Mycorrhiza (1992) 1:137–146

Mycorrhiza



*Original papers*

# Occurrence and expression of acid phosphatase of *Hymenoscyphus ericae* (Read) Korf & Kernan, in isolation or associated with plant roots

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**Summary.** The activity of acid phosphatase produced in pure culture by the endomycorrhizal fungus *Hymenoscyphus ericae* (Read) Korf & Kernan (*H. ericae* LPA 2) was inhibited by high phosphorus levels, alkaline pH, fluoride, molybdate and mannosidase, and activated by concanavalin A. Over 80% of the enzyme activity was due to two wall-bound acid phosphatase isozymes with the characteristics of mannose-rich glycoproteins. Antiserum was raised against the major, low-molecular-weight wall isozyme and its activity tested by immunoblotting and ELISA. The antiserum cross reacted 100% with exocellular (excreted) and 28% with cytoplasmic cellular fractions of *H. ericae* (LPA 2) cultures, and showed high reactivity with other strains of *H. ericae* but not with fungal isolates from *Erica hispidula* L. or *E. mauritanica* L. Ultrastructural localization of acid phosphatase by cytoenzymology and indirect immunogold labelling confirmed its association with the fungal wall in pure culture and showed that the influence of a high phosphorus level, fluoride and molybdate is through inactivation of the enzyme. Intense acid phosphatase activity, sensitive to the latter inhibitors, was also present on external hyphae growing over a host or non-host root but it was weak or absent from intracellular hyphae where these developed within a host root. Indirect immunolabelling confirmed that this acid phosphatase was of fungal origin and that the specific inhibitory effect of host cells is due to inactivation of the enzyme rather than repression of its synthesis. Possible implications of fungal acid phosphatase in ericoid endomycorrhizal infection processes are discussed together with mechanisms that may be regulating the enzyme activity.

**Key words:** Ericaceae – Mycorrhizal fungi – Acid phosphatase – Protein expression – Immunocytochemistry

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## Introduction

Acid phosphatases (EC 3.1.3.2) are generally considered to be involved in the phosphate nutrition of plants and to be key enzymes in the utilization of complexed phosphate esters by ecto- and ericoid mycorrhizal systems (Gianinazzi-Pearson and Gianinazzi 1981, 1989; Mitchell and Read 1981; Mousain 1978, 1989; Mousain and Salsac 1982; Pearson and Read 1973; Stribley and Read 1975; Stribley et al. 1975).

In pure culture, ericoid endomycorrhizal fungi have an acid phosphatase activity which breaks down organic or condensed phosphates and which is stimulated by low levels, but inhibited by high levels, of soluble phosphate in the culture medium (Pearson and Read 1975; Shaw and Read 1989; Straker and Mitchell 1986). This activity is typically inhibited by fluoride and molybdate, and there is preliminary evidence that the enzyme(s) involved may be glycoproteins which are sensitive to glycosidases (Straker et al. 1989).

When ericoid endomycorrhizal fungi grow close to a root surface, intense acid phosphatase activity becomes associated with the fibrillar sheath developing over the hyphal surface. It is absent, however, from hyphae growing at some distance from the root and disappears with colonization of a living host cell (Gianinazzi-Pearson et al. 1986), suggesting that the host plant somehow influences the metabolism of the fungal symbiont.

In order to understand the significance of this enzyme activity in processes determining symbiotic interactions in ericoid endomycorrhiza, acid phosphatase of the ericoid endomycorrhizal fungus *Hymenoscyphus ericae* (Read) Korf & Kernan was extracted, its physiological and biochemical properties investigated and polyclonal antiserum raised against the purified enzyme. Specificity was demonstrated in physiological, biochemical or immunological assays and cross reactivity with other strains of ericoid endomycorrhizal fungi was tested. The localization of acid phosphatase in pure culture and in association with a host or non-host plant was compared cytochemically and by indirect immunolabelling in order to determine whether the enzyme activity

associated with hyphae growing over a host root is of fungal origin, and to investigate the possible mechanism(s) by which a host plant influences the enzyme during the endomycorrhizal symbiosis.

## Material and methods

### *Culture procedures*

A European strain of *H. ericae* (LPA 2), isolated from roots of *Calluna vulgaris* L., was grown on water agar at 23° C; after 20–30 days the margins of the culture were transferred to a modified Norkrans medium (Straker and Mitchell 1985), with $KH_2PO_4$ as the phosphorus source.

*Vaccinium corymbosum* L. and *Nicotiana tabacum* L. seeds were germinated aseptically on water agar. Seedlings were transferred to a sterile soil/water agar mixture in tubes and inoculated with *H. ericae* (LPA 2) (Pearson and Read 1973). Plants were grown for 6–8 weeks under controlled conditions (16 h day, 200 $\mu E\ m^{-2}\ s^{-1}$, 22° C).

### *Methods*

Variations in growth and phosphatase activity of *H. ericae* (LPA 2) were analysed in liquid culture as described by Pearson and Read (1975) at different pH values (3–8), culture times and phosphorus ($KH_2PO_4$) levels.

Acid phosphatase was obtained from total crude extracts of mycelium and from wall, cytoplasmic or extracellular (excreted into the culture medium) fractions as described by Straker and Mitchell (1986), and purified from the wall fraction by gel filtration through a S-400 gel Sephacryl column (Straker and Mitchell 1986). Partially purified wall enzyme extracts consisted of samples which had not been separated by gel filtration. The protein content of extracts was estimated by the Bradford (1976) method. Acid phosphatase activity of the mycelium and extracts was assayed at pH 4.5 using para-nitrophenyl phosphate (pNPP) as the substrate, as described by Bartlett and Lewis (1973). The effect of different concentrations of sodium fluoride, sodium molybdate, α-mannosidase, β-glucosidase and the plant lectin concanavalin A was tested by incubation with the crude extract for 30 min at 37° C before addition of the substrate to the reaction medium. Inhibitor and alkaline pH (9.6) sensitivity of the partially purified wall-bound acid phosphatase of *H. ericae* (LPA 2) were also studied after separation of isozymes by 10% polyacrylamide gel electrophoresis (PAGE). The molecular weight of the low-molecular-weight (LMW) isozyme was estimated using an electrophoretic calibration kit (Sigma) by SDS-PAGE. The glycoprotein nature of isozymes was investigated by affinoblotting with concanavalin A, which detects mannose and glucose residues, as described by Straker et al. (1989).

