Response of tolerant and wild strains of *Scenedesmus biguja* to copper

A.A. FATHI and A.M. El-SHAHED

Botany Department, Faculty of Science, El-Minia University, El-Minia, 61519, Egypt

Abstract

Copper tolerance in *Scenedesmus biguja* was studied by comparing the physiological properties and Cu uptake in wild type and copper tolerant strain. A concentration dependent reduction in growth rate, pigment, protein, sugar, and amino acid contents, oxygen evolution, and alkaline phosphatase activities was noticed in both strains at 1 and 200 μg(Cu) dm^{-3}. The reduction in all parameters was higher in the wild type than in the tolerant strain. The tolerant strain showed also enhanced alkaline phosphatase activity, and insignificant loss of potassium and sodium. The Cu uptake was influenced by the Cu concentrations the algae had been exposed to during their previous growth: the lower the Cu concentration in the culture medium, the higher the activity of the uptake and the capacity of the cells to accumulate Cu.

Additional key words: adaptation, alkaline phosphatase, chlorophyll content, copper uptake.

Introduction

Tolerance to heavy metals in organisms including algae may develop either while growing in metal contaminated natural habitats or through successive cultivation at elevated doses in the laboratory (Rai *et al.* 1991). Cases of acquired resistance to Cu in algae growing in Cu-contaminated fresh water habitats are numerous (*e.g.*, Stokes *et al.* 1973, Stokes 1975, Butler *et al.* 1980, Rai *et al.* 1991, Lombardi and Vieira 1998).

Copper is an essential trace element for plants, because it participates in photosynthetic electron transport and also plays a role as a cofactor of several oxidizing enzymes. However, higher copper concentrations resulted in an inhibition of photosynthetic electron transport (Shioi *et al.* 1978), the destruction of the chloroplast membrane (Sandmann and Böger 1980 a), inhibition of the formation of photosynthetic pigments, and decrease in intracellular K^+ and Na^+ concentrations (De Filippis 1979). A lack of copper, on the other hand, can partially be compensated, for example by a substitution of phycocyanin by cytochrome *c* (Sandmann and Boger 1980 b).

In the present paper we investigate copper tolerance in *Scenedesmus biguja* (isolated from the River Nile) by comparing wild type and tolerant strains exposed to different Cu concentrations.

Materials and methods

Scenedesmus bijuga (Turp.) Lagerh. was isolated from River Nile at El-Minia, Egypt. The alga was grown in Kuhl's medium (1962) at 22 °C, irradiance 60 μmol $m^{-2}s^{-1}$ and 14-h photoperiod. The concentrations of Cu provided to the medium as copper sulfate was 0.5 μg(Cu) dm^{-3} (as in the river water).

Copper tolerant strain of *Scenedesmus biguja* was isolated by successive culturing on agar plates (Kuhl's medium) containing different Cu doses (0.05 to 0.5 mg dm^{-3}) as described by Whitton and Shehata (1982). Colonies appearing on plates containing 0.3 mg dm^{-3} Cu were picked up and transferred repeatedly to agar plates spiked with the same concentration of Cu. Colonies growing successfully on such plates and also in liquid medium containing 0.3 mg dm^{-3} Cu were designated as copper tolerant strain. When this tolerant strain was subcultured in the basal medium that devoid of Cu and then transferred to the medium spiked with

Received 27 January 1999, *accepted* 3 June 1999.
Fax: (+20) 86 343601, e-mail: rumenia@rusys.eg.net

0.3 mg(Cu) dm^{-3}, a gradual loss of tolerance in the strain was noticed after every successive generation (Rai *et al.* 1991). Hence, the strain isolated was not a spontaneous mutant but a physiological adapted one.

A standard initial inoculum of both wild type or tolerant strains were inoculated to culture flasks (250 cm^3 each) that contained 50 cm^3 of nutrient medium supplied with 1.0 and 200 μg(Cu) dm^{-3} and basal medium as a control. At the end of incubation period (7 d) cultures were filtered and washed several time by distilled water for different measurements. At least three replicates for each samples and control were used.

