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Investigations on phosphate uptake and polyphosphate metabolism by mycorrhized and nonmycorrhized roots of beech and pine as investigated by in vivo ^{31}P-NMR

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Abstract. Comparative in vivo ^{31}P-NMR studies of mycorrhized and nonmycorrhized roots of *Fagus sylvatica* and *Pinus sylvestris* and of the fungus *Suillus bovinus* in pure culture have produced interesting new data. With respect to intracellular compartments and pH, ^{31}P-NMR spectroscopy showed that the spectrum of the mycorrhiza results from simple superimposition of the spectra of its symbionts. A special method of cyclic phosphate supply followed by block averaging of the NMR spectra was used to determine the kinetic behaviour of phosphate uptake and storage and its incorporation into polyphosphate at a constant external pH of 5.5. Mycorrhized roots and pure fungus showed transformation of accumulated inorganic phosphate into mobile polyphosphate with a medium chain length. Transformation of mobile into immobile polyphosphate either with a long chain length or in a granular state was also observed. Thus, two different types of fungal polyphosphate could be verified. Deficiency of external phosphate initiated the mobilization of internal phosphate, transforming stored polyphosphate into phosphate. It could be shown that a high fungal mass renders mycorrhizal phosphate metabolism less sensitive to external variation in nutrient concentration. The central role of the fungus in regulating mycorrhizal phosphate metabolism is discussed.

Key words: NMR – Uptake – Storage – Phosphate – Polyphosphate

Introduction

Many ^{31}P-NMR studies on the phosphate metabolism of roots have been published: corn roots (Pfeffer et al. 1986, 1987; Gerasimowicz et al. 1986), root tips of corn (Roberts et al. 1980, 1982, 1984; Reid et al. 1985), pea (Lee and Ratcliffe 1983a,b), bean (Torimitsu et al. 1984) and barley (Jackson et al. 1986). In addition, results have been presented on intact seedlings of corn (Roberts and Testa 1988), *Vigna unguiculata* (Horst et al. 1983), *Catharantus roseus* and *Daucus carota* (Brodelius and Vogel 1985) and *Nicotiana tabacum* (Wray et al. 1985). Investigations of fungi include yeasts: *Saccharomyces cerevisiae* (Den Hollander et al. 1981, 1986; Nicolay et al. 1982), *Zygosaccharomyces bailii* (Nicolay et al. 1982), *Candida utilis* (Nicolay et al. 1982, 1983), and also the basidiomycete *Armillaria mellea* (Cairney et al. 1988). In contrast, ^{31}P-NMR investigations of mycorrhizae are scarce, the only known report being on birch mycorrhizae (Grellier et al. 1989).

Studies on the uptake and storage of phosphate by mycorrhizae have been published. Using $KH_2\ ^{32}PO_4$ as a nutrient medium, ^{32}P tracer experiments with birch mycorrhizae showed a doubling in internal phosphate concentration within 24 h (Harley 1978); the internal phosphate concentration in this case was measured at intervals of several hours. To demonstrate the accumulation of phosphate over a period of several days, ^{31}P-NMR investigations have been done so far with pea root tips (Lee and Ratcliffe 1983b) and birch mycorrhizae (Grellier et al. 1989) using a continuous phosphate supply. Results on phosphate storage by the pure basidiomycete of *Armillaria mellea* have been reported (Cairney et al. 1988).

Materials and methods

Cultures

Mycorrhized and nonmycorrhized, excised roots of *Fagus sylvatica* were removed with ease from forest soil. Nonmycorrhized and mycorrhized seedlings of *Pinus sylvestris* were grown initially in a sterile environment using autoclaved sand, Hoagland solution (Hoagland and Arnon 1950) as a nutrient, and periods of light and dark of 14 h:10 h. After about 2 months, the seedlings were split into two groups, one remaining in the sand and the other prepared for synthetic mycorrhizing.

Synthetic mycorrhizing was performed using Petri dishes with carbon filters fixed to the inside of one half (Kottke et al. 1987).

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Seedlings of *Pinus sylvestris* were passed through a slit in the rim of each Petri dish. Before closing the dishes, the roots were infected with *Suillus bovinus* and then filled with sand. The seedlings were grown vertically in the conditions described above for about 2 months.

Cultures of *Suillus bovinus* (Dr. R. D. Finlay, Department of Microbiological Ecology, University of Lund, Sweden) were grown for 3–4 weeks using MMN (Marx and Bryan 1975).

NMR techniques

^{31}P-NMR investigations were performed using a Bruker AM 360-FT spectrometer and a 20-mm diameter tube. The conditions established for NMR experiments were a frequency of 148.78 MHz, a pulse angle of 60°, an accumulation time of about 3.8 min corresponding to 400 scans, a repetition time of 0.56 s, and a line broadening of 20 Hz. FIDs (free induction decays) measured by NMR were stored in the computer in the form of "serial files" for later processing. All the spectra are in reference to 85% H_3PO_4 present as a parallel tube containing methylene diphosphonate at 17.0 ppm as an external standard.