Rabbit polyclonal antiserum was raised against the purified major acid phosphatase isozyme (LMW), cut as a band from the polyacrylamide gel after PAGE separation and suspended in sterile physiological serum. The protein (100 ng) was injected subcutaneously into a rabbit and a booster injection was similarly made after 4 weeks. The rabbit was bled at 14, 21, 35, and 89 days after the booster injection; control pre-immune serum was taken from the animal before the first injection. The antiserum was partially purified by ammonium sulphate precipitation (Clark and Adams 1977) and was tested against homologous antigens by enzyme-linked immunosorbent assay (ELISA) and immunoblotting (Straker et al. 1989). The effect of the antiserum on fungal acid phosphatase activity was assessed by ELISA: antibodies were incubated at 1:100 dilution for 30 min at 37° C with a crude mycelium extract. The antiserum was tested in ELISA against extracts of wall, cytoplasmic and extracellular fractions of *H. ericae* (LPA 2) and extracts of mycelium of four strains of *H. ericae* (LPA 2, LPA 15, LPA 25, LPA 42), two fungal strains isolated from *Erica hispidula* L. and *Erica mauritanica* L. and three unidentified mycorrhizal ericoid strains from other hosts: LPA 16 (*E. vagans* L), LPA 22 (*V. corymbosum*) and LPA 29 (*E. carnea* L.), the last being very weakly infective only with certain hosts (*Goodyera procumbens* L., *E. carnea* L.) (Gianinazzi-Pearson and Bonfante-Fasolo 1986).

Procedures followed for the ultrastructural localization of enzyme activity and enzyme antigen were those outlined by Straker et al. (1989). Colonies of *H. ericae* (LPA 2) growing in modified Norkrans medium containing either 20 or 150 ppm phosphorus, and excised roots of mycorrhizal seedlings infected by the same fungus were prepared. For cytochemical localization of enzyme activity, 4 mM β-glycerophosphate was used as a substrate and 25 mM Na fluoride or 150 μM Na molybdate as inhibitor controls. For indirect immunolabelling of enzyme antigen, a 1:100 dilution of partially purified serum was used as primary antibody and gold conjugate goat anti-rabbit IgG (GAR 15, Biocell) diluted 1:10 as secondary antibody.

## Results

### *Physiological properties*

The optimum conditions for fungal growth were a 4-week culture period, pH 6 and 20 ppm phosphorus, whilst those for acid phosphatase activity were a 2-week culture period, below pH 6 and 20 ppm phosphorus (Fig. 1), similar to those previously reported (Pearson and Read, 1975; Straker and Mitchell, 1986). Therefore, investigations were carried out on 2-week-old cultures growing in liquid medium at pH 6 with 20 ppm phosphorus. Over 80% of the enzyme activity measured under these conditions came from the wall fraction, whilst that of the cytoplasmic and excreted extracellular fractions was very low (Fig. 2). Activity was strongly inhibited (90%) by 5 mM Na fluoride and 2 μM Na molybdate, as for most acid phosphatases, and up to 17% by mannosidase and 7% by glucosidase at 100 $mU\ ml^{-1}$ (Fig. 3). The latter results indicate that the enzyme responsible is a glycoprotein with predominantly mannose groups, and that the glycosidic part of the protein is involved in the active enzyme function. Enzyme activity was enhanced (27%) in the presence of 60 $\mu g\ ml^{-1}$ concanavalin A (Fig. 4).

### *Biochemical properties*

Partially purified wall-bound proteins of *H. ericae* (LPA 2), separated by 10% PAGE and stained for acid phosphatase activity at pH 4.5, showed two isozyme bands at $R_f$ positions 0.02 and 0.29 (Fig. 6a). The latter disappeared at pH 9.6 and in the presence of fluoride or molybdate (Fig. 6b–d). The isozyme at $R_f$ 0.02, in contrast, was not completely inhibited at alkaline pH, indicating that the wall-bound acid phosphatase activity is determined by two isozymes with slightly different properties. When the two isozymes were separated by gel filtration and tested for activity, that corresponding to the $R_f$ 0.29 band (peak II) was twice as active as the other