Growth was measured in terms of cell number using a haematocytometer, which was used for calculation of growth rate (Nichols 1973). Chlorophyll *a* was estimated in acetone extract according to Metzner *et al.* (1965). Sugar content was determined by the anthrone method (Roe 1955) using glucose as a standard. Total amino acid content was determined according to Moore and Stein (1948). Total protein was measured according to Lowry *et al.* (1951). Photosynthetic oxygen evolution was measured at 27 °C and a irradiance of 450 μmol m^{-2} s^{-1} with a Clark type oxygen electrode (*YSI 4004*) connected with a *Servogor 460* recorder.

Copper uptake by algal cells was measured according to Fathy and Falkner (1997) by a *Perkin-Elmer* Atomic Absorption Spectrometer (*Model 4000,* Norwalk, USA) with graphite furnace (*HGA 500*). The incorporated amount was calculated from the decrease of the copper concentration in the incubation medium, divided by the cell number. The calculated values are the mean of triplicates, the standard deviation was less than 5 % of these mean values.

To known mass of algal cells, cold bidistilled water was added and grinding with acid washed sand was done. The samples were centrifuged for 15 min (g = 2 000 - 3 000), made up to definite volume and used immediately for making assay. Alkaline phosphatase was determined colorimetricaly at 700 nm according to Burton and Riley (1954).

The K^+ and Na^+ present inside the cells were released by heating the washed pellets in boiling water bath. Cells were then removed by centrifugation and amount of K^+ and Na^+ were determined with the help of a clinical flame photometer *Corning 410C* (*Corning Science Products*, Halstead, England) according to Rai *et al.* (1991).

Results and discussion

The data of this study indicated that the amount of copper used (which is higher than that in River Nile, 0.5 μg dm^{-3}) strongly affected the physiological and biochemical processes of the wild type *Scenedesmus bijuga.* Cu supplementation severely reduced the growth (growth rate, dry mass), and chlorophyll *a*, protein, sugar and amino acid contents of the wild type in a concentration dependent manner. However, the impact was insignificant on the tolerant strain (Table 1). A concentration-dependent reduction in dry mass, pigments, protein, sugar and alkaline phosphatase activity was in good agreement with previous findings (Fisher and Frood 1980, Sato *et al.* 1986, Rai *et al.* 1991).

Table 1. Effect of 0, 1.0, and 200 μg(Cu) dm^{-3} on growth rate, dry mass, chlorophyll (Chl) *a* content, oxygen evolution, protein, sugar and amino acid contents, and potassium and sodium contents in wild type and copper tolerant strains of *Scenedesmus bijuga* measured after 7-d treatment. Mean of three replicates ± SE.

Strains Cu concentration	Wild type control	1.0	200	Tolerant control	1.0	200
Growth rate [divisions d^{-1}]	0.26 ± 0.01	0.13 ± 0.00	0.00	0.34 ± 0.01	0.29 ± 0.00	0.30 ± 0.01
Dry mass [mg dm^{-3}(medium)]	3.26 ± 0.01	1.50 ± 0.01	0.02 ± 0.01	4.65 ± 0.03	3.23 ± 0.01	4.00 ± 0.03
Chl *a* [mg dm^{-3}(medium)]	2.06 ± 0.02	1.37 ± 0.05	0.05 ± 0.01	4.64 ± 0.02	3.06 ± 0.02	3.32 ± 0.02
O_2 evolution [μmol(O_2) mg^{-1}(protein) s^{-1}]	0.015	0.008	0.002	0.018	0.013	0.013
Protein [% (d.m.)]	28.65 ± 0.05	20.62 ± 0.03	12.81 ± 0.04	37.22 ± 0.04	[illegible]3.65 ± 0.02	34.82 ± 0.02
Sugars [% (d.m.)]	65.11 ± 0.03	28.98 ± 0.05	10.32 ± 0.02	88.31 ± 0.02	[illegible]7.66 ± 0.04	65.21 ± 0.02
Amino acids [% (d.m.)]	30.26 ± 0.03	22.32 ± 0.04	8.21 ± 0.02	35.32 ± 0.02	[illegible]5.32 ± 0.02	35.41 ± 0.04
K^+ [g g^{-1}(protein)]	0.24 ± 0.05	0.10 ± 0.02	0.10 ± 0.02	0.28 ± 0.03	0.20 ± 0.02	0.22 ± 0.02
Na^+ [g g^{-1}(protein)]	0.12 ± 0.03	0.06 ± 0.00	0.04 ± 0.00	0.15 ± 0.02	0.12 ± 0.02	0.12 ± 0.02

