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Glutathione-Dependent Toxicity of the Algicide 1-Chloro-2,4-dinitrobenzene to *Daphnia magna* Straus

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Conjugation of xenobiotics with glutathione (GSH) is a biotransformation process that generally results in the formation of less toxic products. In higher organisms, GSH S-transferases play a key role in the detoxication of a large number of chemicals (Chasseaud 1979). These enzymes facilitate nucleophilic attack of the sulfhydryl group of GSH on the electrophilic centre of a broad spectrum of compounds. An impressive number of the latter are known as water pollutants (Anonymous 1979). We recently demonstrated that all the freshwater macro-invertebrates investigated contained GSH S-transferase activity (Dierickx 1984). However, the availability of GSH rather than its transfer rate to electrophilic agents may be a limiting factor in cellular protection (Darby 1980).

We now report the importance of the GSH status, modified by two biochemically different ways, on the toxicity of 1-chloro-2,4-dinitrobenzene (CDNB) in the freshwater organism Daphnia magna Straus. We selected this "lower" organism because it is frequently used in ecotoxicological studies, and the test compound CDNB was choosen because it is used as an algicide in cooling waters and because it is a very good substrate for GSH S-transferases (Habig et al. 1974).

MATERIALS AND METHODS

GSH and 5,5'-dithiobis-(2-nitrobenzoic acid) were obtained from Janssen Chimica (Beerse, Belgium), L-buthionine-S,R-sulfoximine (BSO) from Sigma (St. Louis, USA), and bovine serum albumin from Boehringer Mannheim (W. Germany). L-Oxothiazolidine-4-carboxylic acid (OTC) was synthesized by Dr De Keukelere (Rijksuniversiteit Gent, Belgium) by the method of Kaneko et al. (1964) as modified by Shah et al. (1979). All other chemicals, including CDNB, were purchased from Merck (W. Germany). The Daphnia magna Straus were available in our institute; only adult daphnids were used.

Daphnia magna were cultured according to the ISO standard 6341 E (1982). Acute toxicity tests were conducted on adult daphnids. CDNB concentrations ranged from 0.12 to 1.85 mg/L, made up in ISO

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water (ISO standard 6341 E 1982) with a hardness of 250 + 25 mg/L (as CaCO3) and a Ca:Mg ratio of 4:1. 20 Organisms (4 replicates of 5 individuals each) were tested at each concentration. For the calculation of the EC(I)50 (effective concentration of 50% immobilisation) the number of mobile daphnids was counted in each container. The daphnids that were not able to move within the 15 s following a gentle agitation of the test vessel were considered to be immobilized. During the acute test the pH and temperature measurements ranged from 7.0-7.6 and 19.0-20.0 °C, respectively. The EC(I)50 values and the confidence limits were estimated by probit analysis (Finney 1971).

Living daphnids were put in test solutions for the determination of the GSH content. After 24 h the daphnids were homogenized in 100 mM Sørensen phosphate buffer, pH 7.4, in a motor driven Potter-Elvehjem homogenizer, equipped with a teflon pestle (5 up-and-down strokes, 1500 rpm). The homogenate was centrifuged for 15 min at 3000 xg. Part of the supernatant was used for a protein determination according to Bradford (1976) using bovine serum albumin as standard. In another part of the supernatant the proteins were precipitated by addition of an equal volume of 4% sulfosalicylic acid; 0.5 mL of the clear liquid was than used for the GSH determination with 5,5'-dithiobis-(2-nitrobenzoic acid) according to Ellman (1959).

RESULTS AND DISCUSSION

To study the decrease or increase in GSH, BSO and OTC were used. The former is a specific inhibitor of γ-glutamylcysteine synthetase (Griffith 1982) resulting in a decrease of the endogeneous GSH concentration in higher organisms, while the latter functions as a substrate for 5-oxo-L-prolinase (Williamson et al. 1982) resulting in an increase. Treatment of living daphnids with these compounds resulted in the same effect. Culturing the daphnids for 24 h in 0.32 mM OTC resulted in an increase of about 40% in GSH content, while in 0.32 mM BSO a decrease of about 40% was observed (Fig. 1).

Table 1 shows the influence of OTC and BSO on the EC(I)50 values

Table 1. EC(I)50 values (in mg/mL) for adult Daphnia magna, with 95% probability confidence limits in parentheses.

Experiment	Treatment	24h-$EC(I)_{50}$	48h-$EC(I)_{50}$
A	CDNB	0.66 (0.64-0.67)	0.49 (0.48-0.51)
	CDNB + 0.32 mM OTC	0.95 (0.93-0.97)	0.89 (0.87-0.91)
B	CDNB	0.85 (0.83-0.87)	0.59 (0.58-0.61)
	CDNB + 0.32 mM BSO	0.70 (0.68-0.72)	0.51 (0.49-0.53)

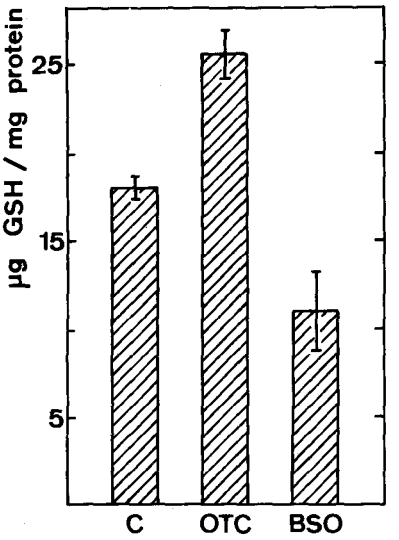

Fig. 1. The influence of OTC and BSO at 0.32 mM on the GSH content in Daphnia magna. The daphnids were incubated for 24 h. C = control. Bars represent 95% confidence intervals limits.

of CDNB. This compound can be considered as very toxic towards Daphnia magna. OTC enhanced the EC(I)50 values by 44% after 24 h and by 80% after 48 h; it thus had a protecting effect. BSO, however, reduced the EC(I)50 values by 18% (24 h) and by 14% (48 h), and thus enhanced the toxicity of the algicide. Neither doubling of the BSO or OTC concentration in the test medium to 0.64 mM, nor a supplementary pretreatment of 24 h with 0.32 mM OTC prior to test A described in Table 1 significantly changed the results reported (results not shown). Neither BSO nor OTC had any effect when tested alone at 0.80 mM.

The combination of both series of experiments clearly demonstrated that the GSH system also functions in Daphnia magna. The effects of OTC and BSO on GSH synthesis do suggest that 5-oxo-L-prolinase and γ-glutamylcysteine synthetase are present in Daphnia magna. These enzymes were shown to be important for the GSH biosynthesis in rats and mice (Griffith 1982; Williamson et al. 1982). As well as in higher organisms, the detoxication of the electrophilic compound CDNB is GSH dependent in daphnids. GSH S-transferase is the enzyme directly involved in the conjugation reaction of GSH with xenobiotics, which is the essential detoxication reaction in the GSH system. Taking into account that all freshwater animals investigated contain this enzyme (Dierickx 1984) and considering the results reported in this study, we conclude that the GSH system can play an important role in the detoxication of aquatic pollutants. These findings could have a practical utility. For example, water polluted by chemicals (causing fish death) might be successfully treated with OTC.

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