Cytoskeletal Structures in *Euglena gracilis* After Triton X-100 Extraction and Dry Cleaving

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Received April 22, 1985
Accepted June 19, 1985

Summary

A three-dimensional network of structural filaments was visible with common electron microscopes in the cytoplasm of *Euglena gracilis* green cells extracted with buffers containing the nonionic detergent Triton X-100. A similar filamentous web was detected at the periphery of critical point dried cells cleaved on grids by means of an adhesive tape. SDS-polyacrylamide gel electrophoresis of the detergent-resistant cytoskeleton showed that actin or actin-like proteins of molecular weight in the range of 43–45 K are not among the components having a structural role in *Euglena*. The significance of these findings was discussed in relation to the capability of the alga to change the cell shape.

Keywords: Cytoskeleton; Triton X-100; Dry cleaving; *Euglena gracilis*.

1. Introduction

Concepts regarding the cytoskeletal constituents of *Euglena gracilis* are fragmentary. In particular, little information is available about the structures implied in motive force development for cell shape changes (euglenoid movement, metaboly) and in the construction of a possible three-dimensional structural system at the cytoplasmic level (see e.g. Bovée 1982 and the references therein). However, based on various indications, the idea that actin microfilaments are important constituents of the *Euglena* cytoskeleton was suggested in recent articles (Hofmann and Bouck 1976, Lefort-Tran *et al.* 1980, Bre *et al.* 1981, Vannini and Poli 1983, Bassi and Donini 1984). Considerable knowledge, instead, has been accumulated on microtubules (MTs) that, at the cell periphery of the alga, form series of helical bands that run along the pellicular strips (Bouck 1982). In the strain Z used here, each band is composed of a small number of tubules; a single MT is generally present on the ridge, two are closely appressed to the notch, and one to five run along the groove (Hofmann and Bouck 1976, Lefort-Tran *et al.* 1980).

In the present study, we described a complex three-dimensional arrangement of filaments detectable in the alga by means of common electron microscopes after the application of the following techniques: i) exposition to nonionic detergent Triton X-100 dissolved in a buffer designed to optimize the preservation of the structural proteins and, at the same time, to remove phospholipids and soluble proteins (Powell *et al.* 1982, Clayton *et al.* 1983, Penman *et al.* 1983); ii) cleaving critical point dried cells on grids by means of an adhesive tape (dry cleaving) (Mesland *et al.* 1981, Traas 1984). Detergent lysis technique were also used in an attempt to identify the cytoskeletal proteins by means of one-dimensional electrophoresis.

2. Materials and Methods

2.1. Organism and Growth Conditions

Green cells of *Euglena gracilis* Klebs, strain Z, were cultured in organotrophic conditions in the liquid medium of Hutner-Provasoli (Wolken 1961), at 26 °C, under constant shaking and illumination (130–150 ft-c).
2.2. Procedures for Transmission Electron Microscopy

A. Extraction with Triton X-100. Logarithmically growing cells were harvested at 800 \times g for 5 minutes and resuspended in a MT-stabilizing buffer (MTSB) containing 100 mM 1,4-piperazinediethanesulfonic acid (PIPES) (pH 6.9), 2 mM ethylene glycol-bis(\(\beta\)-aminoethyl ether)-N,N,N',N'-tetraacetic acid-acetate (EGTA), 1 mM Mg\(^{2+}\). Other samples were placed in the following MTSBs: 100 mM PIPES (pH 6.9), 10 mM EGTA, 1 mM MgSO\(_4\), 300 mM sucrose, 1 mM phenylmethyl-sulfonyl fluoride (PMSF) (PENMAN et al. 1983). One drop of cell suspension was placed on formvar-coated grids covered with a solution (1 mg/ml) of poly-L lysine in distilled water. After 20 minutes, the unattached organisms were washed off by rinsing twice in the MTSB adopted. The grids with adherent cells were then immersed for 5, 10, 20, and 60 minutes in MTSB supplemented with 1.2, 2.5, 5% (v/v) Triton X-100. After lysis, the grids, washed in the buffer without the detergent, were air-dried and then negatively stained with 1% aqueous uranyl acetate for 20 seconds.