The results of this investigation clearly show that increased metal concentration depressed the photosynthetic activity of the two strains of *Scenedesmus bijuga,* causing lower Chl *a* content especially in the wild type. This suppression can be attributed to inhibition of reductive steps in chlorophyll biosynthesis in algae or to the severe interaction with the transport chain, water splitting site and oxidoreductase being the most sensitive steps (De Filippis 1981, Hofner *et al.* 1987a).

Copper treatment resulted in decreased Chl *a* content in both strains coupled with low photosynthetic rate and reduced sugar content. This reduced sugars content perhaps contributed to suppression of protein accumulation by shortage of carbon skeleton. Such results are in accordance with those of Hofner *et al.* (1987a,b).

In tolerant strain at 1.0 and 200 μg(Cu) dm^{-3}, the inhibition of photosynthetic oxygen evolution were only 27.54, and 30.19 %, respectively. But in the wild type the inhibition was 41.81 and 88.44 % at the same copper concentrations (Table 1). The oxygen evolution was more severely affected by Cu treatment in the wild type than the tolerant strain of *Scenedesmus*. The effect of Cu occurred within less than 1 min after addition of it, indicating that this ion passes rapidly into the cell (Fathy and Falkner 1997).

As compared to Na^+, the decrease in K^+ content was more pronounced in both strains. However, the decrease in Na^+ content was more severe in the wild type than in tolerant strain. The low K^+ and Na^+ content in the cells of the tolerant strain after copper treatment supported the opinion that tolerance in this alga was achieved through change in the permeability of plasma membrane (De Filippis 1979).

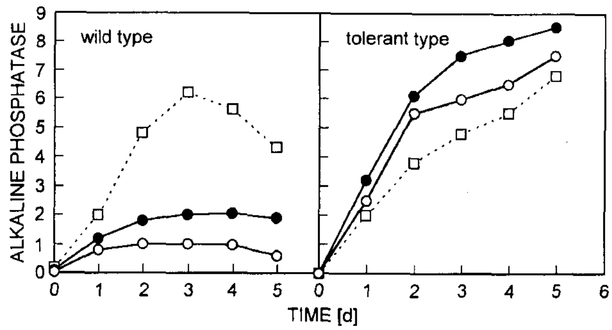

Fig. 1. Alkaline phosphatase activity [nmol μg^{-1}(protein) h^{-1}] of wild type (*open circles*) and tolerant strain (*closed circles*) of *Scenedesmus bijuga* as affected by 1 (*dashed lines*) and 200 (*full lines*) μg(Cu) dm^{-3}.

The alkaline phosphatase activity was suppressed more by Cu in the wild type than in the tolerant strain. At higher Cu concentration the tolerant strain possessed higher activity of alkaline phosphatase than the wild type (Fig. 1). Similar results were found in Cd- and Pb-resistant strains of *Pseudomonas marina* (Chan and Dean 1988). However, the mechanism needs further study.

When algae were washed with a copper free medium and then exposed to different copper concentrations in the incubation medium employed for uptake experiments, both the initial velocities and the amount of copper absorbed during exposure to elevated copper concentration were related to the copper concentrations

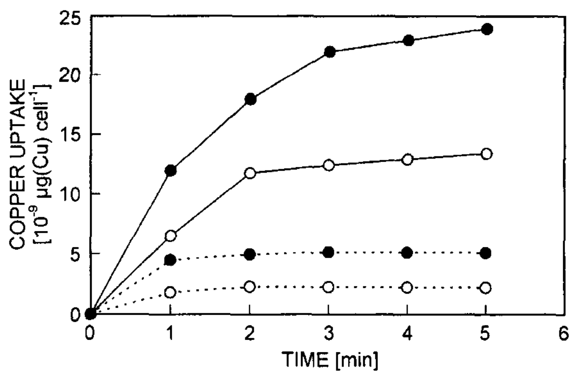

Fig. 2. Influence of the copper concentration (1.0 μg(Cu) dm^{-3} - *dashed lines*, and 10 μg(Cu) dm^{-3} - *full lines*) on the time course of copper uptake [10^{-9} μg(Cu) $cell^{-1}$] of wild type (*open circles*) and tolerant strain (*closed circles*) of *Scenedesmus biguja*.

