Vanadate, an inhibitor of growth, development and endocytosis in

*Dictyostelium discoideum* amoebae

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Summary

Axenic growth of amoebae of the slime mold *Dictyostelium discoideum* was found to be reversibly inhibited by vanadate. Pinocytosis, when measured with fluorescently-labeled dextran as a fluorescent fluid-phase marker was strongly inhibited by vanadate. Inhibition was observable at vanadate concentrations as low as 0.2 mM. Sucrose entry through pinocytosis induced massive cell vacuolation and this effect was blocked by vanadate. Secretion of soluble lysosomal enzymes is another aspect of membrane traffic in *Dictyostelium*. Secretion of two typical lysosomal enzymes, acid phosphatase and hexosaminidase, was inhibited by concentrations of vanadate in the same range as for pinocytosis inhibition. Vanadate also prevented the morphogenetic developmental program that follows nutrient starvation. In contrast, vanadate did not prevent heat-induced spore germination. Vanadate had no significant action on the intracellular nucleoside triphosphate level or on the cytosolic pH. It is suggested that the particular effect of vanadate in *Dictyostelium* is to inhibit the fusion of endosomes with lysosomes. Our results provide a probe that could be useful to clarify the mechanisms of endocytosis.

Key words: *Dictyostelium* amoebae, vanadate, endocytosis, multicellular development, spore germination.

Introduction

Vanadate has been used in a variety of studies as a probe of biochemical functions. All ATPases having a phosphorylated intermediate and several other enzymes such as phosphatases are inhibited by vanadate (Chasteen, 1983; Boyd & Kustin, 1984; Erdmann et al. 1984; Nechay, 1984; Nechay et al. 1986). Another target for vanadate is the membrane P, carrier, through which vanadate is most likely to enter cells; resistance to the toxic action of vanadate has been used to screen for mutants defective in phosphate transport systems (Bowman, 1983; Bowman et al. 1983). One interesting biological action of vanadate is its ability to mimic hormones or growth factors (Nechay et al. 1986).

The cellular slime mold *Dictyostelium discoideum* (Loomis, 1975) is a well-investigated system for the study of endocytosis. Axenic strains show a very high fluid-phase pinocytosis capacity by which they fulfill all their growth requirements. Axenic strains secrete a major portion of their lysosomal hydrolases into the extracellular medium. This process is subjected to specific developmental and environmental controls (Ashworth & Quance, 1972; Loomis, 1975; Crean & Rossmendo, 1979; Dimond et al. 1981). In this paper we report the biological effects of vanadate on the life cycle of *Dictyostelium*. We found that vanadate inhibits vegetative growth and fruiting body formation, but not heat-induced spore germination. Particular emphasis was placed upon two aspects of the endo-/exo cytosis pathways in vegetative cells: fluid-phase pinocytosis and secretion of lysosomal enzymes. Both phenomena are inhibited by vanadate.

Materials and methods

Axenic culture conditions

*Dictyostelium discoideum*, axenic strain AX2 (ATCC 24397) was grown at 22°C in a complex medium containing 18 g l⁻¹ maltose and 0.25 g l⁻¹ dihydrostreptomycin (Watts & Ashworth, 1970). Cell numbers were determined with a Coulter Z4 counter. Cells in their logarithmic phase of growth were used in all the experiments.

Differentiation program

Development was followed on filters as described by Sussman (1987). Amoebae (2×10⁷ to 5×10⁷ cells) were washed twice in...
ice-cold 20 mM-KCl, 55 mM-NaCl, 2.5 mM-MgCl2, 0.5 mg ml⁻¹ streptomycin sulfate, pH 6.4, and distributed uniformly on the surface of black Millipore filters of 25 mm diameter. The filters were supported by pads soaked in the above solution containing 0 to 5 mM-vanadate. Filters were examined and scored after 24 and 48 h of incubation at 22°C.