Phosphate uptake and polyphosphate metabolism

Defined nutrient solution conditions were maintained by inserting two flexible tubes into the NMR tube. The solution to be monitored was transported into and out of the NMR tube by pump at a rate of 500 ml/h.

Before starting, all samples were pretreated as follows. To remove adhering sand, which would give additional field inhomogeneity and thereby large line widths, the roots were rinsed for about 30 min with demineralized water. Subsequently, excised roots and intact seedlings were transferred into the NMR tube and the wash phase was started by supplying demineralized water; all NMR investigations began at the same time. This was called a pilot experiment. To ensure uniform conditions, the pure mycelium was handled in the same way. Preliminary experiments indicated an intact metabolism during the whole wash phase for all samples investigated (not shown). The pilot experiment was completed within about 61 min, corresponding to $16 \times 400 = 6400$ scans. The nutrient supply was started by alternating between two forms of Hoagland solution as described below. After about 5 h, corresponding to eight supply cycles (see below), metabolic steady-state conditions were reached, i.e. conditions of cyclic reproducable in vivo ^{31}P-NMR spectra.

Using the isotope ^{31}P, two kinds of Hoagland solution were used for cyclic experiments, one containing 0.1 mM KH_2PO_4 (1) and the other without phosphate (2). Periodic switching between solutions (1) and (2) at intervals of about 5 and 33 min, respectively, resulted in a cyclic supply. The Hoagland solution was buffered to pH 5.5 using 5 mM 1,4-piperazine-diethanesulphonic acid.

Parallel to the cyclic nutrient supply described above, 160 FIDs of 3.8 min each were taken by the NMR spectrometer. Thus, 10 FIDs over a period of 38 min formed one block, corresponding to one complete nutrient supply cycle during the same time. A total of 16 blocks carried out in this way constituted one experiment on the uptake and storage of phosphate. The FIDs of each block corresponding to the same time period, (i.e. 16 identical types of FIDs) were added by computer. This procedure increased the signal-to-noise ratio by a factor of 4. This summation process resulted in 10 different FIDs corresponding to $16 \times 400 = 6400$ scans each. Calculation of the means of the 10 summated FIDs referred to as block averaging.

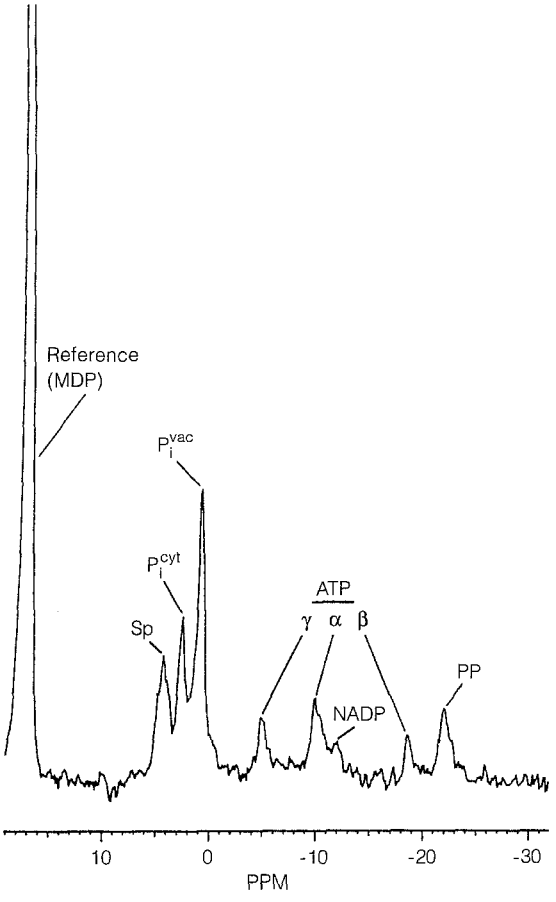

Fig. 1. An analysis of the intracellular phosphate compartments of nonmycorrhized detached roots of *Fagus sylvatica* shown by ^{31}P-NMR. The spectrum corresponds to 85% H_3PO_4 taken at a pulse angle of 60°, an accumulation time of about 61 min (6400 scans), a repetition time of 0.56 s and a line broadening of 20 Hz. *MDP,* Methylene diphosphonate; *Sp,* sugar phosphates; P_i^{cyt}, cytoplasmic anorganic phosphate; P_i^{vac}, vacuolar phosphate; *ATP,* adenosine triphosphate; *NADP,* nicotinamide adenine dinucleotide phosphate; *PP* mobile polyphosphate

Since the area below an NMR peak is proportional to the concentration of the substance in question, the spectra were integrated. Since they reflect internal concentrations of polyphosphate of medium to long chain length and inorganic phosphate, the areas PP_4 and P_i (Fig. 1), respectively, were taken as relative intracellular concentrations and given arbitrary values. Hence in the figures (e.g. Figs. 3–6) each data point corresponds to an internal concentration of P_i and PP_4 up to a maximum which for each supply cycle was defined to be 100%. In all of the ^{31}P-NMR investigations, information about the polyphosphate concentration was obtained by integrating the PP_4 peak, which is characteristic of the medium-chain phosphates of polyphosphate.

