Phosphate transport across biomembranes and cytosolic phosphate homeostasis in barley leaves*


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Abstract. Barley (Hordeum vulgare L.) plants were grown hydroponically with or without inorganic phosphate (P\textsubscript{i}) in the medium. Leaves were analyzed for the intercellular and the intracellular distribution of P\textsubscript{i}. Most of the leaf P\textsubscript{i} was contained in mesophyll cells; P\textsubscript{i} concentrations were low in the xylem sap, the apoplast, and in the cells of the epidermis. The vacuolar concentration of P\textsubscript{i} in mesophyll cells depended on P\textsubscript{i} availability in the nutrient medium. After infiltrating the intercellular space of leaves with solutions containing P\textsubscript{i}, P\textsubscript{i} was taken up by the mesophyll at rates higher than 2.5 I~mol-(g fresh weight)-1.h-1. Isolated mesophyll protoplasts did not possess a comparable capacity to take up P\textsubscript{i} from the medium. Phosphate uptake by mesophyll protoplasts showed a biphasic dependence on P\textsubscript{i} concentration. Uptake of P\textsubscript{i} by P\textsubscript{i}-deficient cells was faster than uptake by cells which had P\textsubscript{i} stored in their vacuoles, although cytoplasmic P\textsubscript{i} concentrations were comparable. Phosphate transport into isolated mesophyll vacuoles was dependent on their P\textsubscript{i} content; it was stimulated by ATP. In contrast to the vacuolar P\textsubscript{i} concentration, and despite different kinetic characteristics of the uptake systems for P\textsubscript{i} of the plasmalemma and the tonoplast, the cytoplasmic P\textsubscript{i} concentration was regulated in mesophyll cells within narrow limits under very different conditions of P\textsubscript{i} availability in the nutrient medium, whereas vacuolar P\textsubscript{i} concentrations varied within wide limits.

Key words: Apoplast – Cytosol – Hordeum (phosphate homeostasis) – Phosphate transport – Plasmalemma – Tonoplast

Introduction

For adequate growth, plants require approximately one molecule of inorganic phosphate (P\textsubscript{i}) for every 500 mole-
cules of carbon fixed in photosynthesis (Marschner 1986). Phosphate is a constituent of nucleic acids and phospholipids, and is also of prime importance in energy metabolism. Phosphate deficiency rapidly reduces plant growth; it decreases photosynthesis and changes assimilate partitioning (Dietz 1989; Sicher and Kremer 1988). Within the cells, a metabolically active P\textsubscript{i} pool can be distinguished from an inactive P\textsubscript{i} pool (Ullrich et al. 1965; see Bieleski 1973 for a review; Rebelle et al. 1983). Phosphate is exchanged between both pools only slowly, depending on metabolic demands (Ullrich et al. 1965; Foyer and Spencer 1986). Whereas the 31P-nuclear magnetic resonance (NMR) technique is capable of distinguishing two P\textsubscript{i} pools in leaves, it does not differentiate between P\textsubscript{i} contained in the cytoplasmic organelles and in the cytosol. Also, the kinetics of P\textsubscript{i} transport cannot be determined with this technique (see Roberts 1984 for a review). Studies with isolated organelles allow the investigation of the intracellular compartmentation and transport of P\textsubscript{i}. However, P\textsubscript{i} may leak from organelles. Cross contamination of organelles, particularly by vacuolar material, results in an overestimation of P\textsubscript{i} when non-aqueous techniques are employed (Dietz and Heber 1984).

This communication describes effects of P\textsubscript{i} nutrition on the P\textsubscript{i} status of barley leaves. Vacuoles can rapidly be isolated from protoplasts. Therefore, isolated vacuoles are used in this communication to distinguish between cytoplasmic and vacuolar P\textsubscript{i} levels. This is possible as the tonoplast of isolated vacuoles has a low permeability to P\textsubscript{i} (Martinoa et al. 1986). We also investigate the role of the apoplast and of the vacuole in maintaining the P\textsubscript{i} homeostasis of the cytoplasm of leaf cells.

