Stress Factors Enhancing Production of Algal Exudates: a Potential Self-Protective Mechanism?

Blahoslav Maršálek* and Renata Rojíčková

Department of Experimental Phylogeny and Ecotoxicology, Institute of Botany, Czech Academy of Sciences, Květná 8, CZ-603 65 Brno, Czech Republic

Z. Naturforsch. 51c, 646–650 (1996); received May 28/June 25, 1996

Algae are known to produce extracellular organic substances under optimum conditions and increase their production under stress. The changes in amount and composition of extracellular carbohydrates and proteins of three green algae Scenedesmus quadricauda, Chlorella kessleri and Raphidocelis subcapitata (known as Selenastrum capricornutum) were studied after a 5-days' cultivation under the influence of different types of stress factors (osmotic, organic, and heavy metal stressors). NaCl enhanced the quantity of carbohydrates more than proteins. A higher increase of proteins than carbohydrides was observed after addition of 3,5-dichlorophenol, glyphosate and cadmium chloride to algal cultures. The production of dissolved organic matter differs from species to species, with the age of a culture and the type of stressor.

Introduction

Bioavailability is one of the main factors that influence the toxic impact of a xenobiotic on an organism. The lipid solubility and the toxicant complexion with natural substances (humic acids, organic acids etc.) plays an important role in the membrane transport (Nelson and Donkin, 1985; Florence et al., 1992). Organisms have evolved different self-protective mechanisms to maintain low intracellular concentrations of toxic substances: (a) active expulsion of toxicants after they have entered the cell, (b) complexation of metals by biologically synthesized ligands, and (c) oxidation, reduction, or chemical modification of the xenobiotics (Folsom et al., 1986).

Algae are an important part of the water food chain and their ability to bioaccumulate or convert pollutants could play a considerable role in water management (Wong et al., 1995). There is clear evidence that algae and cyanobacteria produce extracellular metabolites whose quantity as well as quality is dependent on nutritional status of algae, pH, light intensity, and presence of parasites (Sell and Overbeck, 1992; Maršálek et al., 1992a; Maršálek et al., 1992b).

The composition of algal extracellular products usually varies among species, according to nutrition and the physiological stage or life cycle of algae. Generally, organic acids, amino acids, peptides, sugars, poly- and oligosaccharides are present in the media after algal cultivation (Hellebus, 1974). The extracellular polypeptide of Anabaena cylindrica, for instance, forms complexes with various ions (Fog and Westlake, 1955). With a cell density of 2–4x10^4 cells/ml alga Nitzia closterium produces an exudate which can complex about 0.3 μM of Cu^{2+}. These exudates are produced only in response to copper ions and the excreted amount increases with concentration of copper (Lumsden and Florence, 1983). Vymazal (1987) observed a higher toxicity of Cd^{2+} than of Cu^{2+} for Scenedesmus subspicatus, and Kaplan et al. (1995) described the same effect for Chlorella vulgaris and Chlorella saccharophila. The observed differences in the sensitivity to these two metals may also be due to the differences in the production of metal-binding proteins, which are produced in response to various heavy metals and have been reported for various algae, for example Chlorella spp. (Rauser, 1990; Kaplan et al., 1995).

The objective of this study is to determine the amount and composition of algal exudates under...
3,5-dichlorophenol and glyphosate stress (toxic organic compounds) in comparison with cadmium chloride (heavy metal) and NaCl (osmotic stressor).

Materials and Methods

Common planktonic algae Scenedesmus quadricauda (TURP) Breb. strain Greifswald 15, Chlorella kessleri (FOTT and NOVAK), strain LARG 1, and Raphidocelis subcapitata, known as Selenastrum capricornutum, strain SKULBERG, 1959/1 obtained from the Collection of Autotrophic Organisms at the Institute of Botany, Treboň, were used. Cultures were cultivated in an orbital cultivator (6000 lx, photoperiod 12 h, 24°C, 1 litre of algal suspension in 2 litre bottles, continuous shaking, ISO medium (1989)). At the beginning of the exponential growth phase, stress factors had been applied for 2 days as follows: NaCl 0.6 mM, CdCl₂ 0.03 mM, 3,5-dichlorophenol 0.4 mM, glyphosate 0.2 mM solutions. The algal cultures were harvested at a density of 2x10⁶ cells/ml by centrifugation and the supernatant was used for determination of algal exudates. The total amount of carbohydrates was determined by the phenol-sulphuric method (Safarik and Santrücková, 1992). The total amount of proteins was determined spectrophotometrically at 545 nm using the method with Coomassie blue (Bradford, 1976). Sugars and amino acids were separated after acid hydrolysis on HPTLC plates and determined by the scanning densitometer (Shimazu, Japan; PAG, 1986).

The statistical differences in the production of exudates between controls and stressed algal cultures were evaluated by the non-parametric Wilcoxon test, α=0.1 (Cohran and Cox, 1957). The results come from the mean of three replicates for every experimental variant. If the variability among the replicates exceeded 10% the experiment was repeated.

