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Effects of Monovalent Cations on Phosphate Accumulation and Storage of the Ectomycorrhizal Fungus *Suillus bovinus*

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Abstract—Comparative *in vivo* ^{31}P-NMR studies of the fungus *Suillus bovinus* (L.: Fr.) O. Kuntze in pure culture have produced interesting new data. To investigate the response of phosphate metabolism to a change in external monovalent cations, samples were exposed to a Hoagland solution containing different monovalent cations Li^+, Na^+, K^+, or Rb^+ at 10 mM concentration. A method of nutrient cycling during analysis where the cation was changed and the phosphate kept constant allowed us to determine the kinetics of phosphate accumulation, storage and incorporation into polyphosphate following exposure to the range of test cations. Different external monovalent cations had different effects upon changes in the content of both phosphate and polyphosphate. Treatment with Li^+, Na^+, or Rb^+ resulted in a change in phosphate accumulation to 60, 73, and 107% and in content of the intracellular mobile polyphosphate (polyP) to 119, 112, and 94%, respectively, compared with the control taken as 100%. The effect of each cation is related to its position in the periodic table. Reversing this process, i.e., exchanging with K^+, returned phosphate metabolism to normal. Although, the increase in depolarization of the cell membrane should affect the internal pH, fungal metabolism using energy requiring mechanisms appeared necessary to maintain the intracellular pH. Thus, increasing contents of mobile polyP were the consequence of an increasing energy demand. On the other hand, the increasing depolarization of the cell membrane following the sequence $Rb^+ < K^+ < Na^+ < Li^+$ inhibited the net P_i accumulation. Furthermore, it is postulated that the P_i accumulation was also regulated by the intracellular content in polyP.

Key words: ectomycorrhizal fungus, monovalent cations, NMR, phosphate accumulation, polyphosphate, *Suillus bovinus*

Ectomycorrhizal fungi perform a crucial role in nutrient cycling in forest ecosystems, particularly under conditions of nutrient scarcity [1]. The significance of this symbiosis for the accumulation of nitrogen (N) and phosphorus (P) by forest trees is well recognized [2].

Phosphorus is an important nutrient, and it has already been shown that the internal phosphorus in ectomycorrhizas is regulated particularly by the polyphosphate (polyP) of the fungal partner [3, 4]. The functions of polyP have been shown to be associated with bioenergetic processes [5, 6]. Fungal polyP has been interpreted as an energy source to maintain the ectomycorrhizal metabolism [7, 8]. Besides important soil parameters such as pH and substrate (e.g., phosphate) content, fungal growth may be affected by the concentration of ions in the soil other than the ion being investigated [9]. The effects of monovalent [10, 11] and polyvalent cations [12, 13] upon the kinetics of fungal nutrient accumulation have been shown.

The effects of several cations on substrate accumulation could be interpreted in principle by a change in the kinetics of the uptake mechanism [14]. It has been assumed that the cations screen differently the net negative charge on the surface of the cell membrane [15]. A consequence of a lower net negative charge and a decreased negative surface potential, respectively, is a decreased concentration of substrate cations and an increased concentration of substrate anions near the transport sites [14, 16].

The first objective of the current study was to investigate the effect of various monovalent cations in the nutrient medium upon the mobile polyphosphate (NMR detectable polyphosphates (polyP) with a chain-length $n \leq 100$) contents of the fungus *Suillus bovinus* (L.: Fr.) O. Kuntze, provided by Dr. R. D. Finlay, Department of Microbiological Ecology, University of Lund, Sweden. The second objective was to investigate whether phosphate accumulation and polyphosphate metabolism of

Abbreviations: M^+) monovalent cation; MMN) modified Melin–Norkrans solution; polyP) polyphosphates; PP_4) middle standing phosphate groups of polyphosphates; T) relaxation time.

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the fungus are affected by the different monovalent cations Li^+, Na^+, K^+, and Rb^+.

MATERIALS AND METHODS

Cultures. Cultures of *Suillus bovinus* were obtained from Dr. R. D. Finlay, Department of Microbiological Ecology, University of Lund, Sweden. The mycelia were grown in liquid Modified-Melin–Norkrans (MMN) medium [17], pH 5.5 and harvested after 3.5 weeks to produce a dry-weight of 150-250 mg and a diameter of 4-5 cm. All samples were rinsed with demineralized water before being transferred into the NMR tube.

Nutrition. The nutrients required to maintain fungal metabolism were provided by Hoagland's solution of the following composition: 0.1 mM KH_2PO_4, 5 mM KNO_3, 5 mM $Ca(NO_3)_2 \cdot 4\ H_2O$, 2 mM $MgSO_4 \cdot 7\ H_2O$, 9 μM $MnSO_4 \cdot H_2O$, 0.7 μM $ZnSO_4 \cdot 7\ H_2O$, 0.3 μM $CuSO_4 \cdot 5\ H_2O$, 0.1 μM $(NH_4)MoO_4 \cdot 4\ H_2O$, 46 μM H_3BO_3, 24 μM Fe/Na-EDTA.

NMR techniques. ^{31}P-NMR investigations were performed using a Bruker AM 360-FT spectrometer and a 20-mm diameter tube. The conditions for NMR experiments are described in a previous paper [7].

Polyphosphate kinetics. All experiments were performed in triplicate. The nutrient solution to be monitored was transported in and out of the NMR tube via two flexible tubes at a rate of 500 ml/h. The solution transported out of the NMR tube was collected in test tubes every 3.8 min in synchrony with the accumulation of the NMR spectra. Phosphate content was determined later. All solutions were oxygenated and adjusted to pH 5.5 [18].

