

MAGNETOGRAVIPHORESIS OF STATOLITHS AND ASSESSMENT OF VISCOELASTICITY OF THE *CHARA* CYTOPLASM

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INTRODUCTION: The viscosity of the cytoplasm is crucial for many cellular functions but has not been reliably measured. We measured *in vivo* the viscoelastic properties of the cytoplasm by inducing movements of statoliths [1-6, 14] (BaSO₄-containing vesicles [13]) by ponderomotive forces in High Gradient Magnetic Fields (HGMF) and gravity [7-11].

MATERIALS & METHODS: Statoliths are denser and more diamagnetic than the cytoplasm. They can be displaced by HGMF with a dynamic factor $grad(H^2/2)$ of up to $1.9 \cdot 10^{10}$ Oe²/cm in a custom experimental setup. We measured the HGMF-induced forces on statoliths using magnetograviphoresis of extracted statoliths. For this purpose five rhizoid cells were placed in a 25 μ l drop of aqueous 0.01% SDS and the statoliths were released by severing the rhizoid tip with a scalpel. The density and viscosity of the SDS solution differed from that of distilled water by less than 0.5%. The resulting suspension was collected into a 100 μ m ID capillary. The shear forces during uptake separated the statoliths from

cytoplasmic residue. The capillary was sealed at both ends and positioned between magnetic poles. The dynamic factor of the magnetic field was either zero or $1.9 \cdot 10^{10}$ Oe²/cm. The movement of the particles in the capillary was video-recorded and analyzed by a custom analysis program from the video signal. Individual statolith trajectories were used to calculate their velocity.

Tips of actively growing *Chara* rhizoids were positioned near the upper edge of the same 175 μ m gap between magnetic poles that were used for *in vitro* measurements (Fig. 1). The movements of individual statoliths inside the cells in HGMF and gravity were measured.

RESULTS & DISCUSSION: Based on the sedimentation and the upward velocity in the presence of a HGMF, the difference in magnetic susceptibilities and densities between the extracted statoliths and medium (χ/\bar{n}) was calculated to be $1.3 \pm 0.2 \cdot 10^{-7}$ emu/(g/cm³). This value corresponds to the data for BaSO₄ particles, suggesting that the statoliths contain a significant amount of BaSO₄. The force induced by a magnetic gradient of $1.9 \cdot 10^{10}$ Oe²/cm on *Chara* statoliths was estimated to be 2.4 times stronger, than the gravity force acting on them. The apparent cytoplasmic viscosity in intact rhizoids was about 0.1 Poise, comparable with previous estimations [15]. The contribution of the actin cytoskeleton to cytoplasmic viscoelasticity was assayed before and after application of the actin depolymerizer Latrunculin-B. This drug caused cessation of growth, sedimentation and partial clumping of statoliths. Statoliths in Latrunculin-treated rhizoids can be displaced by HGMF significantly higher than in intact rhizoids (Fig. 2). Analysis of individual statoliths movement indicated 40% increase in apparent cytoplasmic viscosity but elimination of elasticity.

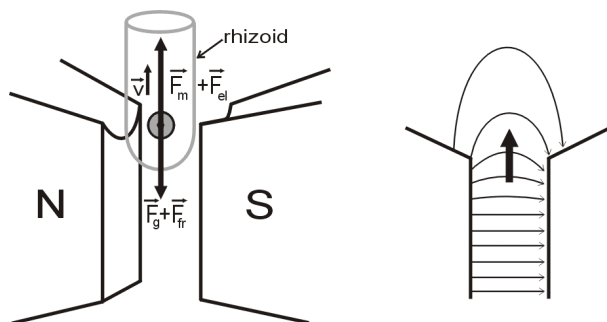


Fig. 1. Schematic representation of the magnetic system used for intracellular magnetophoresis of statoliths in *Chara* rhizoids (left). The statoliths are affected by gravity (F_g), and by the elastic force (F_{el}) of the cytoskeleton. Near the upper edge of the gap diamagnetic statoliths will be pushed up by the ponderomotive magnetic force (F_m). Moving statoliths also experience viscous frictional force (F_{fr}). The distribution of the magnetic field in the gap between the magnetic poles is shown on the right. The bold arrow indicates the direction of the ponderomotive force acting on diamagnetic statoliths.

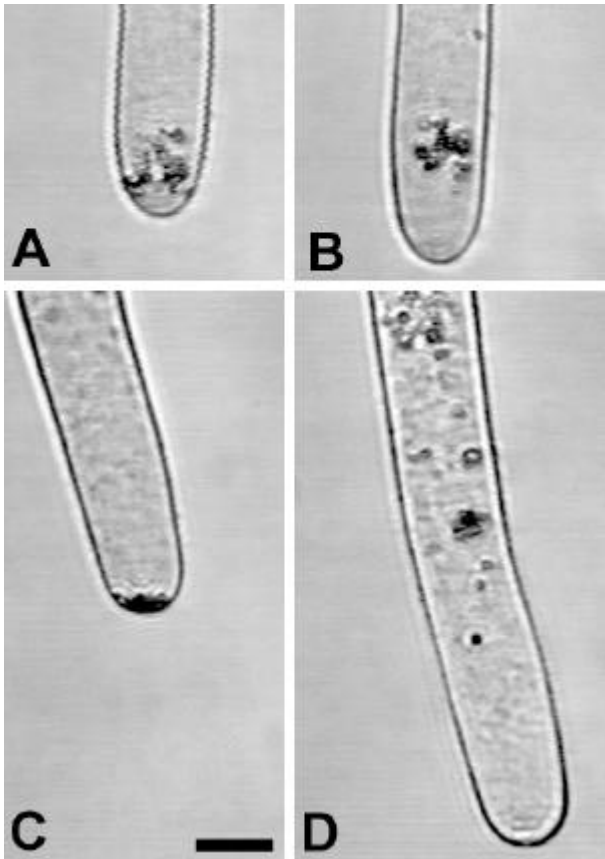


Fig. 2: Vertical displacement of statoliths in HGMF. The initial distribution in untreated rhizoids shows statoliths at a discrete distance from the apex (A). After 12 minutes of HGMF application the statoliths moved upward (B). Longer application of the field did not substantially increase the displacement. Statoliths in a rhizoid treated with 1 μ m latrunculin B sedimented to the rhizoid apex and the rhizoid stopped growing (C). In such rhizoids a much greater displacement of statoliths was possible (D). Bar = 25 μ m.

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