SHORT COMMUNICATION

Factors Affecting Phosphate Uptake in the Marine Fungus *Dermocystidium* sp.

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INTRODUCTION

The basis for the obligately marine nature of certain lower Phycomycetes remains unclear. However, the littoral biflagellate fungus, *Thraustochytrium roseum*, has a specific Na⁺ requirement for phosphate uptake (Siegenthaler, Belsky & Goldstein, 1967*a*). We report below comparable studies on a related primitive marine fungus, *Dermocystidium* sp. (Goldstein & Moriber, 1966).

METHODS

The fungus used in this study was *Dermocystidium* sp. (Vishniac isolate WH49), a non-filamentous, obligately marine phycomycete. The methods were essentially those used in earlier studies of phosphate uptake by this organism (Siegenthaler, Belsky, Goldstein & Menna, 1967*b*). At the conclusion of the incubation period, organisms were removed from incubation mixtures and a portion of the supernatant was analysed for uptake of glucose, inorganic phosphate or glutamate. The glutamate assay was carried out using glutamate dehydrogenase (2 units, Sigma type 1), nicotinamide adenine dinucleotide (NAD), 0.05 M-potassium phosphate buffer (pH 8.5). Initial rates of NAD reduction were calculated from extinction measurements at 340 nm.

Disappearance of inorganic phosphate in nitrogen and in air was measured using organisms starved for 3 days in phosphate-free medium. Air was bubbled for 6 h. through 70 ml. of a suspension of organisms in 0.01 M-tris-0.4 M-sodium chloride-1 mM-potassium dihydrogen phosphate (pH 7.8) mixture in a 200 ml. flask. Nitrogen was bubbled for 4 h. through a duplicate suspension and a sample was removed for measuring oxygen consumption. The remaining organisms in the flask were then aerated for 2 h. Samples for phosphate analyses were removed periodically from both flasks.

Glucose uptake in the presence of various solutes was measured colorimetrically by the method of Nelson (1944). Since arsenate interfered with the colorimetric determination of phosphate, disappearance of ³²P from the supernatant of incubation mixtures with and without arsenate was measured with a gas-flow planchet counting system. Preliminary experiments had established a linear relationship between concentration of fungus and protein at the levels of cellular material used.

RESULTS

Figure 1 shows that phosphate taken up was proportional to NaCl concentration whereas KCl was ineffective. Oxygen consumption was stimulated approximately 50% by 0.4M-NaCl. Considerable glucose (Fig. 2) and glutamate utilization (Table 1) occurred without sodium chloride. There was less oxygen uptake with a mixture of 0.2M-sodium chloride and 0.2M-potassium chloride than with 0.2M-sodium chloride alone. With 0.5 to 1.0mM-potassium dihydrogen phosphate the rate of phosphate disappearance was independent of phosphate concentration. Phosphate below a concentration of 0.1mM disappeared too rapidly for determination of initial uptake

Fig. 1

Fig. 2

Fig. 1. Effect of NaCl on phosphate uptake by *Dermocystidium* sp. (a) ●, no additions; ○, 0.1M-NaCl; ▲, 0.2M-NaCl; ■, 0.4M-NaCl. (b) ●, no additions; ▲, 0.1M-NaCl+0.3M-KCl; ○, 0.3M-NaCl+0.1M-KCl; ■, 0.4M-NaCl. Fungus was grown for 5 days and starved 3 days in phosphate-free growth medium. Basic reaction mixture: tris (pH 7.3); and thalli, 1.3 mg. protein/ml. 1 μmole inorganic phosphate ≡ Δ extinction of 0.37 at 660 nm.

Fig. 2. Effect of additions to incubation medium on glucose uptake by *Dermocystidium* sp. ●, no additions; △, 0.4M-mannitol; ▲, 0.4M-sucrose; ○, 0.2M-NaCl; □, 0.2M-KCl. Fungus was grown for 5 days in complete medium, centrifuged and resuspended in minimal reaction mixture containing 0.05% glucose, 0.1M-tris (pH 8.0), and additions. The concentration of fungus was equivalent to 2.9 mg. protein/ml.

Table 1. Influence of sodium ion on glutamate uptake by suspensions of *Dermocystidium* sp.

