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Cadmium Effects on Oxidative Metabolism in a Marine Seagrass: *Posidonia oceanica*

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Posidonia oceanica, a seagrass in contact with sediment, is an interesting organism for environmental biomonitoring. In fact, up to now, scientists have studied some pollutant effects on growth rate, leaf length, or productivity of this species (Maggi et al. 1977; Pergent 1991). In recent years, the topic of metabolism of xenobiotics in plants has arisen. Many of the environmental chemicals are biotransformed by plants in a manner similar to those in mammalian systems, Studies of the influence of pollutants on biotransformation enzymes in Posidonia oceanica are of great interest in pollution monitoring programs especially in the Mediterranean sea.

In a previous study, we characterized some enzymatic systems involved in the metabolism of xenobiotics (Narbonne et al. 1991; Hamoutene et al. 1992; 1995) in Posidonia oceanica. This study was designed to determine the effects of cadmium on certain biochemical functions in this species.

In some aquatic animals, selected metals have been shown to increase the peroxidation of membrane lipids (Viarengo et al. 1990). In this study, we chose to measure the Thiobarbituric Acid Reactive Species (TBARS) content as an indicator of the lipid peroxidation induced by exposure to cadmium. The TBARS amount is an interesting biomarker of the general oxidative stress due to cadmium. In recent years, the effect of cadmium on cytochrome P-450 monoxygenase activities has been investigated in several species of fish (Forlin et al. 1906; George 1989). The purpose of this study was to determine the effect of Cd on a P-450-dependent activity: ethoxyresorufin O-deethylase (phase I) and on a phase II conjugation enzyme by measuring glutathione S-transferase enzymatic activity.

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MATERIALS AND METHODS

Samples of *Posidonia oceanica* were collected by diving at depths of 3 to 5 m at two sites on the French Mediterranean coast: the Iles de Lerins, small islands off the coast of Cannes, and the bay of Villefranche-sur-mer. In an initial step, experiments were performed only on the non-photosynthetic basal parts of the older leaves; chlorophyll could interefere with the spectrophotometric assay for cytochrome P-450 detection. These etiolated foliar tissues were incubated in aerated artificial seawater containing 5, 10 and 20 µg $CdNO_3$/Liter seawater for 48 hr in the dark. Cadmium incubations were also performed on photosynthetic parts of leaves.

Following incubation with Cd, the basal parts of *Posidonia* leaves were rinsed with distilled water and crushed in a mortar containing liquid nitrogen. The powder obtained was suitable for direct preparation of microsomes (Hamoutene et al. 1992; 1995) or conservation in liquid nitrogen. After homogenization of the samples, a first centrifugation was performed (10,000g), then supernatants (S10) were centrifuged at 100,000g to obtain microsomes (Cl00) and supernatants (Sl00).

For cadmium analysis, basal parts of the leaves were first thawed and dried at 60°C to a constant weight. Digestion of leaves was performed in a microwave oven (CEM MDS8lD). Analyses were determined by atomic absorption spectrophotometry (GBC 904AA) with a graphite furnace (GBC 3000 with PAL 3000 autosampler). The recovery was 98%. The limit of detection was 2 ng Cd/g dry weight.

TBARS amounts were measured in the supernatants (Sl0) and the microsomal pellet (C100) according to the procedure of Buege and Aust (1978); this method allows quantitation of the products of lipid peroxidation reacting with thiobarbituric acid. TBARS formation was measured with or without addition of Fe-ADP in the incubating medium.

Ethoxyresorufin O-deethylase (EROD) activity was assayed using the method of Stegeman et al. (1988) on S10 supernatants. This method is based on spectrophotometric detection of hydroxyresorufin.

Glutathione S-transferase was assayed according to the method of Baars et al. (1978) in supernatants (Sl00), using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. *In vitro* tests were also performed by adding Cd directly to the supernatants; we tested concentrations varying from 2 to 60 ng Cd/ mg of protein in the assay.

Protein concentrations were determined using a Lowry reagent (Lowry et al. 1951) with Bovine Serum Albumin (BSA) as a standard.

RESULTS AND DISCUSSION

The etiolated basal portions of leaves accumulated lower amounts of Cd (Table 1) than the chlorophyllian parts (Table 2). This information is comparable to results of metal contents measured *in situ* in the different parts of *Posidonia oceanica* leaves. Catsiki et al. (1987) have observed a decrease of metal concentration in this order: basal parts < distal parts.

Table 1. Cadmium concentration in non-chlorophyllian leaf basal parts (ng/g dry matter).

	Villefranche-sur-mer		Iles de Lerins	
Cd treatment	July 93	Feb. 94	May 93	May 94
Controls	397.50	116.00	361.60	224.34
5 µg/L	458.69	603.00	396.05	235.36
10µg/L	496.12	1325.00	502.99	384.00
20 µg/L	602.85	861.00	548.79	515.14

Table 2. Cadmium concentration (ng/g dry matter) in chlorophyll-containing leaf parts.