**Spore germination**

*Dictyostelium discoideum*, wild-type strain NC4H (ATCC B/r (Cotter & Raper, 1966). Mature spores of *Escherichia coli* and 48 h of incubation at 22°C. 0 to 5 mM-vanadate. Filters were examined and scored after 24 h. Germination was then followed at 22°C by microscopic examination and counting at regular time intervals (Cotter, 1981; Klein et al. 1988a). Vanadate at concentrations up to 5 mM was added either before or after the heat shock.

**Measurement of fluid-phase pinocytosis**

Pinocytosis was measured with FITC-dextran as a fluid-phase marker as described (Thilo & Vogel, 1980; Klein & Satre, 1986; Klein et al. 1988a). Briefly, *Dictyostelium* amoebae at a concentration of 5 x 10⁶ cells ml⁻¹ were incubated aerobically at 22°C in either axenic medium (Watts & Ashworth, 1970) or 40 mM-Mes-Na buffer, pH 5.3, both containing 2 mg ml⁻¹ FITC-dextran. Samples (1 ml) were taken as a function of time and quenched in 10 ml of ice-cold 20 mM-potassium phosphate (KP) buffer, pH 6.5, containing 0.05 % bovine serum albumin (KP-BSA buffer). Amoebae were collected by centrifugation and washed twice with KP-BSA buffer. Cells were then resuspended in 1 ml 20 mM-KP buffer, pH 6.5, and cell number determined on 50 μl samples. The remaining sample (0.95 ml) was supplemented with 2 ml of 100 mM-NaHPO₄, 0.25% (w/v) Triton X-100. The fluorescence intensity was measured on a Hitachi F-2000 fluorometer (excitation wavelength 470 nm and emission wavelength 520 nm). The amount of pinocytosed FITC-dextran was obtained from a standard fluorescence curve in the same medium. Uptake rates were expressed as endocytic indexes, defined as the volume of incubation medium whose content had been captured by a given number of cells in a given time (μl/10⁶ cells min⁻¹). In view of the reported presence of low molecular weight and reactive contaminants in some commercial FITC-dextrans (Preston et al. 1987), it was determined that identical pinocytosis parameters were obtained with commercial FITC-dextran (Sigma FD-70), FITC-dextran or dextran conjugated with [¹⁴C]phosphatidylcholine, the latter two synthesized in the laboratory by the method of De Belder & Granath (1973) as well as with [³⁵S]dextran (Klein & Satre, 1986). Furthermore, FITC-dextran (2 mg ml⁻¹) was found completely non-toxic towards growth of *Dictyostelium* amoebae in axenic medium or during the starvation-induced differentiation program.

**Secretion of lysosomal soluble enzymes**

Amoebae were collected by centrifugation at 800 g for 4 min and washed twice in ice-cold 20 mM-Mes-Na, pH 6.3. The cells were suspended at 1 x 10⁶ cells ml⁻¹ in 0.1 M-sucrose, 20 mM-Mes-Na buffer, pH 6.3, or in the same medium containing 1 mM-vanadate. Incubation was performed at 22°C and at indicated times, two 1-ml samples were taken for each experimental condition. The first sample was directly supplemented with 20 μl 10 % (v/v) Triton X-100 to give the total activity and the second sample was centrifuged for 1 min in an Eppendorf centrifuge. The supernatant from the second sample containing released activity was collected and supplemented with 20 μl of 10 % (v/v) Triton X-100. These samples were stored on ice and used to measure total and extracellular hexosaminidase and acid phosphatase activities.

**Enzymatic assays**

Hexosaminidase and acid phosphatase activities were assayed at 25°C as described by Wiener & Ashworth (1970), except for the replacement of acetate buffer by 0.1 M-glycine-HCl buffer, pH 5.5, in the case of hexosaminidase. Acid phosphatase was assayed in the presence of 5 mM-EGTA (see below, and Table 1).

**n.m.r. spectroscopy**

³¹P n.m.r. spectra were collected in the pulsed Fourier transform mode at a frequency of 81.01 MHz in a Bruker WM200 WB spectrometer using a 25 mm n.m.r. tube. Experimental conditions were as described (Martin et al. 1987). Chemical shifts were given with respect to orthophosphoric acid at 0 p.p.m. (parts per million), using a capillary containing 50 mM-sodium methylene diphosphonate, pH 8.4, as a standard at +16.4 p.p.m. All spectra shown were plotted with an artificial line-broadening of 15 Hz.