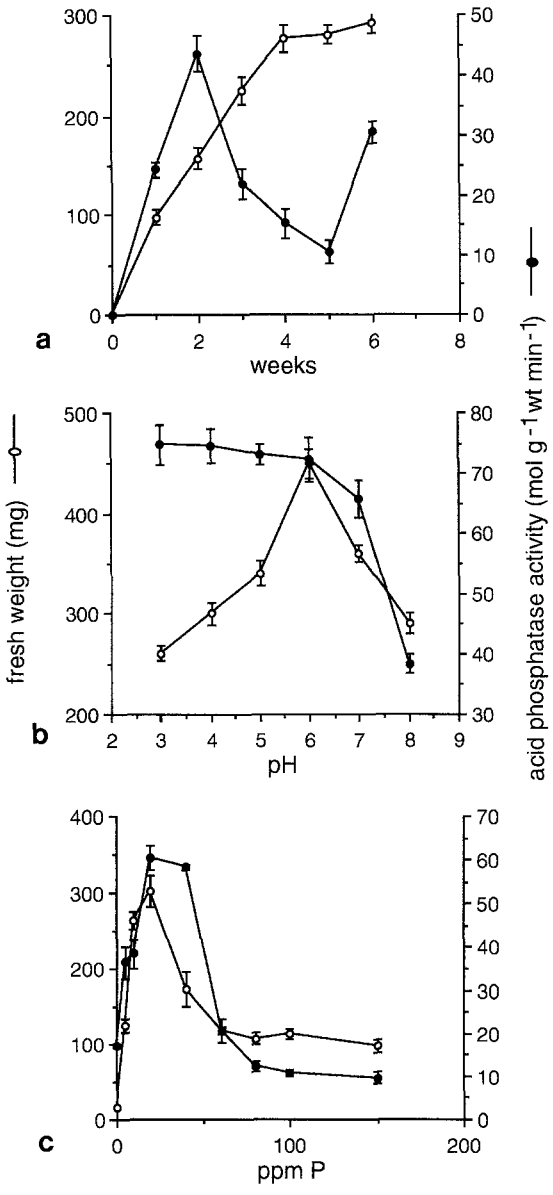



**Fig. 1a–c.** Effect of culture period (**a**), pH (**b**) and phosphorus level (**c**) on fresh weight (*open circles*) and acid phosphatase activity (*closed circles*) of *Hymenoscyphus ericae* (LPA 2). Each point represents the mean of six replicates. *Vertical bars* represent 95% confidence limits

isozyme (peak I). The molecular weight of the former was estimated to be about 78000 da (LMW isozyme) by SDS-PAGE, whilst that of $R_f$ 0.02 was higher [high-molecular-weight (HMW) isozyme].

Affinoblots gave positive reactions with concanavalin A at the $R_f$ corresponding to the two acid phosphatase isozymes (0.02 and 0.29) (Fig. 6e). Mannosidase but not glucosidase suppressed lectin binding (Fig. 6f, g), confirming the mannoprotein nature of the enzymes indicated by the physiological studies.

*Immunological properties*

Antibodies were raised against the purified major LMW wall-bound acid phosphatase isozyme ($R_f$ 0.29) of *H. ericae* (LPA 2). ELISA revealed that antiserum was most active at 35 days, and when diluted 1:100 detected antigen at 10 ng $ml^{-1}$. Immunoblotting showed that the polyclonal antibodies reacted positively with both isozyme bands (Fig. 6h). However, ELISA of the separate wall-bound isozymes after gel filtration gave a two-fold greater response for the LMW (peak II) as compared to the HMW (peak I) isozyme (Table 1). The antiserum gave no reaction with an unrelated commercial acid phosphatase (Sigma).

The antiserum also gave a very strong response with the extracellular enzyme (100%) but a weaker one with that from the cytoplasmic compartment (28%) (Table 1). Cross-reactions with other fungal strains occurred in ELISA; the response was very strong with *H. ericae* isolates (LPA 15, LPA 25, and LPA 42), lower with the unindentified strains LPA 16 and near zero with strains LPA 22, LPA 29 and those from *E. hispidula* and *E. mauritanica* (Table 2).

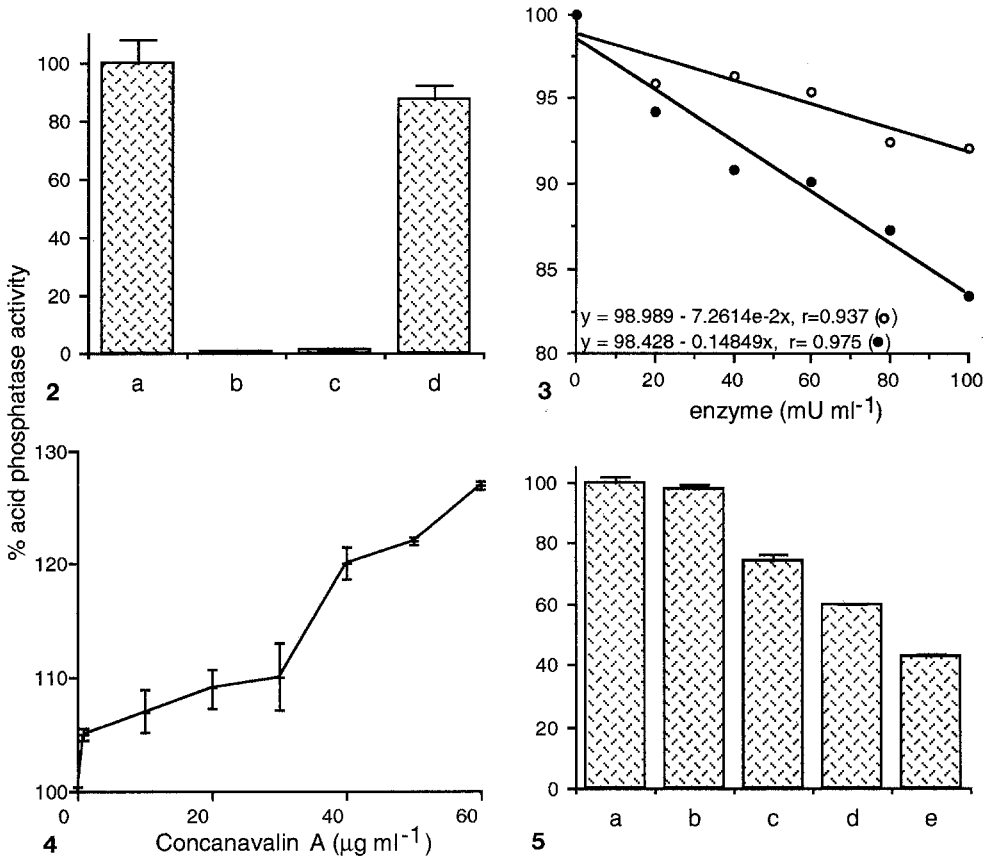
Incubation of crude extracts of mycelium of *H. ericae* (LPA 2) with the antiserum reduced the total acid phosphatase activity of the fungus up to 60% (Fig. 5).

*Cytochemical and immunogold localization of acid phosphatase*

Cytochemical localization of acid phosphatase activity in *H. ericae* (LPA 2) growing in a low-phosphate culture medium was similar to that described by Straker et al. (1989). Acid phosphatase activity was only associated with the surface of hyphae which had living cell contents (Fig. 7a), but it subsequently became detectable in the lumen of those with disorganized cell contents (not shown). The enzyme activity was inhibited by fluoride and molybdate (Fig. 7c, d). Immunolabelling with the antiserum to the LMW acid phosphatase was similarly associated with material covering the hyphal surface and also the fungal wall (Fig. 8a). Scattered labelling sometimes occurred in the living cytoplasm whilst enzyme antigens could be detected within disorganized cell contents in senescent hyphae (not shown).

