Magnesium Starved Cells of *Euglena gracilis* – a Possible Model System for Studying Mg^{2+} Influx?

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This paper is dedicated to Professor Hans Georg Ruppel on the occasion of his 60th birthday

Euglena gracilis, Nutrition, Magnesium Deficiency, Magnesium Transport

In order to obtain a model which allows to directly study Mg^{2+} influx into the cell, Mg^{2+} deficiency was induced in the unicellular photoautotrophic flagellate *Euglena gracilis*. Lack of Mg^{2+} in the culture medium leads to a number of morphological, biochemical, and physiological changes in *Euglena gracilis*. The rate of cell division was reduced under Mg^{2+}-free conditions. Subsequently an enlargement of the cells was observed and they changed from spindle-like to oval shape. The Mg^{2+}-starved cells were well filled with paramylon granules, while their motility and vitality was not impaired. Concurrently with the larger cell size the protein-, carbohydrate-, and chlorophyll content of the cells increased. Further changes were observed in the surface carbohydrates. The proportion of cells with galactose, N-acetyl-galactosamine and mannose on the cell surface rose in the Mg^{2+}-starved cultures, shown in a lectin-binding assay. Fucose was found on the pellicle of Mg^{2+}-starved cells only. Cultivation of *Euglena gracilis* in Mg^{2+}-free medium induced a drastic reduction of the intracellular Mg^{2+} concentration already after 24 h (from 233 nmol/10^6 cells to 82 nmol/10^6 cells). When Mg^{2+} was made available again, the Mg^{2+}-starved cells took them up rapidly and the intracellular concentration of free Mg^{2+} rose. As Mg^{2+} depletion could be induced in *Euglena gracilis* easily by manipulating the culture conditions and as the cells remained viable, it was concluded that this flagellate can be used as a model organism for studying the Mg^{2+} uptake of eukaryotic cells.

Introduction

Magnesium has a central position in the metabolism of pro- and eukaryotes, thus also in the unique eukaryote *Euglena gracilis*. This protozoan shows characteristics of both animal and plant cells. The lack of a cell wall makes *Euglena gracilis* an excellent model organism for studying membrane transport, like Mg^{2+} uptake. This flagellate grows well on defined media and requires Mg^{2+}, amongst several metal ions, for growth (Hilt *et al.* 1987). A large literature also exists concerning its biology and biochemistry (Buetow, 1989). For one, Mg^{2+} ions are structural components of ribosomes and nucleic acids, in photoautotrophic organisms they are the central atoms of chlorophyll, and Mg^{2+} is also needed as a cofactor in a variety of enzymatic reactions, e.g. in all phosphate transferring enzymes. Mg^{2+} is also involved in the regulation of transmembrane channels, specific receptors, and intracellular signalling molecules (Smith and Maguire, 1993). Mg^{2+} modulates the carbon-fixation cycle, oxidative phosphorylation, and is in general necessary for cell growth and its control (Walker, 1986). While the Mg^{2+} transport systems of some prokaryotes (*Salmonella typhimurium, Escherichia coli*) are well understood (Roof and Maguire, 1994; Smith and Maguire, 1993; Snavely *et al.,* 1991), information on the Mg^{2+} transport of eukaryotic cells is still scant, as the technical methods are extremely limited and not sensitive enough (Flatman, 1991; Günther, 1993; Ryan, 1993; Smith and Maguire, 1993; Golf, 1994). The only available radioactive isotope of Mg^{2+}, for instance, is very expensive and has a rather short half-life (21 h). In addition it is very difficult to induce the Mg^{2+} deficiency needed for the measurement of Mg^{2+} uptake in the usual eukaryotic model systems like erythrocytes, certain tumour cell lines, fibroblasts, or squid axons. In Mg^{2+}-deficient culture medium the cells either die or cease

Abbreviations: $[Mg^{2+}]_i$, intracellular Mg^{2+} concentration; $[Mg^{2+}]_o$, extracellular Mg^{2+} concentration.

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to grow (Maguire, 1988). In order to circumvent these difficulties an artificial model system is often used: Erythrocytes can be loaded with Mg^{2+} by incubation with an ionophore at increased external Mg^{2+} concentration (Günther, 1993). Upon removal of the ionophore with serum albumin and transfer of the cells into low Mg^{2+} culture medium, the Mg^{2+} efflux can be measured. It is however uncertain whether the properties of the membrane are changed by the incubation with the ionophore or not and whether the mechanism of the Mg^{2+} uptake can be studied using Mg^{2+} efflux as a model. If a Mg^{2+}-deficient state could be induced in *Euglena gracilis* without manipulating the cytoplasmic membrane only by reducing the Mg^{2+} concentration in the culture medium it should be possible to measure directly Mg^{2+} uptake into the cell. This model should also allow further investigations to determine how cytological and biochemical changes are induced by Mg^{2+} deficiency.

