

SUBCELLULAR LOCALIZATION OF CALCIUM REPOSITORIES IN PLASMODIA OF THE ACELLULAR SLIME MOLD *PHYSARUM POLYCEPHALUM*

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Ultrastructural fibrils consisting of densely packed microfilaments, similar in size to the thin filaments of striated muscle, have been observed in the plasmodium of the acellular slime mold *Physarum polycephalum* (Rhea, 1966; Wohlfarth-Bottermann, 1964). A protein with sedimentation characteristics and ATPase activity typical of muscle myosin is also extractable from slime mold plasmodia (Hatano and Tazawa, 1968, Adelman and Taylor, 1969 b). Additional electron microscope observations made by Anderson (1964) and Wohlfarth-Bottermann (1964) described membrane-bounded vesicles in the vicinity of the microfilamentous bundles of *Physarum*.

This study represents an effort to determine whether these vesicles might serve as repositories

for calcium or other ions that might be involved in the regulation of mechanochemical activity in the microfilamentous bundles.

METHODS

Material

Physarum plasmodia were grown initially from sclerotia by culturing in a moist chamber on filter paper sprinkled with Mother's Oats. When kept in darkness, the plasmodial stage spreads over the filter paper, doubling its mass about every 24 hr, as long as the food supply is maintained. Fresh, nongrowing cultures are established by transferring small pieces of plasmodia to 3% nonnutrient agar in Petri dishes.

Ultrastructure and Ultracytochemistry

Plasmodia were fixed in 5% glutaraldehyde and postfixed in 1% osmium tetroxide in cacodylate buffer, pH 6.5. The fixed material was embedded in Epon, sectioned on a Reichert ultramicrotome, and stained with uranyl acetate and lead citrate. Ultrastructural observations were made with an AEI EM 6B electron microscope. Unfixed frozen sections were also made with an International Electronic Research Corp. (Burbank, Calif.) cryostat. Some living specimens were treated with a 10 mM solution of sodium oxalate before fixation to induce calcium precipitation within organelles that had accumulated calcium ions.

Light microscope observations of the different regions of the plasmodium were made with the Zeiss polarizing and Nomarski differential interference microscopes. The specimens were mounted on a 0.2 mm-thick layer of agar and photographed with a 40 ×/0.85 NA achromat oil immersion objective.

Microprobe-Microanalyzer

Thick frozen sections of plasmodia which had previously been treated with sodium oxalate were viewed with a Cameca electron microprobe operated at 30 kv. X-ray spectral analyses were performed on oxalate deposits within a wavelength range of from 0.981 to 3.922 Å.

RESULTS

Microscopy

When living plasmodia were first treated with a solution of 10 mM sodium oxalate for 10 min at 25°C and subsequently fixed and observed with polarizing optics, the gelled ectoplasmic region would be differentiated from the rest of the cytoplasm by an abundance of light-scattering precipitates within it, clearly outlining endoplasmic channels which noticeably lacked the precipitates (Fig. 2). There was no precipitate formation in the agar substratum other than on the mucus tracks deposited by the advancing plasmodium.

Streaming was slowed by the application of sodium oxalate, but did not stop completely in all cases until after 2 hr of continuous treatment (Figs. 1 a and 1 b). Retraction and detachment from the substratum of the advancing margin also occurred during treatment with the oxalate solution.

Vesicles containing electron-opaque deposits were present only in sections of *Physarum* pre-

viously treated with sodium oxalate (Fig. 3). Treatment with oxalate, fixation, and dehydration did not cause noticeable osmotic deformation of the cytoplasmic organelles. The presence of filaments in association with cytoplasmic vesicles has been demonstrated by Rhea (1966) and Wohlfarth-Bottermann (1964). However, the filaments were not very well defined in specimens treated for electron microscope observation.

Microprobe Microanalysis

The results of the microprobe analyses of the oxalate precipitates in fixed frozen sections indicated a relative abundance of calcium with a very strong (K-alpha) primary X-ray emission and two strong secondary (K-beta) emissions (Fig. 4). The only other element present in concentrations above noise was potassium. Magnesium, which also forms a precipitate with oxalate and has been implicated in *Physarum* actomyosin superprecipitation (Hatano and Tazawa, 1968), was not detectable. When the probe was moved off the precipitate, the calcium and potassium signals fell to noise levels.

DISCUSSION

Electron microprobe analyses of oxalate-induced deposits in the slime mold, *Physarum polycephalum*, show clearly the presence of large amounts of calcium within membrane-limited vesicles.