For each NMR investigation the mycelium was first rinsed with demineralized water to wash off the MMN solution. Otherwise the high amounts of phosphate in the MMN solution would obscure the NMR P_i signals. Moreover, it was necessary to eliminate the high amounts of glucose from the fungal tissue to stimulate the fungal phosphate metabolism. This "wash phase" was completed within 6400 scans (1 h 48 sec).

After the wash phase, the cyclic phosphate nutrient supply was started: two Hoagland's solutions [19] were used to alternate the supply of phosphate, one containing 0.1 mM $KH_2{}^{31}PO_4$ (I) and the other without phosphate but with 0.1 mM KCl (II). All solutions were adjusted to pH 5.5. Periodic alternating supplying with the solutions I for 5 min and II for 33 min, resulted in a cyclic phosphate supply. Due to the lack of glucose in the Hoagland's solutions, the optimal vitality of the fungi within the NMR tube was limited to 28-30 h. This allowed only relatively brief periods of study. After about eight supply cycles, fungal metabolism had equilibrated and the *in vivo* ^{31}P-NMR spectra were reproducible (see [7]).

To study the influence of Li^+, Na^+, K^+, and Rb^+ upon the content of mobile polyphosphate (polyP), another set of nutrient solutions not containing phosphate was used. In the first solution applied, the 5 mM KNO_3 of the original Hoagland's solution was replaced by 10 mM KCl (A) and in the second by 10 mM MCl (B), where M indicates the monovalent cation investigated. A change in external cation was performed through preparing supply pools filled with unbuffered solutions of the respective cation.

Before monitoring, six so-called adjustment cycles of a periodic switching between A and B, i.e., [A], [B], [A], [B], [A], ..., were undertaken at intervals of about 2 × 38 min = 76 min each (Fig. 1). After reaching cyclic reproducible conditions, another 12 cycles of the same type were run but now together with an NMR monitoring of the fungal P compartments. During each cycle of 76 min twenty NMR spectra of 3.8 min duration were taken parallel to this cyclic nutrient supply. These 20 spectra formed one "block", corresponding to one complete nutrient supply cycle during the same time of 76 min. A total of 12 blocks were carried out using this procedure, and constituted one experiment. As an example, Fig. 2 shows the first 10 successive ^{31}P-NMR spectra of these cycles taken within 3.8 min (400 scans) each, representing the influence of an exchange of 10 mM NaCl by 10 mM KCl on the polyP kinetics of *Suillus bovinus*.

On these 12 blocks consisting of 12 × 20 = 240 spectra, a "block averaging" was performed using the follow-

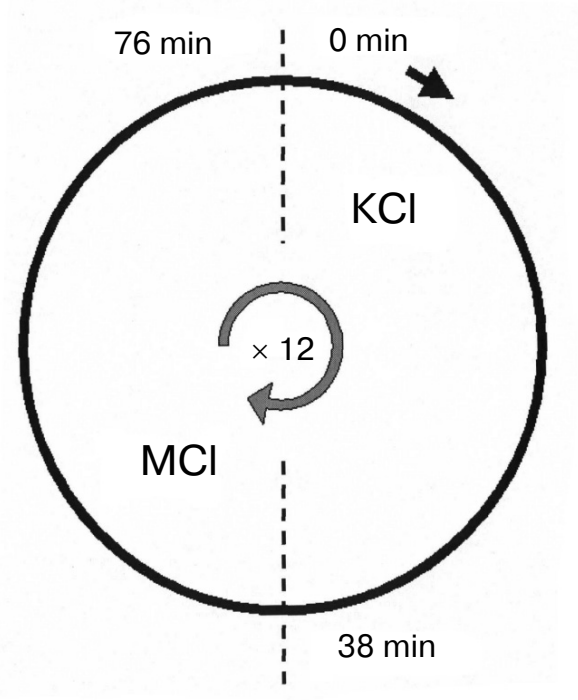

Fig. 1. Cyclic switching between external 10 mM KCl and 10 mM MCl solutions after 38 min each. Each cycle took 76 min to perform, and in total there were 12 cycles of periodic switching between K^+/M^+ solutions.

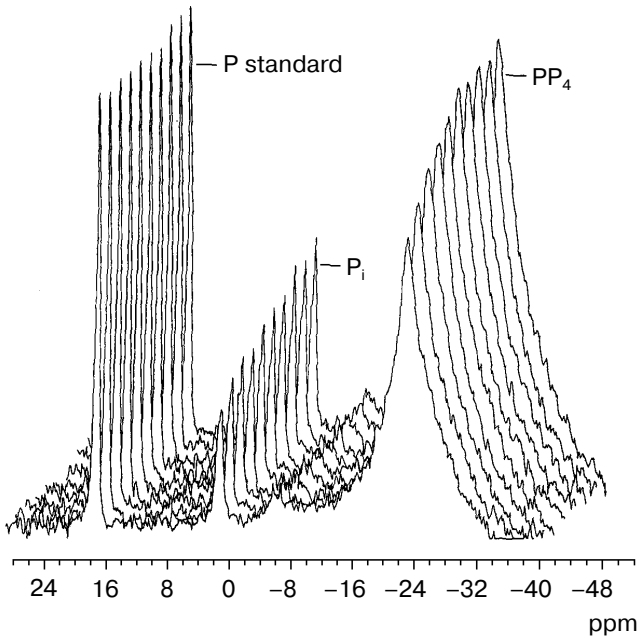

Fig. 2. Influence of external Na^+ on polyP of *Suillus bovinus*. A number of 10 successive 147.7 MHz ^{31}P-NMR spectra of *Suillus bovinus* taken during a supply by Hoagland's solution without phosphate at pH 5.5 under the external replacement of 10 mM K^+ by 10 mM Na^+ within 38 min. The spectra correspond to 85% H_3PO_4, taken at a pulse angle of 60°, an accumulation time of about 3.8 min (400 scans), a repetition time of 0.56 sec, and a line broadening of 20 Hz. Peak intensities: +17 ppm, external standard (methylene diphosphonate); +1.3 and +0.7 ppm, inorganic phosphate of the cytoplasm and vacuole, respectively; −12 ppm, NADP.