Materials and Methods

Cultivation of Euglena gracilis

Euglena gracilis strain Z (Klebs: 1224–5/25) was obtained from the Algensammlung Göttingen, Germany. Liquid cultures were grown at 30° C in 200 ml flasks containing 100 ml of sterile autotrophic medium (Cramer and Myers, 1952), with 3 % CO_2/97 % v/v air bubbled through the culture under a repetitive light-dark cycle (light 14 h/dark 10h). Light was supplied by a bank of 40 W cool-white/40 W warm-white fluorescent lamps at an incident intensity of 7.5 x 10^{19} Quanta/m^2 sec. For maintenance culture cells were spun down at the beginning of the light period every other day and resuspended at about 0.3 x 10^6 cells/ml in fresh sterile normal or Mg^{2+}-free medium. Mg^{2+}-deficient cultures were always produced by transferring normal cells (0.3 x 10^6 cells/ml) into the Mg^{2+}-free medium. For the Mg^{2+}-free medium 0.2 g/l $MgSO_4x7H_2O$ was substituted by 0.14 g/l K_2SO_4.

Growth analysis

Cell number and generation time, protein (Lowry *et al.*, 1951), carbohydrate (Roe, 1955) and chlorophyll (Schmid, 1971) content, respectively, were determined every 24 h at the beginning of the light phase.

Lectin assay

For the detection of glycoresidues at the surface of *Euglena gracilis* the following lectins were used (Sigma):

Lectin	**Specificity**
Solanum tuberosum (SolA)	N-acetyl-α-D-glucosamine
Concanavalin A (ConA)	α-D-mannose, α-D-glucose
Tetragonolobus purpureas (TGA)	α-L-fucose
Lens culinaris (LCA)	α-D-mannose
Helix pomatia (HPA)	N-acetyl-α-D-galactosamine
Bandeiraea simplicifolia (BS-I)	N-acetyl-α-D-galactosamin, α-D-galactose
Ricinus communis (RCA_{120})	β-D-galactose

Lectin assays were carried out according to v. Sengbusch *et al.* (1981). FITC-labelled lectins were dissolved (stock solution: 1mg/1ml) in PBS (140 mM NaCl in phosphate-buffer). Cells were rinsed three times with phosphate-buffer (20 mM KH_2PO_4-Na_2HPO_4, pH 7.2) and resuspended in PBS. For detection of carbohydrate residues on the pellicle of *Euglena gracilis* 200 µl cell-suspension (10^6 cells/ml PBS) were incubated with 20 µl FITC-lectin stock solution for 60 min at 25° C. After rinsing three times with PBS the cells were resuspended in 200 µl PBS and examined with an Orthoplan/Leitz fluorescence microscope (BP 450–490 nm, LP 515 nm). For every sample 10 visual fields were counted.

Isolation of the pellicle of Euglena gracilis

The pellicle of *Euglena gracilis* was isolated as follows: 80 ml of the cell suspension (1.5 x 10^6 cells/ml in PBS) were fractured by five successive 15-sec sonications (Labsonic 1510, Braun 300 w) on ice with a pause of one min after each sonication. After centrifugation (4 500×*g*, 5 min) the pellet was resuspended in 20 ml PBS and homogenised (Potter-homogeniser by Braun). The homogenate was layered onto a discontinuous sucrose gradient consisting of 50/60/70% (w/v) sucrose in PBS at pH 7.2. After centrifugation in a Sorvall ultracentrifuge (1.5 h at 42 000×*g*, 4° C) the pellicle fraction could be collected as a distinct band at the bottom of the 60 % sucrose layer. The

band was diluted and applied to a second sucrose gradient under the same conditions. The collected pellicle fraction was centrifuged for 20 min at 12000×*g* and 4° C. The pellet was resuspended in PBS. Microscopical control for purity and determination of protein content (Lowry *et al.*, 1951) were carried out before further treatment.

