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Correlation between potassium and phosphorus content and their nonuniform distribution in *Acanthamoeba castellanii*

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Summary. Biologically important elements: K, Na, Mg, Ca, Cl, P, and S were analyzed in *Acanthamoeba castellanii*. A higher potassium content, as compared with other cations, was detected. Total content of the cation-forming elements: K, Na, Mg, and Ca was ca. 360 mmoles/kg dry weight of the cells. Phosphorus content was estimated as 492 mmoles/kg dry weight. Content of chlorine, a basic cellular anion, was 173 mmoles/kg dry weight. The low level of chlorine appears not the be sufficient to balance all the cations in *Acanthamoeba*.

Distribution of potassium in *Acanthamoeba* cells was nonuniform and similar to that of phosphorus as shown by X-ray microanalysis technique. Quantitative correlation between phosphorus and potassium as well as the similar distribution of these elements suggests that in *Acanthamoeba* phosphorus is an essential anion which, being nonuniformly distributed in the cell, determines also a nonuniform distribution of potassium.

Introduction

The small, free-living amoeba, *Acanthamoeba castellanii*, will tolerate significant changes in tonicity and in ionic composition of the medium in which it is immersed (Klein 1961; Larochelle and Gagnon 1976). This organism is also characterized by having a high potassium content in comparison with other intracellular cations, and in comparison with the potassium content of the medium (Klein 1959; Larochelle and Gagnon 1976).

According to Klein's experiments cellular potassium may be divided into three fractions: easily diffusible, exchangeable, and bound (Klein 1961). Our preliminary results (Sobota et al. 1981) showed that in *Acanthamoeba* cells potassium was nonuniformly distributed, which may support Klein's conclusion that some part of cellular K exists in bound form.

The present study includes 1) determination of several important cellular elements and 2) evaluation of the correlation between their content and distribution, especially between K and P. In this study we have used analytical methods such as atomic-absorption spectrophotometry, flame-photometry and X-ray microanalysis. The last allows us to examine in situ the elemental composition of the cells, and the compartmentalization of the elements, and also to quantify the elements in microregions of the cells.

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Material and methods

Acanthamoeba castellanii (Neff strain) was cultured in optimal growth medium as described by Klein (1959). Briefly, the medium consisted of (grams per liter): proteose peptone – 15, yeast extract – 1.5, glucose – 10, NaCl – 0.120, KH_2PO_4 – 0.270, $CaCl_2$ – 0.003, $MgCl_2$ – 0.003, $FeSO_4$ – 0.003; pH was adjusted to 6.5 (Optimal Growth Medium 1, OGM-1). The cells were also cultured during several years in the growth medium similar to OGM-1 except for the ionic content which was 10-times higher (OGM-10).

For experiments, the 5-day-old cultures from either OGM-1 or OGM-10 were collected by centrifugation, 2 min, 600 g, 20° C, washed in 240 mM sucrose and pelleted on a small piece of aluminum foil by centrifugation, 5 min, 600 g, 20° C. The foil with the pelleted cells was quenched in liquid propane cooled to about −180° C by liquid nitrogen. Frozen samples were dried under vacuum at −50° C during 3 days and then the samples were slowly warmed under vacuum to room temperature. This kind of preparation of cells for analysis was shown to prevent the loss and redistribution of soluble elements in cells (Burovina et al. 1972; Ingram et al. 1972). The composition, content and distribution of elements in the cells were analyzed using a scanning microscope-microanalyzer, a flame-photometer and an atomic-absorption spectrophotometer.

For X-ray microanalysis, the freeze-dried samples were either embedded in Spurr's resin (Spurr 1969) or used as "cell powder". The embedded cells were cut with a dry glass knife on an LKB ultratome. The 1–2 μm thick sections were mounted on the copper grids and coated with a 20–30 nm carbon layer. The distribution of K, P, S as well as quantiative analysis of these elements in microregions of the cells were performed with a scanning microscope-microanalyzer JSM-U3 (JEOL, Japan). X-ray microanalysis was carried out at accelerating voltage 25 kV, sampl current 5–10 nA. The electron beam was focused to a spot 1–3 μm in diameter.

In case of freeze-dried, non-embedded material, the "cell powder" was placed in deep cavity in carbon holder, coated with 20–30 nm carbon layer, and the quantitative elemental analysis was performed with a microanalyzer JSM-U3 at the similar conditions as described above.

For quantitative X-ray microanalysis the following cristal standards were used: NaCl, $KAlSi_3O_8$, $Ca_3(PO_4)_2$ and FeS. Quantiative data were obtained comparing the counts of X-ray signals excited from a given element in the cell samples with the signals excited from standards (Burovina and Pivovarova 1978). The freeze-dried, non-embedded "cell powder" was also analyzed for Na and K by flame-photometry, and for Mg and Ca by atomic-absorption spectrophotometry. For this purpose, the determined amounts of freeze-dried samples were suspended in 0.1 N HCl,

Table 1. Content of chemical elements in *Acanthamoeba* cells

Growth medium	Method of analysis	Content of elements[a] [mmoles/kg dry weight]						
		K	Na	Ca	Mg	P	S	Cl
OGM-1	X-ray microanalysis	230	79	8	44	492	225	173
	flame-photometry	240	73	–	–	–	–	–
	atomic-absorption spectrophotometry	–	–	5	55	–	–	–
OGM-10	flame-photometry	290	115	–	–	–	–	–
	atomic-absorption spectrophotometry	–	–	11	85	–	–	–

[a] Each value is the mean of three determinations

heated for 10 min to about 60° C and then allowed to stand for 2 h at room temperature.

