Chloride Uptake by Mycelium of *Neocosmospora vasinfecta* and Its Inhibition by Glucose

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Studies of the transport of inorganic ions by fungi have lagged behind those of most other groups of organisms. In the yeast *Saccharomyces cerevisiae*, which is the exception to this statement, most attention has been focused on cation transport: absorption of PO_4^{3-} and SO_4^{2-} has been examined, but the organism 'behaves as though impermeable to Cl^- ' (Rothstein, 1965; cf. Conway & Downey, 1950). However, during K^+ uptake by the mycelial ascomycete *Neocosmospora vasinfecta* net Cl^- absorption also takes place, though not at the same rate (Budd, 1969*a*). This report confirms the absorption of Cl^- in this organism and describes the effect of extracellular glucose on this process.

METHODS

Mycelium of *Neocosmospora vasinfecta* E. F. Smith (ATCC 11681; American Type Culture Collection, Rockville, Maryland, U.S.A.) was grown in submerged culture in the G1 medium of Budd (1969*b*) which contained only contaminant amounts of Cl^- . It was harvested by filtration, washed thoroughly in demineralized water, preincubated in aerated, demineralized water for 3 to 4 h. then filtered and resuspended, at a density of approximately 150 mg. dry wt./l., for 5 h. in aerated 2 mM-K hydrogen phthalate buffer (pH 5.5) alone or with 1 mM-KCl. Chloride absorption was then followed using ^{36}Cl (0.01 mCi/m-equiv. Cl). Successive 100 ml. samples from the suspension were rapidly filtered with suction through Whatman no. 54 paper, washed with demineralized water, and the mycelium extracted for 24 h. at room temperature with 1 N- HNO_3 . A portion of the diluted extract was then counted.

To determine the chemical form of absorbed ^{36}Cl , the mycelium was quickly frozen to -18° after exposure to the isotope, then thawed and refrozen several times and finally extracted in demineralized water. A portion of the extract was counted and the remainder applied to tandem 5 ml. beds of Dowex 50 W (H^+) and Dowex 1 (formate) exchange resins, and followed by several bed vol. of water. The cation exchanger was then eluted with 4 N- NH_4OH : the anion exchanger was eluted successively with 4 N-formic acid and 1 N-HCl. The eluates were taken to small volume *in vacuo* and portions counted.

Na^{36}Cl was purchased from Amersham-Searle Corporation (Canada) and counted with a Packard Tri-Carb model 3001 liquid scintillation spectrometer, using the second solution of Bruno & Christian (1961) as liquid fluor. All chemicals were of analytical grade.

RESULTS

The Cl^- content of freshly harvested mycelium was too small for accurate determination by coulometric titration, but did not exceed 0.5 $\mu\text{equiv.}/100$ mg. dry weight. Freshly harvested mycelium absorbed Cl^- slowly (cf. Budd, 1969*a*), but after several hours aeration in water or buffer at pH 5.5 rapid Cl^- uptake took place on transfer to KCl solutions (Fig. 1).

In other experiments equilibrium with external KCl at 0.1 mM was not attained within 8 h. Loading the mycelium with unlabelled KCl during part of the preincubation period immediately decreased the rate of $^{36}\text{Cl}^-$ uptake (Fig. 1*a*). Virtually all of the isotope recovered from frozen and thawed mycelium was in the form of Cl^- ion, as judged by its binding only to anion exchanger and release by HCl but not by formic acid. Since 100 mg. mycelial dry matter would have contained approximately 0.25 ml. intracellular water, the intracellular Cl^- concentration after 3.5 h (Fig. 1*a*) exceeded the external concentration by 600- to 700-fold.

Fig. 1 (*a*). The effect of glucose on the uptake of chloride by mycelium pretreated in the absence or presence of KCl. After preincubation and pretreatment in 2 mM-K phthalate buffer (pH 5.5) or 2 mM-K phthalate buffer *plus* 1 mM unlabelled KCl, the mycelium was filtered and resuspended in either 0.1 mM-K ^{36}Cl or 0.1 mM-K ^{36}Cl *plus* 0.5% (w/v) glucose. ●, Mycelium pretreated in the absence of KCl and then incubated in K ^{36}Cl ; ○, mycelium pretreated in KCl and then incubated in K ^{36}Cl ; ▲, mycelium pretreated in the absence of KCl and then incubated in K ^{36}Cl *plus* glucose; △, mycelium pretreated in KCl and then incubated in K ^{36}Cl *plus* glucose.