SDS-polyacrylamide gel electrophoresis (SDS PAGE)

Samples of isolated pellicle were solubilised in sodium dodecylsulfat-sample buffer (10 mM tris-[hydroxymethyl]aminomethane/HCl pH 8.0, 2 mM EDTA, 10 % sucrose, 2.5 % SDS, 5 % mercaptoethanol, 0.01 % bromophenol blue) and electrophoresed in a 9–17.5 % gradient polyacrylamide (Serva) gel according to Laemmli (1970). After electrophoresis (24 mA, 4° C for 14 h) gels were fixed and stained with Coomassie blue (0.1 % in 7 % acetic acid) or with 8-anilino-naphthalene sulfonic acid (ANS). Glycoproteins were detected after PAS (periodic acid – Schiff's reagent) staining (Holtzhauer, 1988). The protein pattern was scanned by the SESGA CD60 method.

Determination of magnesium

Magnesium was determined by atomic absorption spectrophotometry (AAS) (Flame technique, Perkin Elmer, model 5 100 Z). All Mg^{2+} influx measurements were done with algae grown under normal or Mg^{2+}-free conditions for 1 d. Cells were harvested and rinsed once with 20 mM phosphate-buffer, pH 7.2 (5 min, 4 500×*g*). Resuspended cells (10^6 cells/ml phosphate-buffer, pH 7.2) to 4 ml or a control without cells were incubated with 10 µl 100 mM $MgCl_2$ (final $[Mg^{2+}]$: 0.25 mM) for several incubation times at 37° C. After incubation cells were centrifuged for 5 min at 4 500×*g*. Supernatant was diluted and $[Mg^{2+}]_o$ was measured by AAS. For determination of the intracellular free Mg^{2+} content ($[Mg^{2+}]_i$) cells were washed three times with 2mM EDTA/20 mM phosphate-buffer, resuspended in 20 mM phosphate-buffer, disrupted by sonication on ice (Labsonic 1510, Braun, 300 W, 3 x 15 sec, interval break 1 min) and immediately centrifuged (20 min, 10 000×*g*, 4° C). Supernatant was diluted and $[Mg^{2+}]_i$ was measured by AAS.

Light microscopy

Cells were examined with an Olympus BH microscope. Images were taken with a 200 ASA Kodak Ektachrom film.

Electron microscopy (EM)

10^7 cells were rinsed three times with EM-buffer (50 mM KH_2PO_4-Na_2HPO_4, pH 7.0) and fixed for 2 h at 4° C in 2.5 % glutaraldehyde (w/v). Samples were rinsed three times with EM buffer and then placed into 2 % Osmium tetroxide (w/v in 50 mM EM-buffer) for two hours. After washing with EM-buffer (three times) samples were dehydrated in a graded ethanol series, transferred into propylene oxide and then into Spurr's (1969) low-viscosity resin. After infiltration for 6 h (four changes of resin under vacuum) the resin was cured for 24 h at 70° C. Ultrathin sections were cut with a diamond knife (Dupont) using a Reichert Ultracut E and put onto 200 mesh copper grids. After counterstaining with 2 % (w/v) uranylacetate and 2 % (w/v) lead citrate, the preparations were examined on a Hitachi H 500 electron microscope.

Results

Growth analysis

When grown in Mg^{2+}-free culture medium, *Euglena gracilis* changes its growth behaviour. After 24 hours, the cell number is significantly smaller than in control culture (Fig. 1). The rate of cell division remains reduced throughout the whole observation time. The initial generation time (Table I) of the Mg^{2+}-starved culture was 26.6 h (normal 16.8 h) . This difference between Mg^{2+}-starved and normal cultures increased drastically with time. After 72 h one cell cycle required 85.7 h in Mg^{2+} deficiency (normal 25.8 h).

Table I. Generation time of normal (N) and magnesium starved ($-Mg^{2+}$) cultures of *Euglena gracilis*.

Conditions	Culture time [h]		
	24	48	72
N	16.8	19.5	25.8
$-Mg^{2+}$	26.6	40.0	85.7