The linear regression equations and correlation coefficients (r) for K, P, and S were calculated according to Texas Instrument SR-51-II programme.

Results and discussion

Table 1 presents the quantitative analysis data for K, Na, Mg, Ca, Cl, P, and S in *Acanthamoeba* cells cultivated in growth media differing in salt concentration (OGm-1 or OGM-10). Different methods used for analysis of the elements: X-ray microanalysis, flame-photometry and atomic-absorption spectrophotometry gave quite similar results for K, Na, Mg, and Ca (Table 1).

A high potassium content, as compared with other cationforming elements, was detected. The average K content was 230 and 240 mmoles/kg dry weight as measured by X-ray microanalysis and by flame-photometry, respectively. The total level of cation-forming elements (K, Na, Mg, and Ca) was ca. 360 mmoles/kg dry weight of *Acanthamoeba* cells.

On the other hand the content of chlorine, a basic cellular anion-forming element, was 173 mmoles/kg dry weight. Such low level of chlorine in *Acanthamoeba* cells suggested that there must be other elements to balance the cellular cations. The phosphorus content in *Acanthamoeba* cells was 492 mmoles/kg dry weight as measured by X-ray microanalysis. The high level of phosphorus indicates that anions of this element may serve as counterions for cations in *Acanthamoeba* cells.

The above data relate to *Acanthamoeba* cells which were cultivated in the essential growth medium – OGM-1. When *Acanthamoeba* was cultivated for 3 years in OGM-10 medium (the growth medium containing a 10-times higher concentration of the salts than OGM-1), the content of K, Na, Mg, and Ca in the cells was 1.2 to 1.5-times higher than in those cultivated in OGM-1 medium (Table 1).

The cells from OGM-1 were also analyzed for distribution of potassium and phosphorus by means of X-ray microanalysis, and the results are presented in Fig. 1. Figure 1 a shows a scanning transmission electron micrograph of the analyzed 2 μm thick section of *Acanthamoeba* cells. The contours of the cells are well outlined and the vacuoles and the nucleus may be distinguished. The distribution of potassium is nonuniform (Fig. 1 b): more intensive X-ray signals characteristic for potassium are detected in the ectoplasmic part of the cells; the lower X-ray-K-signals are seen in vacuoles and endoplasm. The distribution of phosphorus in the same section of resin embedded *Acanthamoeba* cells is very similar to that of potassium (Fig. 1 c). The ectoplasm region and the nucleus are rich in phosphorus while the vacuoles and endoplasm region are poor in this element. The similarity of potassium and phosphorus distribution suggests that there is a correlation between localization of these elements in the cells.

To assess the degree of association between the random values of potassium and phosphorus, quantitative analysis for the two elements was performed in different random microareas of the cells (25 cells, 2 measurements per cell). The cellular microregions analyzed contained varying amounts of K and P ranging from 12 to 86 mmoles/kg wet weight for potassium, and from 30 to 164 mmoles/kg wet weight for phosphorus. A variation in quantity was also observed for sulphur, a basic indicator of organic cellular material, determined in the same microareas of the cells as K. The amounts of sulphur varied from 22 to 133 mmoles/kg wet weight.

In order to establish the relationship between K, P, and S in *Acanthamoeba* cells, the quantities of the elements analyzed were plotted against each other (Figs. 2 and 3). The linear relation between the contents of phosphorus and potassium and the correlation coefficient $r = 0.78$ indicate that potassium fairly well correlates with phosphorus distribution (Fig. 2). Lower relationship between potassium and sulphur was detected. In this case the correlation coefficient was $r = 0.65$ (Fig. 3).

The correlation between potassium and phosphorus in different types of tissues of higher organisms was noted earlier by Williams (1970) and by Burovina et al. (1978). The above presented analysis of two-dimensional distribution of K and P in *Acanthamoeba castellanii* and linear regression analysis of K and P contents in the cells support the hypothesis of phosphorus-potassium relationship.

Other authors indicated the important role of beta- and gamma-carboxyl groups in the binding/adsorbtion of potassium ions in muscle cells (Ling 1952, 1977; Edelmann 1980). Potassium ions and their analogues were found to be selectively bound within A bands in striated muscle cells (Edelmann 1977, 1980; Ling 1977). According to the authors' suggestions, the A band contain high amounts of myosin with higher density of negatively-charged beta- and gamma-carboxyl groups which can adsorb potassium ions. There are also experimental data showing the selective binding of potassium by glycerinated muscle fibers (Shapiro et al. 1980).

In *Acanthamoeba* cells the high content of K and P is seen at the border of the cells, i.e. in the ectoplasm region.

