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Effect of jasplakinolide and cytochalasin D on cortical elements involved in the gliding motility of the eugregarine *Gregarina garnhami* (Apicomplexa)

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Abstract

Since apicomplexans represent exclusively parasitic unicellular organisms with medical and economic impacts, the principles of their motility have been studied intensively. By contrast, the movement in apicomplexan basal groups, such as gregarines, remains to be elucidated. The present study focuses on *Gregarina garnhami* parasitising the digestive tract of the locust *Schistocerca gregaria*, and investigates the involvement of cytoskeletal elements (the ectoplasmic network and myonemes) and the secretion of mucosubstances during eugregarine gliding motility. Combined microscopic analyses were used to verify the role of actin filaments and membranes' organisation in *G. garnhami* motility. A freeze-etching analysis of membranes revealed the size, density, and arrangement of intramembranous particles along with the distribution and size of pores and ducts. Experimental assays using actin-modifying drugs (jasplakinolide, cytochalasin D) confirmed that actin most likely plays a role in cell motility, principally in its filamentous form (=F-actin). Myonemes, localised in the border between the ectoplasm and endoplasm, correspond to the concentric bundles of F-actin. Microscopic analyses confirmed that changes in gamonts motility corresponding to the changes in the organisation and density of myonemes and the ectoplasmic network in drug-treated cells, suggesting that these structures might serve as contractile elements facilitating gliding motility in *G. garnhami*.

Keywords: Ectoplasmic network; F-actin; Gregarine; Motility; Myonemes; Ultrastructure

Introduction

Gregarines are a highly diversified, basal lineage of eukaryotic unicellular organisms belonging to the parasitic group Apicomplexa. Eugregarines are widespread in marine, freshwater, and terrestrial hosts and their development is traditionally considered to be restricted to invertebrate hosts (Schrével and Desportes 2015).

The gregarine pellicle comprises a plasma membrane beneath which an inner membrane complex (IMC), consisting of two closely apposed cortical cytomembranes, is located. The pellicle covering the surface of intestinal

Abbreviations: CLSM, confocal laser scanning microscopy; CYT D, cytochalasin D; DMSO, dimethyl sulfoxide; EF, exoplasmic fracture face; FE, freeze-etching; IMC, inner membrane complex; IMP(s), intramembranous particle(s); JAS, jasplakinolide; Kp, partition coefficient; LM, light microscopy; PBS, phosphate buffered saline; PF, protoplasmic fracture face; SD, standard deviation; SE, standard error; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TRITC, tetramethylrhodamine isothiocyanate.

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eugregarines creates numerous epicytic folds arranged in longitudinal lines separated by grooves (Schrével and Desportes 2015). The organisation of these folds shows an undulating pattern in some species (Valigurová and Koudela 2008; Valigurová et al. 2013; Vávra and Small 1969; Vivier 1968). The usually dilated tips of the epicytic folds comprise 12 nm filaments and ripple dense structures, thought to have a supportive scaffolding function or possibly representing a component of the motility motor (Kováčiková et al. 2017; Schrével et al. 1983; Valigurová et al. 2013; Walker et al. 1984).

In comparison to apicomplexan invasive stages (zoites), in which gliding motility is defined as substrate-dependent and facilitated by an actomyosin motor associated with their pellicle (Heintzelman 2015; Kappe et al. 2004; Keeley and Soldati 2004; Matuschewski and Schüler 2008; Sibley et al. 1998), the exact mechanism of motility in gregarines still remains to be elucidated (Valigurová et al. 2013). Intestinal eugregarines are usually capable of unidirectional progressive gliding with or without obvious changes of cell shape (King 1981, 1988; Kováčiková et al. 2017). The extrusion of a mucous material left to trail behind the gliding gregarines suggests this material to be a part of the gliding machinery (Mackenzie and Walker 1983; Valigurová et al. 2013; Walker et al. 1979). Ultrastructural analysis revealed diverse cortical filamentous structures (e.g. myonemes, ectoplasmic network), assumed to be involved in the motility and cell contraction (Beams et al. 1959; Hildebrand 1980; Valigurová et al. 2013; Walker et al. 1979). A more recent study on *Gregarina* representatives, comprising biochemical analysis, showed these ectoplasmic myonemes to be actin and myosin rich (Heintzelman 2004). Both proteins were also found in the gregarines' cortex (cell envelope comprising the three layered pellicle and associate cytoskeletal structures), following the pattern of longitudinally arranged epicytic folds (Ghazali et al. 1989; Ghazali and Schrével 1993; Heintzelman 2004; Valigurová et al. 2013). Nevertheless, only a few experimental studies have been performed to verify the role of actin filaments in eugregarine gliding motility, using cytoskeletal drugs such as jasplakinolide (inducing actin polymerisation) and cytochalasins (blocking the association or eventual dissociation of actin subunits) (King 1988; Valigurová et al. 2013; Walker et al. 1979).