ing procedure. The spectra of each block, which corresponded to the same time period, i.e., 12 identical types of spectra, were added by computer. This procedure increased the signal-to-noise ratio by a factor of $\sqrt{12}$. This summing process resulted in 20 different spectra corresponding to $12 \times 400 = 4800$ scans each. The calculation of the means of the 20 summed spectra is referred to as block averaging.

The then integrated spectra represented the internal contents of fungal phosphorus compartments [7] (Fig. 3). Information about the mobile polyP was obtained by integrating the PP_4 peak, which is characteristic of the medium-chained phosphate groups of mobile polyphosphate [7]. The peak areas of middle phosphate groups of polyphosphates (PP_4) and P_i were taken to indicate relative intracellular contents and are given in arbitrary values, since a comparative quantitative analysis is only possible in the case of fully relaxed nuclear spins [20]. The relaxation time (T) of the nuclear spins of polyP is very short ($T_{polyP} = 0.05$ sec [21]) and at a repetition time of 0.56 sec [7] the spins are fully relaxed during all the experiments. The relaxation of the other nuclear spins of ^{31}P *in vivo* experiments requires very long time periods T, e.g., $T_{P_i} = 4.0$ sec [21]. The special form of *in vivo* experiment used here does not permit such long periods but requires the choice of a relatively short repetition time. As a result, the NMR yielded relative amounts of internal phosphate components instead of absolute amounts since standards cannot be used.

Phosphate accumulation and polyphosphate metabolism. Investigations on the effect of external monovalent cations upon the accumulation and storage of phosphate were performed on samples of juvenile *Suillus bovinus*. These experiments also began with a wash phase followed by eight phosphate supply cycles, in contrast to the experiment described before, cyclic phosphate supply (see above) was maintained during the whole investigation.

With the aim to reveal the dependence of the fungal phosphate accumulation and storage on external monovalent cation, the effect of Li^+, Na^+, and Rb^+ were related to K^+ as a basis. This divided the investigation into three experiments: 1) K^+/Li^+; 2) K^+/Na^+; and 3) K^+/Rb^+. A change in external monovalent cation was performed easily by preparing supply pools filled with unbuffered Hoagland's solutions of the respective cation adjusted by HCl and NaCl.

After reaching metabolic steady state conditions, the investigation began by running six supply cycles using Hoagland's solutions I and II (see above), both containing

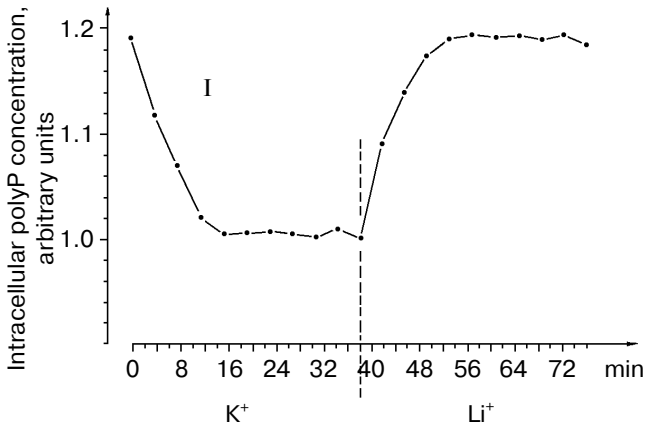

Fig. 3. Influence of external Li^+ and K^+ on polyP of *Suillus bovinus*. Representative excerpt of changes in intracellular content of mobile polyphosphate (polyP) of the mycelium of *Suillus bovinus* in arbitrary units in Hoagland's solution without phosphate and at pH of 5.5 under the external exchange of 10 mM K^+ by Li^+ as a function of time. Hoagland's solution containing K^+ is supplied for 38 min (dotted line) and is then exchanged for an Li^+-containing solution during the next 38 min (total time of treatment is 76 min). Each data point represents the integral of the ^{31}P-NMR peak (see text), respectively, taken in arbitrary units from *in vivo* ^{31}P-NMR spectra of $12 \times 400 = 4800$ scans each. The values are the means of 12 cyclic experiments, run in triplicate. The bar in the upper left corner represents the standard error of the means.

10 mM of K^+. Following this, 13 more supply cycles were run by exchanging 10 mM K^+ with another external monovalent cation M^+ (Li^+, Na^+, or Rb^+) of the same concentration. Finally, seven cycles were run using K^+ again.

Parallel to the cyclic nutrient supply, 6·10 + 13·10 + 7·10 = 260 spectra of 3.8 min each were taken by the NMR spectrometer. Thus, 10 spectra over a period of 38 min formed one "block", corresponding to one complete nutrient supply cycle during the same time. A total of 26 blocks carried out in this way constituted one experiment on the accumulation and storage of phosphate as influenced by exchanging K^+ and M^+.

