PHOTOSYNTHETIC RECOVERY OF CHEMICALLY STRESSED ALGAL CELLS AFTER PHOTOINHIBITION

Laszlo Fodorpataki, Mihai Trifu and Judit Papp
Babes-Bolyai University, Dept. Plant Biology, RO–3400, Cluj, Romania

Key words: abiotic stress, aquatic ecosystems, heavy metals, microalgae, photoinhibition, pigments.

1. Introduction

Chemical stress factors, such as soluble heavy metal ions and herbicides that accumulate in polluted freshwater ponds, may alter the way in which green algae use the incident light energy and protect themselves against photoinhibitory damage (1, 2). Microalgae, as the main primary producers of freshwater ecosystems, are generally known as organisms with a pronounced adaptive plasticity , and many of them are hyperaccumulators of xenobiotics from anthropically affected aquatic environments (3). The impact of external factors on the photosynthesis of aquatic plants is of great interest because the primary production of algal biomass is strongly dependent on the prevailing photosynthetic rates in a dynamic environment. In freshwater microalgae the limitation of photosynthesis oftenly occurs due to the combined effect of low photon flux densities, low concentration of the carbon source and accumulation of soluble xenobiotic compounds (4, 5).
The aim of this work is to reveal some aspects of the interaction of too high photon flux densities and chemical toxicity, as two main stress factors that alter the plants' capacity to cope with oxidative damage and to dissipate safely the excessive excitation energy.

2. Material and methods

Experiments were carried out with axenic monoalgal cultures of *Scenedesmus obliquus* (Turp.) Kutz. and *Chlorella pyrenoidosa* Chick, illuminated continuously with 45 micromole photons $m^{-2}s^{-1}$ (control) or 180 micromole photons $m^{-2}s^{-1}$ (excessive light). The initial density of the cultures was 800 cells per microliter. The cultures grown in the Kuhl-Lorenzen nutrient solution were supplemented with 10 μM and 0.25mM nickel (2+), 10 μM and 0.25mM zinc (2+) or 10μM and 0.25mM sodium gluphosinate (6).
The photosynthetic pigment content of the algal cells was determined spectrophotometrically after extraction with methanol and acetone, performed at $4^{o}C$ in dim light (7). The net oxygen evolution of the cell suspensions was measured

G. Garab (ed.), Photosynthesis: Mechanisms and Effects, Vol. IV, 2685–2688.

polarographically with a Clark-type electrode, in saturating light. The catalase activity was measured titrimetrically on the base of the unsplitted H_2O_2 after 1 hour of incubation with 10 ml H_2O_2 3% (8).
The short-term (1.5s) chlorophyll *a* fluorescence induction transients (10) were measured in samples excited with red light (660 ±10nm) after a dark incubation for 3 minutes.

3. Results and Discussion

The level of light intensity at which photosynthesis becomes saturated because of limitation of energy utilization is influenced by various environmental stress factors. Whenever the input of radiant energy exeeds the alga's capacuty to use it, excess light may lead to the production of dangerous pigment and oxygen species. Accumulation of heavy metals and of herbicides alters the plants' capacity to cope with oxidative damage or to dissipate the excess excitation energy safely as heat, before toxic radicals form.

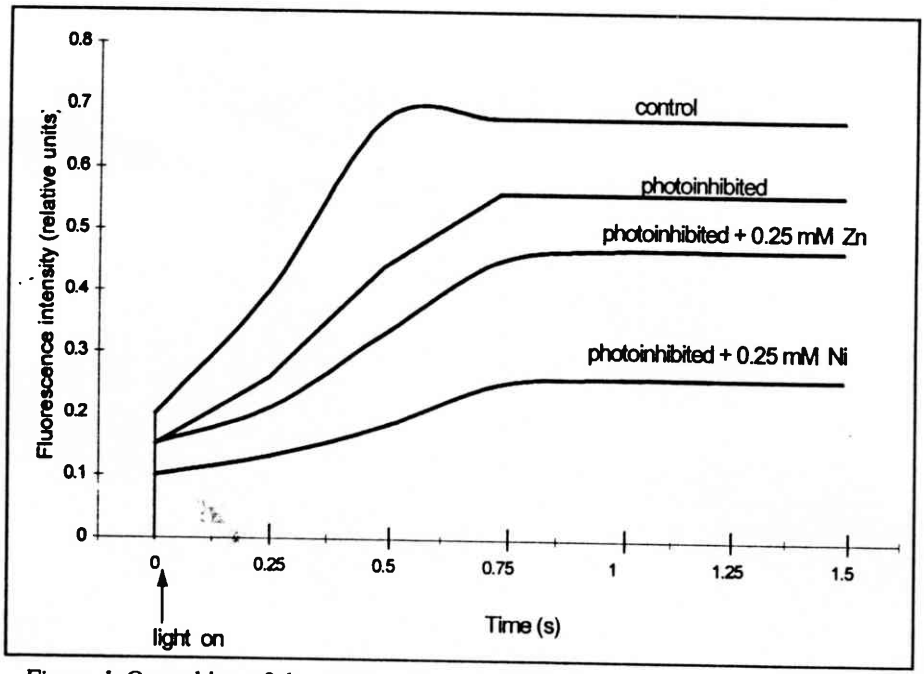

Figure 1. Quenching of short-term chlorophyll fluorescence by photoinhibition and additional heavy metal stress in cultures of *Scenedesmus obliquus.*

Because emission of fluorescence by the photoexcited chlorophylls is in direct competition with the photochemical reactions, the dynamics of the induced fluorescence is an early indicator of damage of the photosynthetic apparatus induces by external factors.
Photoinhibition perturbs the functional organization of the light-harvesting pigment-protein complexes (decreased Fo). Nickel and zinc act synergically with the excessive light intensity during the impairment of photochemical reactions occuring in photosystem II (Fig. 1).
Photoinhibition causes a significant reduction of the chlorophyll content. The amount of chlorophyll *b*, which occurs mainly in the peripheral light-harvesting complex of photosystem II, is reduced by more than 65 %. In the same time, the amount of photoprotective carotenoid pigments becomes 2 times higher. Heavy metal stress enhances the effect of photoinhibition concerning the reduction of the chlorophyll content; e.g. the photoinhibited cells treated with 0.25 mM nickel do not possess chlorophyll *b* at all (Fig.2).