**Reagents**

Sodium monovanadate was obtained from Prolabo (Paris, France) and 4-nitrophenyl phosphate and 4-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside were from Boehringer Mannheim (Grenoble, France). Fluorescein isothiocyanate-labeled dextran (FITC-dextran, average M₉: 70 000, catalog ref. FD-70, lots refs 48F 0067 and 97F 0599), EDTA, EGTA, TTHA, 1, 2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA) were obtained from Sigma (Lyon, France). Tris(hydroxymethyl)aminomethane-N,N,N',N''-hexaethacetic acid (TTHA) was from Fluka (Buchs, Switzerland).

**Results**

Vanadate is an inhibitor of *Dictyostelium* growth

The effect of various concentrations of vanadate from 0 to 5 mM on the growth of *Dictyostelium* amoebae in axenic medium is illustrated in Fig. 1A. Control cultures grew at 22°C with a doubling time of about 8 h and reached a maximal cell density of 2 x 10⁷ to 3 x 10⁷ cells ml⁻¹. Inhibi-

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**Table 1. Reversion of the vanadate inhibitory effect on acid phosphatase enzymatic activity by chelating agents**

<table>
<thead>
<tr>
<th>Concentration of added chelator (mM)</th>
<th>CDTA</th>
<th>EDTA</th>
<th>EGTA</th>
<th>TTHA</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>97</td>
<td>97</td>
<td>97</td>
<td>97</td>
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<tr>
<td>0.5</td>
<td>89</td>
<td>90</td>
<td>87</td>
<td>35</td>
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<tr>
<td>1</td>
<td>92</td>
<td>38</td>
<td>82</td>
<td>28</td>
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<tr>
<td>5</td>
<td>87</td>
<td>29</td>
<td>73</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>82</td>
<td>30</td>
<td>65</td>
<td>20</td>
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In view of the reported presence of low molecular weight and reactive contaminants in some commercial FITC-dextrans (Preston et al. 1987), it was determined that identical pinocytosis parameters were obtained with commercial FITC-dextran (Sigma FD-70), FITC-dextran or dextran conjugated with [¹⁴C]phosphatidylcholine, the latter two synthesized in the laboratory by the method of De Belder & Granath (1973) as well as with [³⁵S]dextran (Klein & Satre, 1986). Furthermore, FITC-dextran (2 mg ml⁻¹) was found completely non-toxic towards growth of *Dictyostelium* amoebae in axenic medium or during the starvation-induced differentiation program.

**Secretion of lysosomal soluble enzymes**

Amoebae were collected by centrifugation at 800 g for 4 min and washed twice in ice-cold 20 mM-Mes-Na, pH 6.3. The cells were suspended at 1 x 10⁶ cells ml⁻¹ in 0.1 M-sucrose, 20 mM-Mes-Na buffer, pH 6.3, or in the same medium containing 1 mM-vanadate. Incubation was performed at 22°C and at indicated times, two 1-ml samples were taken for each experimental condition. The first sample was directly supplemented with 20 μl 10 % (v/v) Triton X-100 to give the total activity and the second sample was centrifuged for 1 min in an Eppendorf centrifuge. The supernatant from the second sample containing
Effect of vanadate on the growth of *Dictyostelium* amoebae in axenic medium. A. Cells were grown at 22°C in the presence of the following vanadate concentrations: 0 mM (■); 0.2 mM (□); 1 mM (▲) and 5 mM (▼). B. Cells were grown at 22°C in the presence of 0 mM (■) or 2.5 mM-vanadate (•). After 8 h of incubation, a sample of the culture containing vanadate was washed and cells were resuspended in vanadate-free medium (▼).

