BIOLOGIA PLANTARUM 34 (3-4): 319-323, 1992

# The development of *Chlorella vulgaris* cells exposed to cadmium at successive stages of their life cycle

U. MAZUREK, A. WILCZOK, D. TYRAWSKA* and B. SWIDERSKA*

*Department of Biochemistry and Biophysics. Silesian Academy of Medicine, Katowice, Poland*
*Institute of Ecology, Polish Academy of Sciences, Warsaw, Poland**

## Abstract

The effect of $CdCl_2$ in a concentration range 0.01-10.0 g $m^{-3}$ on the growth of *Chlorella vulgaris* under synchronous cultivation conditions was determined. The general biological activity, the growth multiplication factor, the cell size and shape and intracellular arrangement showed disturbances of synchronization that depended on $Cd^{2+}$ concentration. The highest inhibition of all mentioned parameters was observed when $Cd^{2+}$ was administered after the second hour of synchronous cultivation, whereas the administration after 6 or 8 h did not induce any significant effect.

## Introduction

Cadmium sensitivity of *Chlorella* cells is an individual feature of the particular strain. Kessler (1985) found that the 211-1e strain of *Chlorella* showed a sensitivity 100 times higher than 13 strains. 0.1 g ($Cd^{2+}$) $m^{-3}$ totally inhibited its development, while other strains grew even at the concentration of 11.2 g $m^{-3}$. Other authors observed the ability of *Chlorella* cells to grow in the medium with $Cd^{2+}$ in a concentration range of 0.1 to 112 g $m^{-3}$ (Anikieva *et al.* 1975, Gipps and Coller 1980, Lue Kim *et al.* 1980). $Cd^{2+}$ sensitivity depends on cultivation conditions, culture medium composition, microelement or nitrogen deficiency. Usually $Cd^{2+}$ caused cell elongation or giant cell formation (Upitis 1983). Vaulina *et al.* (1978) observed a mutagenic $Cd^{2+}$ effect after extended incubation. $Cd^{2+}$ forms complexes with proteins and amino acids, links -SH enzymatic groups and inhibits biological activity.

Based on our previous results (Mazurek *et al.* 1990) and due to the differentiation of biochemical processes during the cell cycle, we suppose that the influence of $Cd^{2+}$ on cell development depends on the phase of the cell growth, when $Cd^{2+}$ is introduced into the cultivation medium. We tested this hypothesis in this experimental series.

*Received* 17 October 1990, *accepted* 5 August 1991.

## Material and methods

*Chlorella vulgaris* Beijerinck cells, strain A-8, were cultivated under the 10/14 h light-dark cycle in conditions described by Wilczok and Mazurek (1987). Cell development in such conditions takes place mainly in the light, while the aplanospores release and other non-photosynthetic processes occur in the dark.

Cadmium as $CdCl_2$ at concentration of 0.01, 0.1, 1.0 or 10.0 g $m^{-3}$ was added at the beginning of the cell cycle (0 h) in order to determine the lethal dose $LD_{50}$ and this dose was used in all experiments concerning the influence of $Cd^{2+}$ at successive stages of the cell cycle.

Mother cells were grown in Petri dishes with agar enriched Lorenzen medium (Kuhl and Lorenzen 1964) and the percentage of cells unable to divide as well as producing 2, 4, 8 or 16 aplanospores and the growth multiplication factor (GMF) were calculated.

To measure the general cell biological activity, the cell suspension absorbance at 680 nm was registered over the whole light period. Changes in size, shape and intracellular arrangement were recorded with the computerized automatic scanning microscope *Morphoquant*. 50 control or cadmium exposed cells were investigated and, according to the programme *Microscan 80*, the obtained data were printed as 21 computerized parameters detailed earlier (Wilczok *et al.* 1985).

## Results and discussion

Cd influence on the growth of *Chlorella vulgaris* cells depending on the phase of their life cycle was tested in a synchronous culture, where within 24 h the development of one generation of cells was completed. We present results obtained only for one cells generation, because the $Cd^{2+}$-affected culture became in successive generations unsynchronous and uncomparable to the control. Disturbances of synchronization of $Cd^{2+}$-treated cells were confirmed morphometrically and with the use of the microcolonies technique.

After administration of $Cd^{2+}$ at concentration of 10, 1.0, 0.1, 0.01 g $m^{-3}$ at 0 h of the life cycle, absorbance at 680 nm increased in all tested cultures. The inhibitory effect of $Cd^{2+}$ was proportional to its concentration (Fig. 1 top). Concentration of 1 g $m^{-3}$ of $Cd^{2+}$ introduced at 0 h decreased the cell biological activity to about 50 % and was estimated as $LD_{50}$. Therefore cadmium toxicity at successive stages of cell development was measured at the concentration equal to $LD_{50}$. The highest inhibition was observed when $Cd^{2+}$ was added after 2 h of cultivation (Fig. 1, bottom.) $Cd^{2+}$ administered after 6 or 8 h of cultivation did not cause any significant changes in the biological activity of the cells. Generally, *Chlorella* cells produce 8 aplanospores. The percentage of cells able to divide into 2, 4, 8 or 16 aplanospores and growth multiplication factors (GMF) are shown in Table 1. In our experiments, control cells produced 8.6 aplanospores at the end of the cell cycle (=GMF). When $Cd^{2+}$ was introduced after 2 to 10 h of cultivation, GMF was 2.7 to 7.3 respectively. Thus, the highest growth inhibition was found when $Cd^{2+}$ was introduced at the 2-nd hour of the synchronous growth.

