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Gravitaxis of *Euglena gracilis* depends only partially on passive buoyancy

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Abstract

In darkness, the unicellular freshwater flagellate *Euglena gracilis* shows a pronounced negative gravitactic behavior, and the cells swim actively upward in the water column. Up to now it was unclear whether this behavior is based on a passive (physical) alignment mechanism (e.g., buoyancy due to a fore-aft asymmetry of the cell body) or on an active physiological mechanism. A sounding rocket experiment was performed in which the effect of sub-1*g*-accelerations (0.05, 0.08, 0.12, and 0.2*g*) on untreated living cells and immobilized (fixation with liquid nitrogen) cells was observed. By means of computerized image analysis the angles of the cells long axis with respect to the acceleration vector were analyzed in order to calculate and compare the reorientation kinetics of the immobilized cells versus that of the controls. In both groups, the reorientation kinetics depended on the dose, but the reorientation of the living cells was about five times faster than that of the immobilized cells. This indicates that in young cells gravitaxis can be explained by a physical mechanism only to a small extend. In older cultures, in which the cells often have a drop shaped cell body, the physical reorientation is considerably faster, and a more pronounced influence of passive alignment caused by fore/aft asymmetry (drag-gravity model) can not be excluded. In addition to these results, *Euglena gracilis* cells seem to respond very sensitively to small accelerations when they are applied after a longer microgravity period. The data indicate that gravitactic orientation occurred at an acceleration as low as 0.05*g*.

Keywords: *Euglena gracilis*; Gravitaxis; Motion analysis; Sounding rockets; Fore/aft asymmetry; Buoyancy

1. Introduction

The unicellular freshwater flagellate *Euglena gracilis* flagellate orients itself in the water column by means of phototaxis (orientation away (negative phototaxis) or toward (positive) a light source) and gravitaxis (movement away (negative) or toward (positive gravitaxis) the center of gravity). Recently the photoreceptor for photoorientation was identified (Iseki et al., 2002; Ntefidou et al., 2003a,b). In contrast, the mechanism of gravitaxis is still not completely understood. Formerly, it was proposed that gravitaxis is a phenomenon which is solely based on a passive physical mechanism like e.g., buoyancy based on a fore-aft asymmetry (Brinkmann, 1968; Roberts, 1970). Passive gravitactic alignment of the cells could be explained by the gravity-buoyancy model and the gravity-drag model (Machemer and Bräucker, 1992; Mogami et al., 2001; Roberts, 1970; Roberts and Deacon, 2002). Both models predicted that the center of mass is not coincident with the geometrical center of the cell (centroid). While gravitactic alignment in the gravity-buoyancy model is based on unequal mass distribution inside the cell (e.g., different density of the cytoplasm between front and rear end) it is caused by fore-aft asymmetry of the cell shape (e.g., rear end thicker than the front end) in the gravity-drag model. The thicker end of the cell sinks faster because of the fluid mechanic of small particles with low Reynolds numbers (Roberts and Deacon, 2002). Later it was found that gravitaxis is impaired by UV radiation or in the presence of heavy metals, which was a first hint that gravitaxis is

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possibly driven by a physiological sensor. It was also found that gravitaxis changes during the life cycle and shows pronounced diurnal changes in synchronized cultures. Young cultures normally show positive gravitaxis (downward swimming) and older a negative one. Synchronized cultures show pronounced negative gravitaxis during the light period and a very much reduced or even no gravitaxis in the dark period. Negative gravitaxis often converts into a positive one when exposed to environmental stressors like high light radiation or increased salinity (Richter et al., 2002b, 2003a,c). This phenomenon is probably triggered by reactive oxygen species. Research over the past few years revealed that gravitaxis is most likely based on complex physiological mechanisms in which calcium (Richter et al., 2001b, 2003b), cAMP (Streb et al., 2002; Tahedl et al., 1998) and possibly the membrane potential are involved (Richter et al., 2001a). The subsequent elements in the assumed signal transduction chain have not yet been identified.

