CYTOKININS AND MAGNESIUM IONS MAY CONTROL THE FLOW OF METABOLITES AND CALCIUM IONS THROUGH FUNGAL CELL MEMBRANES.

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SUMMARY

Compounds that are known to display cytokinin activity in green plants function as allosteric regulators of metabolite and ion transport in fungal cells. These hormonal compounds stimulate a massive release of calcium bound to a glycoprotein localised on the membrane surface, and simultaneously, activate the transport of calcium into the cells. The energy-linked import of sugars, nucleosides, and amino acids is inhibited by the cytokinins. The activating effect of cytokinins is neutralised by magnesium ions. Calcium and metabolite import in the fungi studied appear to depend upon a delicate balance between the concentrations of cytokinins, calcium, and magnesium ions.

What the precise mechanism of action of biological compounds generally classed as cytokinins is, has been an enigma to plant scientists for almost 2 decades. The systems in which these 'hormonal' compounds have been implicated are diverse (1). But, one thing is clear, as Steward (2) has maintained, that 'no single substance unlocks the door of cell division'.

We wish to report here some experimental data in support of yet another concept that, at least in fungal cells, cytokinins may regulate the transport of metabolites (sugars, nucleosides, and amino acids) operating in harmony with Mg^{++}, and Ca^{++}. Cytokinins, it seems, may play a crucial role in cell differentiation and development at the level of metabolite transport.

MATERIALS AND METHODS

Organism. Achlya sp. (1969) was used in these studies. Some of the effects have been confirmed on a taxonomically-unrelated fungus, Blastocladiella emersonii, but those results will be presented when a comprehensive taxonomic study is completed.

Transport Studies. Osmotically-shocked and unshocked cells were treated in the same way before use in determining the initial velocity of transport. Details of osmotic shock treatment and initial reaction rate transport assay procedures are given in other communications (3, 4). The basic incubation medium consisted of 5 mM Tris-acetate, 1 mM KCl, 1 mM NaCl, and 5 mM D-glucose when the transport of this metabolite was not being examined. Cycloheximide (10 μg/ml) was included in the assay systems of normal cells to inhibit macromolecular synthesis as defined in a previous report (5).

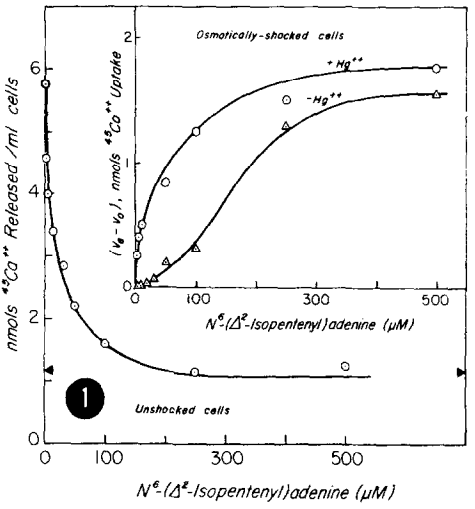
Chemicals. Radioactive isotopes were obtained from Amersham/Searle and biochemicals from Sigma Chemical Co.

RESULTS

Cytokinin and Ca^{++} Efflux by Unshocked Cells. Normal Achlya germlings sequester large quantities of Ca^{++} when a thiol-reactive agent such as Hg^{++}, I_2, Ag^{+} or an organic mercurial is added to the transport medium. The Ca^{++} taken up in the presence of Hg^{++}, I_2 or Ag^{+} has been shown to bind to a Ca^{++} sequestering glycoprotein (mol wt 6,000) that is present on the surface of the plasma membrane and releasable by cold osmotic shock (4). The cells so stripped of the glycoprotein do not respond to Hg^{++} stimulation during Ca^{++} uptake (6). This technique permitted us to study the in vivo responses of the two components of the Ca^{++} transport system independently.

Unshocked cells induced to bind $^{45}Ca^{++}$ in the presence of Hg^{++} released the glycoprotein-bound Ca^{++} when exposed to cytokinins. The rate of $^{45}Ca^{++}$ efflux as a function of cytokinin concentration is shown in Fig. 1. N^6-(Δ^2 isopentenyl)adenine (6ipAde) was the most effective cytokinin studied and the 3-isomer, triacanthine, which was so effective in the in vitro system (7) was practically inert. Xanthine, not generally regarded as a cytokinin, was almost as effective as 6ipAde. Zeatin, the hydroxy-derivative of 6ipAde, was a strong inhibitor as were N^6-(dimethyl and methyl substituted)adenines.

Although some 30 compounds of which 8 have been established as plant



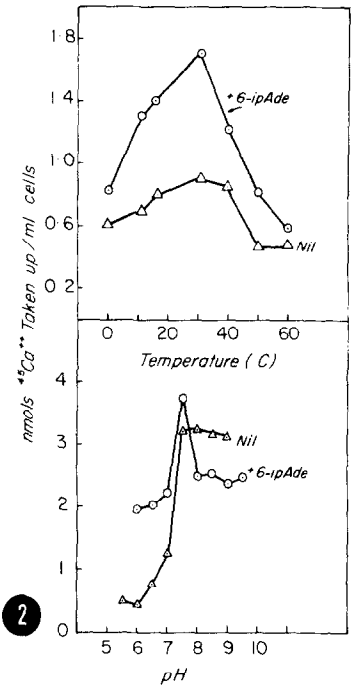


FIG. 1. Influence of N^6-(Δ^2-isopentenyl)adenine on (a) $^{45}Ca^{++}$ release from normal Achlya germlings treated with 10 μM $HgCl_2$ and (b) on $^{45}Ca^{++}$ transport into osmotically-shocked Achlya germlings with and without 10 μM $HgCl_2$ present. The symbols (◀ ▶) designate the level of $^{45}Ca^{++}$ intake not affected by $HgCl_2$. v_e represents initial reaction rate in the presence of cytokinin, and v_o the rate in its absence.