As a result of the exchange of external monovalent cations, the samples required a period of about half an hour to reach cyclic reproducible metabolic states anew. Thus, after every cation change, the first 10 spectra of K^+/M^+ and the first 10 spectra of M^+/K^+ could not be used. It turned out that the same metabolic conditions were reached after the exchange with K^+ as before, when exposed to M^+, requiring a period of about half an hour, i.e., 10 spectra = 1 block. Therefore, six blocks taken before and the remaining six blocks taken after exposure were identical. On the remaining 6 + 12 + 6 blocks consisting of 240 spectra, the block averaging was performed by the following procedure: the spectra corresponding to the same period and taken during exposure to K^+ (i.e., six identical types of spectra before and 6 after exposures to M^+) were added using the computer. This procedure also increased the signal-to-noise-ratio by a factor of $\sqrt{12}$ (see above). The 12 spectra taken during the exposure of the fungus to M^+ were treated in the same way. Thus, this summing process resulted in two different spectra corresponding to 12 × 400 = 4800 scans each. Equal treatment of all the spectra taken by NMR, finally led to three sets of summed spectra. Each set reflected the maximum level of accumulation and storage of phosphate by the fungus (see [18]) at an external monovalent cation concentration as related to the accumulation and storage at the same level of external K^+.

The integrated spectra reflected internal contents of P_i and polyP [7]. Information about the mobile polyP was obtained by integrating the PP_4 peak, characteristic of medium chained polyphosphate. The areas of PP_4 and P_i were taken then, as relative intracellular contents, and given in arbitrary values related to those found in the presence of K^+.

The results of P_i and polyP as related to the respective monovalent cation were compared through calibrating the levels found in the presence of K^+ and defining these maxima as 100%. Thus, it was possible to interpret all the amounts in arbitrary units.

RESULTS

Polyphosphate kinetics. The influence of the three monovalent cations (Li^+, Na^+, and Rb^+) on the changes in polyP content of *Suillus bovinus* in relation to that polyP content measured in the presence of K^+ (Figs. 3-5) is represented by the integration of the PP_4 peaks of the 20 successive NMR spectra. For clarity, the results in the presence of K^+ are always presented to the left of the dotted line, while the results in the presence of Li^+, Na^+, and Rb^+, respectively, are given to the right (Figs. 3-5). To enable a comparison between the effects of the various monovalent cations upon the fungal polyP content, the steady state polyP content under external K^+ conditions (Figs. 3-5, at t = 38 min) was defined as 100%. In all the experiments on the polyP kinetics no detectable change in intracellular P_i content was found (data not shown).

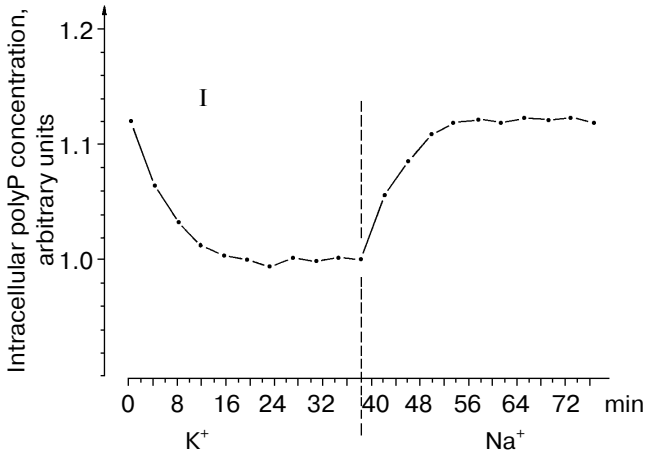

Fig. 4. Influence of external Na^+ and K^+ on polyP of *Suillus bovinus*. Same conditions as in Fig. 3 but exchanging K^+ by Na^+ after 38 min.

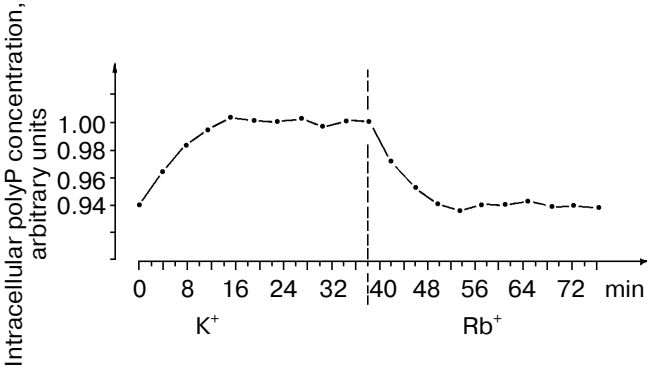

Fig. 5. Influence of external Rb^+ and K^+ on polyP of *Suillus bovinus*. Same conditions as in Fig. 3 but exchanging K^+ by Rb^+ after 38 min.

After replacing LiCl by KCl solution (Fig. 3, at $t = 0$ min) a rapid decrease in the content of internal polyP from about 119 to 102% of steady state occurred within the first 11 min (Fig. 3) in each of the 12 cycles. At $t = 26$ min, the steady state value specified as 100% was reached. On renewed transfer to LiCl the fungal polyP content rose rapidly from 100 to 118% within the first 15 min, then reaching a plateau at its initial value 119%.

