CALCIUM IS A PROMINENT CONSTITUENT OF THE GAMMA PARTICLE IN THE ZOOSPORE OF BLASTOCLADIELLA EMERSONII AS REVEALED BY X-RAY MICROANALYSIS

by

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SUMMARY. Energy selective X-ray elemental microanalysis was used to demonstrate the presence of Ca, K and P (relative amounts about 1 : 1 : 4, respectively) in the virus-like Gamma Particles found in the zoospores of the aquatic fungus Blastocladiella emersonii. Some Gamma Particles, however, lack detectable amounts of K. The possible significance of these observations for understanding the triggering of encystment and the initiation of wall synthesis is underscored.

INTRODUCTION. The Gamma Particles (1) in the zoospores of Blastocladiella emersonii (2) are thought to play a direct, indispensible and causal role (3) in encystment and, thereby, to provide an explanation* for the initiation of cell wall synthesis, hence they have been designated a type of 'encystosome' (5). The isolation, enzyme activity, structure, function, genesis, and decay of these DNA-RNA-containing particles -- virus-like in some respects -- have been reviewed (6); additional details about their formation were published recently (7). We wish to call attention here to the following correlations:

(a) Under certain conditions, B. emersonii zoospores can be prevented from encysting, i.e., stabilized, with Ca^{+2}; conversely, zoospores can be triggered to encyst with K^{+} (3,8,9,10,11,12).

(b) When zoospores pre-labeled with ^{45}Ca are triggered to encyst, ^{45}Ca is released; however, it is not released from non-encysting spores (10).

* It may not, however, be a sufficient explanation (4).

(c) When zoospores are triggered to encyst, the Gamma Particles begin to 'decay' (11), this being one of the first detectable morphological changes associated with encystment (3).

Our principle purpose in this report is to provide data on the elemental composition of the Gamma Particle, and evidence that this organelle contains Ca, the amount being greater than that found in either the background cytoplasm or any other discrete structure examined thus far in the zoospore. Accordingly, the Gamma Particle could be the source of the Ca released when zoospores are triggered to encyst with K^+.

MATERIALS AND METHODS. Evenly dispersed, synchronous, single generation thalli of our original strain (13) of B. emersonii were grown in Petri plates on peptone-yeast extract-glucose media. Zoospores derived therefrom were pre-fixed directly on the plates with glutaraldehyde, and processed and embedded in low viscosity resin, all as described previously (14). Additionally, reference spores were also post-fixed in 2% OsO_4 (14). The unstained (test) spores and reference spores were rinsed three times in the cacodylate buffer, dehydrated, and embedded in low viscosity resin (14).

Populations of spores prepared in this way generally consist primarily of flagellated zoospores, but they may include variable quantities of spores which have just retracted their flagella, judging (for reasoning, see 3,6) from the presence of internal peripheral axoneme sections and the onset of decay among some Gamma Particles, but an absence of detectable cyst wall material around the plasmamembrane.

For the elemental analyses, sections of various thicknesses (100 - 200 nm) were used. The Gamma Particles were easily recognized from their shape, size, and electron opaqueness.

The instrumentation consisted of a JEOL 100B transmission electron microscope, its scanning attachment, and a KEVEX-LINK X-ray energy selective analysis system, located in the Electron Microscope Unit, Department of Physiology, University of Glasgow, Scotland. The standard JEOL microanalysis stage was modified by replacing the copper anti-contamination device with low atomic number metals in order to reduce the bremsstrahlung background. X-ray spectral lines from the copper specimen grids did not interfere with the analysis.

The microscope was operated with an electron beam of 80 kV accelerating potential (JEOL spot size 3) and a beam current of 100 μA, measured at the electron gun. The size of the electron probe was adjusted to uniformly cover the organelle under analysis, however spot sizes approaching 25 nm were obtainable. Since no Faraday cup was used, no direct beam current measurements at the specimen were possible.