Material and methods

Plant growth. Barley (Hordeum vulgare L., cv. Gerbel) was grown in hydroponic culture. The standard medium contained 9 mmol-1-1 KNO\textsubscript{3}, 6 mmol-1-1 Ca(NO\textsubscript{3})\textsubscript{2}, 3 mmol-1-1 MgSO\textsubscript{4}, 1.5 mmol-1-1 KH\textsubscript{2}PO\textsubscript{4}, 0.13 mmol-1-1 Fe-ethylenediaminetetraacetate (EDTA) and micro nutrients. Plants used for the preparation of xylem sap grew in a medium which contained 5 mmol-1-1 KNO\textsubscript{3} and 4 mmol-1-1 KCl instead of 9 mmol-1-1 KNO\textsubscript{3}. Levels

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of P, were increased as indicated below to obtain plants with elevated P levels. Phosphate-deficient plants were grown in the absence of P. Ten days after sowing, the primary leaves and the seeds were removed from the plants to minimize transfer of endogenous P to young tissue.

Isolation of protoplasts and vacuoles. Mesophyll protoplasts were prepared from primary or secondary leaves 10 or 21–23 d, respectively, after sowing. The procedure was essentially as described by Kaiser et al. (1982), but sucrose and glycine betaine substituted for sorbitol and Percoll in the isolation media. The enzymes for protoplast isolation were freed from P by gel filtration.

Protoplasts from the cells of the epidermis had a low density and were separated from heavier mesophyll protoplasts by flotation. After complete digestion of the leaves, protoplasts from primary leaves of 10-d-old plants were resuspended in sorbitol medium (450 mmol l⁻¹ sorbitol; 20 mmol l⁻¹ 2-(N-morpholino)ethanesulfonic acid/KOH, pH 6.5; 20 mmol l⁻¹ potassium gluconate; 3 mmol l⁻¹ CaCl₂; 0.1% polyvinylpyrrolidone) and overlaid with glycine betaine medium (450 mmol l⁻¹ glycine betaine instead of sorbitol). A fraction consisting of the large protoplasts from the upper epidermis and of light mesophyll protoplasts was recovered from the interphase. The epidermal protoplasts were further purified by gradient centrifugation: sorbitol medium was added to the protoplast suspension (equal volumes) and overlaid with a mixture of glycine betaine medium and sorbitol medium (3:2, v/v) and then with glycine betaine medium. The gradient was spun at 600 g for 10 min. The pale, chlorophyll-less protoplast fraction was removed and Percoll was added to a final concentration of 10% (v/v). A mixture of sorbitol and glycine betaine medium (7:3, v/v) was layered on the suspension and glycine betaine medium added on top. The gradient was spun as above and the chlorophyll-free band of intact epidermal protoplasts was recovered. Protoplast numbers and volumes were determined by microscopic analysis. Intactness was demonstrated by accumulation of neutral red in the large central vacuole and by the ability of the protoplasts to incorporate 32P into intact epidermal protoplasts was recovered. Protoplast isolation were freed from P by gel filtration.

Vacuoles were isolated by the method of Martinoia et al. (1982). For measuring P uptake into vacuoles in vivo, isolated protoplasts were labelled with 32P (S.A. = 15 MBq μmol⁻¹; Amersham) and vacuoles were prepared by the fast method of Kaiser et al. (1982). This method yields intact vacuoles within less than 1 min.

Xylem sap collection. Xylem sap was isolated from 21-d-old barley plants grown in the presence of varying P concentrations. The plants were prepared and xylem sap isolated with a Scholander pressure chamber as described by Wolf and Jeschke (1987).

Infiltration of leaves and preparation of infiltrate. Ten-day-old primary leaves were detached and infiltrated in vacuo by immersion in a solution containing 100 mmol l⁻¹ sorbitol and 1 mmol l⁻¹ CaCl₂ (or Ca-lactate when chloride was to be determined). After the surface of the leaves had been carefully dried, infiltrate was extracted by centrifugation (1000 g; 2 min). To measure P uptake, 12 mmol l⁻¹ KH₂PO₄, pH 6.5 and 2 mmol l⁻¹ KHCO₃ were included in the solution used for infiltration. (In the colorimetric determination, the solution gave a reading corresponding to 11.3 mmol l⁻¹ P.) In these experiments, the infiltrated leaves were illuminated at a fluence rate of 8 W m⁻². After specified times, infiltrate was prepared as described above. The calculation of apoplastic soluble concentrations was based on the following relationships: 1 g of primary leaf tissue contains 80 μl of apoplastic and may be infiltrated with 300 μl of solution (Pfanz 1987).