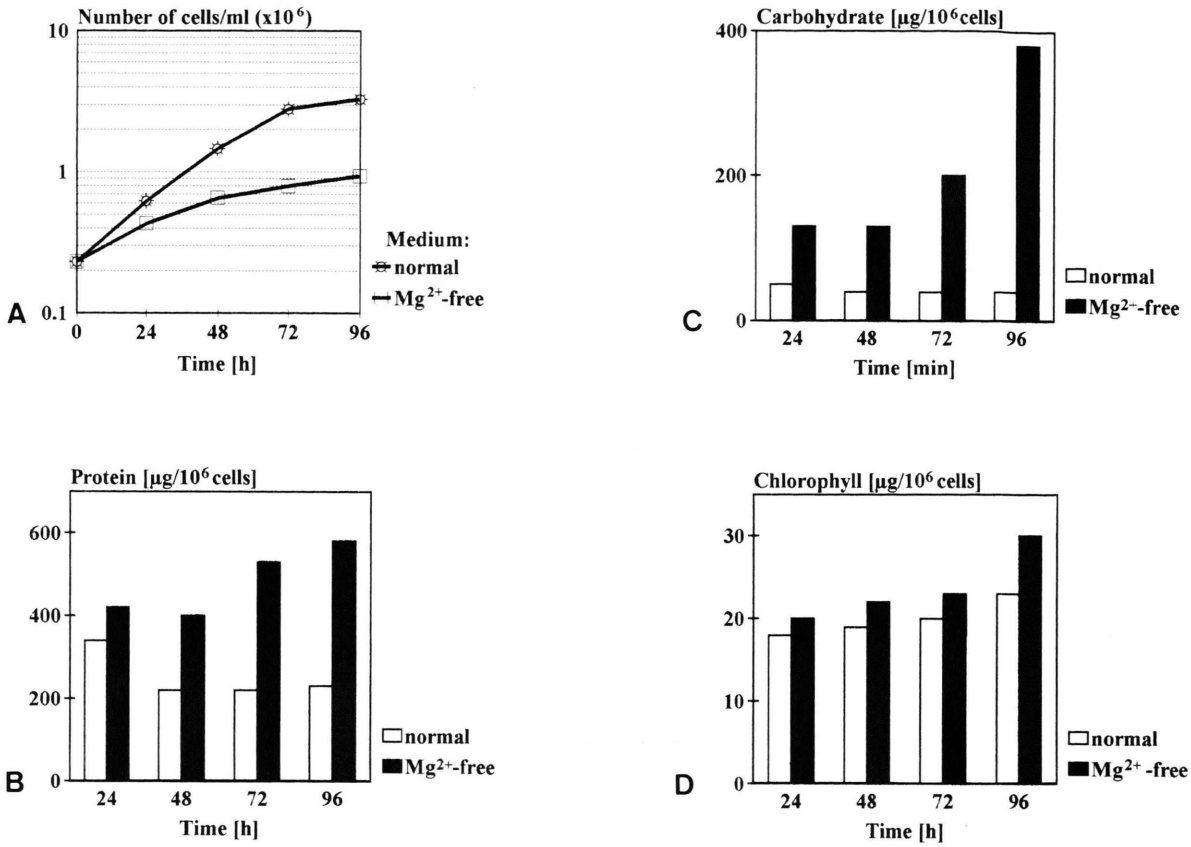

Fig. 1. Growth of *Euglena gracilis* cultures under normal or Mg^{2+}-free conditions. Number of cells (A), content of protein (B), carbohydrate (C) and chlorophyll (D) (n = 6–12).

The cell content of protein, carbohydrate and chlorophyll increased in Mg^{2+}-starved culture compared with normal culture after 24 h (Fig. 1). These differences increased with time in all three parameters, especially for carbohydrate.

Morphology

At 24 h the Mg^{2+}-starved cells did not show any morphological differences to the normal cells, however upon further cultivation at 48 h the Mg^{2+}-starved cells became larger and oval in shape. After 72 h the differences were even more remarkable (Fig. 2). The number of mitochondria, paramylon granule, and vacuoles increased under Mg^{2+} deficiency. Nucleus and pellicle remained unchanged (Fig. 2 C and D). The enlargement of the Mg^{2+}-starved cells was obviously due to rises in the number of paramylon granule and in the content of protein and carbohydrate per cell, as shown above.

Cell surface

Vitamin B_{12} deficiency leads to alterations in the carbohydrate residues on the cell surface of *Euglena gracilis* (Bré *et al.*, 1986), so by using FITC-conjugated lectins of different specificity it was studied whether Mg^{2+} starvation, too, leads to changes in the presentation of carbohydrate residues on the cell surface. In normal cultures, 13 % of the cells had β-D-galactose (RCA) on the surface whereas the other sugars studied were only found on 1–3 % of the cells. α-L-Fucose (TGA) was not found at all (Table II). On Mg^{2+}-starved cells the proportion of cells staining positive was increased for all sugars studied, α-D-mannose (LCA), and N-acetyl-α-D-galactosamine (HPA,