Biochemical and cytological studies indicate that *Physarum* possesses proteins with physical and chemical properties similar to those of muscle actin and myosin (Hatano and Oosawa, 1966 a, 1966 b; Hatano and Tazawa, 1968; Adelman and Taylor, 1969 a, 1969 b). Thus, the localization of calcium stores in the plasmodium suggests a mechanism for control of the contractile process of the slime mold that resembles, to a certain extent, that of striated muscle, where a network of calcium-releasing and calcium-sequestering vesicles controls the levels of calcium available to the actomyosin-ATPase system.

The plasmodial vesicles could conceivably simultaneously release calcium through a depolarizing potential as is the case in striated muscle (Rhea, 1966). In fact, Kamiya and Abe (1950), Tauc (1953), and Kishimoto (1958 a) have demonstrated the existence of a cycle of bioelectric potentials superimposable on the cyclic pattern of cytoplasmic streaming with a slight

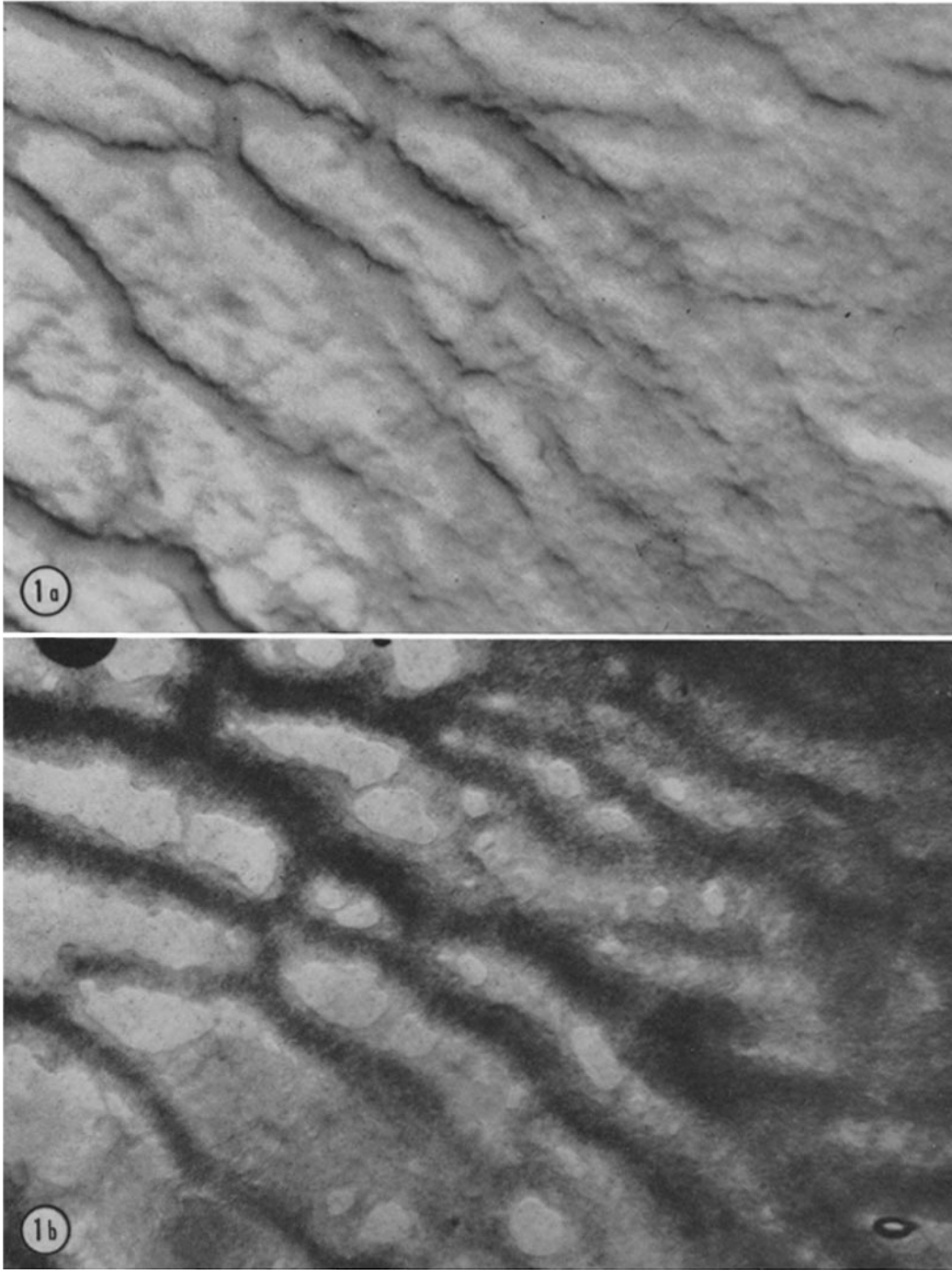


FIGURE 1a Light micrograph of normal, streaming plasmodium of *Physarum* showing large cytoplasmic channels. $\times 150$.