	Villefranche-sur-mer	Iles de Lerins
Cd treatment	July 93	May 93
Controls	719.00	734.21
5 µg/L	2639.55	1439.77
10µg/L	1492.09	3404.00
20 µg/L	8292.23	4661.36

A series of measurements was also made concerning the Cd concentration per mg protein following subcellular fractionation of *Posidonia* leaf basal parts (Table 3). The protein percentages of each fraction are indicated in brackets. The fractions were: 1) the 10,000g pellet (C10) which contained the nucleus, the mitochondria, the chloroplasts and the cell wall fragments; 2) the 100,000g supernatant (Sl00) representing the cytosol fraction and 3) the 100,000g pellet (Cl00) containing the microsomes.

Table 3. Subcellular distribution of cadmium in *Posidonia oceanica* leaf basal parts expressed as ng Cd/mg protein. and in % Cd in each fraction. (-: not detected, H: homogenates).

Fraction		H(100%)	C10(13.98%)	S100(76.61%)	C100(5.37%)
Controls	ng Cd/mg protein	4.36	0.97	5.32	-
	% Cd	100	2.73	93.48	-
5 µg/L	ng Cd/mg protein	6.68	6.40	5.86	-
	% Cd	100	11.76	67.20	-
10 µg/L	ng Cd/mg protein	7.43	5.50	8.26	-
	% Cd	100	9.09	85.17	-
20 µg/L	ng Cd/mg protein	12.59	17.45	11.45	-
	% Cd	100	17.02	69.67	-

These data revealed that a major portion of the Cd accumulated by the plant was found in the soluble fraction (Sl00) which consisted essentially of cytosol and other soluble compounds resulting from destruction of the various cellular compartments. In contrast to our results, Fabris et al. (1982) showed that in the seagrass Heterozostera tasmanica a considerable amount of Cd was bound to the solid structure (fiber fraction) of the leaves after Cd treatment. Nevertheless, we observed an increase in the percentage of Cd in the Cl0 fraction with the increase of Cd concentrations, showing perhaps a progressive binding to membranes. No Cd was detected in the microsomes.

For lipid peroxidation tests, both non-induced peroxidation (lipids that are already peroxidized or that peroxidize during the reaction) and peroxidation initiated by Fe-ADP were measured. For both batches (2 sampling locations) of plants, the amount of TBARS measured in the Sl0 fractions without incubation with Fe-ADP decreased following incubation of Posidonia leaf basal parts with Cd (Figure 1). A two -way ANOVA (incubation with Cd/origin of Posidonia specimens) demonstrated a highly significant decrease in non-induced TBARS content ($p < 0.05$) after Cd treatment. In addition, there was no interaction between the origin of the plants and exposure to Cd. A decrease in non-induced lipid peroxidation was also observed in the microsomes (Cl00) of the plants from the Iles de Lerins, but was not confirmed by the measurements on the Cl00 of Posidonia samples from Villefranche-sur-mer. After in vitro initiation of peroxidation with Fe-ADP, the TBARS content in the supernatants Sl0 and the microsomal pellets varied with the origin of the plants.

Viarengo et al. (1990) found that exposure of mussels to Cd or Zn did not affect the TBARS (measured without in vitro initiation of peroxidation) levels in the examined tissues. In contrast, Cu and Fe were responsible for an increase of TBARS amount. As Fe and Cu ions are thought to be involved in redox reactions, they may participate both in the initiation and propagation steps of lipid peroxidation; Cd and Zn which do not undergo redox cycling were found unable to stimulate the lipid peroxidation process in mussel tissues (Viarengo et al. 1990). In contrast, an increase of in vitro lipid peroxidation was observed in incubates of microsomes prepared from the livers of croaker (Micropogonias undulatus) exposed to Cd in the water (Wofford and Thomas 1988).

As EROD activity in Posidonia oceanica is unstable due to difficulties inherent in measuring EROD, the results obtained in this study must be interpreted with caution (Table 4).