In general, the transmission mode of operation was used to locate organelles of interest. However, when contrast was extremely low, the scanning transmission mode was used with its inherent loss in resolution. The anti-contaminator was vital since hydrocarbon contamination build-up on the specimen occurred after a short period of time when it was not in use. The distance between the X-ray detector and the specimen was generally about 20 mm.

The counting rate for X-ray photons varied with thickness of the specimen but was, in general, between 500 and 1000 counts/second in the entire spectrum. The data, consisting of the number of X-ray photons collected vs energy, were

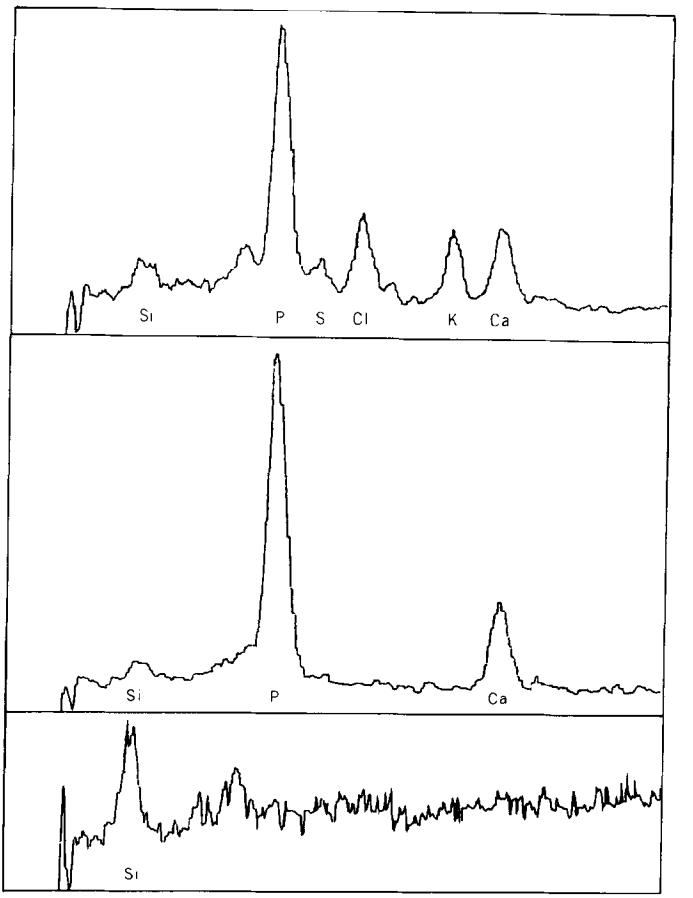

Fig. 1. Elemental analysis of two Gamma Particles. The number of X-ray photons (vertical axis) is plotted against X-ray photon energy (horizontal axis). Top: scan of a Gamma Particle, Expt. #44. Center: scan of a Gamma Particle, Expt. #47. Bottom: scan of embedding medium background, Exp. #51 (unsmoothed plot).

recorded in three ways: (a), data were read onto floppy disc magnetic storage; (b), energy vs number of photons was plotted by an XY recorder; (c), a teletype printed out the integrated number of photon counts under specified spectral peaks, corrected for background. The latter data provided the basis for semi-quantitative comparisons of the relative concentrations of elements. For additional discussion of the elemental microanalysis techniques used herein and related matters, see (15).

RESULTS. Electron microscopic scans of typical Gamma Particles (for profiles and morphology, see 3,6,7,11) yielded the spectra shown in Fig. 1. No attempt has been made to fully quantify elemental concentrations within particular specimen areas since the number of specimens analyzed was limited.

TABLE I

Comparative amounts of Ca, K and P in two Gamma Particles*

	(p - b)**		
	Ca	K	P
Exp. 44	721	765	2794
Exp. 47	829	0	3527
Embedding medium	0	72	0

* The same Gamma Particles used for scans shown in Fig. 1

** (p - b) = integrated number of photon counts under specified spectral peaks, minus bremsstrahlung backgrounds.

In addition, uncertainties about section thicknesses leaves some question as to the absolute concentrations, and radiation-induced losses of particular elements from the specimens were not calibrated. The data presented here should therefore only be taken to indicate the presence of elements within the region analyzed, allowing semi-quantitative estimates of the ratios of elements present within the Gamma Particles to be made. We have not compensated for detector efficiency differences among elements since errors introduced by omission of this correction are small in comparison with the scatter in the data themselves.