To obtain estimates of phosphate uptake, the effluent from the NMR tube was carefully collected into several pools. Comparison of the solutions pumped into and out of the NMR tube showed a decrease in the concentration of phosphate as a consequence of cellular uptake. The phosphate uptake demonstrated by this method was of the same order as that shown by in vivo NMR, thus validating NMR methods for such kinetic studies.

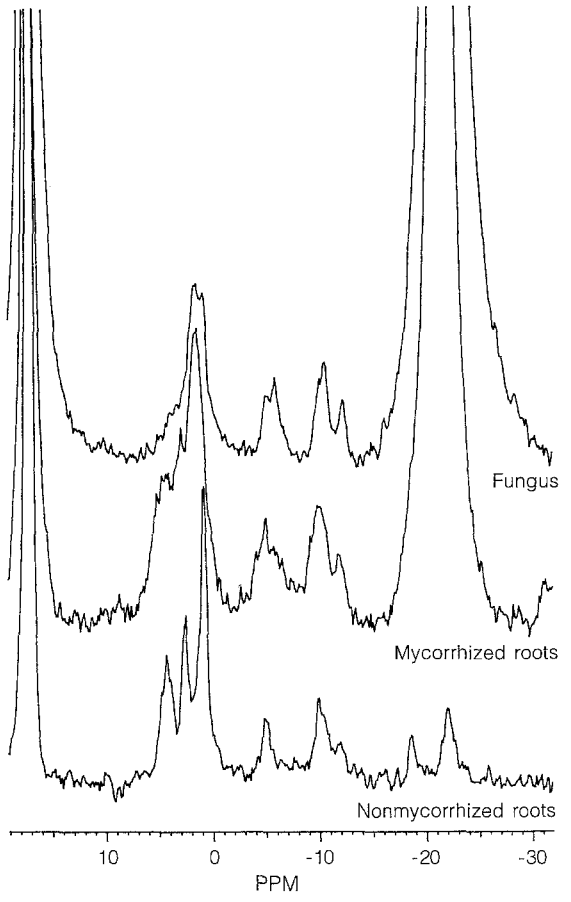

Fig. 2. 148.78 MHz ^{31}P-NMR spectra of nonmycorrhized (see text), and mycorrhized detached roots of *Fagus sylvatica* and pure mycelium of *Suillus bovinus*. NMR conditions as in Fig. 1. Peak identities: +17 ppm, external standard (MDP); +5 ppm, sugar phosphates; −5, −10 and −18 ppm, γ-, α-, β-ATP; −12 ppm, NADP. For nonmycorrhized roots, peaks at +1.0 and +2.6 ppm indicate vacuolar and cytoplasmic P_i, respectively, of *Fagus sylvatica*. For the mycelium, peaks at +0.7 and 1.3 ppm are P_i of the vacuole and cytoplasm, respectively, of *Suillus bovinus*

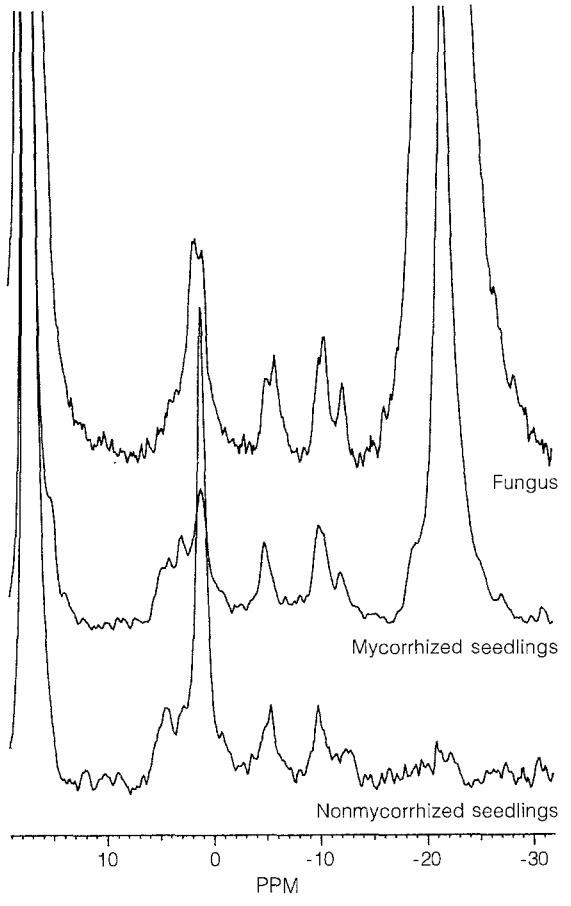

Fig. 3. 148.7 MHz ^{31}P-NMR spectra of nonmycorrhized (see text) and mycorrhized roots of intact seedlings of *Pinus sylvestris* and pure mycelium of *Suillus bovinus*. NMR conditions as in Fig. 1. Peak identities: +17 ppm, external standard (MDP); +5 ppm, sugar phosphates; −5, −10 and −18 ppm: γ-, α-, β-ATP; −12 ppm, NADP. For nonmycorrhized roots, +1.0 and +2.6 ppm indicate vacuolar and cytoplasmic P_i, respectively, of *Pinus sylvestris*. For the mycelium, +0.7 and 1.3 ppm are P_i of vacuole and cytoplasm, respectively, of *Suillus bovinus*