Results and Discussion

The results of the experiments with three different species of algae Scenedesmus quadricauda, Chlorella kessleri and Raphidocelis subcapitata, show that the production of extracellular substances can vary from species to species. However, generally the amount of extracellular proteins and carbohydrates increased significantly under the stress conditions (Table I). However, these results are from the young cultures (in the middle of the exponential growth phase). The cultures at the end of the active growth may contain a 3–12 times higher amount of exudates (Marsálek et al., 1992a).

The composition of carbohydrates as well as proteins was determined spectrophotometrically at 545 nm using the method with Coomassie blue (Bradford, 1976). Sugars and amino acids were separated after acid hydrolysis on HPTLC plates and determined by the scanning densitometer.

| Table I. The composition of selected compounds in the medium after the cultivation of algae. All values are in μg/l and represent average of three replicates. A = Scenedesmus quadricauda, B = Chlorella kessleri, C = Raphidocelis subcapitata. |
|----------------------------------|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|
considerable increase of total carbohydrates, whereas the total protein content was affected only slightly (Fig. 1). On the contrary, the production of extracellular proteins was more enhanced after addition of the toxic organic compounds (dichlorophenol and glyphosate) and cadmium. The lowest amount of carbohydrates was produced during cadmium exposure, while the same stress factor led to the highest excretion of proteins of each alga. Thompson and Couture (1991) observed in their study that *Selenastrum capricornutum* (*Raphidocelis subcapitata* in our study) produces three times more proteins than carbohydrates when exposed to cadmium ions. In our case this ratio was much higher (nearly 5 times).

The results presented in the tables are similar to the data of Eberhard and Wegmann (1989) who reported that algae increased the production of proline under stress. However, they focused their attention on the cellular content under cold or drought stress. Wu *et al.* (1995) supported the idea that proline is a compound accumulated in algal cells as a osmoprotectant in relation to the level of salinity, osmolarity and drought as well as to the high levels of intracellular metals. Their experiments showed that a more tolerant species to copper or cadmium accumulates more proline than a sensitive one does. An experiment conducted with a supply of exogenous proline indicated that proline can significantly lower the toxicity of copper to *Anacystis nidulans*.

According to our experience, the quantity of compounds released from algal cells depends on the physiological stage of cultures and thus the algal culture should not be expressed generally as a number of cells per ml. Frequently, a higher amount of exudates per cell is released from cultures at the beginning of exponential growth phase than during the stationary phase. The intensity and type of stress or the physiological stage of the cultures seem to be the main factors increasing the extracellular algal production.

**Fig. 1.** The amount of total extracellular proteins and carbohydrates in the medium after the 48-h cultivation of algae under stress. □ Total proteins, ■ Total carbohydrates.
As mentioned above the intracellular accumulation of algal metabolic products plays also an important role in the protection of algae against the stress factors (Kaplan et al., 1995). One of the most widely spread metal detoxicants are polypeptides, phytochelatins, which were isolated not only from algae but from higher plants and fungi as well (Ahner et al., 1995; Ahner and Morel, 1995).

The natural phytoplankton populations are also discussed as the producers of the algal extracellular substances. There exists a recently published opinion proposing that a reason for algal exudation is to reduce virus infection by encouraging bacterial growth, which in turn may support flagellates. This theory is based on the fact that phytoplankton cells exude dissolved organic matter that supports growth of local bacterial community and consequently heterotrophic flagellates. Both bacteria and flagellates remove more than 50% of viruses before they have a chance to infect their host phytoplankton cell (Murray, 1995). The fact that extracellular products of phytoplankton are utilized by heterotrophic bacteria has been known for a long time. For example, this phenomenon was observed in algal blooms that have higher exudation rates and are closely associated with bacterial development (Nalewajko et al., 1980). However, the relationship is more complicated when one considers that the significance of phytoplankton extracellular products to bacteria differs among environments and time, moreover, the nutritional relationship between algae and bacteria is influenced by zooplankton. In addition, bacteria themselves lyses algal cells and their number increase after the collapse of algal population. On the contrary, they can be suppressed by antibacterial substances produced by algae. Nevertheless, the antiviral protection can be one of the reasons for exudation.

Three algae, whose extracellular production is documented in this paper, are frequently used as test organisms for toxicity assessment (ISO, OECD, U.S.EPA). In connection with the discussed extra- and intracellular products it is an open question whether an algal organism can influence the impact of a toxicant during an algal assay period and change its sensitivity. This phenomenon will be investigated further in a more detailed study.

Acknowledgements

A part of this study was supported by the grant of European Community (Mobility scheme) to B.M. We thank J. Damborský, M.Sc. (Masaryk University, Brno, CZ) for the pragmatic discussion and the critical remarks on the manuscript.