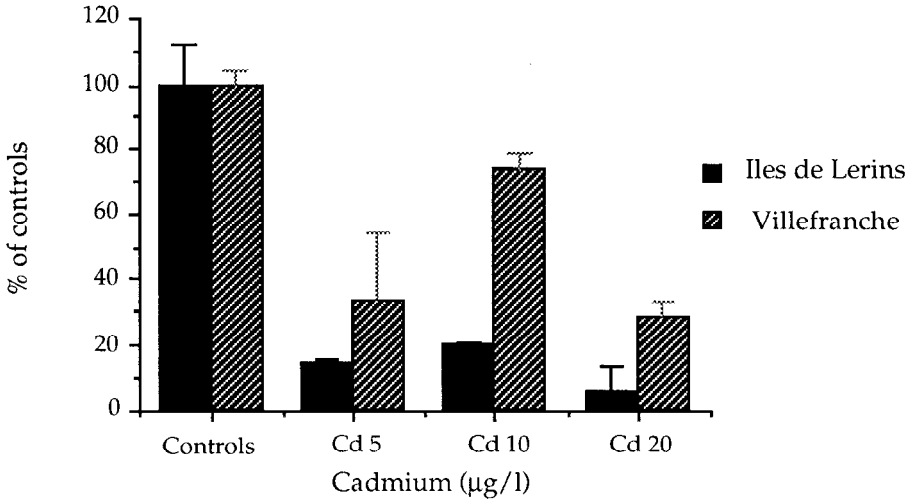

Figure 1. TBARS content (without Fe-ADP) in S9 fractions (basal parts of leaves) as a percentage of controls (all the values are significantly different compared to controls).

For the Posidonia samples from Villefranche-sur-mer, EROD activity was no longer proportional to the quantity of proteins after incubation with 5 and 10 µg/L of Cd; no EROD activity was detectable after incubation with 20 µg/L of Cd. For the Posidonia samples from Iles de Lerins, a decrease of EROD activity was already observed with 10 µg Cd/L.

Table 4. EROD activity in S9 fractions (basal parts of leaves) expressed in pmoles/min/mg protein.
(±: activities not proportionnal to the quantity of proteins in the assay).

	Villefranche-sur-mer	Iles de Lerins
Controls	38.59	381.03
5 µg/L	±	±
10µg/L	±	0
20 µg/L	0	0

Inhibition of cytochrome P-450-dependent enzyme by Cd has previously been described in fish; authors observed a significant decrease in EROD activity after treatment with Cd (Forlin et al. 1986; George 1989). In contrast, in the case of plants, incubations with Cd resulted in an increase of some P-450-dependent enzymatic activities (Batard et al. 1995).

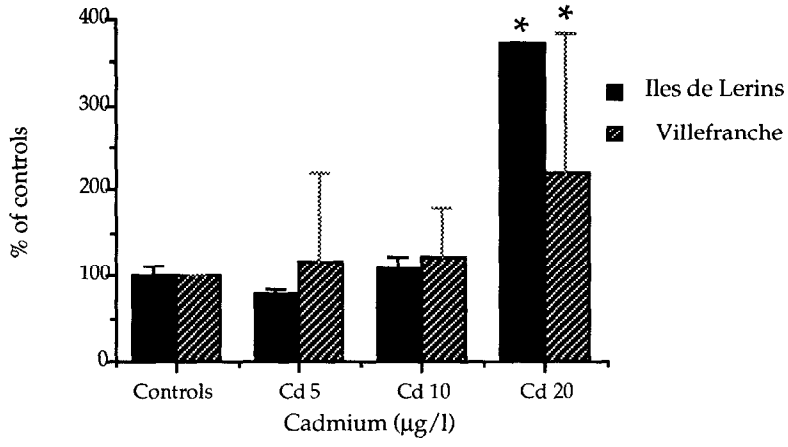

Figure 2. GST activities as a percentage of controls (* statistically significant different compared to controls).

Exposure to 5 and 10 µg/L Cd did not have any significant effect on the glutathione S-transferase activity in the supernatants S100. By contrast, a significant increase in activity was observed with both batches of Posidonia treated with 20 µg/L (Figure 2). An increase of GST activity was also observed after in vitro tests with concentrations varying from 2 to 60 ng Cd/ mg protein. Both results suggest that this increase could be due to a direct effect of Cd on enzyme activity. The binding of metallic cat ions to enzymes can alter their activity, not only by inhibiting, but also by stimulating the catalytic function of the enzymes (Eichhorn et al. 1969).

Apart from their role in the metabolism and transport of endogenous compounds, the glutathione S-transferase isozymes play an essential role in protecting organisms from peroxidative damage (Mannervik 1985). The decrease in the peroxidative phenomena in Posidonia (without in vitro initiation) following exposure to Cd could perhaps be associated with the development of a protection system based on enzymes (GST) or active molecules like glutathione or ascorbic acid. Glutathione, one of the most important soluble compounds involved in cellular protection, appears to be associated in plants with the production of phytochelatins, molecules which play a similar role as animal metallothioneins (Gekeler et al. 1989).

This study on the effect of Cd on certain aspects of the xenobiotic metabolism in Posidonia oceanica provides a

number of indications concerning possible defense systems in this plant. The study of inhibition or induction phenomena of this plant enzymatic system could allow the use of Posidonia in pollution monitoring programs. In fact, monitoring the response of different species at different trophic levels for impact assessment can help to establish the importance of different routes of exposure (Livingstone 1993). Additional experimental studies are required to elucidate the numerous factors that can modify the enzymatic systems of Posidonia.

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