While it is unlikely that passive buoyancy is exclusively responsible for gravitactic alignment of the cells, it possibly contributes to gravitactic orientation. In earlier experiments the angle of the long axis of cell immobilized with liquid nitrogen was analyzed during sedimentation. The data showed that in the cell shape seems to play an important role in gravitactic alignment (Richter et al., 2002c). On the other hand it was also shown that cells, which did not show gravitaxis, aligned their cell long axis parallel to the vector of acceleration within a relatively short time. These observations indicated that cell shape and gravitaxis are not necessarily linked with each other. To quantify the contribution of cell shape in gravitaxis, a sounding rocket experiment was performed, which supplied about 6 min of microgravity. During this time the reorientation kinetics of living and immobilized cells under sub-1*g*-acceleration (achieved by a centrifuge) was monitored.

2. Materials and methods

2.1. Cell growth

Euglena gracilis Z was obtained from the collection of algal cultures at the University of Göttingen (Schlösser, 1994). The cells were grown in a mineral medium as described earlier (Checcucci et al., 1976; Starr, 1964) in stationary cultures in 100-ml Erlenmeyer flasks at about 20 °C under continuous light of about 18 W m^{-2} from mixed cool white and warm tone fluorescent lamps. The culture age was about 3 weeks.

2.2. Space experiment

The experiments were performed during the sounding rocket campaign TEXUS 40 in spring 2003. All experiments were carried out with cells from the same culture. A fraction of the cell culture was immobilized by means of liquid nitrogen. Aliquots (about 500 µl) of the cell culture were filled into Eppendorff tubes and transferred into a Dewar vessel with liquid nitrogen. The rapid fixation resulted in a very well conserved cell shape. The samples were thawed and filled into two custom-made glass cuvettes with a frame of stainless steel (Astrium, Bremen, Germany). The diameter of the cuvette was 50 mm and the depth 100 µm. Shortly before take off (late access) the cuvettes were mounted on modified microscopes bolted on a centrifuge stage inside the payload of the rocket (Skylark rocket engine). The centrifuge allowed the application of sub-1*g* accelerations (0.05, 0.08, 0.12, and 0.2*g*) during the microgravity phase of the experiment. After lift off the engine accelerated the rocket with 9*g* for about 90 s. After burnout the payload was separated from the engine and flew a steep parabolic curve to about 100 km height. The total microgravity time was about 367 s. The video images of the cells (see Section 2.4) were down-linked to the ground by means of telemetry. The experimenters on ground could control the rotation speed of the centrifuge.

2.3. Acceleration profile during microgravity

The acceleration profile during the microgravity phase achieved by centrifugation was as follows: Lift off: 70 s 9*g*, µg 35 s, 0.05*g* 79 s, 0.08*g* 71 s, 0.12*g* 94 s, and 0.2*g* 88 s. Between the subsequent acceleration steps the cuvettes were shortly rotated to avoid accumulation of cells due to gravitactic stimulation from the previous acceleration step.

2.4. Motion analysis

The swimming cells were recorded by a 4× objective in dark field mode by a b/w CCD camera. Illumination was obtained from a 50 W halogen lamp. The monitoring light was filtered by an RG 715 filter (Schott & Gen., Mainz, Germany) to prevent induction of phototactic and photophobic responses. The immobilized cells were recorded by means of a color camera. The video signals were stored on a video recorder (Philips VR948/02M, The Netherlands) and analyzed later by means of a custom-made image analysis system (WinTrack 2000, Real Time Computer, Möhrendorf, Germany). Subsequent video frames were digitized with a Matrox Meteor II analog/digital converter (Matrox, Quebec, Canada) in an IBM-compatible computer (3 GHz Pentium II, 1024 MB RAM). The program used is written in Visual Basic 6.0 (Microsoft, Redmond, USA). A general cell tracking algorithm (Häder and Lebert, 1985; Häder and Vogel, 1991) is used to detect and follow cells in a given sequence of images. Cells can be tracked either for a short period or as long as they are in the field of view. Time, position, speed, orientation, area and form factor for each movement vector, at a maximum time resolution of 40 ms, are stored and can be used for further analysis. Additionally, a procedure was developed which determines the orientation of the length axis of the cells. The software calculates rectangles around each cell,