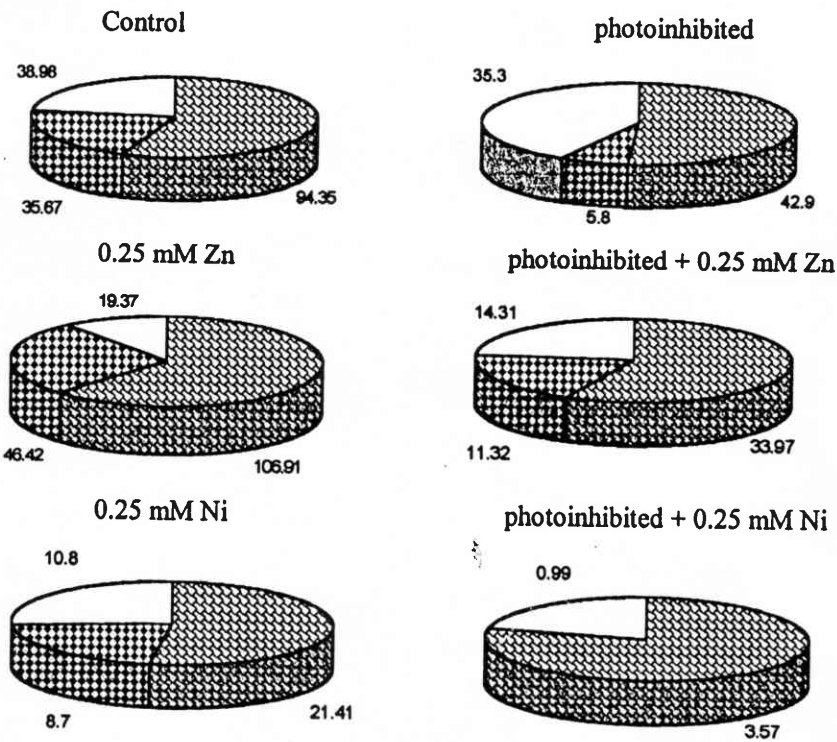

Figure 2. Photosynthetic pigment content of *Scenedesmus obliquus* (mg g^{-1} d.w.) in photoinhibited and chemically stressed cultures

(▨ - chlorophyll *a;* ▩ - chlorophyll *b* ; □ - carotenoids)

Net O_2 production of algal cells reflects the overall efficiency of the conversion of light into chemical energy stored in the cells. The herbicide gluphosinate, which interferes with the photorespiratory metabolism, increases the net oxygen evolution of the algal cells, while heavy metal stress predisposes to photoinhibition of energy-converting light reactions and lowers by 40-60% the photosynthetic oxygen production.
The steady state level of catalase activity appears to change significantly whenever stress effects either excessively enhance deleterious oxidative conditions and degradation, or inhibit repair mechanisms. Functional disturbance of catalase and of photosystem II represent specific and widely occurring early symptoms of incipient damage to the algal cells, indicating stress conditions where the repair capacity is not sufficient.

4. Conclusions

Chemical stress factors, which restrain the rate of photosynthesis, predispose the algal cells towards photoinhibition, and inhibit or delay the recovery of light-harvesting and light energy-converting systems from damages caused by excessive photon flux densities.
Photoinhibition enhances the enzymatic activity of catalase, but this effect cannot be exhibited when photoinhibition is combined with the action of sodium gluphosinate, which is a herbicide that disturbs the metabolism of ammonium and interferes by feed-back inhibition with the photorespiratory carbon oxidation cycle.
The presence of 0.25 mM Zn^{2+} compensates the increase in the carotenoid pigment content caused by photoinhibition, leading to a less effective photoprotection of the algal cells.
In both algal species the amount of chlorophyll *b*, which occurs mainly in the peripheral light-harvesting complex of PS II, is decreased by more than 65% under photoinhibitory conditions, and this reduction becomes maximal when excessive photon flux densities interact with millimolar amounts of nickel ions.

References

1 Aro, E.-M., McCaffery, S. and Anderson , J.M. (1993) Plant Physiol. 103, 835-843
2 Horton, P. and Ruban , A.V. (1992) Photosynth. Res. 34, 375-385
3 Őquist, G., Chow, W.S. and Anderson, J.M. (1992) Planta 186, 450-460
4 Barber, J., Guerrero, M.G. and Medrano, H. (1992) Trends in Photosynthesis Research, Intercept Ltd., London
5 Liker, E. and Garab, Gy. (1995) Physiol. Plant. 93, 187-190
6 Nagy-Tóth, F., Péterfi, L. and Fodorpataki, L. (1992) Acta Bot. Hung. 37, 295-316
7 Kubin, S. (1991) Arch. Protistenkd. 139, 111-116
8 Zhang, J. and Kirkham, M.B. (1994) Plant Cell Physiol. 35, 785-791
9 Horváth, G., Droppa, M., Fodorpataki, L., Istokovics, A., Garab, Gy. and Oettmeier, W. (1996) Proc. Natl. Acad. Sci. USA, 93, 3876-3880
10 Schweiger, J.,Lang,M. and Lichtenthaler, H.K. (1996) J. Plant Physiol. 148, 537-547