PLASMALEMMA INVAGINATIONS OF PHYSARUM DEPENDENT ON THE NUTRITIONAL CONTENT OF THE PLASMODIAL ENVIRONMENT

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SUMMARY

Quantitative estimates of plasmalemma invaginations in plasmodial veins of Physarum polycephalum were made under different conditions of nutrition. Pronounced differences were observed dependent on the nutritional content of the substrate. There was a decided increase in the number of plasmalemma invaginations in plasmodial veins grown on substrates containing absorbable food substances compared to veins migrating on non-nutrient substrates. This observation supports the proposition that the nutritional content, rather than the physical properties, of the substrate is the decisive factor for the formation of plasmalemma invaginations. The invaginations are believed to be concerned with the uptake of non-particulate food substances.

INTRODUCTION

Plasmalemma invaginations are characteristic constituents of the plasmodial stage of the acellular slime mould Physarum polycephalum (Wohlfarth-Bottermann, 1963; Rhea, 1966; Usui, 1971; Daniel & Jarlfor, 1972; Stiemerling & Stockem, 1975; Wohlfarth-Bottermann, 1974). The plasmodial stage is one of the vegetative stages of this organism. In general, plasmalemma indentations in eukaryotic cells are involved in the uptake of nutrients, transport of water and/or ions, extrusion of excrements, secretion of slime, as well as in other events, e.g. cell locomotion. Many cell types show an enlargement of cell surface area during increased cellular activity. This enlargement of the outer membrane can be provided by formation of pseudopodia or microvilli, i.e. extensions, or of invaginations of the membrane. In many cases plasmalemma invaginations are more or less permanent constituents of the cell (e.g. basal labyrinth in ion-transporting cells (Komnick, 1977)) but often they are temporary in nature, e.g. pinocytotic channels in amoeboae (Stockem, 1977).

In Physarum polycephalum, plasmalemma invaginations can be observed in all plasmodial forms, irrespective of their size or the nutrient substrate on which they grow (agar or filter paper). However, the number of invaginations as well as their volume show variations according to the environment, i.e. the substrate. The aim of the present work was to reveal a possible relationship between nutritional factors and the quantity and shape of the invaginations.
MATERIAL AND METHODS

Physarum polycephalum was grown in axenic cultures containing a semi-defined medium (SD-medium) (Daniel & Rusch 1961). Cultures on solid substrate were started by transferring axenically grown microplasmodia to wet filter paper and rolled oats (Camp, 1936), on which they were allowed to fuse to macroplasmodia. Parts of the macroplasmodium cultivated by this method were transferred to different substrates: (a) 1% agar without nutrients; (b) 1% agar containing yeast extract, tryptone, glucose and haemoglobin (SD-medium) (Daniel & Baldwin, 1964); (c) filter paper without nutrients; and (d) filter paper soaked with SD-medium.

Plasmodial strands were fixed in situ 16 h after transfer to agar with 1% OsO4 + 1% K2Cr2O7, pH 6.4–7.2 (Wohlfarth-Bottermann, 1957), dehydrated in a graded series of ethanol and embedded in styrene methacrylate.

For light-microscopic examinations, semithin sections of 2–4 μm thickness were cut on an LKB Ultrotome. No poststaining procedures were performed.

Morphometric measurements were performed by counting points of intersection of the plasma membrane with a lined screen (distance of lines, 5 mm) in 3 different directions (0°, 45°, 90°) on veins cut in cross-section (enlargement factor, 100 x) (Sitte, 1967). The results of the counting procedures were expressed in percentages.

RESULTS

All cross-sections of protoplasmic strands of Physarum polycephalum exhibit the typical differentiation into ectoplasmic tube and endoplasmic core. Within the ectoplasmic tube, the plasmalemma invaginations can be identified in low-magnification phase-contrast microscopic pictures. For morphometry, higher magnifications were employed (Figs. 1, 2).

The gross morphology of veins from 'normal', i.e. from non-nutritive agar, and from agar containing nutritive substances (SD-medium), is strikingly similar. Differences can be detected, however, when cross-sections of veins from different substrates are examined with respect to their plasmalemma invaginations.