Morphometric analysis with the application of *Microscan 80* computing programme revealed that cell development of young aplanospores to mother cells capable to release eight daughter cells took place during the 10 h light period of cultivation. In this time the cell size described by POLC parameter (cell surface), OBWC (cell outline) and WYMA (cell axis length) increased considerably, however the mean cell size value of $Cd^{2+}$ affected cells was about two times smaller than of that of control cells.

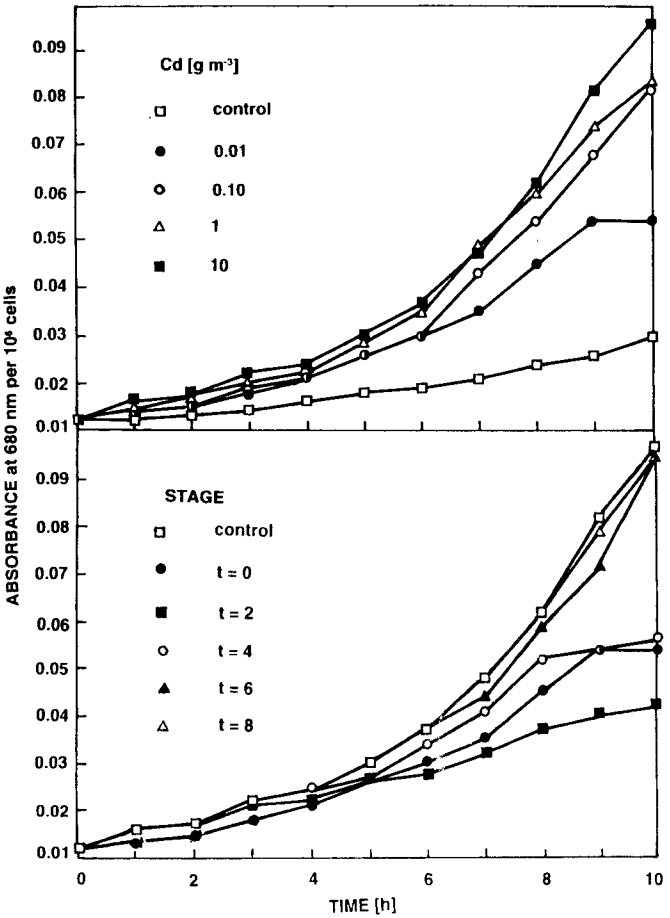


Fig. 1. Absorbance at 680 nm during synchronous growth of *Chlorella vulgaris* after the administration of various $Cd^{2+}$ concentrations at the beginning of the cell cycle (0 h) (*top*) or after administration of 1 g $m^{-3}$ at successive stages of cell cycle (*bottom*).

This phenomenon can be easily explained analysing a typical histogramme of the cell size of synchronously growing *C. vulgaris*, exposed to 10.0 g $m^{-3}$ of $Cd^{2+}$ and determined 24 h after a $Cd^{2+}$ administration (Fig. 2). As compared with the control not exposed to $Cd^{2+}$, more than 50 % of exposed mother cells were not able to

release aplanospores. After 24 h all unexposed cells were found as newly formed aplanospores.

All other morphometrically described parameters demonstrated destructive $Cd^{2+}$ action on the cells. The higher the $Cd^{2+}$ concentration, the higher the destruction. The changes in algae size were closely correlated with absorbance changes, described by 12 other parameters which were synthesized by point by point cell inside absorption measurements and their computed integration. The mean value of the sum of absorbance of all measured points of the cell image (SUEX) for normally growing cells during 10 h of cultivation increased from 133 at 0 h to 1043 at 10 h, while for the $Cd^{2+}$ exposed cells only to 877. The reason of this decrease was a changed cell size distribution at the same mean cell size (Fig 2). Therefore all kind of determinations carried out in similar experiment, which lead to mean value only, where the size, shape and intracellular absorbance distribution was not taken into account, should not be taken into consideration.