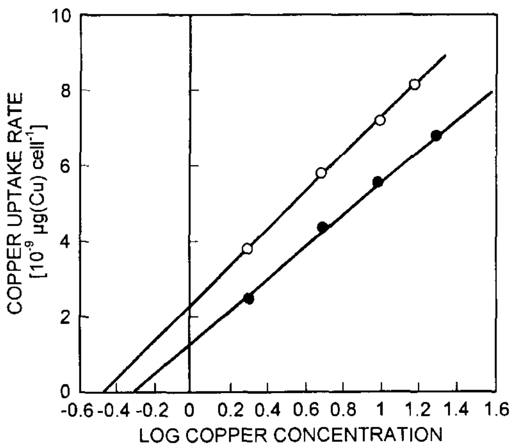

Fig. 3. Thellier plot of the initial velocities of copper influx, with the wild type (*A*) and tolerant strain (*B*) of *Scenedesmus biguja*. Copper concentrations in the uptake experiment were 1, 2, 5, 10, and 20 μg(Cu) dm^{-3}.

the algae had experienced during preceding growth (Fig. 2). The higher the copper concentration in the growth medium, the lower the observed rate of net uptake under the experimental conditions. A Thellier plot (Thellier 1970, 1993) of uptake data obtained with the wild type and tolerant strains, followed straight lines that intercept the log Cu concentration axis at the concentrations 0.33 and 0.5 μg(Cu) dm^{-3} for the wild type and tolerant strains, respectively (Fig. 3). The mentioned concentrations represent those below which the net uptake of Cu becomes negative and growth ceases. The Thellier plot of the concentration dependence of the rate of copper absorption showed that during this physiological adaptation to elevated copper concentrations the uptake system adopted characteristic properties such that there was an extended range of validity of the flow-force relationship (Fathy and Falkner 1997). Generally the data of the uptake experiments show that copper toxicity for algae depends on the environmental copper levels the algae have experienced during preceding growth. A border-line between toxic and non toxic copper concentrations can therefore not be given in terms of fixed, species dependent parameters but is rather a function of the copper concentrations to which the algae are capable to adapt during growth.

References

Burton, J.O., Riley, J.P.: Determination of soluble phosphorus and total phosphorus by sulphite metol method. - In: Paech, K., Tracey, M.V. (ed.): Modern Methods of Plant Analysis. Vol. 2. P. 370. Springer-Verlag, Berlin 1954.

Butler, M.A.E., Haskew, E.J., Young, M.M.: Copper tolerance in the green alga *Chlorella vulgaris*. - Plant Cell Environ. **3**: 119-126, 1980.

Chan, K.Y., Dean, A.C.R.: Effects of cadmium and lead on growth, respiration and enzyme activity of the marine bacterium *Pseudomonas marina*. - Chemosphere **17**: 597-607, 1988.

De Filippis, L.F.: The effect of heavy metal compounds on the permeability of *Chlorella* cells. - Z. Pflanzenphysiol. **92**: 39-49, 1979.

De Filippis, L.F., Hampp, R., Ziegler, H.: The effects of sublethal concentrations of zinc, cadmium and mercury on *Euglena*. Growth and pigments. - Z. Pflanzenphysiol. **101**: 37-47, 1981.

Fathy, A.A., Falkner, G.: Adaptation to elevation of the concentration of trace element copper during growth of *Scenedesmus bijuga* is reflected in the properties of the copper uptake system. - J. Trace Microprobe Techniques **15**: 321-333, 1997.

Fisher, N.S., Frood, D.: Heavy metals and marine diatoms: influence of dissolved organic compounds on toxicity and selection for metal tolerance among four species. - Mar. Biol. **59**: 85-93, 1980.

