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Growth, photosynthetic and respiratory responses to sub-lethal copper concentrations in *Scenedesmus incrassatulus* (Chlorophyceae)

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Abstract

In the present paper we investigated the effects of sub-lethal concentrations of Cu^{2+} in the growth and metabolism of *Scenedesmus incrassatulus*. We found that the effect of Cu^{2+} on growth, photosynthetic pigments (chlorophylls and carotenoids) and metabolism do not follow the same pattern. Photosynthesis was more sensitive than respiration. The analysis of chlorophyll *a* fluorescence transient shows that the effect of sub-lethal Cu^{2+} concentration *in vivo*, causes a reduction of the active PSII reaction centers and the primary charge separation, decreasing the quantum yield of PSII, the electron transport rate and the photosynthetic O_2 evolution. The order of sensitivity found was: Growth > photosynthetic pigments content = photosynthetic O_2 evolution > photosynthetic electron transport > respiration. The uncoupled relationship between growth and metabolism is discussed.

Keywords: Algae; Copper toxicity; Metabolism; Chlorophyll *a* fluorescence; JIP-test

1. Introduction

Among the great varieties of modern pollutants interfering with photosynthetic organisms metabolism at different stages, heavy metals are one of the most common non-biodegradable pollutants reported at elevated concentration in many parts of the world (Mallick and Mohn, 2003).

Copper(II) is the most commonly used toxic heavy metal for industrial purposes and its presence in aquatic systems arises from both naturally occurring and man-made origin. Various sources of Cu, including industrial and domestic wastes, agricultural practices, copper mine drainage, copper-based pesticides, and antifouling paints, have contributed to a progressive increase in Cu concentrations in aquatic environments (Ma et al., 2003; Andrade et al., 2004). Estimates of total anthropogenic discharge of copper to surface waters ranges from 35×10^3 to 90×10^3 metric tons per year worldwide (Nriagu and Pacyna, 1988). Application of copper sulfate remains the most commonly used method for controlling nuisance algae in lakes and water reservoirs. These copper treatments can result in potentially high levels of Cu in the surface waters, accumulation of copper in the sediments, and water quality problems (Haughey et al., 2000).

Copper can be good and evil to algae as it is an essential micronutrient for algal growth, participating in important biological reactions as an enzymatic cofactor and electron carrier in the photosynthetic and respiratory processes

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(Andrade et al., 2004) but, when in excess, it becomes highly toxic (Dewez et al., 2005). Copper reduces growth as well as photosynthetic and respiratory activities (Nalewajko and Olaveson, 1995). The photosynthetic apparatus is particularly susceptible to this cation, resulting in a decrease in the activity of photosystem II and electron transfer rates (Fernades and Henriques, 1991; Mallick and Mohn, 2003). Toxicity of Cu^{2+} might also result from the oxidation of sulphydryl groups of enzymes leading to their inhibition (Teisseire and Guy, 2000). Because of its redox properties, copper induces oxidative stress by generating reactive oxygen species like superoxide and hydroxyl radicals via Haber-Weiss and Fenton reactions. Oxidative stress directly damages proteins, amino acids, nucleic acids, and membrane lipids (Nagalakshmi and Prasad, 1998).

Although the effect of copper on growth and the metabolism of microalgae have been extensively studied, little information is available about the relationship between the growth and metabolism at sub-lethal concentrations of copper.

2. Material and methods

2.1. Alga culture

All the experiments were conducted with axenic cultures of the green alga *Scenedesmus incrassatulus* (Chlorophyta, Chlorococcales), obtained from the Laboratorio de Hidrobiología Experimental del Instituto Politécnico Nacional, México.