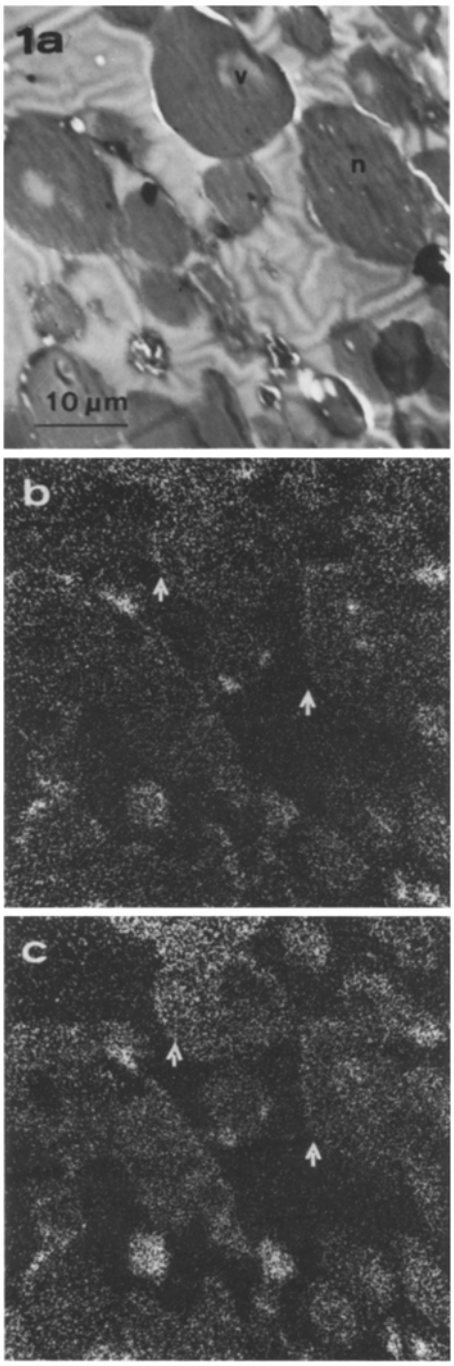

Fig. 1 a–c. Distribution of potassium and phosphorus in *Acanthamoeba* cells. **a** Scanning transmission electron micrograph of the analyzed cells (2 µm section). **b** and **c** X-ray image of the distribution of potassium and phosphorus, respectively. *Arrows*, the ectoplasm regions of the cells rich in K and P; *v*, vacuole; *n*, nucleus

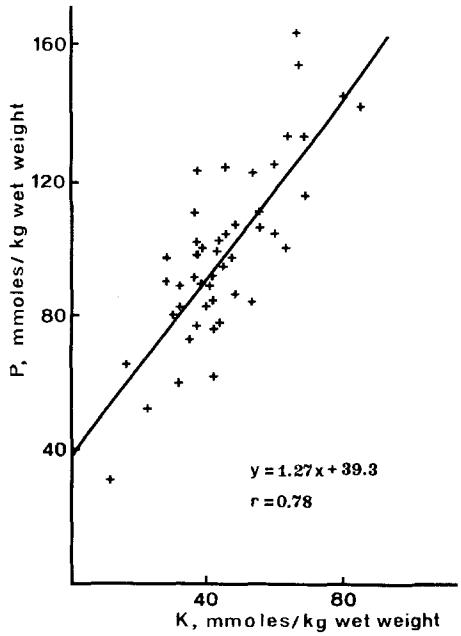

Fig. 2. Relationship between phosphorus and potassium content in random microareas of *Acanthamoeba* cells

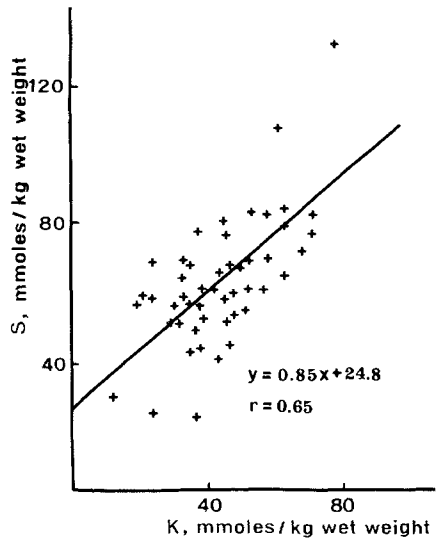

Fig. 3. Relationship between sulphur and potassium content in random microareas of *Acanthamoeba* cells

This part of cell is mainly occupied by glycogen particles and a microfilament network (Bowers and Korn 1968; Sobota et al. 1981). Therefore, the high content of phosphorus in ectoplasm region seems to be related to the presence of microfilaments which are known to contain one mole of ADP per mole of actin subunit, as well as to the presence of the phosphorylated forms of many contractile proteins (Kasai and Oosawa 1969; Perry 1979).

Such higher content of phosphate compounds in *Acanthamoeba* ectoplasm would cause the appearance of higher negative charges in this cellular region and potassium ions as positively-charged would be adsorbed/bound on/by these negatively-charged phosphate groups. A nonuniform distribution of potassium thus is observed in *Acanthamoeba* cells.

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