The present study provides a complex microscopic analysis of the cell cortex in the eugregarine *Gregarina garnhami* (Gregarinidae Labbé, 1899) parasitising the intestine of the desert locust *Schistocerca gregaria* (Forskål, 1775) (Orthoptera, Acrididae). Using the combined approaches of light, electron and confocal microscopy, supplemented by experimental motility assays, we focused on structures that appear to be responsible for gliding motility in *G. garnhami*. A gliding force derived from the putative actomyosin system was already proposed by King (1988); however, the exact mechanism involved in eugregarine motility is still poorly characterised (Heintzelman 2004; Schrével and Desportes 2015; Valigurová et al. 2013). The main purpose of this study was to perform experimental analyses comparable to those published on other apicomplexan species, to evaluate newly acquired data using different microscopic techniques and to link these data with already known fragmentary information about motility and related structures in *G. garnhami*. We have chosen this species for experimental purposes because it represents a model parasite from a widely available laboratory insect. Data obtained on this species is therefore verifiable and suitable for comparison with data published on other *Gregarina* spp. (e.g. Heintzelman 2004; Valigurová et al. 2013). For the first time, gamonts of *G. garnhami* were experimentally treated with jasplakinolide and cytochalasin D to observe their effect on eugregarine survival, motility and changes in cortical filaments of actin nature (myonemes and ectoplasmic network). Phalloidin, in comparison to actin antibodies, specifically binds to F-actin and provides the proof of drug-induced changes in organisation of actin filaments. This study also deals with a drug-induced changes of epicytic folds, the undulating pattern of which remains preserved, and with the role of mucus in gregarine gliding. Detailed FE analysis of gregarine cortex facilitated the visualisation and identification of structures that are difficult to observe under TEM (e.g. ectoplasmic network) and allowed us to distinguish between the types of pores. Additionally, complex statistical analysis of size/distribution of IMPs and pores' diameters was performed and compared with other *Gregarina* representatives (personal non-published data obtained during previous studies).

Material and Methods

Gamonts of *G. garnhami* were collected from the midgut and caeca of *S. gregaria* (Insecta, Orthoptera). After narcosis, decapitation, and dissection of the host, parasites were isolated from the host intestine using Ringer's saline solution (0.75% [w/v] NaCl, 0.035% [w/v] KCl, 0.021% [w/v] $CaCl_2$; pH = 7.2). Gregarine gamonts were then transferred to embryo dishes and carefully washed with Ringer's solution. The manipulation and observation of parasites were performed using an Olympus SZX7 stereomicroscope (Olympus).

For experimental assays, gregarines were divided among four embryo dishes with a 30 mm cavity. Afterwards, the parasites were treated with commercial membrane-permeable drugs influencing the polymerisation of actin: jasplakinolide (JAS, Invitrogen, Czech Republic) and cytochalasin D (CYT D, Invitrogen, Czech Republic). Both drugs were reconstituted in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Czech Republic) to prepare a 1 mM stock solution and, prior to each experimental assay, diluted in Ringer's saline to prepare a working solution with a final concentration of 30 μM. For controls and each experimental assay with cytoskeletal drug, approximately 200 individuals of *G. garnhami* were used. The monitoring of parasite motility was performed hourly using a stereomicroscope. After 7 h of each experi-