Results

NMR spectra

In all five preparations investigated by in vivo ^{31}P-NMR – pure mycelium of *Suillus bovinus*, mycorrhized and nonmycorrhized detached roots of *Fagus sylvatica*, mycorrhized and nonmycorrhized roots of intact seedlings of *Pinus sylvestris* – the ^{31}P-NMR spectra (Figs. 2, 3) include signals for several metabolites. In each case the peaks −5, −10 and −18 ppm are derived from γ-, α- and β-ATP, while sugar phosphates are at 5 ppm and nicotinamide adenine dinucleotide phosphate at −12 ppm. The spectra of the nonmycorrhized detached roots of *Fagus sylvatica* and roots of intact seedlings of *Pinus sylvestris* indicate a vacuolar inorganic phosphate concentration of 1.0 ppm and a cytoplasm concentration of 2.6 ppm (Figs. 2a, 3a). In contrast, the signals for vacuolar and cytoplasmic inorganic phosphate of the mycelium of *Suillus bovinus* are 0.7 ppm and 1.3 ppm, respectively (Figs. 2c, 3c). In addition, an intense signal resulting from the medium-chain phosphate groups PP_4 of mobile polyphosphate was given by the mycorrhized roots and the mycelium.

It can be seen that the NMR spectra of the mycorrhized roots arise by superimposition of those of the two symbionts, namely from root and mycelium (Figs. 2b, 3b). Hence in the spectrum of mycorrhized roots of *Fagus sylvatica* (Fig. 2b) at 0.7 ppm and 1.3 ppm, only a shoulder is visible instead of distinct peaks, and any analysis of fit for revealing the chemical shifts is difficult. Compared to the beech roots, the spectrum of the nonmycorrhized roots of *Pinus sylvestris* (Fig. 3a) shows a difference with respect to resolution of the inorganic phosphate peaks. Here the signal of vacuolar inorganic phosphate is so intense that the cytoplasm signal is obscured.

To determine the internal pH, an in vitro pH versus chemical shift plot, carried out using a phosphate solution, was taken as the basis (Roberts et al. 1980, 1981; Lee and Ratcliffe 1983b). *Suillus bovinus* had a vacuolar pH of 5.4±0.1 (0.7 ppm) and a cytoplasmic pH of

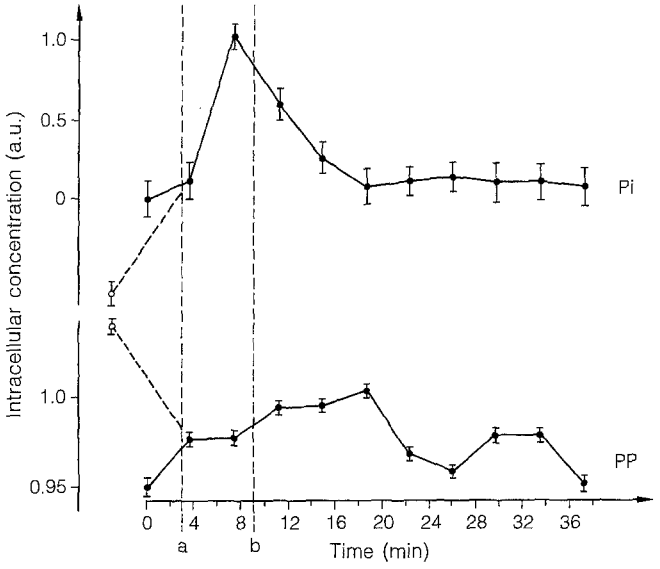

Fig. 4. The intracellular concentrations of inorganic phosphate (*Pi*) and mobile polyphosphate (*PP*) of *Suillus bovinus* in arbitrary units (a.u.) as a function of time in the presence of an external phosphate supply at a constant pH 5.5. The supply period of Hoagland solution containing 0.1 mM KH_2PO_4 is marked by the interval *a–b*. Each data point represents the integral of the peak taken in arbitrary units from in vivo ^{31}P-NMR spectra of $16 \times 400 = 6400$ scans each. The first data points (joined to the others by *dotted lines*) are results from the wash phase before the start of the cyclic experiments