The integrated number of X-ray photons (corrected for background) characteristic of the three Gamma Particle elements of particular interest here are presented in Table I. The ratios Ca:K and P:K differ greatly in the two particles. This is clearly seen in Fig. 1. Energy peaks due to several elements are visible in the traces (top and center, Fig. 1) for two separate Gamma Particles; the background trace (bottom) for an adjacent area in the embedding medium is included. Physiologically important elements were not detected in the embedding medium; the presence of Si is probably due to deposition of products from the vacuum system.

Only P and Ca were found in one Gamma Particle (Fig. 1, center) whereas other Gamma Particles (e.g. Fig. 1, top) also contained K, S and Cl. The presence of S and Cl may have resulted from stray radiation striking specimen areas outside the Gamma Particles, since regions of cytoplasm ground substance just outside of this organelle contain significant amounts of both elements. However, the difference in K may be significant and suggests that Gamma Particles may actually vary in their content of K. The ratio of Ca to P was essentially the same in the Gamma Particles examined.

Based upon our studies thus far (unpublished data), the concentration of Ca in the Gamma Particle is much higher than that in the nuclear apparatus, the single mitochondrion, and other regions of the cytoplasm.

DISCUSSION. Gamma-like particles have been seen recently in other aquatic fungi (5,16), those in Rozella allomycis having been analyzed by X-ray techniques (17) and shown to contain P. Our findings now demonstrate that the Gamma Particles in B. emersonii also contain high levels of P, a fact predictable from their content of nucleic acids (6)†, thus strengthening existing similarities (5,16,17) between the two kinds of particles. Additionally, however, our report apparently provides the first firm evidence for the presence of Ca* in a clearly defined sub-cellular organelle of an aquatic fungus. Indeed, the amount of Ca in the Gamma Particle greatly exceeds that detected anywhere else in the zoospore (unpublished data).

The mechanism by which encystment is initiated in the B. emersonii zoospore is attracting increasing attention for several reasons, perhaps the most interesting being that encystment does not require concomitant protein

† The Gamma-like particles in R. allomycis contain polyphosphate (17) but whether or not they contain nucleic acids is not known. The Gamma Particles in B. emersonii contain DNA-P and RNA-P, but whether or not they contain P in some other chemical form is not known. However, the concentration of total P in Gamma Particles can be roughly estimated from our elemental microanalysis to be in the range 10-100 mM; the concentration of nucleic acid-P in Gamma Particles can be estimated from their DNA and RNA content to be about the same, i.e., ca. 50 mM.

* Small Ca signals were apparently detected in some Gamma-like particles in R. allomycis (17) but a firm conclusion could not be drawn about the presence or absence of Ca because of equipment insensitivity.

synthesis (18,19,20,21). With respect to the role of K as a triggering agent for encystment, it has been hypothesized** that K might function by displacing Ca at some unknown sites within the cell. In the light of the evidence we have presented here (not only the abundance but also the apparent absence of K in the Gamma Particles examined), these organelles could be the sites in question. Movements of Ca and K into and out of Gamma Particles, together with other factors (3,4,21), might thus be involved in triggering activity in the Gamma Particle -- an activity characterized morphologically by formation and release of membrane vesicles which migrate from the Gamma Particles to the plasma membrane and fuse with it.

In summary, if our interpretation is basically valid, it would be via the Gamma Particle that Ca and K exert control over the first stages of encystment in this fungus and, thereby, over the regulation of chitin synthesis and cell differentiation in B. emersonii.

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** This idea was introduced by D.R. Soll (PhD Thesis, 1970, Univ. of Wisconsin, Madison) but apparently it has not been emphasized further because some of the experimental results (10) could not easily be reconciled with it.

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