The cells were autotrophically grown in batch cultures (250 ml) using an EDTA (ethylenediaminetetraacetic acid) free medium, called PCG (Perales-Vela, 2004). The growth conditions were: 22 °C with a photon flux density of 200 μmol m^{-2} s^{-1}, and a 14-h light/10-h dark cycle. The cultures were aerated with filtered air (200 ml/min) to provide a constant concentration of CO_2. The culture medium and all the other solutions were prepared with ultrapure water (ELIX 10 Millipore, USA). Stock solution of $CuSO_4 \cdot 5H_2O$ (Sigma–Aldrich) was filter-sterilized by passing through a Millipore membrane filter (0.22 μm) before supplementing to the experimental systems (Mallick and Mohn, 2003). All glassware used were soaked for at least 24 h in 10% HNO_3, and thoroughly rinsed with ultrapure water.

2.2. Growth and metabolic measurements

Photosynthetic oxygen evolution and respiratory oxygen uptake rates were measured with a temperature controlled Clark-type oxygen electrode (YSI, USA) in exponentially growing cultures. The cells were washed in air-equilibrated buffer (KH_2PO_4 100 mM, pH 7.0). The metabolic activity was measured in the same buffer by turning on (photosynthesis) and off (respiration) a white actinic light (700 μmol/m^2 seg). Chlorophyll *a* and *b* and total carotenoids were determined spectroscopically with 100% (v/v) methanol extracts, according to the equations of Wellburn (1994).

Growth was determined as dry weight. A known volume of each treatment was filtered on a 8 μm pore membrane and dried at 80 °C for 72 h to constant weight. The concentration that leads to 50% growth reduction was calculated according to OECD-guideline 201 (OECD, 1984) and was recorded as EC_{50}.

Chlorophyll *a* fluorescence transients were measured at room temperature by a Handy-Plant Efficiency Analyzer (HPEA, Hansatech, Instruments Ltd., Norfolk, UK) as described by Strasser et al. (1999). Each chlorophyll *a* fluorescence induction curve was analyzed according to the so-called JIP-test using the BiolyzerHP3 software (Strasser and Strasser, 1995; Maldonado-Rodriguez et al., 2003). The following data from the original measurements was used: (1) the minimal fluorescence yield F_0 at 50 μs when all reaction centers (RCs) are open, (2) the maximal fluorescence yield, F_m, (3) the initial slope at the beginning of the variable fluorescence, $M_0 = (F_{300\ \mu s} - F_0)/(F_m - F_0)$, (4) the relative fluorescence at 2 ms (phase J) and (5) the variable fluorescence at phase J, $V_J = (F_J - F_0)/(F_m - F_0)$. Using these data, the following fluxes ratios or yields were calculated (Appenroth et al., 2001):

1. The maximum quantum yield of PSII for primary photochemistry; $\varphi P_0 = F_v/F_m$.
2. The efficiency with which a trapped exciton, having triggered the reduction of Q_A to Q_A^- can move an electron further than Q_A^- into the electron transport chain; $\psi_0 = 1 - V_J$.
3. The quantum yield of electron transport; $\varphi E_0 = F_v/F_m \cdot \psi_0$.
4. The quantum yield of dissipation excitation energy in the antenna; $\varphi D_0 = 1 - \varphi P_0$.

Using the same data, the total number of active reaction center per absorption (RC/ABS) was calculated as follow:

$$RC/ABS = [1 - (F_0/F_m)]/(M_0/V_J)$$

The electron transport rate (ETR) and non-photochemical quenching (NPQ) were measured with a PAM fluorometer (FMS 2 Hansatech Instruments Ltd., Norfolk, UK) as described by Nielsen and Nielsen (2005) and Masojídek et al. (1999). All measurements were performed at room temperature (25 °C) in the dark. The algae samples were adjusted at same optical density ($0.4_{680\ nm}$) before the chlorophyll fluorescence study. Each cell suspension was filtered through a Millipore filter (8 μm) and then they were dark adapted for 5 min. This was the time needed to reduce PSII to a constant fluorescence level (F_0) (Mallick and Mohn, 2003). The Chlorophyll *a* fluorescence signals were detected directly from the surface filter. The diameter of the irradiated sample area was 4 mm.

All experiments were repeated al least three times. The results were verified statistically by Student's *t*-test ($P < 0.05$) using the program Sigma-Plot-Stat.