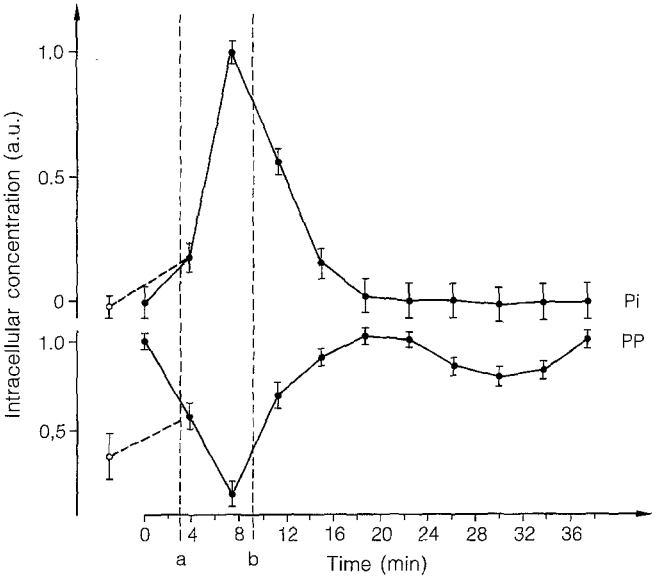

Fig. 5. The same experiment illustrated in Fig. 4 but with nonmycorrhized detached roots of *Fagus sylvatica*

6.5 ± 0.1 (1.3 ppm). For the roots of *Fagus sylvatica* and *Pinus sylvestris* the same method gave values of pH 6.1 ± 0.1 (1.0 ppm) for the vacuole and pH 7.7 ± 0.1 (2.6 ppm) for the cytoplasm. The field inhomogeneity produced by the investigated samples resulted in NMR peaks with line widths of up to 60 Hz.

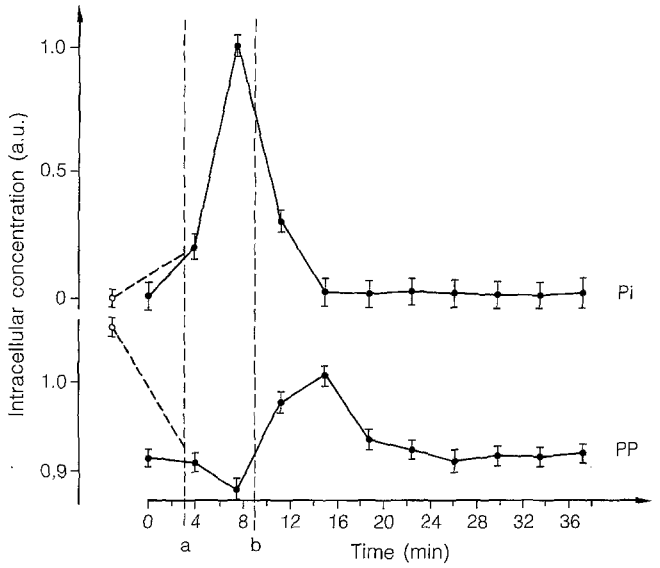

Fig. 6. The same experiment illustrated in Fig. 4 but with mycorrhized detached roots of *Fagus sylvatica*

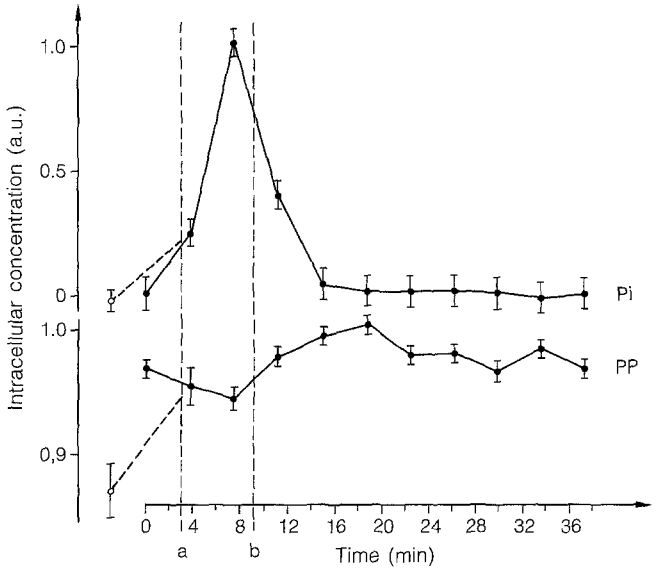

Fig. 7. The same experiment illustrated in Fig. 4 but with mycorrhized roots of intact seedlings of *Pinus sylvestris*

Phosphate uptake and polyphosphate metabolism

For clarity, the data on phosphate uptake and storage of the samples investigated are divided into four figures: mycelium of *Suillus bovinus* (Fig. 4), detached roots of *Fagus sylvatica* (Fig. 5, nonmycorrhized) and (Fig. 6, mycorrhized) and mycorrhized roots of intact seedlings of *Pinus sylvestris* (Fig. 7). The period of external phosphate supply is shown by the vertical lines in each figure. The first point (joined to the others by a dotted line) is the so-called pilot experiment prior to the cyclic experiments.