To obtain relative estimates of the total area of invaginated plasmalemma, morphometric measurements were performed using a lined screen and a counting procedure based on intersection points (Sitte, 1967). The total number of intersection points of the lined screen with both the 'outer' plasmalemma (Pₒ) and all invaginated plasmalemma membranes (Pᵢ) was calculated (Pₒ + Pᵢ) and assigned a value of 100%. The percentage value of invaginated membranes (Table 1 A, B) was thus calculated.

Each xᵢ represents an average value derived from at least three measurements of one single vein, i.e. the values in Table 1 are based on a total of 15 veins and at least 45 different measurements. For morphological studies, an additional 30–40 veins were investigated. The morphometrically revealed differences between veins on nutrition-containing substrates and non-nutrition substrates are clearly corroborated morphologically (compare cross-sections depicted in Figs. 1 and 2).

Fig. 1. Unstained cross-section of a protoplasmic vein grown on non-nutritional agar. Phase-contrast: ec, ectoplasmic tube; en, endoplasmic core; arrows, plasmalemma invaginations.

Fig. 2. Unstained cross-section of a protoplasmic vein grown on agar containing SD-medium. Phase-contrast: Labelling as Fig. 1.
Plasmalemma invaginations of Physarum
DISCUSSION

As a possible explanation for the difference in appearance of plasmodial invaginations, Wohlfarth-Bottermann (1974) proposed a predominantly transporting type of vein on the one hand, and a nutrition-absorbing and excretory type of vein on the other hand. Analysing correlations between contraction-dependent ectoplasmic surface movements and endoplasmic flow phenomena, Hülsmann & Wohlfarth-Bottermann (1978) found further support for the existence of two vein types in one and the same plasmodium, one of them being engaged mainly in protoplasmic transport.

The present investigation shows a significant decrease in the invaginated surface area in those veins which are not involved in food uptake (e.g. in transporting veins). In contrast to this, veins on nutrient-rich substrates show a striking increase in the number of plasmalemma invaginations. On an average, in nutritional veins, 80% of the total plasmalemma area was invaginated, whereas in 'non-nutritive', i.e. transport veins, the corresponding value was 40%. These values indicate that a functional connexion exists between the extent of the invaginated plasmalemma area and nutritional activity of the plasmodial veins.

This view is supported by the fact that no corresponding difference was found between veins grown on SD-agar and those grown on filter paper soaked with the SD-medium. This means that differences in the number of plasmalemma invaginations are not, as could be argued, predominantly determined by the physical properties of the substrate, but by the availability of absorbable food substances.

Veins derived from starved plasmodia reduce their invaginations to small and narrow lacunae-like indentations, which are often difficult to detect (compare figs. 4 and 9 in Wohlfarth-Bottermann, 1974). Less than 40% of the total plasmalemma surface is invaginated into the cytoplasm (Fig. 1). In contrast to this, in absorbing veins (grown on nutritive agar) more than 80% of the plasma membrane is invaginated (Fig. 2).

The results of the morphometric measurements strongly favour the assumption that the invagination system of Physarum polycephalum is highly dynamic and

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**Table 1. Results of morphometric measurements:** A, veins on substrates containing nutritive medium; B, veins from non-nutritive substrates.

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Plasmalemma invaginations of Physarum

enables the plasmodium to adapt its surface to the varying conditions of the environment, and consequently to its own physiological state.

We conclude that the uptake of food substances in non-particulate form is favoured by an increase in the number of plasmodial invaginations. This is valid for the ectoplasmic tube of plasmodial veins. In this context it should be mentioned that the front of a migrating plasmodium represents a more or less compact protoplasmic sheet, i.e. the plasmodial area mainly responsible for food uptake does not initially appear to be differentiated in the form of veins. Light-microscopic investigation of this region, however, reveals that (a) compact protoplasmic sheets also contain endoplasmic channels (veins), and (b) according to previous investigations (fig. 5, Wohl- farth-Bottermann, 1974) these sheets do not represent homogeneous protoplasmic layers, but also contain, as do ectoplasmic tubes of the veins, a well developed system of plasmalemma invaginations.

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REFERENCES


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