The surface material of pure cultured mycelium of *H. ericae* (LPA 2) and associated acid phosphatase activity was greatly reduced with a high level of soluble phosphate in the medium (Fig. 7b). Intense immunogold labelling with LMW antiserum was, however, still associated with the fungal wall and occasional scattered gold grains occurred within the living cytoplasm (Fig. 8c). A similar density of gold particles was found with a low or high level of phosphorus in the medium, indicating that although enzyme activity changed antigen frequency did not differ.

Electron microscope observations of the mycorrhizal association showed that the outer wall of external hyphae of *H. ericae* (LPA 2) developed a typical organized fibrillar sheath in the presence of a host root (*V. corym-*




**Figs. 2–5.** Acid phosphatase activity of *H. ericae* (LPA 2). **Fig. 2.** Association with different fractions of cultures: *a* crude; *b* extracellular; *c* cytoplasmic; *d* wall. **Fig. 3.** Effect of glucosidase (*open circles*) and mannosidase (*closed circles*). **Fig. 4.** Effect of different concentrations of concanavalin A. **Fig. 5.** Effect of low-molecular-weight acid phosphatase antiserum: *a* without antiserum; *b* pre-immune serum 1:100; *c* antiserum 1:500; *d* antiserum 1:100; *e* antiserum 1:10. Each point represents the mean of six replicates. *Vertical bars* represent 95% confidence limits

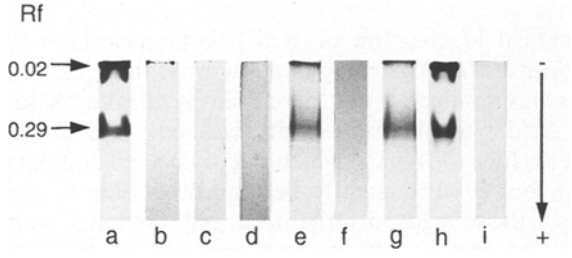



**Fig. 6.** Polyacrylamide gel electrophoresis of wall-bound acid phosphatase of *H. ericae*. *Lane a* pH 4.5 isozyme activities. *Lanes b–d* Effect of pH 9.6 (*b*), 20 mM fluoride (*c*) and 150 M molybdate (*d*). *Lanes e–g* Concanavalin A affinoblots vizualizing glycoprotein (*e*) and the effect of incubation with 100 mU $ml^{-1}$ α-mannosidase (*f*) or β-glucosidase (*g*). *Lanes h–i* Immunoblots with LMW acid phosphatase antiserum 1:100 (*h*) or with pre-immune antiserum 1:100 (*i*)

*bosum*) and that acid phosphatase activity became localized over the fibrils as hyphae grew close to the root surface (Fig. 9a). As in pure culture mycelium, this enzyme activity was inhibited by fluoride and molybdate (Fig. 9c, d). After hyphal penetration into living host cells, the fibrillar sheath of *H. ericae* disappeared and the acid phosphatase activity was not, or only weakly, detectable (Fig. 9a). Activation of fungal acid phosphatase also occurred in association with non-host tobacco roots, but this was not repressed when hyphae grew within the non-host cells (Fig. 9b). Phosphatase antigens were associated with the fungal wall and the fibrillar sheath of external hyphae (Fig. 10a). Although enzyme activity was greatly inhibited when hyphae grew within host cells, immunogold localization revealed the constant presence of enzyme antigen in the wall of these hyphae (Fig. 10), indicating that phosphatase activity rather than synthesis was affected. No significant immunolabelling was observed in any tissue with pre-immune antiserum (Figs. 8b, d, 10b).

## Discussion

Physiological and biochemical studies of the wall-bound acid phosphatase extracted from a European strain of *H. ericae* (LPA 2) and separated by PAGE have shown the presence of two isozymes (LMW and HMW), the activity of which is inhibited by molybdate, fluoride and alkaline pH. These results are similar to those reported for a South African fungus from *E. hispidula* (Straker et al. 1989). Furthermore, they confirm the glycoprotein nature of the isozymes (Straker et al. 1989), and indicate that mannose is the principle sugar associated with wall-bound acid phosphatase of ericoid endomycorrhizal fungi and is involved in the active properties of the enzyme. The cross-reaction of *H. ericae* (LPA 2) phosphatase antiserum with other isolates of *H. ericae* indicates that these isozymes are common features of this fungal species. In addition to their physiological significance, they may also be useful for identification of these fungi in mycorrhizal associations: the lack of serological relationships with other unidentified isolates suggests that these are not taxonomically closely related to *H. ericae*

**Table 1.** Enzyme-linked immunosorbent assay (ELISA) response with low-molecular-weight (LMW) acid phosphatase isozyme antiserum, corrected for pre-immune serum, of the two wall-bound, LMW and high-molecular-weight (HMW) isozymes, the cytoplasmic fraction and the extracellular enzyme of *Hymenoscyphus ericae* (LPA 2). Antibodies were diluted 1:100, protein was concentrated to 100 ng $ml^{-1}$ and each value is the mean of four replications. Values in columns followed by the same letter are not significantly different ($P=0.05$, Newman-Keuls test)

| Cellular fraction | ELISA response | |
|---|---|---|
| | % | OD (450 nm) |
| Wall-bound | | |
| peak I (HMW) | 44 | 0.734b |
| peak II (LMW) | 100 | 1.675a |
| Cytoplasmic | 28 | 0.469c |
| Extracellular | 100 | 1.659a |