Hofner, W., Naguib, M.I., Kobbia, I.A., Zeinab, K.H.I.: Use of laboratory culture of some algae to predict heavy metals toxicity. I - The role of some heavy metals in the growth, pigmentation, carbohydrate and protein synthesis in *Chlorella fusca* and *Spirulina maxima*. - Egypt. J. Microbiol. **22**: 213-226, 1987a.

Hofner W., Naguib, M.I., Kobbia, I.A., Zeinab, K.H.I.: Use of laboratory culture of some algae to predict heavy metals toxicity. II - Studies on the activities of malate and glutamate dehydrogenase in *Chlorella fusca* and *Spirulina maxima* as affected by some heavy metals. - Egypt. J. Microbiol. **22**: 227-234, 1987b.

Kuhl, A.: Zur Physiologie der Speicherung kondensierter anorganischer Phosphate in *Chlorella*. - In: Beiträge zur Physiologie und Morphologie der Algen. Pp. 157-166. Fischer-Verlag, Stuttgart 1962.

Lombardi, A.T., Vieira, A.A.H.: Copper and lead complexation by high molecular weight compounds produced by *Synura* (*Chrysophyceae*). - Phycologia **37**: 34-39, 1998.

Lowry, O.H., Rosebroug, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Metzner, H., Rau, H., Senger, H.: Untersuchungen zur Synchronisierbarkeit einzelner Pigmente-Mangel-Mutanten von *Chlorella*. - Planta **65**: 186-194, 1965.

Moore, S., Stein, W.: Photometric ninhydrine method for use in the chromatography of amino acids. - J. biol. Chem. **17**: 367-388, 1948.

Nichols, H.W.: Culture methods, and growth measurements. - In: Stein, J.R. (ed.): Handbook of Physiological Methods. Pp. 7-24. Cambridge University Press, Cambridge 1973.

Rai, L.C., Mallick, N., Singh, J.B., Kumar, H.D.: Physiological and biochemical characteristics of a copper tolerant and a wild type strain of *Anabaena doliolum* under copper stress. - J. Plant Physiol. **138**: 68-74, 1991.

Roe, J.H.: The determination of sugar in blood and spinal fluid with anthrone reagent. - J. biol. Chem. **212**: 335-343, 1955.

Sandmann, G., Böger, P.: Copper deficiency and toxicity in *Scenedesmus*. - Z. Pflanzenphysiol. **98**: 53-59, 1980a.

Sandmann, G., Böger, P.: Copper-induced exchange of plastocyanin and cytochrome *c*-553 in cultures of *Anabaena variabilis* and *Plectonema boryanum*. - Plant Sci. Lett. **17**: 417-424, 1980b.

Sato, C., Schnoor, J.L., McDoland, B.: Characterization of effects of copper, cadmium and nickel on the growth of *Nitrosomonas europae*. - Environ. Toxicol. Chem. **5**: 403-416, 1986.

Shioi, Y., Tamai, H., Sasa, T.: Effects of copper on photosynthetic electron transport systems in spinach chloroplasts. - Plant Cell Physiol. **19**: 203-209, 1978.

Stokes, P.M.: Adaptation of green algae to high levels of copper and nickel in aquatic environments. - In: Hutchinson, T.C. (ed.): International Conference on Heavy Metals in the Environment. Vol. II. Pp. 137-154. Toronto 1975.

Stokes, P.M., Hutchinson, T.C., Krauter, K.: Heavy metal tolerance in algae isolated from contaminated lakes near Sudbury, Ontario. - Can. J. Bot. **51**: 2155-2168, 1973.

Thellier, M.: An electrokinetic interpretation of the functioning of biological systems and its application to the study of mineral salt absorption. - Ann. Bot. **34**: 983-1009, 1970.

Thellier, M., Ripoll, C., Vincent, J.C., Mikulecky, D.C.: Interpretation of the fluxes of substances exchanged by cellular systems with their external medium. - In: Greppin, H., Bonzon, M., Degli, R. (ed.): Some Physicochemical and Mathematical Tools for Understanding of Living Systems. Pp. 221-227. University of Geneva, Geneva 1993.

Whitton, B.A., Shehata F.H.A.: Influences of cobalt, nickel, copper and cadmium on the blue green alga *Anacystis nidulans*. - Environ. Pollut. **27**: 275-281, 1982.