Regional Difference in Oscillatory Characteristics of *Physarum* Plasmodium as Revealed by Surface pH

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**Abstract.** The surface pH of a *Physarum* plasmodium advancing on an agar plate with a definite antero-posterior polarity was measured with a small antimony pH combination electrode. There was a clear pH gradient over the plasmodium surface from the advancing front (pH 6.6±0.1) to the rear (pH 5.6±0.4), whereas the surface pH of freshly formed endoplasmic drops was 6.9±0.3. In addition to this regional pH difference, an oscillation of pH was detected over the entire plasmodium surface. In the front zone this oscillation did not disappear even when a small area surrounding the electrode tip was disconnected from the rest of the plasmodium, but in the strand region the oscillation disappeared as soon as the strand was disconnected near the electrode tip from the rest of the plasmodium. It took 10–20 min before the oscillation began again in the disconnected strand. This shows that the active site of oscillation is only in the front region and that the rest of the plasmodium is somehow under the control of the front part.

An acellular slime mold advancing on the surface of a solid substratum shows various regional differences according to its antero-posterior polarity; these include its morphological and cytoskeletal organization (3, 5, 6), cation content (1) and its physiological functioning (10). Recently we measured the surface pH of the plasmodium by developing a suitable antimony electrode (7, 8). Here we consider the regional characteristics of *Physarum* plasmodium in terms of surface pH.

Plasmodia of *Physarum polycephalum* (strain Ng-1) were cultured by a slightly modified version of Camp’s method (2), as described (7). The surface pH of the plasmodium was measured with a specially designed small antimony combination electrode (8, 9). This electrode eliminates possible differences in local membrane potentials and allows the detection, with a sufficiently high time resolution, of just the pH of the slime layer on the surface of the plasmodium.

At the front edge of a plasmodium of about 20 cm in length advancing on a solid substratum with a definite antero-posterior polarity, the average surface pH was 6.6±0.1 (for 5 specimens). However, the surface pH decreased posteriorly. In the region 2–3 mm from the advancing edge, the surface pH was 6.0±0.5 (for 8 specimens); in the network and dendrite regions behind, it was 5.6±0.4 (for 5 specimens). The pH of the slime left behind the rear terminal region was 5.0±0.6. Slight differences in the absolute level of the surface pH were observed among specimens, but a definite pH gradient was invariably found on the surface of an advancing plasmodium from its anterior to posterior region. The pH gradient was steep (ca.
0.2 pH/mm) in the front zone, but this gradient became much more gentle (ca. 0.04 pH/cm) in the network region.

When the plasmodium was pricked with a needle, its endoplasmic sol spurted out and formed a drop. The surface pH of these endoplasmic drops was 6.9±0.3 (for 12 specimens), which coincided well with the cytoplasmic pH (7). This pH value of the drop did not differ significantly with the region of the plasmodium where the drop was obtained.

Besides the regional pH difference, an oscillation of the surface pH with a period of about 2 min and a peak-to-peak amplitude of less than 0.1 pH unit was immediately noticeable when the antimony pH electrode came in light contact with the area 2-3 mm behind the advancing edge. This oscillation was not disturbed even when an area of about 5 mm×5 mm around the electrode tip was dissected to disconnect it from the surrounding area (Fig. 1). Thus this area contains an independent oscillator. The descending trend of the wave train may be explained by the increase in acidity
due to the rearward shift of the electrode relative to the plasmodium as the plasmodium migrated forward, although the electrode itself remained stationary relative to the agar substratum.

When the antimony electrode was placed in contact with the surface of the plasmodial strand forming the rear network region of the plasmodium, an oscillation in pH was detected just as in the case of the front region. If the strand was disconnected from the rest of the plasmodium about 5 mm from the electrode, however, this oscillation immediately stopped, and then re-commenced about 10–20 min later (Fig. 2). This may mean that the rear strand region does not oscillate spontaneously when it constitutes the rear part of the whole, but has the potential to oscillate if isolated. When a part is isolated from the plasmodium, some time may be necessary for it to reorganize itself into an independent plasmodium.

Our observations agree with those of Yoshimoto and Kamiya (10), who showed that the active site of the contraction-relaxation oscillation in the advancing plasmodium is restricted to its anterior region. This conclusion was based on tensiometric experiments. They removed a slender, rectangular piece of cytoplasmic gel and compared its contractile properties with those of a strand segment isolated from the network region of the same plasmodium. The anterior piece began to contract and relax without an appreciable lag phase after it had been excised, while the segment of the strand from the network behind the advancing front showed no significant oscillation until after a lag period of about 10–20 min.

Using a sensitive polarizing microscope, Kamiya (4) showed that birefringence of the cytoplasmic fibrillar structure (bundles of F-actin) in the anterior zone of a fan-like expanse of the plasmodium changes cyclically, so that the birefringent fibrils appear clearly when the endoplasm moves away from the front (contracting phase) and then almost disappear in the opposite phase (relaxing phase). The rear network region has many strongly birefringent fibrils, but they show little cyclic change (unpublished data).

These earlier observations indicated the existence of a regional difference in the organization of the plasmodial body when it advances in one direction with antero-posterior polarity, and are consistent with the present observations. The fact that oscillation of the surface pH on the strand ceases when it is disconnected from the frontal region suggests the importance of the shuttle endoplasmic streaming as the carrier of a putative oscillation-inducing factor or factors from the front.

REFERENCES


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