The effects produced by the monovalent cation Na^+ (Fig. 4) were similar but less pronounced than those in the K^+/Li^+-experiment. When the Na^+ is replaced by K^+ (Fig. 4, at $t = 0$ min) fungal polyP content decreased always from about 112 to 100% within 19 min. Substituting Na^+ for K^+ after completing 38 min made the polyP content return to its initial value of about 112% within the next 19 min (Fig. 4, $t = 57$ min).

In comparison with the results under exposing the mycelia to LiCl and NaCl, respectively, a different reaction occurred on exposure to Rb^+ (Fig. 5). After transferring the mycelia from RbCl into KCl environment (at $t = 0$ min in Fig. 5) an increase in fungal polyP occurred instead of the expected decrease as shown in Figs. 3 and 4. The level of polyP recovered to 100% within approximately 16 min when K^+ was restored. When reverted to RbCl in the external nutrient supply ($t > 38$ min), the level of polyP decreased to approximately 94% of its initial level in the next 16 min ($t = 54$ min).

Accumulation and storage. The graphical representations of the influence of monovalent cations upon the accumulation of phosphate by *Suillus bovinus* (Fig. 6-9) show at least slight changes in the kinetics of P_i content and internal polyP. The contents of external inorganic phosphate contents in the test tubes were regularly measured to ensure that the internal P_i contents were actually due to a P_i accumulation.

When phosphate was supplied to the fungus in Hoagland's solution I (0 min), phosphate content (indicated by P_i) rose showing to a maximum change in the content at 7.6 min (Figs. 6-9). When exposed to M^+, the fungus yielded a maximum phosphate change in the content of about 60% of the K^+ value in the presence of Li^+ (Fig. 6). The other maxima were about 73% in the presence of Na^+ (Fig. 7), 100% in the presence of K^+ (Fig. 8), and about 107% in the presence of Rb^+ (Fig. 9). After the maximum, changes in the phosphate content fell within approximately 12 min to its initial value 0%.

The polyP kinetics (Figs. 6-9) showed generally an increase to an absolute maximum after 19 min, a relative minimum after approximately 27 min, and a relative maximum at around 34 min, and reached its initial value after 38 min. The fungal polyP content increased slowly on exposing the *Suillus bovinus* to Li^+ from about 119% of the K^+ value to a maximum of about 124%, decreasing then to its initial content (Fig. 6). When the fungus was exposed to Na^+, similar kinetics was found starting at 112% of the K^+ value with a maximum polyP content of approximately 118% (Fig. 7). In contrast, the polyP contents reached more intense maxima and relative minima during exposure to K^+ and to Rb^+, respectively. The initial polyP content in the presence of K^+ was 100% and reached its maximum at around 110% (Fig. 8), whereas in the presence of Rb^+, an initial value of about 94% and a maximum of 107% (Fig. 9) were found.

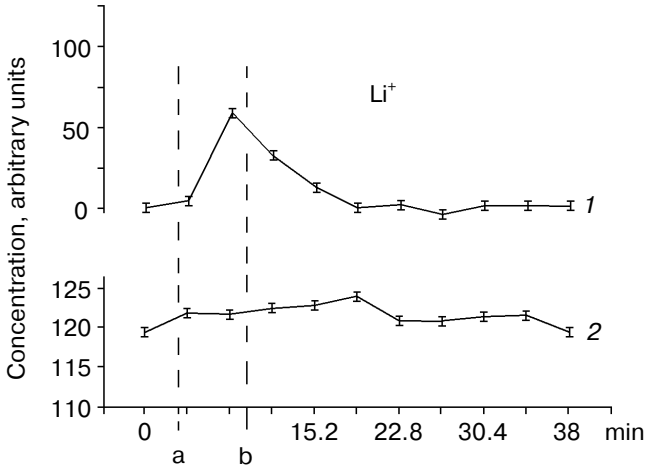

Fig. 6. Effects of external Li^+ on accumulation and storage of phosphate in *Suillus bovinus*. Comparison of inorganic phosphate accumulation (*1*) and polyphosphate kinetics (*2*) of *Suillus bovinus* in arbitrary units in the presence of 10 mM LiCl using periodic supply by Hoagland's solutions I and II at pH 5.5 (see text). The period of supplying Hoagland's solution with 0.1 mM $KH_2{}^{31}PO_4$ is marked by "a" and "b". Each data point represents the integral of the ^{31}P-NMR peak (see text), respectively, taken in arbitrary units from *in vivo* ^{31}P-NMR spectra of $12 \times 400 = 4800$ scans each. The values are the means of 12 cyclic experiments, run in triplicate. The bars represent the standard error of the means.

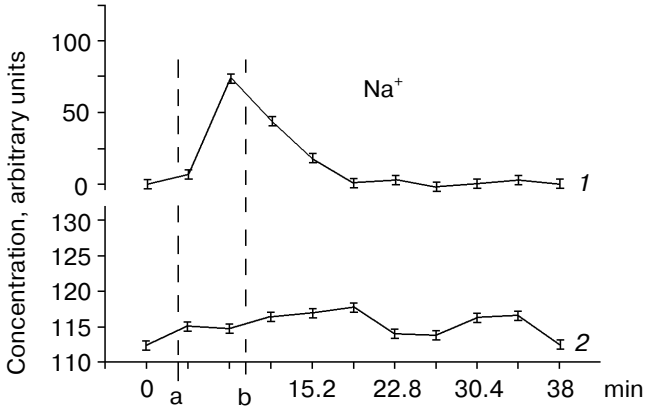

Fig. 7. Effects of external Na^+ on accumulation and storage of phosphate in *Suillus bovinus*. Same conditions as in Fig. 6 but exchanging Li^+ by Na^+.