Additions	Moles/l.	Glutamate uptake (μmoles/5 h.)
None	—	0.21
Sodium chloride	0.2	0.36
Potassium chloride	0.2	0.00
Sucrose	0.4	0.06

The incubation mixture contained tris-HCl (0.1M, pH 8.0), L-glutamic acid (10^{-2} M), and fungal thalli (2.4 mg. protein/ml.)

rates. Arsenate was a potent inhibitor of phosphate uptake; the degree of inhibition depended on the ratio of arsenate to phosphate. Azide and phenylmercuric acetate abolished phosphate uptake. Indeed, phenylmercuric acetate caused leakage of phosphate from the organism. An initial inhibition of phosphate uptake by 2,4-dinitrophenol disappeared after 4 h. A 20% increase in oxygen consumption and a marked inhibition of phosphate uptake occurred with 0.2 mM-2,4-dinitrophenol. In *Thraustochytrium roseum* (Siegenthaler *et al.* 1967*b*), puromycin abolished the lag period associated with phosphate uptake. In *Dermocystidium* sp., phosphate uptake was enhanced but to a lesser extent than in *T. roseum*.

Fig. 3. Phosphate uptake by *Dermocystidium* sp. in a nitrogen atmosphere and in air. ■ Indicates nitrogen for 4 h. followed by introduction of air; ●, air only. The fungus was grown for 5 days and starved 3 days in phosphate-free medium. The reaction mixture contained 0.1 M-tris (pH 8.0), 0.4 M-NaCl, 1.0 mM-KH₂PO₄, and fungus equivalent to a concentration of 5.0 mg. protein/ml. (■) and 4.8 mg. protein/ml. (●), 1 μmole inorganic phosphate ≡ Δ extinction 660 nm. of 0.37.

No significant phosphate uptake occurred under nitrogen; this inhibition was completely reversed by aeration (Fig. 3). Glucose (a component of the growth medium) stimulated phosphate and oxygen uptake whereas a variety of Krebs cycle intermediates stimulated phosphate uptake alone.

DISCUSSION

That Na⁺ is required absolutely for phosphate uptake but not for oxygen, glucose or glutamate metabolism implies direct participation of Na⁺ in phosphate transport; Na⁺ stimulation of other phenomena may be indirect responses to Na⁺-dependent reactions. As with *Thraustochytrium roseum*, the obligately marine nature of ocean-inhabiting lower phycomycetes is related to a need for Na⁺ for phosphate transport at the concentration of Na⁺ in littoral waters. Goldstein, Belsky & Chosak (1969) showed that sucrose or other osmotically active substances permitted optimal growth with as little as 0.1% sodium chloride compared with 2.4% needed in their absence. No growth occurred without sodium chloride. Shapiro (1967) reported that mixed cultures of diverse micro-organisms release a large part of their inorganic phosphorus

into the medium in anoxic conditions. *Dermocystidium* sp. did not; neither phosphate release nor uptake occurred under nitrogen. Uptake could subsequently be induced by aeration, indicating that neither viability nor the phosphate transport system was permanently impaired by anoxia. These data and the effects of the electron-transport inhibitors phenylmercuric acetate and azide support the hypothesis that phosphate uptake in *Dermocystidium* sp. is an active, energy-requiring process. Shapiro's results imply that phosphate retention is also energy-dependent, but this does not apply to *Dermocystidium*. His use of mixed cultures hampers interpretation since one particular strain in the mixture of micro-organisms could account for the phosphate liberation.

The need for concentrations of inhibitors greater than those which elicit comparable effects on isolated mitochondria probably results from our use of intact organisms (Siegenthaler *et al.* 1967*b*). At effective concentrations these metabolic inhibitors affect respiration and transport in *Dermocystidium* sp. as they do in plant and animal mitochondria. A specific function of Na⁺ in physiological systems is emerging. Christensen, Liang & Archer (1967) have reported a distinct Na⁺ requirement for amino acid transport in Ehrlich ascites cells, and Shah & Wedding (1968) have shown that 1.0 mM-sodium chloride stimulates threefold phosphate esterification at one of the phosphorylation sites associated with succinate oxidation by turnip root mitochondria. Their findings are consistent with the observation that Na⁺ is required for dinitrophenol stimulation of mitochondrial respiration (Siegenthaler *et al.* 1967*b*). Both reports support the hypothesis that Na⁺ regulates the formation of a non-phosphorylated high-energy intermediate during oxidative phosphorylation. We have not studied mitochondria from *Dermocystidium* sp. because they have proved difficult to isolate.

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