The intracellular concentration of P_i and PP_4 measured during the wash phase (pilot experiment) is

mostly different from that found at the beginning and end of each reversible cycle. While the concentration of inorganic phosphate in the roots during the pilot experiment is in agreement with that at the beginning of each cycle (Figs. 5–7), that in the mycelium is much lower, namely about −50% with respect to the cycle start value (Fig. 4). Polyphosphate in the pilot experiments with *Suillus bovinus* (Fig. 3) and mycorrhized detached roots of *Fagus sylvatica* (Fig. 6) was found at concentrations of 105% and 108%, respectively. This is in contrast to concentrations in nonmycorrhized detached roots of *Fagus slyvatica* (Fig. 5) and mycorrhized seedlings of *Pinus sylvestris* (Fig. 7) of about 30% and 87%, respectively. Thus the first pair showed higher and the second pair lower concentrations of PP_4 during the wash phase than at the start of the supply cycle.

When the external supply was in the form of Hoagland solution containing KH_2PO_4, phosphate uptake rose. After approximately 8 min, a maximum in uptake was achieved, defined here as 100%. Termination of the external phosphate supply by switching to Hoagland solution whithout KH_2PO_4 resulted in a decrease in the rate of phosphate uptake. After 20 min the P_i uptake already returned to its starting value, here defined as 0% concentration of intracellular phosphate (Figs. 4–7).

With respect to the kinetics of change in internal polyphosphate, the samples showed characteristic differences. In the case of *Suillus bovinus* at each cycle the PP_4 concentration increased up to the maximum (100%) within 20 min, before decreasing in oscillations to the original value (Fig. 4). In contrast, the PP_4 concentration of the roots first showed a decrease with the minimum already reached after 8 min (Figs. 5–7). Subsequently, the concentration rises and tends to a final value of the same order as that at the start of each cycle. Although the kinetics of the PP_4 concentration of the nonmycorrhized detached roots of *Fagus sylvatica* was more marked, it is in principle comparable to that of the mycorrhized seedlings of *Pinus sylvestris* (Figs. 5, 7). The mycorrhized detached roots of *Fagus sylvatica* nevertheless showed the same characteristics, but not before an increase in PP_4 concentration up to a maximum higher than the value at the start and end of the cycle and reached within 16 min (Fig. 6).

Discussion

NMR spectra

In in vivo NMR spectroscopy of roots and pure fungus, the difference in chemical shift permits characterization of both an identification of the intracellular compartments and the intracellular pH. As described above, the chemical shift is related to the pH by a pH versus ppm plot, which is determined in vitro (not shown). The validity of the plot, however, is affected by the intracellular environment. Previous investigations tested the influence of ions such as KCl and $MgCl_2$ on in vitro ^{31}P-NMR of a phosphate solution (Roberts et al. 1981; Lee and Ratcliffe 1983b). The results show only minor differences between the pH/ppm plots of pure of K_3PO_4 and those of the same solution with other ions added; hence, the in vitro plot of K_3PO_4 is reliable. A further source of inaccuracy is the use of a pH electrode to determine the pH/ppm dependence. The reliability of the intracellular pH values obtained by in vitro ^{31}P-NMR is about ±0.1.

Using an in vitro pH versus ppm plot, the vacuolar and cytoplasmic pH of roots of different species varied in the range of 5.5–6.1 and 7.1–7.7, respectively. The pH fungal vacuole and cytoplasm is also species dependent and has been reported to be in the range of 6.0–6.5 and 7.3–7.5, respectively.

For the investigated nonmycorrhized detached roots and roots of intact seedlings of *Fagus sylvatica* and *Pinus sylvestris*, the chemical shift and the pH of the cytoplasm (2.6 ppm/pH 7.7±0.1) and the vacuole (1.0 ppm/pH 6.1±0.1) is in good agreement with that of mung bean root tips (Torimitsu et al. 1984) and of the roots of intact corn seedlings (Roberts and Testa 1988). In contrast, the vacuolar pH of corn roots was found to be 5.5±0.1 (Roberts et al. 1982), and the pH of the cytoplasmic of corn roots (Roberts et al. 1980) and *Nicotiana tabcum* (Wray et al. 1985) was reported to be 7.1±0.1.

The mycelium of *Suillus bovinus* shows lower chemical shift and thus a more acid pH in the cytoplasm (1.3 ppm/pH 6.5±0.1) and the vacuole (0.7 ppm/pH 5.4±0.1) than other fungi. The cytoplasmic pH of *Saccharomyces cerevisiae* (Den Hollander et al. 1981, 1986; Nicolay et al. 1982) was about 7.5, that of *Zygosaccharomcyes bailii* (Nicolay et al. 1982) and *Candida utilis* (Nicolay et al. 1982, 1983) was of 7.3±0.5, and a value of 7.5±0.5 was found in the basidiomycete *Armillaria mellea* (Cairney et al. 1988). In general, this means a difference of approximately 1 pH unit more alkaline than the cytoplasmic pH of *Suillus bovinus*. The vacuolar pH of the yeasts and the basidiomycete are also alkaline, i.e. for the yeasts 6.0±0.2 and for the basidiomycete 6.5±0.5.