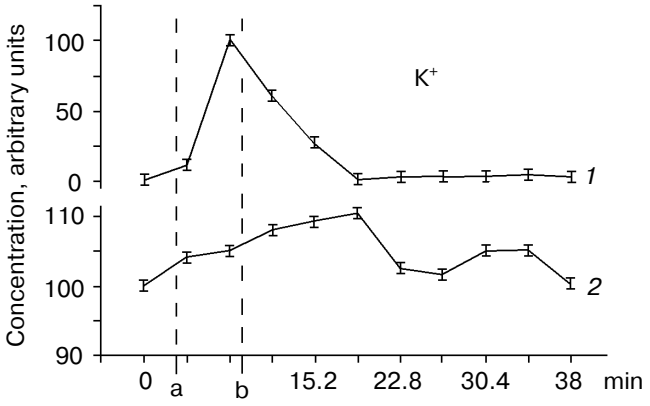

Fig. 8. Effects of external K^+ on accumulation and storage of phosphate in *Suillus bovinus*. Same conditions as in Fig. 6 but exchanging Li^+ by K^+.

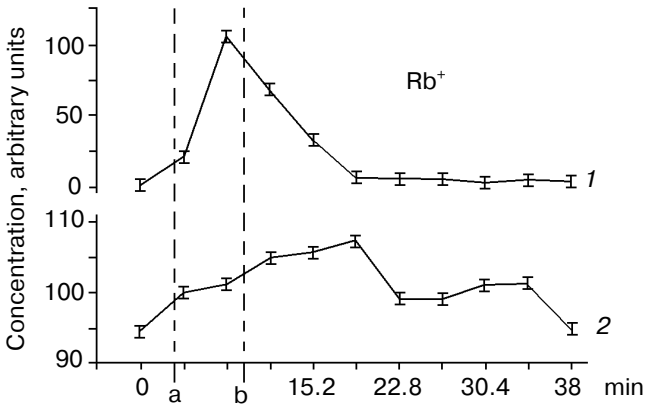

Fig. 9. Effects of external Rb^+ on accumulation and storage of phosphate in *Suillus bovinus*. Same conditions as in Fig. 6 but exchanging Li^+ by Rb^+.

DISCUSSION

Polyphosphate kinetics. The aim of the current work was to investigate the effects of monovalent cations on the content of mobile polyP in *Suillus bovinus*. Effects of the ion radius will be important if in the external medium one monovalent cation is exchanged for another of the same concentration. Then, this effect should be due to the physical properties of these ions.

The results of our experiments showed a significant increase in mobile polyphosphate (polyP) during exposure to the monovalent cations Rb^+, K^+, Na^+, Li^+ related to their position in the periodic table. PolyP is an important regulatory molecule [22]. It is not only considered as a phosphorus storage pool, but the functions are, moreover, associated with bioenergetic processes [5]. Since fungal cells contain very limited amounts of organic energy sources, the breaking-up of the energy-rich bonds of the phosphate groups obviously provides the fungus with enough energy and P_i to phosphorylate ADP into ATP [23] in absence of external energy sources like glucose. There are mainly two different forms of fungal polyphosphate: the mobile polyP is of middle-chain-length ($n \leq 100$) and *in vivo* ^{31}P-NMR-detectable, the immobile polyP is of long-chain-length ($n >> 100$) or of granular form, and not detectable by *in vivo* ^{31}P-NMR [7, 20, 24]. The degradation from immobile into mobile polyP provides energy to the fungus [3, 7]. Thus, the relative amount of mobile polyP in fungal cells can be regarded as an equivalent for the metabolic activity of the fungus. Therefore, a change in the internal polyP content points to a cellular response to environmental changes. A rise in mobile polyP with external cations Rb^+, K^+, Na^+, Li^+ (Figs. 3-5) then indicates an increased energy demand. The presence of monovalent cations as well as of Cl^- the external medium at a concentration of 10 mM represents a stressful situation.

Several authors have reported stimulation of plasma membrane H^+-transport activity by salt stress [25, 26]. The H^+ efflux and the related metabolic activities (e.g., those involved in the control of the cellular pH) will increase in the order $Rb^+ < K^+ < Na^+ < Li^+$ when metabolic cells are exposed to these cations [27]. An increase in H^+-ATPase activity indeed causes an increase in energy consumption. Since mobile polyP is postulated to be a fungal energy source, besides ATP, the increase in mobile polyP content in *Suillus bovinus* in the sequence $Rb^+ < K^+ < Na^+ < Li^+$ can be due to such an energy requiring process (Figs. 3-5).

The cell pH is one of the factors regulating ion transport in fungi [28, 29]. When monovalent cations are added to the medium, the pH of metabolizing cells generally increases [30, 31]. If cations are transported into the cell, protons will be transported out of the cell as a consequence of maintenance of the electronegativity, and the cell pH will increase. The transport mechanism involved may be active or passive. If the entry of cations into the cell is very slow, it can be neglected, and the external effects at the cell surface can lead to a change in intracellular pH [32].

Generally, biological membranes have a net negative surface charge and, consequently, give rise to a surface potential. The value of this potential depends on the ionic strength of the surrounding medium [33]. Cations screen the negative charges on the surface causing the intrinsic charge on the surface to become less negative [15, 16]. Moreover, the strength of depolarization also depends on the cation properties [32]. A decreasing net negative charge will decrease the proton concentration near the surface and thus increase the external pH.