Growth inhibition of growth was observable at a concentration of 0.2 mM-vanadate. The generation time was increased to 20 h and only two doublings occurred before growth stopped completely. When the culture medium contained 1 mM-vanadate no growth occurred and the cell number of the inoculum was maintained for at least 4 days without cell lysis. At 5 mM-vanadate, the cell number remained constant for two days and cells then started to lyse slowly with a half-time of about 24 h. The growth inhibitory effect of vanadate on *Dictyostelium* was due to growth stasis and not to cell death, for if the cells were removed from the axenic medium containing 2.5 mM-vanadate after 8 h of incubation and suspended in medium without added vanadate, they resumed growth with a generation time of 11 h, very similar to that of the control without vanadate (Fig. 1B).

Vanadate is an inhibitor of FITC-dextran pinocytosis

Fluid-phase pinocytosis in *Dictyostelium* amoebae was measured with FITC-dextran as a fluid-phase marker. In 40 mM-Mes-Na buffer, pH 5.3, FITC-dextran was internalized with an initial fluid influx rate of $3.3 \times 10^{-3} \mu l/10^6$ cells min$^{-1}$, up to a final plateau equivalent to an apparent volume of 0.3 $\mu l/10^6$ cells (Fig. 2A). When 0.2 mM-vanadate was added to the incubation medium simultaneously with FITC-dextran, pinocytosis started with the same kinetics as in a control experiment for a period of about 50–60 min. In a following phase, pinocytosis slowed down, stopped, and internalized FITC-dextran was re-exported with an efflux rate of $0.9 \times 10^{-3} \mu l/10^6$ cells min$^{-1}$. In the presence of 1 mM-vanadate, influx of FITC-dextran was immediately slowed down to $1.5 \times 10^{-3} \mu l/10^6$ cells min$^{-1}$ and the internalization reached a plateau at 0.07 $\mu l/10^6$ cells. Experiments were also conducted in nutritive medium (see Materials and methods), as fluid entry has been suggested to proceed in this case through both receptor-mediated endocytosis and simple pinocytosis (Rossomando et al. 1981; Maeda, 1983; Maeda & Kawamoto, 1986). In axenic medium, FITC-dextran was internalized with an influx rate of $5.5 \times 10^{-3} \mu l/10^6$ cells min$^{-1}$ up to a final plateau corresponding to an apparent volume of 0.5 $\mu l/10^6$ cells. In the presence of 1 mM-vanadate, both the initial influx rate and the final plateau were reduced by a factor of 3, similar to what was observed in non-nutritive Mes–Na buffer (Fig. 2B).

The effect of vanadate on fluid-phase pinocytosis was also determined with amoebae in which the endosomal compartment was previously loaded with FITC-dextran in 40 mM-Mes–Na, pH 5.3, for 100 min in the absence of vanadate. Then, as shown in Fig. 3, upon addition of vanadate and still in the presence of extracellular FITC-dextran, intracellular FITC-dextran was rapidly released into the medium after a lag period of 30–50 min depending on the vanadate concentration. Vanadate-induced FITC-dextran efflux kinetic rates were always lower than or at the most equal to the efflux rate determined with amoebae loaded with FITC-dextran in the first stage, washed and then resuspended in medium.

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Fig. 3. Effect of vanadate on the pinocytosis plateau. Dictyostelium amoebae were incubated at 22 °C at a cell concentration of $5 \times 10^6$ ml$^{-1}$ in 40 mM-Mes-Na buffer, pH 5.3, containing 2 mg ml$^{-1}$ FITC-dextran. At $t = 100$ min, a portion of the cells was collected by centrifugation and washed twice in ice-cold NaP$_2$ buffer, pH 6.3, containing 0.5 mg ml$^{-1}$ BSA. The washed cell pellet was then resuspended at 22 °C in the initial volume of 40 mM-Mes-Na buffer, pH 5.3, but without added FITC-dextran. At the same time, the remaining unwashed portion (in FITC-dextran loading medium) received 0, 0.2 or 1 mM-vanadate. Intracellular FITC-dextran ($\mu$g per 10$^6$ cells) was measured for the various conditions at the indicated times. The following symbols were used: (•) no added vanadate; (O) +0.2 mM-vanadate; (▲) +1 mM-vanadate; (Δ) washed cells, no vanadate added.