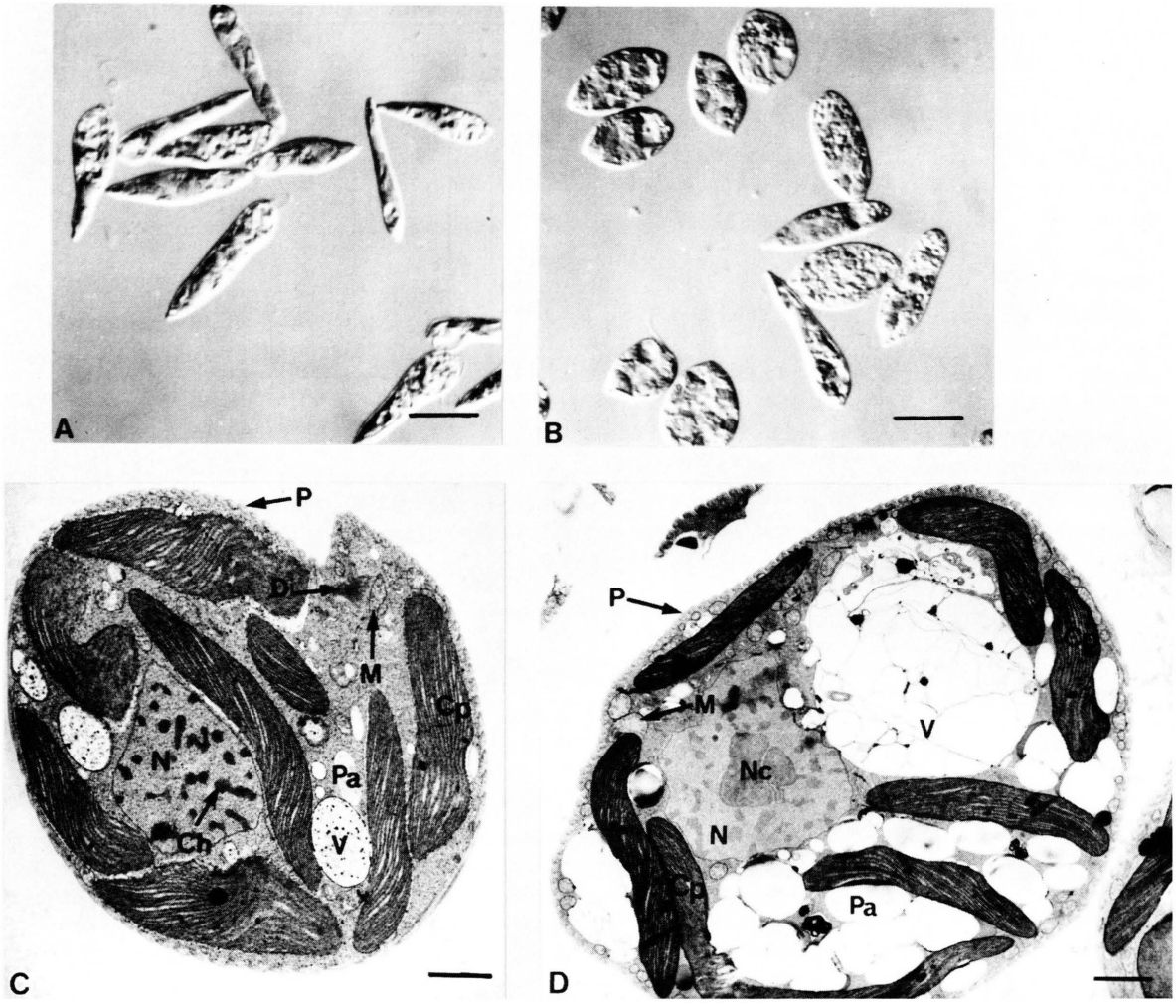

Fig. 2. Cells of *Euglena gracilis* grown in normal (A,C) or Mg^{2+}-free (B,D) medium for 72 h. A,B: Light microscopy, bar: 20 μm. C,D: Electron microscopy, bar: 2 μm. Ch: Chromosome, Cp: Chloroplast, Di: Dictyosome, M: Mitochondria, N: Nucleus, Nc: Nucleolus, P: Pellicle, Pa: Paramylon, V: Vacuole.

Table II. Labelling of *Euglena gracilis* cells grown under normal (N) or Mg^{2+}-free ($-Mg^{2+}$) conditions using FITC-labelled lectins.