Since the intracellular pH is affected by a decreasing net negative charge due to the presence of different monovalent cations, the properties of these cations are of

a central importance. A principal physical property is the ionic radius. Since the hydration energies of the cations decrease as their non-hydrated ionic radii increase from Li^+ to Rb^+, the number of water molecules in the first hydration layer decreases, too. Following statistical calculations on the hydrated state of the cations Li^+, Na^+, K^+, Rb^+ [34], it can be shown that a constant radius can be supposed from the ion's charge center to the outer sphere of the first hydration shell [35]. Neglecting chemisorption, the probable distance between the charge center of the electrostatically adsorbed cation and a negative charged surface can be expected to decrease with decreasing ionic radius [35]. This phenomenon is due to the fact that the smallest cation with its center of charge nearest to the adsorption site will experience the largest attractive force. Outweighing the hydration forces causes a decrease in the affinity with growing ionic radius in the sequence $Li^+ < Na^+ < K^+ < Rb^+$. This statement has been proven experimentally on ion exchange resins containing ionized carboxylate groups [36]. Nevertheless, lower charged surfaces can cause a change in the cation affinities [37, 38]. The consequence of reducing the distance between the positive charge center of the cations and their adsorption position on the membrane surface is an increase of the screening of the negative fixed charges. This causes the net negative charge (zeta potential, see [39]) to become less negative [15, 16].

When the net negative charge of the surface decreases in the sequence $Rb^+ > K^+ > Na^+ > Li^+$, the effective extracellular concentration of the free H^+ ions near the surface decreases also following the simple Gouy–Chapman theory [40]. Then, the resulting proton gradient across the cell membrane into the cell inclines. This implies an efflux of intracellular H^+ and stimulates the metabolism of *S. bovinus* to maintain the internal pH. Thus, the energy required will increase with decreasing cation radius. Since polyP is the most important fungal energy source, an increase of mobile polyP should be expected in order to compensate the increased energy demands. Indeed, the results of our experiments showed such an increase in the content of mobile polyP. We postulate that these requirements of energy may be a stress response due to the active maintenance of the internal cell pH.

Accumulation and storage of phosphate. Another objective of our studies was to determine the dependence of the accumulation and storage of phosphate by *Suillus bovinus* upon monovalent cations. In our experiments, the inorganic phosphate accumulation increased with an increase in pure ionic radii of external monovalent cations in the series $Li^+ < Na^+ < K^+ < Rb^+$ (Figs. 6-9).

Many studies of the accumulation and storage of phosphate by ectomycorrhizal fungi have been published [41, 42]. An important factor for the phosphate accumulation is the proton concentration near the cell surface. In yeast cells, it has been demonstrated that the transport of phosphate (P_i) is accompanied by an influx of two-to-three H^+ [32, 43]. A decrease in the negative surface potential (e.g., produced by external monovalent cation Rb^+, K^+, Na^+, Li^+) will cause a decrease in the interfacial proton concentration. It seems that both depolarization of the cell membrane and decreasing the pH gradient across the yeast cell membrane lead to a decrease in the changes in the phosphate content energized by the proton motive force [14]. This agrees well with the results of our study, where polyP contents also decreased in the sequence $Li^+ > Na^+ > K^+ > Rb^+$ (Figs. 6-9).

It has been discussed [7] that the intracellular pools of mobile polyP and P_i are limited to defined amounts; MacFall and coworkers [3] demonstrated also that the sum of the P_i and mobile polyP contents is maintained in fungal cells, while the individual contents of both can vary. The transformation of intracellular inorganic phosphate into polyphosphate is advantageous to further the accumulation of phosphate by clearing the cellular pools of inorganic phosphate ready for storage. Obviously, a similar mechanism is involved in the transformation from mobile polyP into immobile [7]. Thus, mobile polyP might regulate the intracellular level of P_i that, in turn, may regulate the accumulation of phosphate. Holzer [44] indeed has shown the rate of phosphate entry in yeast is regulated by the intracellular orthophosphate content. When the cellular orthophosphate content was relatively high, no net accumulation occurred. In contrast, a decrease in intracellular orthophosphate content was followed by an increase in phosphate accumulation [44]. This would mean for our results that by exposure to Li^+ (Fig. 6), the limited possible level of intracellular polyP was nearly reached. It was demonstrated by a low level in P_i accumulation and a high level in mobile polyP, whereas by exposure to Rb^+ (Fig. 9), an accumulation of higher amounts of P_i due to low amounts of polyP was possible.

REFERENCES

1. Read, D. J., Francis, R., and Finlay, R. D. (1985) in *Ecological Interactions in Soil* (Fitter, A. H., ed.) Blackwell Scientific Publications, Oxford, pp. 193-217.
2. Harley, J. L., and Smith, S. E. (1983) *Mycorrhizal Symbiosis*, Academic Press, London, p. 483.
3. MacFall, J. S., Slack, S. A., and Wehrli, S. (1992) *Plant Physiol.*, **100**, 713-717.
4. Mousain, D., and Salsac, L. (1985) in *Mycorrhizae: Physiology and Genetics* (Gianinazzi-Pearson, V., and Gianinazzi, S., eds.) Institut National de la Recherche Agronomique, Paris, pp. 357-361.
5. Beauvoit, B., Rigoulet, M., Guerin, B., and Canioni, P. (1969) *FEBS Lett.*, **252**, 17-22.
6. Felter, S., and Stahl, A. J. C. (1973) *Biochemie*, **55**, 245-249.
7. Gerlitz, T. G. M., and Werk, W. B. (1994) *Mycorrhiza*, **4**, 207-214.

