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ScienceDirect

European Journal of Protistology 50 (2014) 248–257

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Further investigations on the polypeptides and reconstitution of prasinophycean ejectisomes

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Received 6 January 2014; received in revised form 25 February 2014; accepted 27 February 2014
Available online 18 March 2014

Abstract

Ejectisome fragments were isolated from the prasinophyte *Pyramimonas grossii* and subjected to different treatments, i.e. Percoll density gradient centrifugation, incubation at pH 2.5 or at pH 10.8, or incubation in 6 M guanidine hydrochloride. Sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed that Percoll density gradient centrifugation did not improve the purity of the ejectisome fragment-enriched fractions. The ejectisome fragments withstood pH 2.5 and pH 10.8 treatment, and no loosely bound polypeptides became detached. The disintegration of ejectisome fragments was achieved in 6 M guanidine hydrochloride, and reassembly into filamentous, ejectisome-like structures occurred after dialysis against distilled water. Fractions enriched either in ejectisome fragments or in reconstituted ejectisome-like structures were dominated by three polypeptides with relative molecular weights of approximately 12.5–19 kDa and two additional polypeptides of 23 and 26 kDa. A polyclonal antiserum directed against an ejectisome fragment-enriched fraction weakly cross-reacted with these polypeptides, and no significant immuno-labelling of ejectisome fragments was registered. A positive immuno-label was achieved using immunoglobulin (IgG) fractions which were gained by selectively incubating nitrocellulose stripes of these polypeptides with the antiserum.

Keywords: Ejectisome; IgG fractions; Prasinophyte; *Pyramimonas grossii*; Reconstitution experiments; Transmission electron microscopy

Introduction

Prasinophytes comprise a heterogeneous group of primitive, unicellular green algae. Beside motile, flagellated, scale-bearing genera, non-motile coccoid genera without body scales have been described. Phylogenetic analyses of SSU rRNA sequences of 13 prasinophyte species representing eight genera revealed four independent clades with the phylogenetic position of *Pycnococcus provasoli* being unresolved (Nakayama et al. 1998).

Based on ultrastructural data, six subgenera are actually believed to constitute the genus *Pyramimonas*, i.e. *Pyramimonas* McFadden, *Vestigifera* McFadden, *Trichocystis* McFadden, *Punctatae* McFadden, *Hexactis* Hori, Moestrup et Hoffman, and *Macrura* Hori et Moestrup (Hori et al. 1995; McFadden et al. 1986, 1987; Pienaar and Sym 2002). A common feature of species of the subgenera *Trichocystis* and *Hexactis* is that they harbor projectile organelles, i.e. ejectisomes which closely resemble R-bodies of several bacteria as both are built up by single coiled ribbons composed of protein. More complex ejectisomes have been described for cryptophytes and kathablepharids (Clay and Kugrens 1999a,b; Kugrens et al. 1994; Okamoto and Inouye 2006; Okamoto et al. 2009; Santore 1985; Vørs 1992; Wehrmeyer 1970; and references cited therein).

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http://dx.doi.org/10.1016/j.ejop.2014.02.001
0932-4739/

The ejectisomes of the prasinophyte *Pyramimonas grossii* have been first described by Manton (1969). Non-discharged ejectisomes were located at the cells anterior ends and consisted of coiled ribbons which measured 5 nm in thickness and 500 nm in width. After discharge, the ejectisomes formed left-handed, single-coiled hollow tubes which measured up to 35 μm in length and 100 nm in width. Morrall and Greenwood (1980) investigated the ejectisomes of *Pyramimonas parkeae* and found that non-discharged coils exhibited ribbon-like layers with thicknesses of 4.9–5.5 nm. Each ribbon consisted of highly ordered subunits which were obvious as light and dark substructures. The authors measured subunit intervals of 2.8–3.3 nm for both, non-discharged and discharged ejectisomes. These data have been confirmed in a recent study (Rhiel et al. 2013).