Fig. 4. Inhibition of sucrose-induced vacuolation by vanadate. Cells were incubated in 20 mM-Mes-Na buffer, pH 6.3, containing 0.1 M-sucrose for 90 min and photographed (A); vanadate was then added to the suspension at a final concentration of 5 mM and incubation continued for an additional 120 min after which samples were photographed (B). Cells were incubated in 20 mM-Mes-Na buffer, pH 6.3, containing 0.1 M-sucrose and 5 mM-vanadate for 90 min and photographed (C). Bar, 10 μm.

Vanadate is an inhibitor of sucrose-induced cell vacuolation
Sucrose can also be used as a tool for studying endocytosis, since it enters cells exclusively by pinocytosis and, in turn, leads to vacuolation of Dictyostelium amoebae, as has been observed in other cell types (Cohn & Ehrenreich, 1969). Upon incubation of amoebae in the presence of 0.1 M-sucrose, vacuoles progressively appeared in the cytoplasm. By about 90 min an extensive vacuolation state was observed, which remained stable for at least 4–5 h (Fig. 4A). The cells remained healthy under these conditions. If 5 mM-vanadate was added to amoebae that had been vacuolated by a 90 min-incubation with 0.1 M-sucrose, vacuolation disappeared within 2 h, even though the external medium still contained 0.1 M-sucrose (Fig. 4B).

In another set of experiments in which 5 mM-vanadate was added together with sucrose at zero-time, vacuolation was prevented (Fig. 4C). Some rounding of the cells was observed at this high concentration of vanadate, but no cell lysis occurred during several hours of incubation.

Vanadate is an inhibitor of lysosomal enzyme secretion
The extracellular release of mature lysosomal enzymes in Dictyostelium amoebae was measured in order to explore the intracellular pathways following the fusion of primary lysosomes with incoming endocytic vesicles. The release is selective for lysosomal enzymes and occurs without cell lysis (Crean & Rossomando, 1979; Dimond et al. 1981; Seshadri et al. 1986; Klein et al. 1988a). Two typical lysosomal enzymes, hexosaminidase and acid phosphatase, were chosen for these experiments as they were shown to be representative of different secretion patterns in Dictyostelium (Dimond et al. 1981). One difficulty in measuring the effect of vanadate on acid phosphatase secretion is that the enzymatic activity of Dictyostelium acid phosphatase is itself inhibited by vanadate, as has been shown for human liver and wheat germ enzymes (VanEtten et al. 1974). In the experiments reported here, this problem was overcome by reversing the inhibition through the formation of a complex between vanadate (VO$_2^+$) and aminocarboxylate chelating agents (Kustin & Toppen, 1973; Bowman & Slayman, 1979; Gibbons, 1982). As shown in Table 1, TTHA and EDTA were the most active in reversion and yielded maximal reversion levels of 80 and 70%, respectively. EGTA and CDTA were less effective and at a concentration of 10 mM produced 27 and 14% reduction of acid phosphatase inhibition. In control experiments, chelating agents had no effect by themselves at concentrations up to 10 mM on the enzymatic activity of acid phosphatase.

Secretion of acid phosphatase and hexosaminidase proceeded almost linearly in a medium containing a metabolized sugar such as maltose at rates of 12 and 18%
of the total activity per hour, respectively. In the presence of vanadate, secretion leveled after one hour at 14% for acid phosphatase and 36% for hexosaminidase (Fig. 5A,B). In the presence of a non-metabolized sugar, sucrose, which stimulated secretion in *Dictyostelium* (Crean & Rossomando, 1979; Seshadri et al. 1986; Klein et al. 1988a), kinetic curves showed an initial slow phase of about 1 h followed by a rapid secretion phase, after which secretion proceeded at a lower rate. The amount of secreted acid phosphatase or hexosaminidase activities reached more than 80% of the total enzymatic activity after 4 h of incubation (Fig. 5C,D). Vanadate had a major inhibitory effect on this secretion process. After the initial slow phase, the rapid phase of secretion was completely blocked and only 15% of the acid phosphatase or about 35-40% of the hexosaminidase activities were secreted, respectively, after 4 h of incubation. A consistent increase in the total activity of hexosaminidase was observed, reaching 1.4-fold after 4 h, while the activity of acid phosphatase remained stable. A similar observation was reported in *Tetrahymena pyriformis* (Banno et al. 1987). The addition of 1 mM-vanadate completely prevented the rise in the total hexosaminidase activity (Fig. 5B,D).