B. Dry cleaving. Cell from the same samples used for Triton X-100 extraction were fixed for 30 minutes in a solution of 3% glutaraldehyde and 1% tannic acid in a 0.5 M sodium cacodilate buffer (pH 7.2). After two rinses in distilled water, a drop of cell suspension was placed on grids covered with 5 mg/ml of poly-L lysine in distilled water. After washing with water, the specimens were postfixed with a buffered 1% OsO\(_4\) for 30 minutes, and subsequently dehydrated in the acetone series. After critical point drying, the cell were cleaved by inverting the grids on a common Scotch tape and by removing them with a forceps (MESLAND et al. 1981, TRAAS 1984).

The extracted and cleaved cells were observed and photographed with a Zeiss 109 R electron microscope operating at 80 kV.

2.3. Scanning Electron Microscopy

Cells adherent to poly-L lysine-coated coverslips were extracted with Triton X-100 as described above for the grids. After fixation with 3% glutaraldehyde in MTSB for 20 minutes and critical point drying, the samples were coated with gold and examined with a Siemens Autoscan (Electron Microscopy Center of Ferrara University).

2.4. Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis (PAGE)

The cells were deprived of the flagellum by a cold shock at 0–4 °C for 1–2 hours, as described by ROGALSKY and BOUCK (1980), and then extracted with Triton X-100 following the procedures adopted for the grids. The soluble and structural fractions were analyzed by slab gel electrophoresis in the presence of SDS following the method of LAEMMLI (1970). The concentration of acrylamide was 10%. SDS-PAGE was also carried out for the supernatants (cytoplasmic extracts) obtained by high-speed centrifugation of cellular homogenates made as indicated by POLLARD (1976). The gels were stained with Coomassie blue using the FAIRBANKS (1971) method.

3. Results

The pellicle membrane of Euglena gracilis, formed by an alternation of ridges, organized in a quasi-crystalline fashion, and normally structured grooves (LEFORT-TRAN et al. 1980), opposed a strong resistance to briefly protracted extractions with low concentrations of Triton X-100. By applying stronger conditions, such as exposition to 2.5 and 5% Triton X-100 for 1 hour and 20 minutes, respectively, the pellicle of most cells broke into separate strips, probably because of the complete destruction of the grooves (Fig. 1). In this way, the internal, detergent-insoluble architectural components of the alga became visualizable in the thinnest portions of the negatively stained whole mounts.

The most noticeable feature consisted of an extensive network of filaments, 5–8 nm in diameter, often assembled in bundles whose thickness changed significantly throughout their variable length (Fig. 2). Locally this meshwork was interrupted by electron-dense zones probably corresponding to the aggregation of several bundles (Fig. 3). Regular arrangements of MTs were not detectable in these specimens.

Scanning electron microscope examination of critical-point dried cytoskeletons confirmed the presence of pellicular strips and filament bundles (Fig. 4). The substitution of a buffer with another one among those designed to preserve the structural proteins did not change the cytoskeletal features.

Using the dry cleaving procedure, cell periphery layers of different size and thickness were left on the polylysine-coated grids. In several cases, a complicated network of filaments was seen which—in terms of size, thickness and interaction pattern of its constituents—resembled the fibrous web appearing after Triton X-100 extraction (Fig. 5).

The protein composition of the detergent-insoluble structures, investigated by SDS-PAGE, revealed that one of the major components was a 55 K peptide (co-migrating with glutamate dehydrogenase), probably corresponding to tubulin. Several other minor bands were also present, but no peptide with migration characteristic identical or similar to that of the rabbit muscle actin was detectable (Fig. 6). Proteins having the molecular weight in the same range (43–45 K) of the subunits which form the various actsins isolated from different organisms (METCALF et al. 1980) were not found in the detergent soluble fraction (containing more than 60% of the total protein content) nor in the cytoplasmic extracts.

Changes in Triton extraction length and buffer composition did not modify the electrophoretic pattern of Euglena structural proteins (Fig. 6).

Recently it was reported that a web of filaments is detectable in a bleached strain of Euglena gracilis