Uptake of P from the transpiration stream. Primary leaves were cut and immersed in solutions containing 1 mmol l⁻¹ CaCl₂ and various concentrations of KH₂PO₄ (KOH, pH 6.0). The leaves were illuminated for 4 h. The amount of P, taken up by the leaves was determined. After feeding P, infiltrate was prepared as described above.

Transport of P into protoplasts and vacuoles. Uptake of P by protoplasts and vacuoles was measured by the method of Martinoia et al. (1987). The incubation medium used for measuring P uptake by protoplasts contained 0.6 mol l⁻¹ glycine betaine, 1 mmol l⁻¹ CaCl₂ and 10 mmol l⁻¹ 2-(N-morpholino)ethanesulfonic acid (adjusted to pH 6.0 with 2-aminooxy-ethanol)-1,3-propanediol (Tris). The incubation medium used for measuring P uptake by vacuoles consisted of 0.3 mol l⁻¹ sucrose, 0.2 mol l⁻¹ glycine betaine, 2 mmol l⁻¹ EDTA, 6 mmol l⁻¹ MgCl₂, 1 mmol l⁻¹ MnCl₂, 30 mmol l⁻¹ K-glucuronate, 30 mmol l⁻¹ 4-(2-hydroxyethyl)-piperazinesulfonic acid, 0.2% (w/v) bovine serum albumin, 1 mmol l⁻¹ dithiothreitol and 10 mmol l⁻¹ ATP (when indicated). The pH of the medium was adjusted to 7.8 with Tris.

Phosphate uptake by protoplasts was initiated by the addition of protoplasts to the 32P-containing incubation medium and terminated by centrifugation of the protoplasts through a layer of silicone oil. After centrifugation, the protoplasts were washed three times with water. After washing, protoplasts were treated with 1 M HCl for sorbitol and Percoll in the isolation media. The enzymes for protoplast isolation were freed from P by gel filtration.

Measurement of oxygen evolution. Photosynthetic oxygen evolution by the leaves was measured with an air-phase oxygen electrode at saturating CO₂ (Delieu and Walker 1981). The irradiance was 300 W m⁻².

Determination of P, and of anions. Inorganic phosphate in leaves was extracted in 5–7% (v/v) perchloric acid. After neutralization of the solution with 3 mol l⁻¹ K₂CO₃, P was measured by the method of Fiske-Subbarow (1925). Phosphate in protoplasts and vacuoles was determined by a one-step spectrophotometric assay (Bencini et al. 1983). In the latter case, bovine serum albumin was omitted from isolation media. Alternatively, anion contents were determined by anion-exchange chromatography as described by Schröppel-Meier and Kaiser (1988a).

Results

Phosphate uptake into leaf cells. To estimate the P concentration in the apoplast, primary barley leaves were infiltrated with a Pₙₙₙ free solution and infiltrated, termed intercellular washing fluid (IWF), was obtained from the leaves by mild centrifugation. Apoplastic P concentration were between 0.5 and 2 mmol l⁻¹. Calculations were based on an estimated apoplastic volume of 80 μl per g leaf fresh weight (Pfanz 1987). There was the possibility that part of the P, detected in the IWF may have been due to contamination by cytosolic and vacuolar material released from broken cells. However, measurements of the activity of cytosolic, chloroplastic and vacuolar enzymes in the IWF showed that contamination with intracellular material was well below 0.5% (data not shown).

Depending on growth conditions, the predominant apoplastic anion was either nitrate or chloride; their concentrations ranged from 3 to 25 mmol l⁻¹. Even in the presence of excess P in the nutrient solution, for example