FIG. 2. Influence of N^6-(Δ^2-isopentenyl)adenine on $^{45}Ca^{++}$ uptake by osmotically shocked Achlya germlings at (a) different temperatures (b) different pH values.

cytokinins were tested in this system, only a few agents are mentioned here. A comprehensive analysis will be reported elsewhere. But, as in the in vitro system, purine and pyrimidine nucleosides, pyrimidines, benzimidazole derivatives, caffeine, and various methylxanthines (except theophylline), proflavins etc., were ineffective. Nucleotides, AMP, ADP, GMP, GDP, cyclic AMP and cyclic GMP were also inert. We therefore conclude that this is a specific system that interacts largely with cytokinins and may be of physiological importance. The Ca^{++} release effect could not be due to simple chelation.

Cytokinin and Ca^{++} Uptake by Osmotically-Shocked Cells. Further support for the conclusion that chelation is not involved was obtained when the effects of cytokinin on the transport of Ca^{++} by shocked cells was studied. The rate of $^{45}Ca^{++}$ intake into these cells increased significantly in the presence of cytokinins. Unlike the unshocked cell system, Hg^{++}, Ag^{+}, or I_2 acted synergistically to facilitate cytokinin activation (Fig. 1, inset). This implies that there is a close connection between Ca^{++} release from the glycoprotein by cytokinin when its thiol groups are oxidised, and the import of Ca^{++} by a separate membrane transport system whose interaction with cytokinins is facilitated when thiol reacting agents are present.

Cytokinin-assisted Ca^{++} transport can be antagonised by Mg^{++}. As shown in the inset of Fig. 1, the activation plot with cytokinin as the varied ligand is sigmoidal and hyperbolic in the presence of Hg^{++}. If Mg^{++} is included in the transport medium, the activation plot again becomes cooperative implying that Mg^{++} was displacing Hg^{++}. The binding loci for Hg^{++} and cytokinin may therefore be distinctly different.

Activation of Ca^{++} intake into shocked cells by 6ipAde is temperature and pH dependent (Fig. 2) indicating that a physiological process may be involved.

Influence of Cytokinin on Metabolite Uptake. Evidence has been presented elsewhere (3, 8) that nucleosides, sugars, and amino acids are concentrated by an active transport system in Achlya. The osmotically-shocked cells are unable to concentrate metabolites, but this ability reappears when the cells recover and resume growth following a lag period of about 12 hr. Normal cells are inhibited by cytokinins in their ability to concentrate these metabolites. As shown in Fig. 3, the rate of metabolite uptake decreases at almost the same rate at which Ca^{++} is released from the sequestering glycoprotein (Fig. 1).

DISCUSSION

The data presented in this communication support the concept that

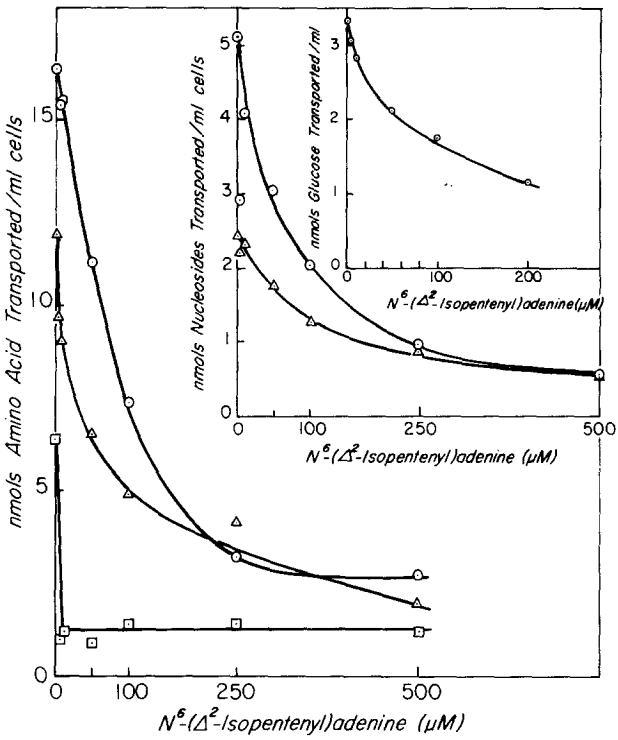


FIG. 3. Influence of metabolite uptake by normal Achlya germlings by N^6-(Δ^2-isopentenyl) adenine. In the outer illustration, the effects on (⊙) methionine, (△) phenylalanine, and (⊡) glutamic acid are shown. In the inner illustration, the effects on (⊙) uridine, and (△) adenosine are shown. In the innermost illustration, the effect on D-glucose is shown.

cytokinin-like compound(s) may regulate the rate of inflow of metabolites and Ca^{++} into the cells of some fungi.

Cytokinins appear to have a dual function in fungal transport activities initiating Ca^{++} release from a glycoprotein present on the surface of the cell membrane, and stimulating Ca^{++} intake into cells. Whether cytokinins directly interfere with an energy-generating system thereby reducing the ability of the cells to concentrate metabolites, or whether the inhibition is indirect through cytokinin interaction with the Ca^{++} transport system, is unclear. Experiments designed to elucidate the probable mechanism of cytokinin action in these fungal cells are currently in progress.

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