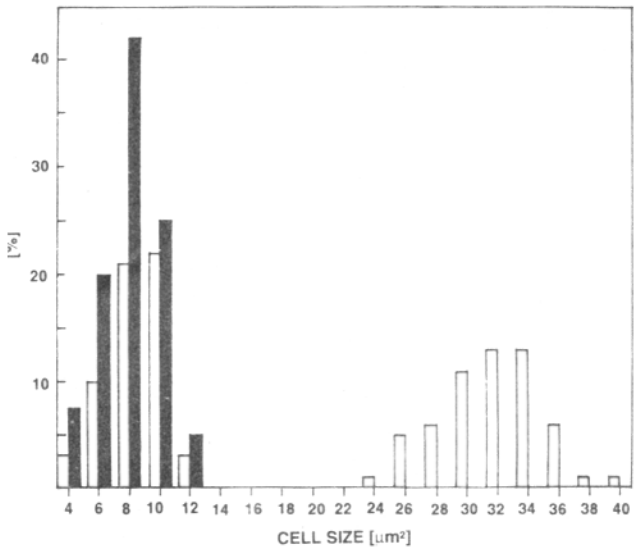


Fig. 2. Histogramme of the cell size distribution of synchronously growing *Chlorella* cells exposed to 10 g $m^{-3}$ of $Cd^{2+}$ determined 24 h after a $Cd^{2+}$ administration. *Full columns* - control; *empty columns* - 10 g(Cd) $m^{-3}$.

Under our cultivation conditions the cell cycle began from young fully synchronous aplanospores (0 h). After two hours of cultivation DNA synthesis began and lasted until the end of the cell division with the peak at 5 h. Aplanospores release started at 11 h and within 30 min 80-90 % of all mother cells were divided into 8 aplanospores. Then the cells were kept in the dark till the next cycle and mainly water uptake and swelling occurred (Wilczok and Mazurek 1987). $Cd^{2+}$ administered after 2 h of cultivation caused a stronger destructive effect than when added at 0 h. Probably $Cd^{2+}$ at this time forms complexes with the newly synthesized S-phase

enzymes affecting the whole cell development. In context of known mechanisms of $Cd^{2+}$ action and the described biochemical processes in *Chlorella* cells, it was not a surprise that the $Cd^{2+}$ administration at 8 or 10 h, when the S-phase was almost completed, did not cause significant changes in *Chlorella* cell development.

Table 1. Percentage of aplanospores released from *Chlorella vulgaris* mother cells exposed to $CdCl_2$ (1 g $m^{-3}$) administered at successive stages of the cell cycle and the growth multiplication factor (GMF).

| Time of $Cd^{2+}$ addition [h of cell cycle] | Number of aplanospores in microcolonies [% of divided cells] 0 | 2 | 4 | 8 | 16 | GMF |
|---|---|---|---|---|---|---|
| 0 | 29 | 15 | 47 | 9 | 0 | 3.2 |
| 2 | 45 | 10 | 38 | 7 | 0 | 2.7 |
| 4 | 32 | 2 | 22 | 44 | 0 | 4.8 |
| 6 | 10 | 2 | 31 | 55 | 3 | 6.3 |
| 8 | 10 | 1 | 18 | 71 | 0 | 6.5 |
| 10 | 5 | 0 | 14 | 79 | 2 | 7.3 |
| control | 0 | 0 | 10 | 78 | 12 | 8.6 |

## References

Anikieeva, I.D., Vaulina, E.N., Kogan, I.G.: [Effect of $Cd^{2+}$ ions on *Chlorella*. 1. Growth, viability and mutability of *Chlorella*.] - Genetika **11**: 12-78, 1975. [In Russ.]

Gipps, J.F., Coller, B.: Effect of physical and culture conditions on uptake of $Cd^{2+}$ by *Chlorella pyrenoidosa*. - Aust. J. Mar. Freshwater Res. **31**: 747-755, 1980.

Kessler, E.: An extremely $Cd^{2+}$-sensitive strain of *Chlorella*. - Experientia **41**: 1621-1622, 1985.

Kuhl, A., Lorenzen, H.: The handing and culturing of *Chlorella*. - In: Prescott, D.M. (ed.): Methods in Cell Physiology. Pp. 159-189. Academic Press, New York - London 1964.

Lue-Kim, H., Wozniak, P.C., Flechter, R.A.: $Cd^{2+}$ toxicity on synchronous population of *Chlorella ellipsoidea*. - Can. J. Bot. **58**: 1780-1788, 1980.

Mazurek, U., Naglik, T., Wilczok, A., Latocha, M.: Effect of $Cd^{2+}$ on photosynthetic pigments in synchronously growing *Chlorella* cells. - Acta biochim. pol. **37**: 391-394, 1990.

Upitis, V.: [Macro-and Microelements for Optimal Mineral Nutrition of Microalgae.] - Zinatne, Riga 1983. [In Russ.]

Vaulina, E.N., Anikieeva, I.D., Kogan, I.G.: [Induced Mutagenesis and Selection in *Chlorella*.] - Nauka, Moskva 1978. [In Russ.]

Wilczok, A., Mazurek, U.: The role of water in size and shape characteristics of newly formed aplanospores of *Chlorella vulgaris*. - Studia biophys. **122**: 237-248, 1987.

Wilczok, T., Wacławek, R., Wilczok, A., Mazurek, U.: Morphometric analysis of *Chlorella* cells used as a biotest. - Wiss. Hefte päd. Hochsch. Köthen **1**: 63-64, 1985.