Lectin	Specificity	Stained cells [%] N	$-Mg^{2+}$
SolA	N-acetyl-β-D-glucosamine	1	2
ConA	α-D-mannose, α-D-glucose	1	5
TGA	α-L-fucose	0	5
LCA	α-D-mannose	3	14
HPA	N-acetyl-α-D-galactosamine	2	26
BSI	N-acetyl-α-D-galactosamine, α-D-galactose	2	32
RCA	β-D-galactose	13	87

BSI), now ranging from well over 10 % to over 30 %, and β-D-galactose (RCA) reaching 87 %. In summary, Mg^{2+}-starved *Euglena gracilis* presented mainly β-D-galactose, N-acetyl-α-D-galactosamine, α-D-mannose and α-D-glucose on its cell surface. In addition α-L-fucose was found.

Pellicle

The lectin-assay showed that the carbohydrate pattern on the cell surface of *Euglena gracilis* was changed by Mg^{2+} starvation. This raised the question if the glycoprotein composition of the cyto-

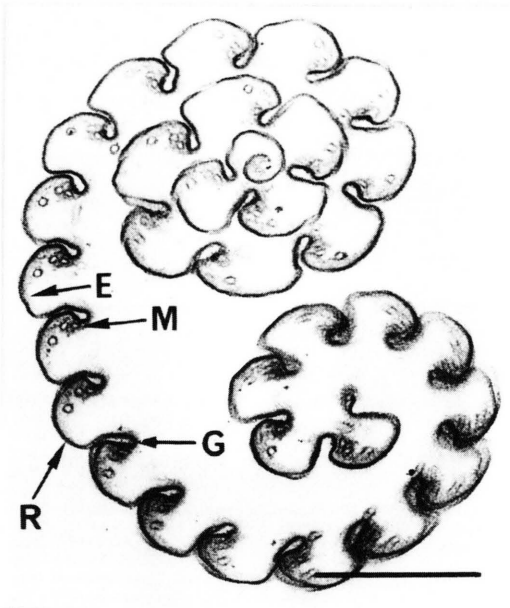

Fig. 3. Ultrathin section of isolated pellicle from normal *Euglena gracilis*. E: Epiplasmic layer, G: Groove, M: Microtubuli, R: Ridge. Bar: 0.5 μm.

plasm membrane was also changed. Accordingly, a protein analysis of the isolated pellicle (Fig. 3) was performed.

a) Coomassie Blue-staining

The separation of the pellicle proteins in the SDS-gel showed no qualitative difference between the protein pattern in Mg^{2+}-starved and normal cells. Densitometrically 31 bands were found in either test, 10 of them more pronounced.

b) PAS-staining

Five glycoprotein bands (MW: 50–200 kDa) are detectable upon PAS-staining, and were also found upon silver nitrate staining. No differences between normal and Mg^{2+}-starved cultures were observed. Thus Mg^{2+} deficiency had not any effect neither on the whole protein pattern nor on the glycoprotein pattern of the pellicle.

Intracellular Mg^{2+} content of Euglena gracilis

Normal cells of *Euglena gracilis* showed a free intracellular Mg^{2+} content ($[Mg^{2+}]_i$) of 233 nmol/10^6 cells, while cells cultured under Mg^{2+}-free conditions contained only $[Mg^{2+}]_i$ = 82 nmol/10^6 cells

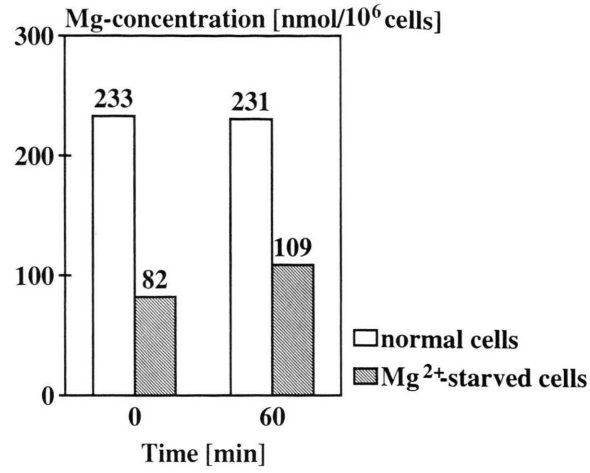

Fig. 4. Intracellular free Mg^{2+} ($[Mg^{2+}]_i$) in *Euglena gracilis* before (t_0) and after incubation (t_{60}) of normal or Mg^{2+}-starved cells in a Mg^{2+}-containing test medium (0.30 mM) at 37 °C. n = 9–11. U-test: normal cells: p = 0.859; Mg^{2+}-starved cells: p = 0.005.