**Table 2.** ELISA response with LMW acid phosphatase isozyme antiserum of *H. ericae* (LPA 2), corrected for pre-immune serum, against crude phosphatase extracts from other fungal strains. Antibodies were diluted 1:100, protein was concentrated to 100 ng $ml^{-1}$ and each value is the mean of four replications. Values in columns followed by the same letter are not significantly different ($P=0.005$, Newman-Keuls test)

| Strains | Response (%) | DO (450 nm) |
|---|---|---|
| Hea LPA 2 | 100 | 1.665a |
| Hea LPA 42 | 98 | 1.637a |
| Hea LPA 15 | 98 | 1.635a |
| Hea LPA 25 | 80 | 1.341b |
| LPA 16 | 34 | 0.567c |
| LPA 22 | 3 | 0.044e |
| LPA 29 | 5 | 0.090d |
| *E. hispidula* | 3 | 0.044e |
| *E. mauritanica* | 2 | 0.039e |

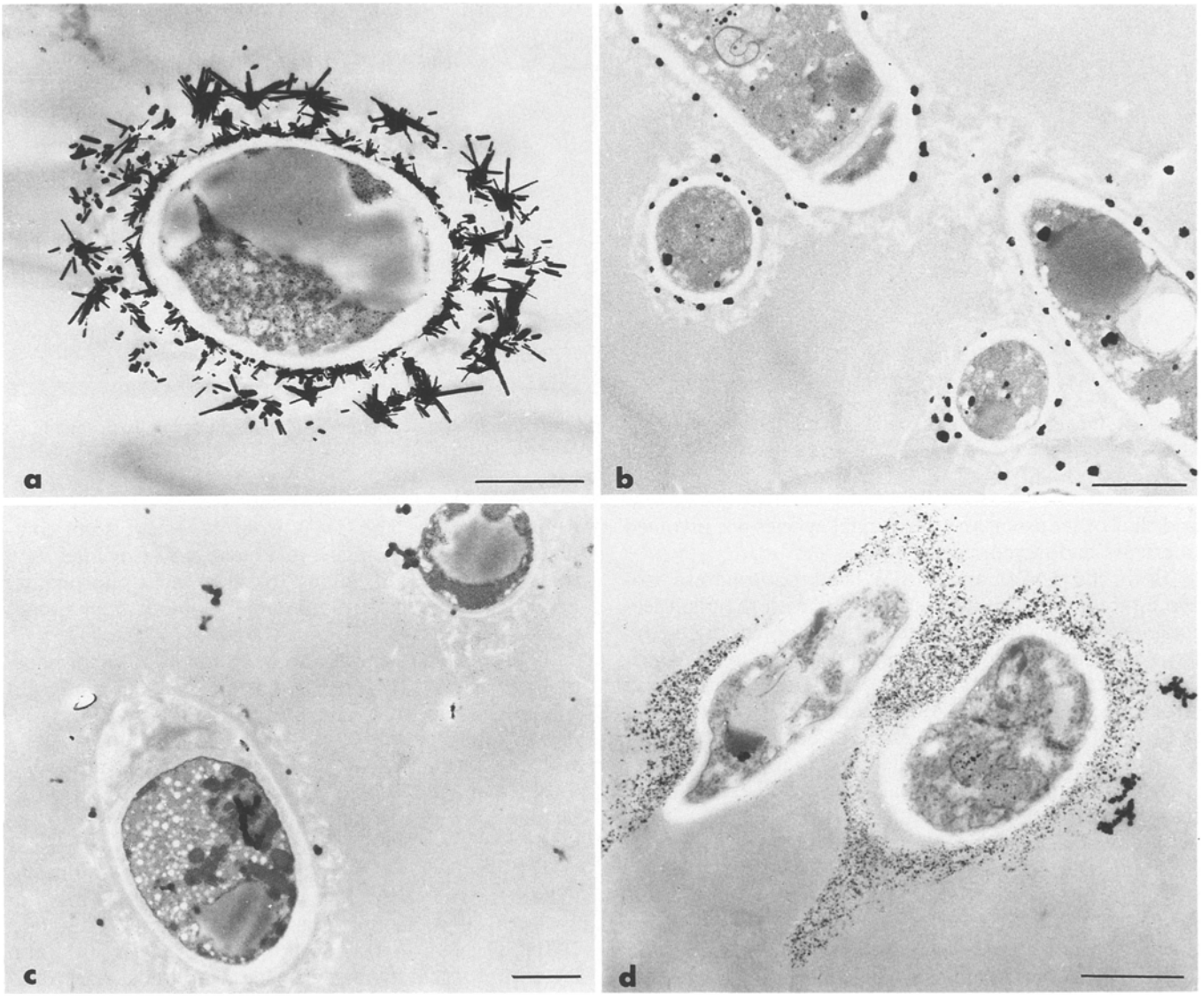



**Fig. 7a–d.** Cytochemical localization of acid phosphatase activity in pure culture mycelium of *H. ericae* (LPA 2) growing in low (**a**) or high (**b**) phosphate medium, and the effect of fluoride (**c**) and molybdate (**d**) on activity in low phosphate medium