Figures 2 and 3 show that the spectra of the mycorrhized roots (b) originated from the spectra of the two symbionts (a, c), which was verified by computer fit (results not shown). In fact, the resolution of all of the in vivo spectra was affected firstly by the irregular orientation of the probes inside the NMR tube, producing considerable magnetic field inhomogeneity. Secondly, a line broadening of 20 Hz was used, and as a consequence of both these factors a resulting line width of about 60 Hz prevents resolution of the signals of ATP and ADP, e.g. distinction of γ-ATP/β-ADP (≈ -5 ppm) and α-ATP/α-ADP (≈ -10 ppm) (Figs. 2, 3). Thus, the only method for estimating the ATP concentration where needed is to use the ^{31}P-NMR peak of β-ATP (≈ -18 ppm), which in the case of the mycelium and mycorrhized roots here was completely obscured by the intense PP_4 peak (b, c in Figs. 2, 3). Comparable results from in vivo ^{31}P-NMR investigations of other fungi are to be found elsewere: *Saccha-*

romyces cerevisiae (Den Hollander et al. 1986), *Zaccharomyces bailii* and *Candida utilis* (Nicolay et al. 1982, 1983) and *Armillaria mellea* (Cairney et al. 1988). In most cases the β-ATP was covered by the PP_4 peak, although Den Hollander et al. (1981) presented ^{31}P-NMR spectra of *Saccharomyces cerevisiae* in which it is possible to distinguish between the peaks of β-ATP and PP_4, but both with relatively narrow line widths. This implies a lower concentration of polyphosphate, possibly as the consequence of either a sparing phosphate supply or the termination of phosphate supply. In our experiments, the spectra of nonmycorrhized roots (Figs. 2a, 3a) both contained small but characteristic peaks of PP_4 at around -22 ppm as a result of existing intracellular polyphosphate. This suggests either adhering hyphae or a freshly initiated mycorrhizal infection of the nonmycorrhized roots taken from the forest soil. In addition, this result furnishs proof of the sensitivity of NMR. Hence the term nonmycorrhized roots may no longer be completely correct. Further in vivo ^{31}P-NMR experiments performed using intact pine seedlings, especially those grown in sterile conditions, show spectra in which the peak usually seen at about -22 ppm is absent, i.e. polyphosphate is absent from these genuine nonmycorrhized roots (data not shown).

Phosphate uptake and polyphosphate metabolism

The results illustrated in Figs. 4–7 show that polyphosphates play a central role in phosphate storage.

Considering the kinetics of intracellular P_i and phosphate uptake in response to repeated cycles of external phosphate supply, the fluctuations in phosphate uptake were determined by the extracellular P_i concentration, and in this respect all the samples investigated showed comparable behaviour. With the exception of the mycelium, at the start of each cyclic experiment the internal P_i concentration was the same as that found before starting the experiment, i.e. during the wash phase (Figs. 5–7). The stability of intracellular P_i concentration is possibly due to the conversion of PP_4 into P_i by the symbiotic fungus as a consequence of the absence of external phosphate during the wash phase. Prior to the cyclic experiments the mycelium of *Suillus bovinus* indeed showed a P_i concentration about 50% lower than at the start of each of the cyclic experiments (Fig. 4). Loss of cellular phosphate is excluded, since there was no indication of phosphate in the solution of the NMR tube. The possible basis of this behaviour is intracellular conversion of P_i into polyphosphate during the wash phase and minimal supply to the cell due to the absence of any phosphate in the external medium. In fact, during the wash phase the polyphosphate is at a higher concentration than at the start of each supply cycle, in relative units about 105% and 95%, respectively (Fig. 4). Further discussion, therefore, is limited to intracellular polyphosphate concentration.

In the mycorrhized and nonmycorrhized roots investigated, P_i was also related to PP_4. As previously mentioned in the case of nonmycorrhized detached roots of *Fagus sylvatica* and roots of intact seedlings of *Pinus sylvestris,* the pilot experiment showed lower PP_4 concentrations at the start of each cycle (Figs. 5, 7). In contrast, the pure mycelium of *Suillus bovinus* and the mycorrhized detached roots of *Fagus sylvatica* both showed higher concentrations of polyphosphate during the pilot experiment than at the start of each supply cycle (Figs. 4, 6). This behaviour will be first explained for the pure fungus and mycorrhized detached roots.

A deficiency in external P_i supply possibly results in reduced metabolic activity in the mycelium and a decrease in the concentration of intracellularly available phosphate by conversion to polyphosphate, which thus increases in concentration (Fig. 4). The mycorrhized detached roots show the same behaviour in terms of polyphosphate, and indeed show a constant P_i concentration during the change from the wash phase to the cyclic experiments. But this implies that the "mobile" polyphosphate detectable as the PP_4 peak on NMR is in part transformed intracellularly into "immobile" polyphosphate not detectable by ^{31}P-NMR. Thus for the first time two different types of polyphosphate become evident: mobile polyphosphate of medium chain length ($n \lesssim 100$) and immobile polyphosphate of longer chain length ($n \gg 100$).