(*b*). The effect of glucose on the outflow of chloride. After harvesting and 3.5 h. aeration in water, mycelium was incubated in 1 mM-K ^{36}Cl for 2 h. at 25°. Replicate samples were then filtered, washed by resuspension in water, filtered and resuspended in one of the following solutions: demineralized water (●), 1 mM-KCl (▲), 0.5% (w/v) glucose (○) and 0.5% (w/v) glucose *plus* 1 mM-KCl (△). The mycelium at the beginning of the outflow period contained 15 μequiv. Cl^- /100 mg. dry wt.

When glucose was in the uptake medium, $^{36}\text{Cl}^-$ uptake immediately decreased, whether or not the mycelium was loaded with unlabelled Cl^- during preincubation. In Fig. 1*a*, comparison of the third curve with the first curve, and the bottom curve with the second curve, shows that over the first 30 min. glucose decreased net $^{36}\text{Cl}^-$ uptake by approximately 60% in both the loaded and non-loaded mycelia. Mannitol at the same concentration (0.5%, w/v) as glucose in Fig. 1*a* had no effect on Cl^- uptake for at least 90 min. Fructose, however, acted like glucose.

In other experiments similar to that of Fig. 1*a*, uptake of $^{36}\text{Cl}^-$ by non-loaded mycelium was reversed after 1 h. by the addition of 0.5% (w/v) glucose. The retention of absorbed $^{36}\text{Cl}^-$ by the mycelium was therefore examined (Fig. 1*b*). Negligible $^{36}\text{Cl}^-$ outflow took place to water only, and only about 25% to 1 mM unlabelled KCl after 5 h. Glucose at 0.5% (w/v),

in the presence or absence of 1 mM unlabelled KCl, promoted rapid $^{36}\text{Cl}^-$ loss, which was almost quantitative after 5 h. Mannitol at 0.5% (w/v) had no effect in a parallel experiment (not shown).

DISCUSSION

The negligible Cl^- content of freshly harvested mycelium in these experiments ensures that $^{36}\text{Cl}^-$ uptake is net and does not include significant isotopic exchange. Chloride is evidently accumulated from dilute KCl and, assuming an internal negative membrane potential at the mycelial surface (cf. Slayman, 1965*a, b*), this represents active transport. The decrease in Cl^- uptake by glucose, which is the carbon source used for growth, is, therefore, unexpected. Glucose could inhibit net Cl^- uptake either by decreasing Cl^- influx or by promoting Cl^- outflow. That Cl^- influx is decreased is indicated by the observation that glucose inhibits $^{36}\text{Cl}^-$ uptake over the first 30 min. to the same extent in mycelium loaded with unlabelled Cl^- as in mycelium initially almost devoid of Cl^- . If glucose acted only by causing outflow of Cl^- ions after their entry, the isotopic dilution of entering $^{36}\text{Cl}^-$ in the loaded mycelium should slow the loss of isotope as compared to non-loaded controls. The apparent glucose inhibition would therefore be smaller in loaded mycelium. This is not observed.

The results of Fig. 1*a* do not exclude an influence of glucose on Cl^- outflow, and Fig. 1*b* shows that this is promoted in the presence of glucose. Inhibition of net Cl^- uptake by glucose is therefore the sum of its effects on entry and outflow. How these effects are mediated is not known, but they cannot be simply an osmotic effect, since mannitol at the same osmolarity as glucose has no influence on Cl^- entry or outflow for at least 90 min. The inhibition of net Cl^- uptake might be due to the sugar molecule itself or to some metabolite.

The phenomenon of inhibition of halide uptake by glucose could be a general one but to the authors' knowledge it has previously been reported only for *Chlorella* (Paschinger & Broda, 1967).

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