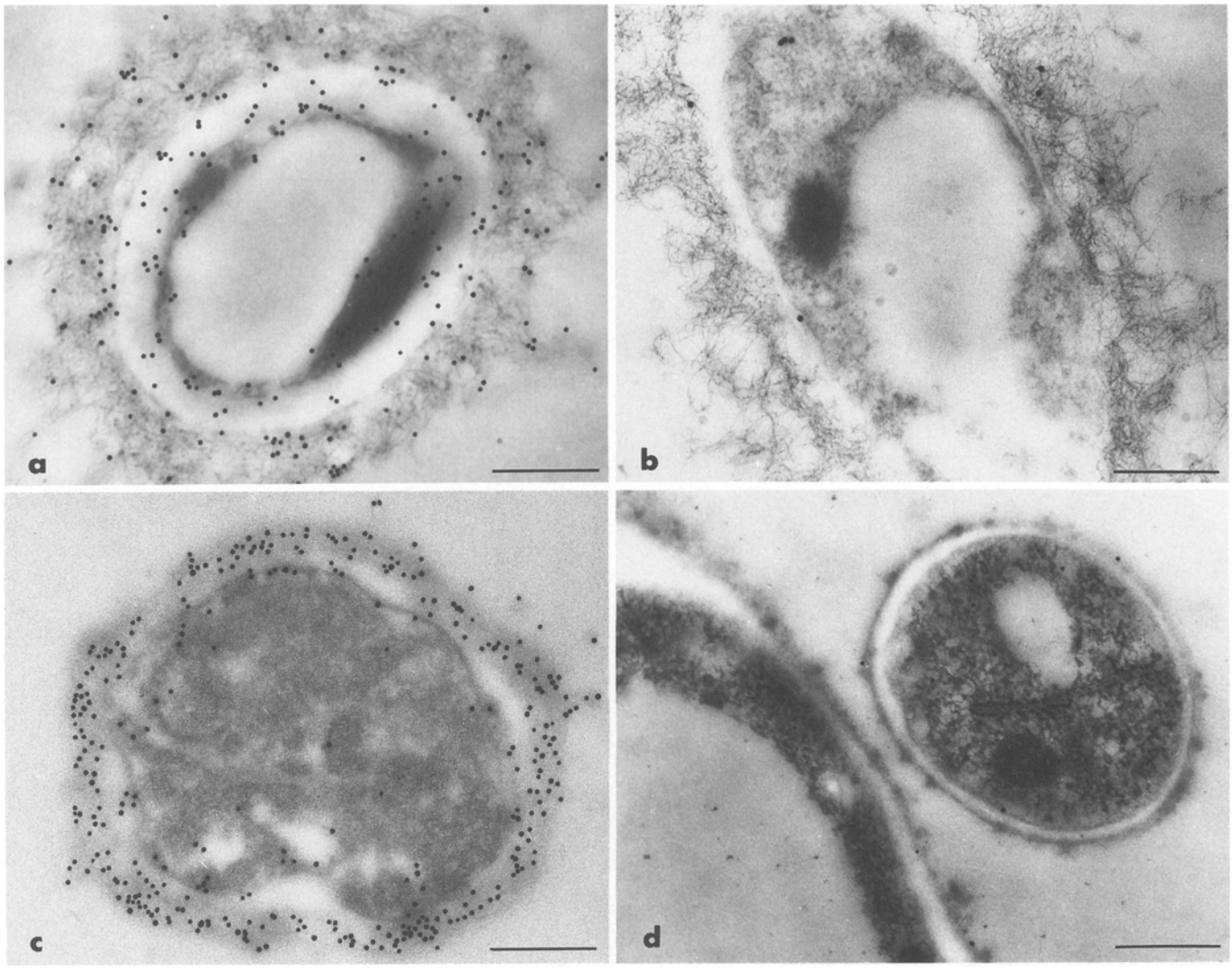



**Fig. 8a–d.** Immunogold localization of acid phosphatase in pure culture mycelium of *H. ericae* (LPA 2) growing in low (**a, b**) or high (**c, d**) phosphate medium. **a, c** 1:100 LMW phosphatase antiserum; **b, d** 1:100 pre-immune serum. *Scale bar* = 0.2 μm

and that other ascomycetous fungal species are involved in ericoid endomycorrhiza.

Both the LMW and HMW acid phosphatase isozymes are mannoproteins which react with the plant lectin concanavalin A. Although virtually no enzyme activity is associated with the extracellular fraction of *H. ericae* cultures, the LMW-specific antiserum gives a very strong response in ELISA, indicating that this isozyme is excreted into the culture medium but under an inactive form. The strong and identical immunological responses obtained with both wall-bound and extracellular fractions suggest that the LMW isozyme is predominantly associated with the external acid phosphatase activity at the hyphal surface. It could, therefore, be involved in phosphate release and/or host recognition at the root surface. The HMW isozyme is less active than the LMW isozyme. ELISA showed only a 40% response of the LMW antiserum with the HMW isozyme, suggesting that the latter is not excreted into the medium, since a mixture of the isozymes would have otherwise given less than a 100% response to ELISA in the extracellular fraction. The HMW isozyme, which is not completely inhibited at higher pH, may be associated with the cytosol fraction, giving the weak acid phosphatase activity and occasional immunolabelling of hyphal contents.

Cytochemical localization has shown that acid phosphatase activity in *H. ericae* is mainly associated with the wall surface of hyphae in pure culture and of external hyphae growing close to roots. Immunolabelling with the LMW fungal phosphatase isozyme antibodies localizes antigens with a similar distribution to the phosphatase activity, indicating that they correspond to the same enzyme. The fungal enzyme activity associated with external hyphae no doubt contributes to the enhanced surface phosphatase activity detected on endomycorrhizal roots (Gianinazzi-Pearson and Gianinazzi 1981). The fact that acid phosphatase activity is stimulated on external hyphae growing near a host root, inhibited on hyphae developing in living host cells but unaffected when fungi grow within non-host roots, suggests that the host plant somehow controls expression of the

