PHYTOCHELATIN SYNTHESIS BY MARINE MACROALGAE LIVING UNDER NATURAL HEAVY METAL CONCENTRATIONS IN THESSALONIKI BAY, NORTHERN GREECE

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Abstract

Thioles are sulfuric analogous of alcohols and include phytochelatins (PT) with cystein and glutathione (GSH) being the most common peptides in plants. When metals (Hg, Pb, Cu) are present, thioles form chelating complexes. Photo-autotrophs are the main entrance for heavy metals in the trophic chain with humans and animals as the final receivers. Phytochelatins are polypeptides, different from the metallothioneis (MT) of mammals, and their synthesis is linked to the presence of metals. Biosynthesis of MT is connected to the metabolism of glutathione, and only limited information exists about their role in macroalgae. An innovative HPLC method for measuring micromoles of the peptides is presented in this study.

Key words: algae; metals; analytical methods; Greece

Introduction

The reduced form of glutathione, GSH, is a tripeptide (γ-glu-cys-gly) that exists interchangeably with the oxidized form, GSSG. In plants, the physiological importance may be divided in two categories: sulphur metabolism and defence. GSH is the predominant non-protein thiol (1) and it regulates sulphur uptake at root level in the plant. It is also the precursor of the phytochelatins, which are essential in sequestering heavy metals (2). Heavy metal toxicity poses major environmental and health problems. Cadmium, for example, is a non-essential heavy metal, which is toxic to cells at very low concentrations. Cadmium ions displace Ca++ or Zn++ in proteins and may cause oxidative stress (3). Furthermore the concentration of essential, but at high concentrations toxic, metals such as Cu++, Zn++, Fe++ is strongly controlled. We present data on phytochelatin concentrations in natural samples of dominant macroalgae species from Thessaloniki Bay, a body of water under the impact of increasing pollution levels.

Methods - materials

Algae specimens of the dominant species in the Thermaicos Gulf were collected on a monthly basis from inshore of a heavily industrialized area. Reduced, oxidized, and free glutathione and cystein were determined in algal tissue with post column derivatization with monobromobimane and liquid chromatography. The chromatography system was composed of a Hewlett-Packard Series 1100, fluorescence detector Hewlett-Packard 1046A and Hewlett-Packard CHEM Station for LC Version A.04.02 (1996). Chromatography columns for inverse phase chromatography were packed with 3_m Hypersil ODS at 9000 p.s.i. Heavy metal concentrations in the algal samples were measured with AAS (6).

Results and discussion

Seasonal variation of the oxidized, reduced and total cystein in Chlorophyceae showed an inverse pattern. The concentration of the reduced form was only high during spring 1994.

Reduced and total glutathione exhibited a similar fluctuation and the highest concentrations coincided with that of cystein. The oxidized forms showed the opposite temporal trend.

Environmental conditions caused much higher synthesis of glutathione than cystein in Chlorophyceae where metals accumulated to a greater degree than in Rhodophyceae (7). Further statistical analysis (multi-factor linear correlation) showed that 90% of the concentration variation of all forms of glutathione was due to the combined presence of Cr, Pb, Cd and Zn. Synthesis of glutathione was even better attributed to the synchronous effect of Cu, Pb, Cd and Zn and copper ions were suspected for causing extended production for the peptide (8).

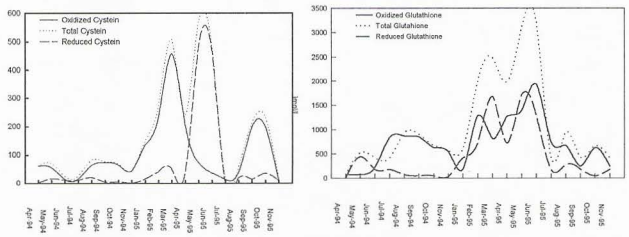

Fig. 1: Oxidized, reduced and total cystein and glutathione concentration (µmol/l) over time in the dominant Chlorophyceae.

Table 1: Uptake of Pb, Zn, Cu, Cr and Cd and glutathione and cystein biosynthesis in Chlorophyceae and Rhodophyceae.

	Rhodophycea				Chlorophyceae		
	mean value (µg/g dry wt)	min-max	SD		mean value (µg/g dry wt)	min-max	SD
Pb	3.29	1-18.33	3.62	Pb	3.6	1.5-5.9	1.42
Zn	3.10	0.7-7.03	1.73	Zn	6.36	2.7-10.9	3.04
Cu	0.49	0.15-1	0.22	Cd	55.17	2-18	50
Cr	552.72	53.33-1966	531.92	Cr	477.2	10-1766	547.3
Cd	65.9	4-705	149.2	Cu	683.7	0.324-1	0.3
	(µg/l)				(µg/l)		
Oxy-Cys	103.74	13.1-456.76	111,8		4.5	1,7-5.64	1,03
_xy-Glu	710.4	68.9-1928.5	497.5		50.18	29.7-71.1	4,6
Tot-Cys	5.3	23.1-566.4	175.8		5.3	1.7-6.3	1.248
Tot-Glu	1094	70-3213	961.3		54.1	32.6-75.8	15.96
Red-Cys	64.4	0.1-473	144.2		1.25	0.3-2.01	0.47
Red-Glu	441.2	1.6-1706.1	544.8		3.82	1.4-5.98	1.4

References

1. Rennenberg H., 1982. Glutathione metabolism and possible biological roles in higher plants. *Phytochemistry* 21:27771-81.
2. Grill E., S. Löffler, E. Winnacker, M.H. Zenk 1989. Phytochlelatins, the heavy metal binding peptides of plants are synthesized from glutathione by a specific γ-glutamylcysteine dipeptidyl transpeptidase (phytochelatine synthetase). *Proc. Natl. Acad. Sci USA* 86:6838-42.
3. Goyer, R.A., (1997). Toxic and essential metal interactions. *Annu. Rev. Nutr.*, 17, 37-50
4. Svardal M. A., M. A. Mansoor P. M. Ueland, 1990. Determination of Reduced, Oxidized, and Protein-Bound Glutathione in Human Plasma with Precolumn Derivatization with Monobromobimane and Liquid Chromatography. *Analytical Biochemistry*, 184, 33-346.
5. Determination of the in vivo redox status of cystein, cysteineglycine, homocysteine and glutathione in human plasma, 1992. *Analytical Biochemistry*, 200, 21-229.
6. Atomic Absorption Spectrometry, 1979 version. An essay review, K.C. Thompson. Methods for the Examination of Waters and Associated Materials, Dept. of The Environment, Standing Committee of Analysis, HMSO 1980, pp. 1-50.
7. Ahner B. A., N. M. Price, F. M. M. Morel, 1994. Phytochelatin production by marine phytoplankton at low free metal in concentrations: Laboratory studies and field data from Massachusetts Bay. *Proc. Nat Acad. Sci. USA*, vol. 91, 8433-8436.
8. Noctor G., C. H. Foyer, 1998. Ascorbate and Glutathione: keeping active oxygen under control. Annu. Rev. Plant Phsyiol. *Plant Mol. Biol.* 49: 249-279.