FIGURE 1b The same plasmodium after treatment with 10 mM sodium oxalate for 10 min. Precipitates of calcium oxalate have been deposited throughout the cytoplasm. $\times 150$.

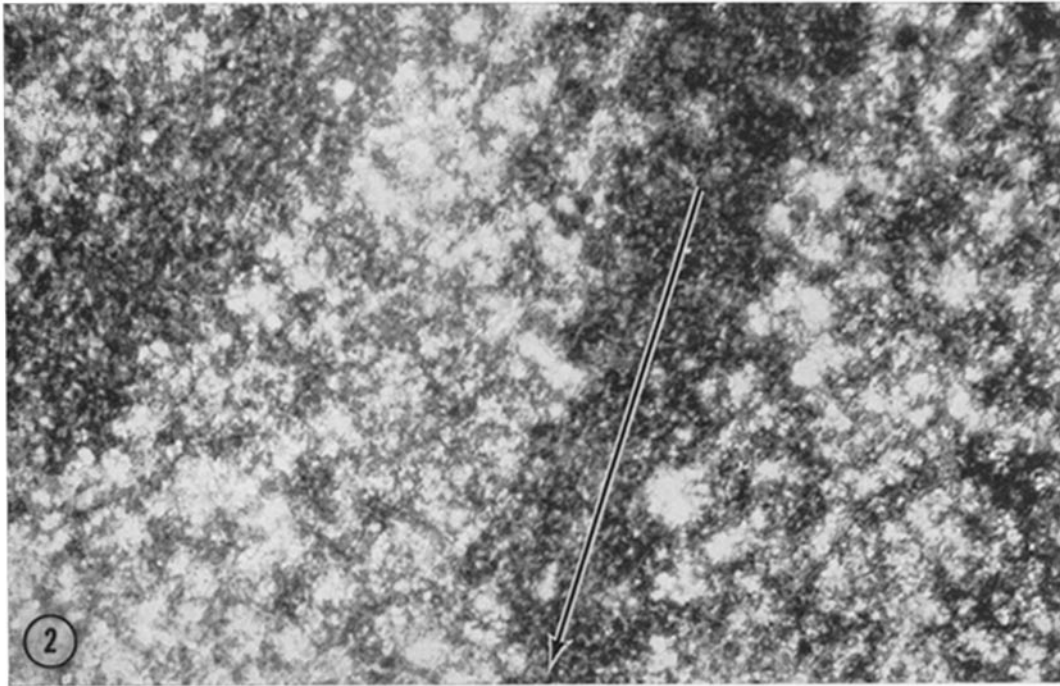


FIGURE 2 Polarized light micrograph of an oxalate-treated, fixed *Physarum* plasmodium. Large, light-scattering precipitates of oxalate are located within the ectoplasmic gel. The arrow points along the axis of one of the channels which remain visible because of the low distribution of light-scattering crystals within them. $\times 190$.

phase shift. Because the potential changes persist when streaming is stopped by applied physical force equal and opposite to the hydraulic pressure gradient in the channels, it is assumed that they are not a result of forces generated during streaming but a rather independent event related to the motive force for streaming (Kishimoto, 1958 b), which might possibly serve as triggers for vesicular release of calcium and thus regulate the levels of calcium in different regions of the plasmodium.

Electron micrographs of oxalate-treated plasmodia show calcium oxalate precipitates and a detectable concentration of potassium within some vesicles.

Anderson (1964) found a gradient in the concentration of labeled potassium (K^{42}) from the advancing front of a migrating plasmodial mass to the trailing end. His experiments indicated that the organism was constantly depositing the labeled potassium on the agar substratum as it advanced.

It is also probable that the vesicles which

accumulate calcium and potassium are the same vesicles which are involved in the redistribution and extracellular deposition of these two ions as observed by Anderson (1964).

One cannot overlook the possibility of calcium oxalate accumulation through surface membrane invagination. However, there was no evidence of membranous inpocketings typical of pinocytotic processes. Treatment of the agar substratum with 10 mM sodium oxalate did not result in calcium oxalate deposition in any way comparable to what is observed when the same treatment is applied to the plasmodium. The evidence overwhelmingly indicates that the vesicular precipitates represent internal calcium stores.

Plasmodia of *Physarum* possess calcium-containing vesicles which may be in close association with microfibrils (Wohlfarth-Bottermann, 1964). In this way, *Physarum* bears some resemblance to striated muscle, where a more elaborate system of vesicles controls contraction through a calcium-release and calcium-sequestering mechanism.