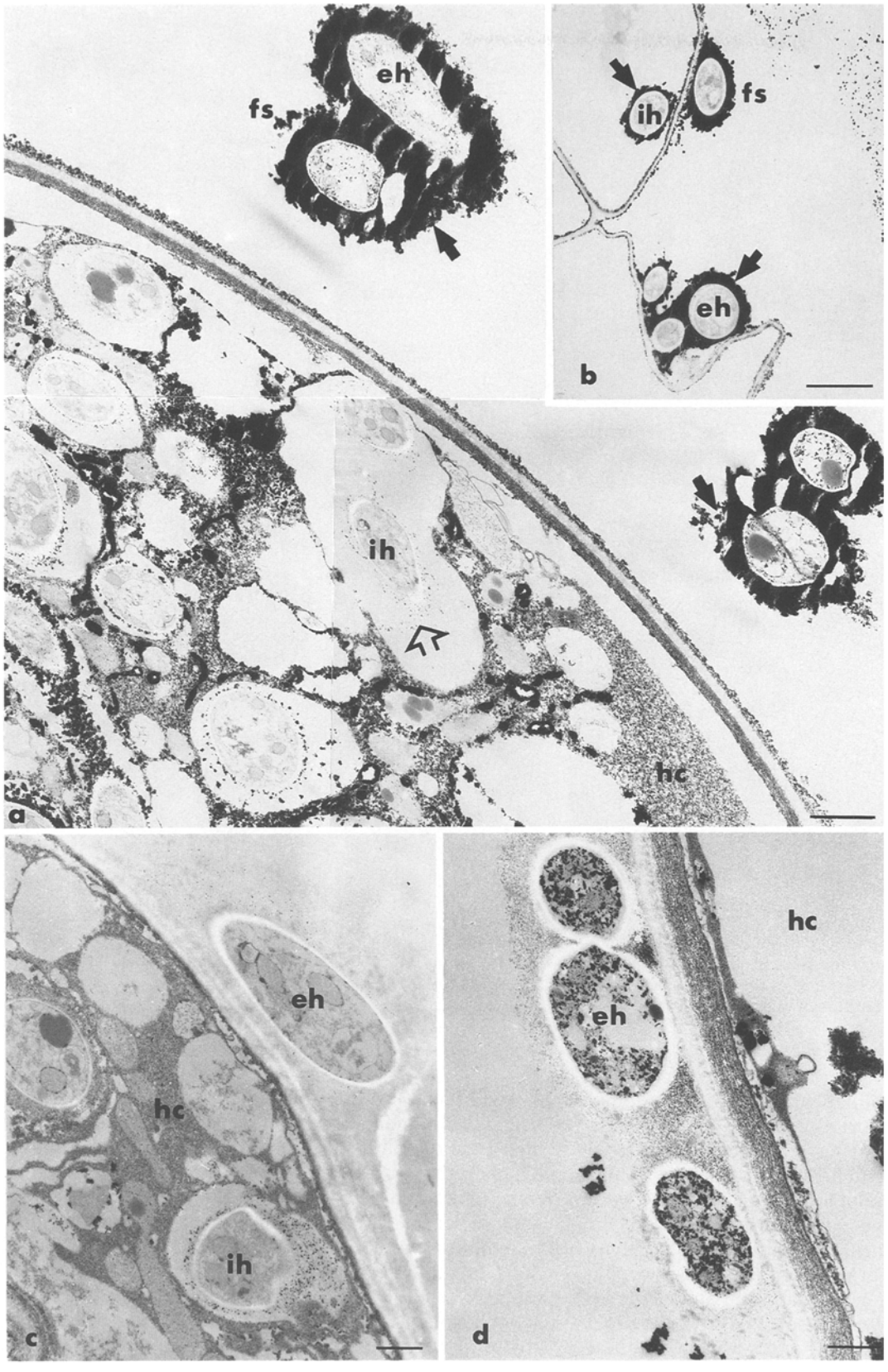



**Fig. 9.** Cytochemical localization of acid phosphatase activity (*closed arrows*) in mycelium of *H. ericae* (LPA 2) growing in association with roots of the host *V. corymbosum* (**a, c, d**) or non-host *N. tabacum* (**b**), and inhibition (*open arrows*) by fluoride (**c**) or molybdate (**d**). *eh*, External hyphae; *ih*, internal hyphae; *fs*, fibrillar sheath. **a, c, d** *Scale bar* = 1 μm, **b** = 2.5 μm