**Effect of vanadate on cytosolic pH and on the energy status of Dictyostelium cells**

Monomeric vanadate is a structural analog of P<sub>i</sub> and this could account for some of its biological effects. For example, all the inhibitory effects of vanadate described above might be related to a decreased level of nucleoside triphosphates in the amoebae. *In vivo* 31P n.m.r. was thus used to assess the action of vanadate on the P<sub>i</sub> and the phosphorylated metabolite content in *Dictyostelium* amoebae (Fig. 6). The 31P n.m.r. spectra of aerobic cells were obtained in the presence of 5 mM-vanadate, a concentration that maximally inhibits fluid-phase pinocytosis and lysosomal enzyme secretion. The nucleoside triphosphate level and the nucleoside di- to triphosphate ratio were maintained at the same value as in control samples. Cytosolic pH was deduced from the chemical shift of the resonance line of cytosolic P<sub>i</sub>. Its value was close to pH 7.4 in the controls and remained unchanged in the presence of vanadate. The most significant change noticed in the 31P n.m.r. spectra was the progressive appearance of a new resonance at ~23 p.p.m., corresponding to the central phosphate atoms of polyphosphate chains. When cells incubated in 5 mM-vanadate for 2.5 h were pelleted, the 31P n.m.r. spectrum of cells resuspended in vanadate-free medium was identical to that of control cells and all the n.m.r.-detected polyphosphates were found in the extracellular medium. The presence of polyphosphates on the outside of the plasma membrane has been reported in yeast (Tijsen et al. 1981).

**Vanadate is an inhibitor of Dictyostelium morphogenesis**

To determine whether vanadate interfered with *Dictyostelium* development, morphogenetic behavior was followed by starving amoebae on Millipore filters placed on absorbent pads saturated with solutions containing increasing amounts of vanadate from 0.1 to 5 mM. Control samples completed differentiation and produced fruiting bodies in about 24 h. No significant difference was observed for samples containing up to 0.25 mM-vanadate (Fig. 7). In the presence of 0.5 mM-vanadate, fruiting body formation was grossly perturbed and only aggregates of small size and aberrant morphology were formed. At higher vanadate concentrations, no aggregation occurred and cells remained at the surface of the filters as completely smooth lawns.

**Vanadate has no effect on heat-induced spore germination**

Dormant spores constitute a stage in the life cycle of

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Fig. 6. Effect of vanadate on the $^{31}$P n.m.r. spectrum of aerobic *Dictyostelium* amoebae. Amoebae were suspended at a concentration of $5 \times 10^8$ cells ml$^{-1}$ and bubbled with oxygen in 40 mM-Mes-Na buffer, pH 5.3, and in the same medium containing 5 mM-vanadate. n.m.r. spectra corresponded to a 15 min accumulation between incubation times of 60 and 75 min. The upper trace (A) is the control spectrum and the lower trace (B) is the spectrum in the presence of vanadate. PME, phosphomonoesters; cytPi, extPi, cytosolic and extracellular $P_i$; NTP, NDP, nucleoside tri- and diphosphate; polyP, central phosphate atoms of polyphosphate chains; IP$_6$, inositol hexa(kis)phosphate.

*Dictyostelium* with low metabolic activity. Upon appropriate stimulation, a complex sequential germination process begins with the reactivation of metabolism and ends with the release of prevegetative amoebae. The effect of vanadate on *Dictyostelium* spore germination after heat-induced activation was investigated. Dormant spores were activated by heat shock at 45 °C for 30 min; this was performed either with or without vanadate. Germination was then followed in the presence of the same concentrations of vanadate. In all cases, vanadate at concentrations up to 5 mM was found to have essentially no effect on the entire germination sequence, including the early swelling stage as well as the emergence stage. Some rounding of the nascent amoebae was noticed in the presence of vanadate at concentrations higher than 1 mM. Lysis of nascent amoebae began after several hours in 5 mM-vanadate.

