT2 in roots and leaves was similar to that of potato phosphate transporters described by Leggewie et al. (1997). The induction of LePT1 and LePT2 in response to Pi starvation correlates well with published reports of increased phosphate uptake rate of roots and cell cultures subjected to Pi deprivation (Clarkson and Scott good, 1982; Drew and Saker, 1984; Katz et al., 1986). This enhanced Pi absorption following Pi starvation has been proposed to be associated with a larger capacity for Pi transport, possibly by the formation of additional carriers for Pi (Anghinoni and Barber, 1980; Lelebreve and Glass, 1982; Drew et al., 1984; Furihata et al., 1992; Shimogawara and Usuda, 1995). In this study the transcript levels of both LePT1 and LePT2 showed a significant increase within 1 d after the transfer to Pi-deficient medium, and reached a maximum after about 5 d of starvation. Resupplying Pi to tomato plants starved for 5 d repressed the LePT1 and LePT2 transcripts back to their uninduced levels within 2 d, suggesting a role for these two transporters in the uptake of Pi under Pi-limiting conditions.

In situ localization of LePT1 and LePT2 in roots and leaves correlated with accumulation of message in specific tissues under phosphate starvation. Localization of transcripts in the epidermis of roots and pronounced induction of message in response to Pi starvation suggest that epidermally localized transporters play a significant role in phosphate acquisition under natural conditions. As Pi moves through the apoplastic pathway, the exclusion of anions, including Pi, from the narrow pores in the cell wall was similar in both halves. The amount of these transcripts, both in roots and leaves, was also comparable to that found in the plants provided with 250 μM Pi. The expression pattern of TPSII (Liu et al., 1997), another phosphate-starvation-induced gene, also indicates that tissues of divided-root plants have sufficient phosphorus to repress phosphate-starvation-induced gene expression. Furthermore, the total phosphorus content in leaves and roots of Pi-sufficient (250 μM) plants was comparable to that of leaves and roots provided with no Pi in divided-root plants (data not shown). These results suggest that signals for increased uptake of Pi by roots under starvation are likely due to changes in internal Pi concentrations. Earlier reports (Lelebreve and Glass, 1982; Drew et al., 1984) have showed that the supply of Pi to a part of the root system may partly or fully compensate for the deficiency in other parts of the root system by greater rates of nutrient uptake. In their study a localized supply of Pi resulted in greater translocation of labeled Pi to the remainder of the root system exposed to a phosphate-deficient solution. Higher mobility of Pi in both xylem and phloem may lead to rapid equilibration of Pi concentration in tissues farther away from the source of Pi supply. As long as the phosphate requirement of plants is met, the expression of phosphate transporters in the entire root system appears to be depressed.

Leaf phosphorus status may regulate uptake of Pi by the roots (Marschner, 1995). The location of regulation of phosphate uptake away from roots should allow plants to absorb the required amounts of the nutrient to maintain a physiologically constant concentration in the cytoplasm. The proposed control of phosphate uptake, directly or indirectly, by the internal concentration of Pi should lead to a regulated expression of phosphate-transporter genes in roots despite wide variations in the availability of phosphorus under natural conditions. At present the nature of the phosphate-starvation signals are not clear. There are reports suggesting a role for ethylene in the phosphate-starvation response (Drew et al., 1989; He et al., 1992). Temporary deprivation of phosphorus has been shown to decrease C2H4 production and enhance the sensitivity of roots to ethylene during aerenchyma formation (Drew et al., 1989; He et al., 1992). Although no direct evidence is available at present, it is likely that ethylene may play a role as a component of the phosphate-starvation response mechanism. Under phosphate starvation significant amounts of carbohydrates are translocated from shoots to roots. Even though there is no experimental evidence for a role for sugar-mediated signals during Pi starvation, the existence of sugar phosphate molecules in the carbohydrate pools acting as phosphate-starvation signals cannot be ignored.
could decrease its concentration to the submicromolar range (Clarkson, 1991). This interaction between Pi and the apoplastic pathway could be a significant factor in maintaining the concentration of Pi in a reasonable range for uptake by phosphate transporters. Under these conditions the epidermis will be exposed to a relatively higher concentration of phosphorus, as compared with other tissues away from the surface. Higher expression of Pi transporters in root epidermis is most likely an adaptive mechanism to enhance and optimize Pi uptake by roots under Pi-deficiency conditions. The contribution of epidermal uptake of nutrients in general and Pi in particular under deficiency conditions may be a significant component of nutrient acquisition by plants. The uptake of NO₃ under low concentration is also suggested to occur at the epidermis (Rufy et al., 1986). It was elegantly shown by Rufy et al. (1986) using the induction of NO₃ reductase as an indicator of NO₃ uptake by different root tissues. A distinct induction of NO₃ reductase activity was noticed in epidermal cells under micromolar concentration. As the concentration of NO₃ increased in the bathing medium, the enzyme activity was also noticed in other cell types, including the endodermis.

Plants have developed several adaptive mechanisms to cope with limiting concentrations of Pi available in soils, most of which focus on increasing the availability of external Pi and efficiency of Pi uptake and utilization inside. However, in the constantly fluctuating environment surrounding roots the most important response is fine tuning of Pi uptake rate relative to the availability of Pi. Transcriptional regulation of Pi-transporter gene expression, regulated directly or indirectly by internal Pi, appears to be a very important part of this control mechanism in roots.

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LITERATURE CITED