already after 24 h (Fig. 4). Such Mg^{2+}-deficient cells should take up Mg^{2+}, so Mg^{2+}-deficient as well as normal cells were transferred into test medium with 0.30 mM Mg^{2+}. The change of Mg^{2+} concentration was determined by decrease in extracellular Mg^{2+} and by increase of cellular Mg^{2+}. As expected, the free intracellular Mg^{2+} content of the Mg^{2+}-deficient cells increased from 82 nmol/10^6 cells to 109 nmol/10^6 cells after an incubation time of 60 min at 37 °C. With 231 nmol/10^6 cells,

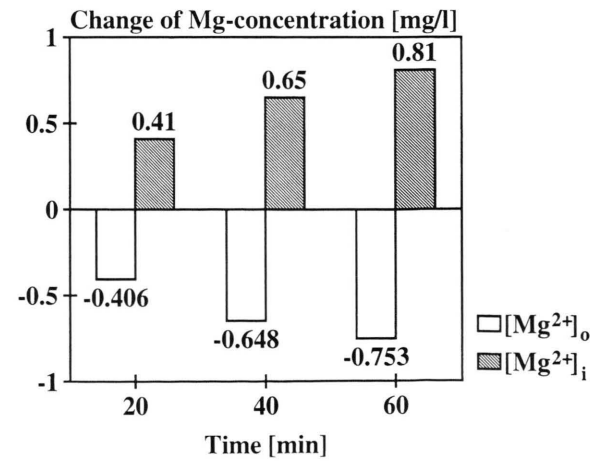

Fig. 5. Changes of Mg^{2+}-concentration in the test medium ($[Mg^{2+}]_o$) and in Mg^{2+}-starved cells ($[Mg^{2+}]_i$) after incubation for several time periods (20, 40, 60 min) at 37 °C. n = 4–5. All changes are significant (p = 0.04).

the free intracellular Mg^{2+} content of the normal cells remained nearly constant (Fig. 4), and no Mg^{2+} decrease in the test medium was observed. In contrast, the Mg^{2+} concentration in the test medium ($[Mg^{2+}]_o$) decreased in the presence of Mg^{2+}-starved cells by 0.75 mg/l. The time course of Mg^{2+} uptake into cells is shown in Fig. 5. After an incubation time longer than one hour (2, 3 and 4h), no further decrease in Mg^{2+} concentration of the test medium ($[Mg^{2+}]^o$) was observed (data not shown).

Discussion

Mg^{2+} deficiency rapidly induces an inhibition of cell division in *Euglena gracilis*, accompanied by strong morphological and physiological changes. This phenomenon can also be observed in *Euglena gracilis* if the cells are cultured under vitamin B_{12}-deficient conditions or in aging cultures (Carell, 1969; Carell *et al.* 1970; Gomez *et al.*, 1974a, 1974b; Lefort-Tran *et al.*, 1980). Along with the increase of paramylon granules in the cytoplasm cell size increases steadily (Leedale, 1982). It is not yet clear whether the paramylon accumulation is only an effect of increased synthesis of the ß-1,3-glucan or also of an inhibition of paramylon catabolism.

The metabolism as a whole is affected by Mg^{2+} deficiency. If Mg^{2+} is not available in sufficient quantities the photosynthesis of the cells is disturbed. As almost all enzymatic reactions of the glycolysis and the citric acid cycle perform optimally only in the presence of Mg^{2+} ions (Heaton, 1990), disturbances can also be expected in the dissimilation of *Euglena gracilis* (data not shown).

The inhibition of cell division of Mg^{2+}-deficient cells can be explained if the existence of a Mg^{2+}-dependent DNA polymerase is supposed in *Euglena gracilis* (Maguire,1990), as has been discussed for e.g. *Chlorella* (Galling, 1963) and found in *Escherichia coli* (Griep and McHenry, 1988), leading to disturbances of the DNA-replication. This hypothesis is supported by two observations: 1. The ultrastructural investigations do not show any changes in the nucleus. 2. Upon specific nuclear staining with DAPI only one nucleus can be found in Mg^{2+}-deficient *Euglena gracilis* (Vossiek, personal communication), whereas upon manganese depletion two nuclei are observed (Hilt *et al.*, 1987). Inhibition of cell division and cell enlargement have to be considered in relation. As long as a sufficient amount of Mg^{2+} is available the metabolism functions normally, i.e. syntheses are performed which prepare for eventual cell division. If the cell cannot divide, all the synthesis material remains in the cell.