In a previous study, the ejectisomes of three cryptophyte species have been isolated and the first data on the constituting polypeptides were presented. Furthermore, the chemical stability of cryptophycean ejectisome fragments was investigated, and they were successfully isolated by Percoll gradient centrifugation. Afterwards they were disassembled and reconstituted by treatment with guanidine hydrochloride followed by dialysis. Finally, an antiserum against the polypeptides was raised which immuno-labelled discharged ejectisomes of the cryptophytes (Ammermann et al. 2013). Investigations on the stability of the ejectisomes of *Pyramimonas grossii* have not been performed, and Percoll gradient centrifugation was not applied in our previous study (Rhiel et al. 2013). Additionally, no antiserum had been raised against the constituting polypeptides, and no dissociation/reconstitution experiments had been performed. Therefore, the current work focuses on the following three topics. First, the protocol for the isolation of discharged ejectisomes of *Pyramimonas grossii* was modified, and Percoll density gradient centrifugation and pH treatments were applied. Second, experiments with guanidine hydrochloride were performed to see if ejectisomes of *Pyramimonas grossii* could be successfully disintegrated and afterwards reassembled. Third, an antiserum was raised against a fraction enriched in ejectisome fragments of *Pyramimonas grossii* and used in immunogold-labelling electron microscopy experiments.

Material and Methods

Cultures and growth conditions

The prasinophyte *Pyramimonas grossii* was obtained from the Scandinavian Culture Collection of Algae and Protozoa (SCCAP, University of Copenhagen, Denmark, strain-no. K-0253) and was cultured as described by Rhiel et al. (2013).

Isolation of ejectisome fragments and sodium dodecyl sulfate (SDS)-solubilisation

The isolation and SDS-solubilisation of ejectisome fragments followed the protocols outlined by Rhiel et al. (2013) with some modifications. The cells (200–250 ml culture volume, 7–10 d culture age) were harvested by centrifugation at 3220 g for 15 min in an Eppendorf 5810R refrigerating centrifuge equipped with an A-4-62 swinging bucket rotor (Eppendorf, Hamburg, Germany). The cells were resuspended in 9 ml buffer (5 mM Tris/HCl, pH 7.5) and lysed by adding 1 ml of 10% (v/v) Triton X-100 (1% final concentration). The insoluble material consisting of contaminating bacteria, starch grains, body scales, and ejectisome fragments was pelleted from the supernatant by centrifugation at 3220 g for 15 min, resuspended in 4 ml buffer and again centrifuged. After incubation in 2–4 ml of 5% (w/v) EDTA, pH 7.1 for 4 h at room temperature, the body scales were dissolved and removed by two subsequent washing/centrifugation steps (washing in distilled water). The resulting ejectisome fragment-enriched fractions were kept either frozen or immediately used for experiments.

For SDS solubilisation, ejectisome fragment-enriched fractions were pelleted by centrifugation and resuspended in 200–400 μl distilled water. Fifty to one hundred (50–100) μl aliquots of 10% (w/v) SDS were added (2% final concentration). Then, the mixtures were centrifuged (Heraeus Biofuge pico table top centrifuge for 10 min at 16000 g, Kendro Laboratory Products GmbH, Langenselbold, Germany). The SDS-insoluble pellets were subjected to two additional washing/centrifugation steps with distilled water before they were used for negative staining transmission electron microscopy and SDS-PAGE, whereas the supernatants were subjected to SDS-PAGE.

Percoll density gradient centrifugation

Percoll density gradient centrifugation followed the protocol outlined by Ammermann et al. (2013). An ejectisome fragment-enriched fraction was diluted with 6 ml buffer consisting of 5 mM Tris/HCl pH 7.5 and 150 mM NaCl. Then, a mixture of 4.5 ml Percoll (Sigma, Munich, Germany) with 0.5 ml of 1.5 M NaCl was added. The solution was filled into a re-sealable polyallomer ultracentrifugation tube (Science Services, Munich, Germany), and the tube was sealed and centrifugated in a Beckman L8-55M ultracentrifuge (Beckman Coulter, Munich, Germany) equipped with a NVT65 rotor for 1 h at 27400 g and 20 °C. The ejectisome fragment-containing bands were collected using needle tipped syringes, diluted with distilled water, and subjected to two washing/centrifugation steps (Heraeus Biofuge pico). Then, the fractions were used for SDS-PAGE.

pH treatment

Five hundred (500) μl of an ejectisome fragment-enriched fraction was pelleted by centrifugation (Heraeus Biofuge pico, 10 min, 16000 g) and incubated either in 500 μl of 100 mM Tris/HCl pH 2.5 or in 500 μl of 100 mM Tris/HCl pH 10.8. Then, the mixture was centrifuged, and the pellet was