Discussion

In this paper the effects of vanadate on the major stages of the life cycle of *Dictyostelium* were investigated. The results demonstrate that vanadate is an inhibitor of the vegetative growth of *Dictyostelium* amoebae, acting efficiently at concentrations as low as 0.2 mM. The same concentrations of vanadate were reported to be toxic in other simple eucaryotes: yeast (Willsky *et al.* 1984) and microscopic fungi (Bowman, 1983). The vanadate-induced growth inhibition of *Dictyostelium* amoebae is fully reversible as intoxicated cells rapidly resume growth when vanadate is removed from the medium. An identical situation was reported in the case of the yeast *Saccharomyces cerevisiae* (Willsky *et al.* 1984).

Fig. 7. Effect of vanadate on *Dictyostelium* morphogenetic development. Photographs were taken after 48 h of development. A. Control; B, 0.25 mM-vanadate; C, 0.5 mM-vanadate; D, 1 mM-vanadate. Bar, 1 mm.
Acanthamoeba (Hohman & Bowers, 1984). Alternatively, the pinocytosis pathway and lysosomal secretion as measured with the fluorescent dye Lucifer yellow CH in the yeast S. cerevisiae (Riezman, 1985). As our results in Dictyostelium show, vanadate has a strong inhibitory effect on the influx of the fluid-phase marker FITC–dextran and on its plateau level, but does not block FITC–dextran efflux from loaded cells. A simple explanation for these effects could be that vanadate prevents the fusion of incoming endosomal vesicles with lysosomes, as originally proposed by Seglen (1983) for hepatocytes.

Inhibition of endosome–lysosome fusion is in agreement with the experiments using sucrose to probe endocytic compartments, assuming that vacuolation corresponded to osmotically swollen secondary lysosomes. The fact that lysosomal enzyme secretion is inhibited by vanadate is also consistent with this hypothesis. Dictyostelium cells, like those of the related slime mold Polysphondylium pallidum (O’Day, 1973) and other lower eucaryotes such as Acanthamoeba (Hohman & Bowers, 1984) or Tetrahymena (Florin-Christensen et al. 1989), efficiently secrete their lysosomal enzymes. This behavior is in contrast to that of mammalian cells, which secrete lysosomal hydrolases in a much smaller amount and as a diversion from a prelysosomal compartment (for review, see Storrie, 1988). Pinocytosis and lysosomal enzyme secretion in Dictyostelium could be directly linked through membrane recycling, as proposed in the case of Acanthamoeba (Hohman & Bowers, 1984). Alternatively, the pinocytosis pathway and lysosomal secretion process could be independent, as shown in Tetrahymena (Florin-Christensen et al. 1989).

Vanadate has an inhibitory effect on the development of Dictyostelium. At low concentrations (0.25–0.5 mM), the compound affects the formation of fruiting bodies and at high concentrations (>0.5 mM) it blocks the aggregation process. The action of vanadate on differentiation might be understood within the framework of the above hypothesis and could be linked to an inhibition of the autophagic processes needed to provide the energy required for fruiting body formation (Loomis, 1975).

Spore germination after heat activation is highly resistant to vanadate at concentrations as high as 5 mM. However, the emerging amoebae round up, lose viability and begin to lyse within several hours at such concentrations. In a parallel study, we also found that microcysts of Polysphondylium pallidum would germinate in 5 mM-vanadate solutions (unpublished results). Like those of Dictyostelium, the emerged amoebae of P. pallidum begin to lyse after several hours in the presence of 5 mM-vanadate. The apparent lack of effect of vanadate on cysts and spores is in keeping with the idea that resistant forms of the slime molds may not be in intimate contact with the environment until just before completion of the emergence stage of germination.

In conclusion, vanadate appears to be an interesting tool for probing the mechanisms of endocytosis in Dictyostelium amoebae and their fluid-phase pinocytosis pathways.

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