Vannini *et al.* (1981) showed that culture conditions can affect the expression of surface glycoresidues on *Euglena gracilis*. Under photoheterotrophic conditions conA-receptors (glucose, mannose) were found not only on the pellicle but also on the membrane of the flagellum. In accordance with the study of Bré and Lefort-Tran (1984) I found conA-receptors only on 1 % of the normal cells of *Euglena gracilis* (photoautotrophic conditions). Under Mg^{2+}-free conditions the proportion of lectin-binding cells increased for all carbohydrate moieties studied, especially for N-acetyl-α-D-galactosamine (26 %), α-D-galactose (32 %) and β-D-galactose (87 %). α-D-mannose, α-D-glucose (ConA-receptors) and α-L-fucose were only found in 5 % of the Mg^{2+}-starved cell population.

Regarding to the pellicle proteins I looked especially for glycoproteins because some other deficiencies, for example Vitamin B_{12} deficiency (Bré *et al.*, 1986), lead to alterations in the glycoprotein pattern and because it was possible to show that under Mg^{2+}-free conditions carbohydrate residues of the cell surface were changed. At least 31 proteins in the pellicle were found, 5 of them glycoproteins, but no differences between normal and Mg^{2+}-starved cells were detected. Studied by lectin-assay the carbohydrate pattern on the cell surface, however, changed under Mg^{2+}-deficient conditions. This raised the question which membrane components were responsible for the changed carbohydrate residues. One possibility are the glycolipids, because the beginning enlargement of the cells caused by Mg^{2+} deficiency leads to tensions in the pellicle, uncovering structures which were inaccessible before. This is especially feasible in the region of the grooves (Fig. 3). A further class of compounds which could contribute to the change in the pattern of surface carbohydrates are the polysaccharides of mucin. It is widely assumend that mobile cells of *Euglena gracilis* are unveiled and have only few mucin fibres at the posterior end of the cell (Rosowski, 1977). As Mg^{2+} deficiency is an unfavourable environment for *Euglena gracilis*, it cannot be excluded that the cells try to enter a palmelloid stage by secreting mucin

(Gomez *et al.*, 1974a). The fact that the cells do not form a continuous mucin layer could be explained by the permanent aeration in the given culture conditions which would strip the mucin from the cells. Nevertheless Mg^{2+}-deficient cells may bear remains of mucus on their surface owing to an increased production of mucin causing a reaction with FITC-lectins. As the possibility of mucin secretion was hitherto not taken into account, it appears worthwhile to ascertain that Mg^{2+}-deficient *Euglena gracilis* are mucin-free before assaying the glycolipids. This could be done with alcian blue or ruthenium red, both dyes staining specifically polysaccharide of mucin (Gomez *et al.*, 1974b; Triemer, 1980). On the other hand it should be possible to isolate and analyse the polysaccharide mucus if it should be present on the cells (Cogburn and Schiff, 1984).

Whereas normal cells contain 233 nmol/10^6 cells of free Mg^{2+}, the deficient cells contain only 82 nmol/10^6 cells. Wherever Mg^{2+} needs as a structural component during cell growth, it is lost irrevocably from the cytosolic pool. If there is no intracellular Mg^{2+} depot, this pool can only be replenished from extracellular Mg^{2+} (Günther, 1990). As expected, the intracellular Mg^{2+} content of Mg^{2+}-deficient cells increased in 60 min from 82 nmol/10^6 cells to 109 nmol/10^6 cells.

The intracellular Mg^{2+} content of heterotrophic *Euglena gracilis* determined by Price and Vallee (1962), Falchuk *et al.* (1975) and Hilt *et al.* (1987) was markedly lower (10 nmol/10^6 cells) in spite of a higher Mg^{2+} concentration in the medium. Kempner and Miller (1972), too, found values much smaller (33 nmol/10^6 cells) than in this investigation, but nevertheless Cameron *et al.* (1986) found comparable intracellular Mg^{2+} contents for *Euglena gracilis* under autotrophical culture conditions (total: 250 nmol/10^6 cells; free: 100–120 nmol/10^6 cells). Possibly the methods for determining Mg^{2+} were not sensitive enough, or the differences are due to variations in the culture conditions (heterotrophic versus autotrophic in the present investigation).

In the present study it is shown that Mg^{2+}-deficient conditions can easily be induced in *Euglena gracilis* by variation of the culture conditions already within 24 h without afflicting vitality and cell size, and a time-dependent Mg^{2+} uptake can be measured, so this flagellate lends itself as a well studied model organism (Buetow, 1989) for the elucidation of the Mg^{2+} uptake of eukaryotes.

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