It remains to be shown that calcium is re-

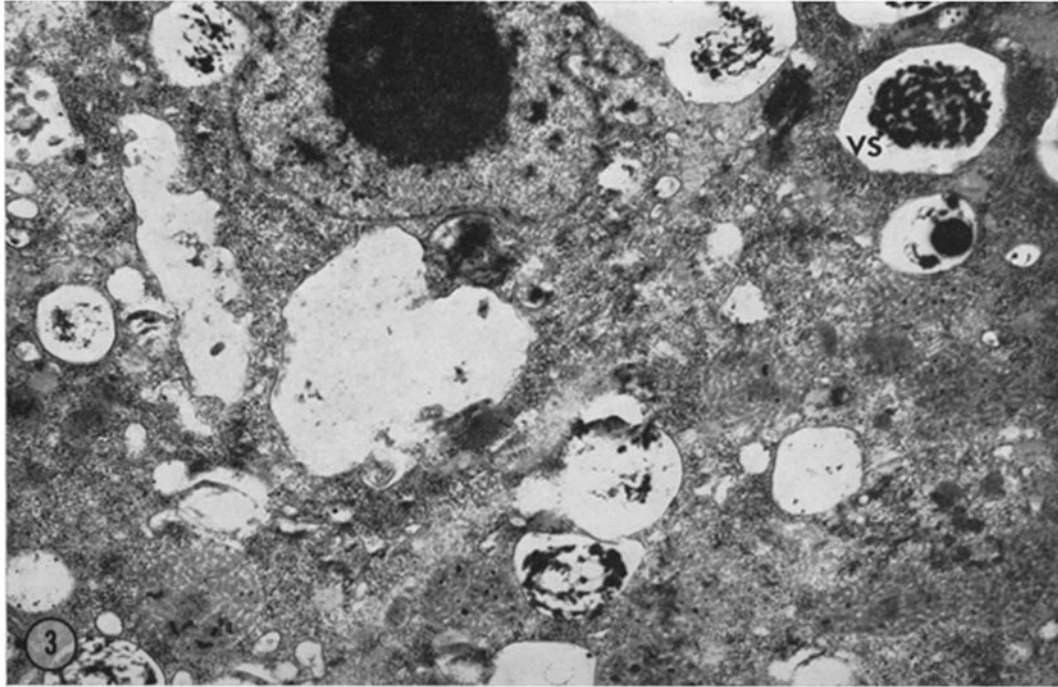


FIGURE 3 Region of a *Physarum* plasmodium treated with oxalate. Oxalate precipitates are seen in vesicles (Vs) which appear throughout the plasmodium. $\times 10,000$.

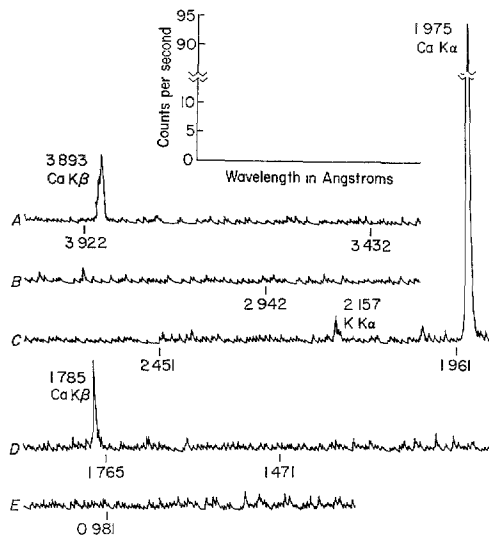


FIGURE 4 Continuous spectral readout (lines A through E) of a thick, frozen section of a *Physarum* plasmodium with the microprobe microanalyzer. Lines A and D show two strong secondary (K_{β}) emissions for calcium X-rays. Line C shows the primary (K_{α}) emission for calcium as well as a weak K_{α} emission for potassium.

leased from these plasmodial vesicles as part of the control of the plasmodial contraction cycle.

SUMMARY

Ultrastructural and cytochemical studies of the slime mold *Physarum polycephalum* show numerous membrane-limited vesicles which, when treated with sodium oxalate, accumulate an electron-opaque precipitate in the plasmodia

When analyzed with the electron microprobe, the precipitates are shown to be abundant in calcium, probably as calcium oxalate, with relatively small concentrations of potassium. It is likely that the vesicles, which have been shown by others to be often associated with fibrils, participate in the mechanochemical events associated with streaming in *Physarum* by regulating the concentration of free calcium in the plasmodium.

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