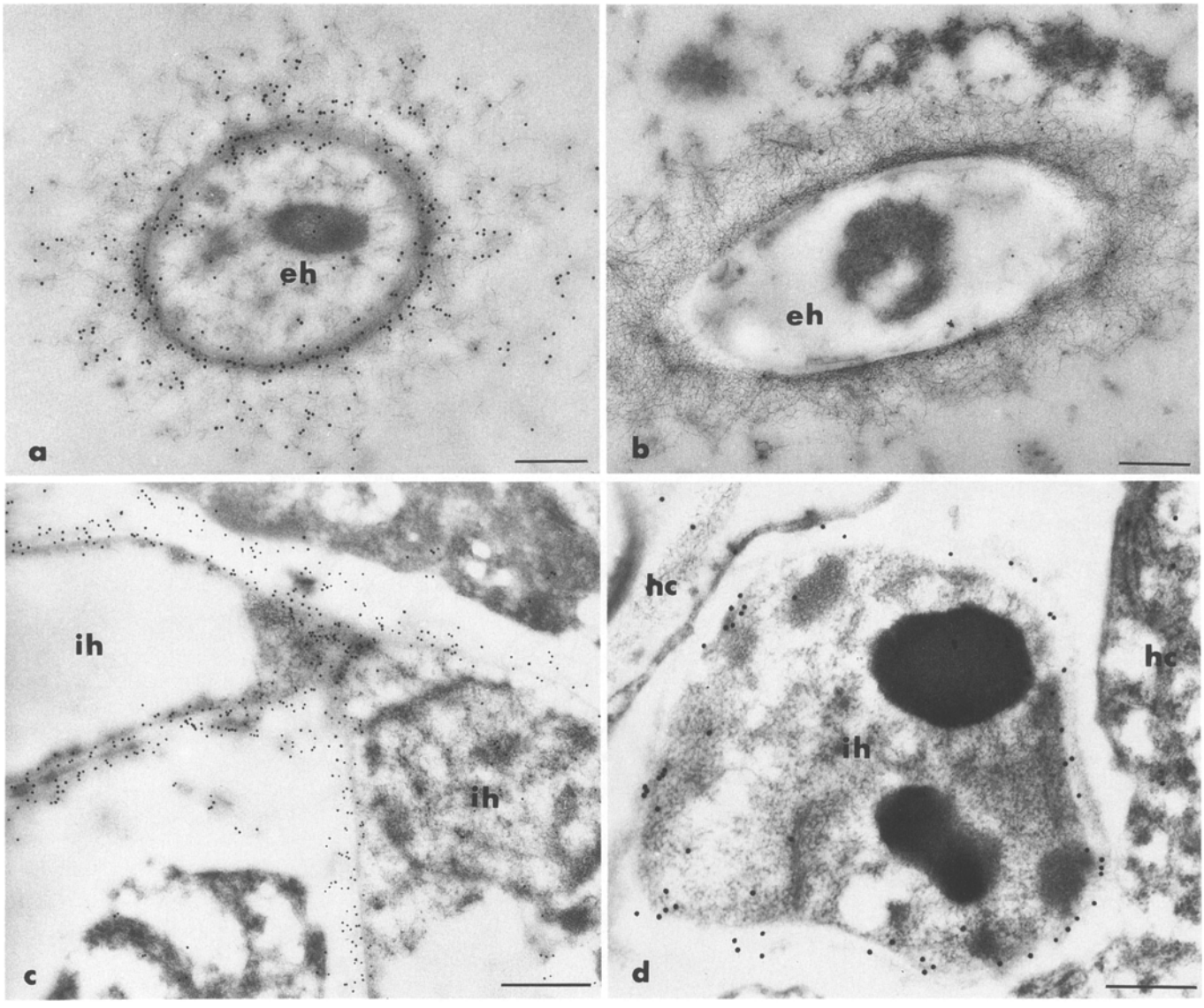



**Fig. 10a–d.** Immunogold localization of acid phosphatase in hyphae of *H. ericae* (LPA 2) growing close to the root surface (**a, b**) or in cells of *V. corymbosum* (**c, d**). **a, c, d** 1:100 LMW phosphatase antiserum; **b** 1:100 pre-immune serum. *eh*, External hyphae; *ih*, internal hyphae. *Scale bar* = 0.3 μm

enzyme protein during cellular interactions in endomycorrhizae. Bonfante-Fasolo et al. (1986, 1987) and Gianinazzi-Pearson et al. (1986) have previously suggested that host control over fungal metabolism may be important for cellular and physiological compatibility between host roots and fungi. This is particularly relevant for the specific repression of fungal acid phosphatase activity within host cells. Such hydrolytic enzyme activity would be incompatible with host cell metabolism and its persistence in non-host cells may be associated with the rapid disorganization observed during colonization of these tissues (Bonfante-Fasolo et al. 1984). The mechanisms regulating the fungal acid phosphatase activity in the mycorrhizal association are not known but these are presumably complex, and depend not only on plant factors but also soil properties (nutrient levels, pH).

Wall-bound acid phosphatase from *H. ericae* (LPA 2) is stimuated by a low level, and inhibited by a high level of soluble phosphate in the medium; thus as hyphae grow into the phosphate depletion zone at the root surface, enzyme activity could be enhanced. Alternatively, or in addition, the host plant may somehow affect the fungal acid phosphatase through lectin interactions so that enzyme activity is enhanced as the fungi grow close to the root. The binding of *H. ericae* (LPA 2) acid phosphatase to concanavalin A and the resulting increase in enzyme activity suggest a stabilization of the most active enzyme conformation by the lectin. Such enzyme-lectin interactions have also been reported by Ferens and Morawiecka (1985) for the acid phosphatase of rye germ. The physiological and biochemical properties of the ericoid fungal phosphatase (optimum activity at low pH and P) would make it particularly well adapted

to the typically poor, acid soils of heathlands where soluble forms of phosphate are very scarce. Since acid phosphatase of ericoid endomycorrhizal fungi can break down complex phosphate esters (Pearson and Read 1975; Straker and Mitchell 1986), stimulated enzyme activity could release phosphate from the less available complexed sources which predominate in heathland soils (Cosgrave 1967) and so increase the phosphorus assimilation of the host plant, thus conferring an important advantage in such ecosystems. In addition to improving phosphate availability to the host, the acid phosphatase of *H. ericae* may also be involved, as a wall-bound glycoprotein, in the cellular process of infection. Such a role has been suggested for acid phosphatase in host infection by *Candida albicans* (Tronchin et al. 1980). Furthermore, glycoproteins are believed to be involved in pistil-pollen recognition processes (Calzoni and Speranza 1989; Sharma and Shivanna 1983; Sharma et al. 1985).

Acid phosphatase activity declines on hyphae developing within living host cells but the enzyme molecule, as detected by immunogold localization of its antigen persists, i.e. phosphatase activity rather than synthesis in inhibited. The host factor(s) responsible for inhibition of the fungal phosphatase activity are not known, but may include the higher pH and phosphate concentration in the host cells, since these have a similar effect on enzyme activity and distribution in hyphae growing in pure culture. However, glycoproteins of eucaryotes which are rich in mannose residues (Faye et al. 1989), as is the LMW phosphatase of *H. ericae*, have a common precursor [polypeptide (N or O), (GlcNAc)2, (mannose 9)] modifications of which are determinate for different functions such as biological activity (Tsaftari et al. 1980), post-translational maturation (Fay and Chrispeels 1987), secretion and transport (Driouich et al. 1989), solubility and stability (Driouich et al. 1989; Prives and Olden 1980; Sly and Fischer 1982; Winkler and Segal 1984). In ericoid endomycorrhiza, the fact that immunoserological reactions of acid phosphatase antigen in the fungal wall are associated with the greatly reduced or inhibited enzyme activity of hyphae growing in host cells could be due to isozymes having conformational modifications, for example on the glycosidic part of the molecule which we have shown to be necessary for enzyme activity. Removal of mannose residues from the enzyme molecule or interference with glycosylation of a precursor by the host cell would expose the N-acetyl glucosamine moiety, so inactivating the enzyme and eventually giving the protein another function (Tsaftari et al. 1980). The possibility of modification of the enzyme on its glycosidic groups is supported by the observation of Bonfante et al. (1987) that the plant lectin concanavalin A, which detects mannose residues, binds with external hyphae but not internal hyphae, which only react with wheat germ agglutinin for *N*-acetyl glucosamine detection. If mannose removal from the enzyme molecule by the host is the mechanism involved in inactivation, it is interesting to note that mannose has been reported to be a particularly effective agent for the sequestration of cytoplasmic orthophosphate (Sheu-Hwa et al. 1975). In this context, such an enzyme control mechanism could be important in the phosphate physiology of the ericoid endomycorrhiza symbiosis, particularly in mechanisms of secretion and transport related to phosphate mobilization to the plant.

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