BIOCHEMICAL ANSWER OF POSIDONIA OCEANICA TO A METALLIC STRESS

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Abstract
In order to demonstrate a biochemical answer to a metallic stress in *Posidonia oceanica* (L.) Delile, GST activity variations and isoenzyme A1/A1 induction have been studied for a mercuric contamination. Sampling is realized over an annual cycle in (i) an anthropized site, with highly mercuric contamination, and (ii) a reference site. Sites contaminated by mercury were those for which the highest enzyme activities were recorded, and mercury seems to increase GST activity, particularly in the bases of *P. oceanica* leaves. Moreover, A1/A1 isozyme is revealed only in bases and seems to be induced by mercury.

Key words: Phanerogams , mercury, enzymes, bio-inidicators

Introduction

Glutathione S-transferases (GSTs) are a group of dimeric enzymes catalyzing the conjugation of electrophilic xenobiotics with the endogenous glutathione (GSH) (1, 2).The effect of this reaction is generally to convert a reactive lipophilic molecule into a water soluble, non-reactive conjugate which may easily be excreted or stored (2). Plant GSTs can be induced by biotic stimuli such as pathogen invasion and abiotic stimuli, such as herbicides and heavy metals (3). Authors indicate that the level of expression of GST is a crucial factor in determining the sensitivity of cells to a broad spectrum of toxic chemicals and that GST induction is part of an adaptive response mechanism to chemical stress (4).

Material and methods

Shoots of *P. oceanica* were collected (i) in an anthropized (A) site subjected to mercury industrial wastes, in Rosignano (Italy), and (ii) in a reference (R) site in Lérins Islands (France). Only bases and blades from adult leaves were analyzed. Mercury concentrations were measured, after digestion of the tissues, by CVAA (Perkin Elmer FIMS 100). GST activities were determined in the post-mitochondrial fraction, by UV-visible spectrophotometry (Uvikon 930, Kontron) with reduced Glutathione (GSH) and Chlorodinitrobenzene (CDNB) as substrates (5) and expressed according to protein levels (determined following 6). Proteins from Rosignano and Lérins shoots were separated on SDS PAGE 12% and transferred to Hybond membrans. Blots were blocked in skimmed milk 5%, incubated overnight with 6 $\mu g.mL^{-1}$ purified GST A1/A1polyclonal antibody (provided by 3) and then with a 1:15000 dilution of antichicken IgG-HRPeroxidase and finally revealed by ECL method.

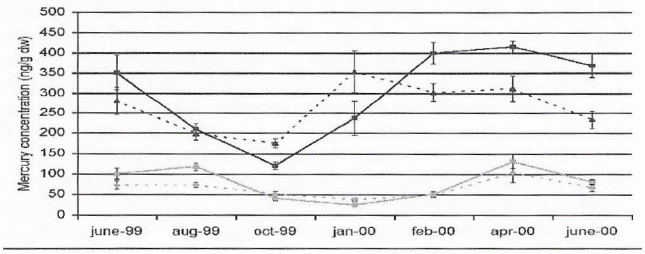

Figure 1: Mercury concentration in *P. oceanica* bases and blades from stations R and A.

Results

Mercury concentrations (fig.1) are significantly higher at station A (264 and 299 $ng.g^{-1}$ y^{-1}dw on average, for blades and bases, respectively) as compared to R (63 and 77 $ng.g^{-1}$ y^{-1} dw), regardless of the period. GST activity (fig.2) is higher at station A, regardless of the period (120 and 178 $nmol.min^{-1}.mg^{-1}$ prot., for R and A, respectively).

Enzyme activity in adult leaves was always higher in the bases, regardless of station or period (110 and 190 $nmol.min^{-1}.mg^{-1}$ prot. for blades and bases, respectively). ECL revelation (fig.3) demonstrates the presence of a 31kD band (A1/A1 isoenzyme) which is only present in the bases of *P. oceanica* and more accentuated from the anthropized site

Discussion and conclusion

GST activity was observed to be higher in anthropised site, it would appear that an elevated metal content is responsible for higher GST activity as yet demonstrated (5). GSTs are known to play a role in the protection of the cell against oxidative stress through the metabolism of glutathione (7). According to these authors, metals may evoke a decrease in glutathione content which could be mainly related to a stimulation of GST activity. The increase in GST activity, observed in *P. oceanica*, may thus be considered as an indirect or secondary effect of mercury.

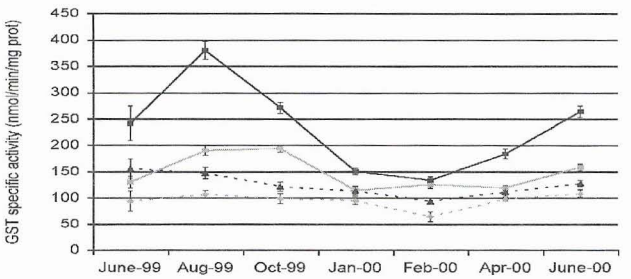

Figure 2: GST activity in *P. oceanica* bases (full line) and blades (dotted line) from stations R (grey) and A.

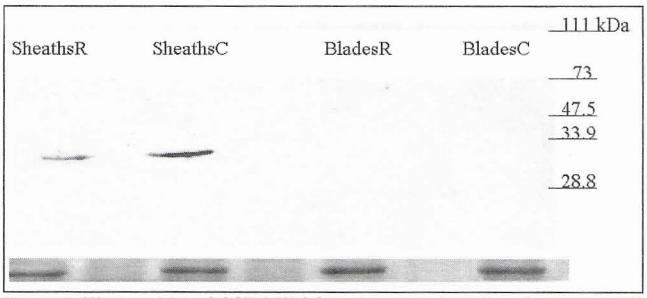

Figure 3: Western blot of GST A1/A1 from bases and blades of reference (R) and anthropized (A) stations. The amount of protein per lane was 8.6 µg.

Contrary to the bases, blades exhibited very low activity levels and thus provide very little information concerning variations in GST activities between seasons and between stations. These observations can be explained with the presence of A1/A1 isozyme only in the bases of *P. oceanica*, this isozyme being characterized by an high CDNB activity (3). The results, provided here, reveal that GST activity may represent a valuable marker for mercury contamination. Interpretation of GST activities along with mercury concentration data associated with isoenzymes induction can allow us to conclude that this metal induces GST activity, at least for A1/A1 isoform. It is therefore possible that another isoform, exhibiting a low CDNB activity, but high activity for an other substrate (ethacrynic acid, metolachlor...) is present, for instance in the blades. In fact, in a given organism, specific GST activities may be used as a criterion for distinguishing GST iosenzymes (3, 8). Only separation and characterization of all the isoenzymes will be necessary to determine whether or not the induction of one or several of these isoenzymes can be attributed to mercury contamination in *P. oceanica*.

References

1. Cummins I., Cole D.J., Edwards R., 1997. Purification of multiple glutathione transferases involved in herbicide detoxification from wheat (Triticum aestivum L.) treated with the safener fenchlorazole-ethyl. *Pest. Biochem. Physiol.*, 59: 35-49.
2. Clark A.G., 1989. The comparative enzymology of the glutathione S-Transferases from non-vertebrate organisms. *Comp. Biochem. Physiol.*, 92B:419-446.