The solution pumped out of the NMR tube did not have a higher concentration of phosphate than the ingoing solution, which verifies that the nondetectable, immobile polyphosphate is localized inside the fungal cells. In fact, electron microscopy has shown polyphosphate granules localized in the sheath of ectomycorrhizae (e.g. Chilvers and Harley 1980; Lapeyrie et al. 1984; Grellier et al. 1989). However, Grellier et al. (1989) do not distinguish between mobile, NMR-detectable polyphosphate and the granular polyphosphate which is immobile and thus not detectable by NMR (Gadian 1982). Indeed Grellier et al. (1989) reported an increase in the concentration of polyphosphate granules during external phosphate supply. Possibly the reaction converting mobile polyphosphate into P_i in mycelium is faster and more favourable than the conversion of immobile polyphosphate into P_i directly. Thus it may be postulated that there is a special pool containing polyphosphate, at a concentration dependent an a given situation, which is regulated by change in concentration of free or stored intracellular P_i concentration within certain limits.

The behaviour of the mycorrhized roots follows a similar scheme, except that the symbiontic fungus has to supply P_i to both the roots and itself. Hence, any deficiency in external phosphate concentration, induces the fungal cells to partly mobilize immobile polyphosphate to mobile, medium-chain-length polyphosphate. This is manifested by a higher concentration of NMR-detectable polyphosphate than in the presence of external P_i (Fig. 6). With regard to the sum of the symbionts alone, the P_i required by the mycorrhiza is of a higher concentration; this behaviour possibly ex-

plains the finding that the intracellular P_i is constant under the change between the pilot experiment and the start of each supply cycle.

Mycorrhizal nutrient transfer between the symbionts must certainly be considered, especially carbon, which is connected to the phosphate transfer (Woolhouse 1975; Harley and Smith 1983; Clarkson 1985, 1988; Dexheimer et al. 1985; Smith and Smith 1985, 1989; Kottke and Oberwinkler 1987; Farrer and Lewis 1987).

The behaviour of nonmycorrhized detached roots and synthetic mycorrhized roots of intact seedlings differs from that of the two cases considered above. Both have lower mobile polyphosphate concentrations during the pilot experiment than at the start of each supply cycle (Figs. 5, 7). This difference from pure mycelium and mycorrhized detached roots grown and mycorrhized in a natural environment is remarkable considering that the roots have a lower fungal mass. The reserves of polyphosphate storage intracellularly are very small and exhausted much earlier during a period of deficiency, and hence show a low PP_4 concentration during the pilot experiment (Figs. 5, 7). The pools of phosphate are only replenished on the completion of several supply cycles (not shown). The result is an increase in mobile polyphosphate concentration to that found at the start of each cycle.

With respect to the concentration of mobile polyphosphate and the storage of phosphate at each cycle, the four cases investigated also demonstrated distinctly different behaviour. In particular, *Suillus bovinus* differs markedly from the roots since the concentration of PP_4 increases with external phosphate supply (Fig. 4). After removing external P_i, a maximal polyphosphate concentration was reached within 19 min. Since the change in internal P_i concentration actually indicates phosphate uptake, it may be seen that a finite period is required to convert mobile polyphosphate into immobile (and thus not NMR-detectable) polyphosphate. During the period when no external phosphate was available, the intracellular mobile polyphosphate decreased to exactly the same concentration found at the start of each cycle. Each cycle requires approximately 38 min. External phosphate accumulated by the fungus is completely converted indirectly into immobile polyphosphate which cannot be detected by NMR.

As to the fungus, all the roots investigated are completely different with regard to mobile polyphosphate. As described above, in response to increase in intracellular P_i concentration the roots react by a decline in mobile polyphosphate (Figs. 5–7). One possible explanation is as follows: Since both the mycorrhizal fungus and the root accumulate P_i from the external medium, the intracellular P_i concentration of the mycorrhiza increase more rapidly than that of the symbiontic fungus alone. Consequently, by conversion into immobile polyphosphate the pool of mobile polyphosphate of the symbiontic fungus decreases to an extent greater than that of the pure fungus alone. On the other hand, P_i stored by both symbionts is partly converted into mobile polyphosphate and the concentration of the latter increases. Finally, the concentrations of both the intracellular P_i and the mobile polyphosphate reach the value recorded at the start. Since the fungus-to-root ratio is very low for the nonmycorrhized detached roots, the fall in mobile polyphosphate concentration here is more distinct (Fig. 5) than in the mycorrhized detached roots (Fig. 6). For the mycorrhized seedlings of *Pinus sylvestris* there is indeed a decrease, since transport of part of the mycorrhizal P_i into the needles produces a decrease in intracellular P_i concentration in the mycorrhiza.

Previous investigations of the uptake and storage of phosphate by mycorrhizae differ somewhat from our work. However, the previous experiments involved time periods of hours (Harley 1978) and days (Pearson and Tinker 1975; Lee and Ratcliffe 1983b; Grellier et al. 1989). Consequently, any observation of the conversion of P_i into mobile polyphosphate and mobile polyphosphate into immobile polyphosphate was impossible, since the time resolution required is in the order of a few minutes (≈ 10 min).

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