8. Harley, J. L., and McCready, C. C. (1981) *New Phytologist*, **89**, 75-80.
9. Thompson, G. W., and Medve, R. J. (1984) *Applied and Environmental Microbiology*, **48**, 556-560.
10. Calahorra, M., Ramírez, J., Clemente, M., and Peña, A. (1987) *Biochim. Biophys. Acta*, **899**, 229-238.
11. Peña, A., Uribe, S., Pardo, J. P., and Borbolla, M. (1984) *Arch. Biochem. Biophys.*, **231**, 217-225.
12. Borst-Pauwels, G. W. F. H. (1993) *Biochim. Biophys. Acta*, **1145**, 15-24.
13. Theuvenet, A. P. R., and Borst-Pauwels, G. W. F. H. (1976) *Biochim. Biophys. Acta*, **426**, 745-756.
14. Borst-Pauwels, G. W. F. H. (1981) *Biochim. Biophys. Acta*, **650**, 88-127.
15. Theuvenet, A. P. R., and Borst-Pauwels, G. W. F. H. (1976) *Bioelectrochem. Bioenerg.*, **3**, 230-240.
16. Theuvenet, A. P. R., and Borst-Pauwels, G. W. F. H. (1976) *J. Theor. Biol.*, **57**, 313-329.
17. Marx, D. H., and Bryan, W. C. (1975) *Forest Sci.*, **21**, 245-254.
18. Gerlitz, T. G. M., and Gerlitz, A. (1997) *Mycorrhiza*, **7**, 101-106.
19. Hoagland, D. R., and Arnon, D. I. (1950) *Circulations of the California Agriculture Experimental Station*, United States Department of Agriculture, Berkeley, CA, p. 347.
20. Gadian, D. G. (1982) *Nuclear Magnetic Resonance and Its Applications to Living Systems*, Oxford University Press, New York.
21. Kugel, H. (1988) *^{31}P- und ^{23}Na-Kernspinresonanzspektroskopische Untersuchungen des Energiemetabolismus der Euryhalinen Mikroalge Platymonas subcordiformis*: Ph.D. Thesis [in German], University of Bremen, Bremen.
22. Kulaev, I. S. (1979) *Biochemistry of Inorganic Polyphosphates*, J. Wiley and Sons, Chichester, New York.
23. Kortstee, G. J. J., Appeldoorn, K. J., Bonting, C. F. C., van Niel, E. W. J., and van Veen, H. W. (2000) *Biochemistry (Moscow)*, **65**, 332-340.
24. Vagabov, V. M., Trilisenko, L. V., and Kulaev, I. S. (2000) *Biochemistry (Moscow)*, **65**, 349-354.
25. Yamashita, K., Kasai, M., Yamamoto, Y., and Matsumoto, H. (1994) *Soil Sci. Plant Nutr.*, **40**, 555-563.
26. Yamashita, K., Yamamoto, Y., and Matsumoto, H. (1996) *Plant Cell Physiol.*, **37**, 949-956.
27. Sacchi, G. A., Espen, L., Nocito, F., and Cocucci, M. (1997) *Plant Cell Physiol.*, **38**, 282-289.
28. Rodríguez-Navarro, A., Blatt, M. R., and Slayman, C. L. (1986) *J. Gen. Physiol.*, **87**, 649-674.
29. Ryan, J. P., and Ryan, H. (1972) *Biochem. J.*, **128**, 139-146.
30. Ryan, H., Ryan, J. P., and O'Connor, W. H. (1971) *Biochem. J.*, **125**, 1081-1085.
31. Zlotnikova, I. F., and Vakhmistrov, D. B. (1982) *Fiziol. Rast.*, **29**, 1012-1016.
32. Borst-Pauwels, G. W. F. H. (1993) *Biochim. Biophys. Acta*, **1152**, 201-206.
33. Vos, J., Kuriyama, K., and Roberts, E. (1968) *Brain Res.*, **9**, 224-230.
34. Azzam, A. M. (1954) *Zeitschrift für Elektrochem.*, **58**, 889-899.
35. Gerlitz, T. G. M. (1984) *Zur Struktur der Elektrolytischen Doppelschicht*: Ph.D. Thesis [in German], University of Oldenburg, Oldenburg.
36. Gregor, H. P. (1956) *Trans. N. Y. Acad. Sci.*, **18**, 667-692.
37. Eisenman, G. (1961) *Biophys. J.*, **2**, 259-323.
38. Eisenman, G., and Conti, F. (1965) *J. Gen. Physiol.*, **48**, 65-73.
39. Obi, I., Ichikawa, Y., Kakutani, T., and Senda, M. (1989) *Plant Cell Physiol.*, **30**, 129-135.
40. Bockris, J. O'M., and Reddy, A. K. N. (1970) *Modern Electrochemistry*, Plenum Press, New York, pp. 724-732.
41. Cairney, J. W. G., Jennings, D. H., Ratcliffe, R. G., and Southern, T. E. (1988) *New Phytologist*, **109**, 327-333.
42. Martin, F., Marchal, J. P., Tyminska, A., and Canet, D. (1985) *New Phytologist*, **101**, 275-290.
43. Roomans, G. M., and Borst-Pauwels, G. W. F. H. (1979) *Biochem. J.*, **178**, 521-527.
44. Holzer, H. (1953) *Biochem. Zeitschrift*, **324**, 144-155.