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In vitro inhibition of camel hepatic glutathione transferases by selected organic azides

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Abstract

Glutathione S-transferases (GST) are a group of multifunctional enzymes, widely distributed in both animal and plant kingdom. The present study was carried out to investigate the inhibitory potential of three structurally related organic azides (n-propyl azide, n-butyl azide, and heptyl azide) on camel hepatic GST activity *in vitro*. The mean inhibition constants (*Ki*) were estimated to be 0.419 ± 0.068, 0.501 ± 0.068, and 0.563 ± 0.036 mM for n-propyl azide, n-butyl azide, and heptyl azide respectively, using Lineweaver-Burk plots. These results indicated that hepatic GST was sensitive to the organic azides used.

Keywords: propyl azide, butyl azide, heptyl azide, GST, CDNB, kinetics

Introduction

Every organism is continuously exposed to hazardous agents in its environment. As a result, organisms have evolved sophisticated pathways, environmental response machinery, that can minimize the biological consequences of these agents. The Glutathione S-transferase (GST, EC 2.5.1. 18) family are phase II biotransformation enzymes that detoxify carcinogens and their reactive intermediates, by facilitating their conjugation to endogenous tripeptide glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine), and the product is more water soluble, less toxic and more readily excreted from the host (Commandeur *et al.* 1995, Luchmann *et al.* 2007, Rueff *et al.* 2002, Sheehan *et al.* 2001).

Like other mammals, the camel exhibits GST activity in the liver and extrahepatic tissues (Hunaiti & Abukhalaf 1987, Hunaiti & Asa'd 1989, Hunaiti & Sarhan 1987). Camels are of great interest: although characterization of classical detoxification systems in such animals is not well known, they have unusual biochemical characteristics, differing from other mammals in a number of biochemical and physiological properties. For example, the inherent capacity to withstand water deprivation for long periods and to drink large amounts of water in a short time has been known for some time. In view of these unusual characteristics of camels, it is possible that GST may differ from those of other mammals (Faye & Bengoumi 1994).

Azide is a broad-spectrum biocide showing bactericidal, fungicidal, insecticidal and nematocidal activity (Pollock *et al.* 1977, Singh *et al.* 2008). Inorganic azide compounds such as sodium azide (NaN_3) are potent seed mutagen also shown to be mutagenic in bacteria, *Neurospora*, *Arabidopsis*, barley and *Drosophila* (Owais *et al.* 1981, Owais *et al.* 1983, Owais *et al.* 1986, Owais & Kleinhofs 1988, Owais 1993). The interest in azide is not just because they are mutagenic, but the problem of significant azide release into the environment is not a hypothetical one. Azide is readily protonated in the aqueous environment to yield volatile hydrazoic acid (HN_3) that can then pose an airborne hazard (Chang & Lamm 2003, Singh *et al.* 2008). The high solubility of sodium azide implies that spills could potentially migrate into sewers, streams, lakes and groundwater systems(Smith & Wilcox 1994, Weiss 1996). Other azides including compounds such as organic azides (compounds replaced by a hydrocarbon group - general formula RN_3) may be metabolized and converted to toxic or mutagenic compounds. Sarhan (2007) showed that organic azides (n-butyl and n-hexyl azides) possess mutagenic activity based on his-→ his+ reversions, in a TA100 tester strain. Some organic azide compounds were found to be detoxified by one of the detoxifying systems present in the animal tissue, such as glutathione S-transferase, and excreted in the urine.

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Therefore the determination of azide and its derivatives in environmental and biological samples is of important practical significance.To understand the role of such molecules in an organism it is necessary to characterize its interactions with cellular proteins like glutathione S-transferase (Hunaiti *et al.* 1988). Therefore, the present study aimed to investigate the inhibitory potential of three new structurally related organic azides (n-propyl azide, n-butyl azide and heptyl azide) on camel hepatic Glutathione S-transferase activity *in vitro*.

Materials & Methods

GSH and CDNB were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). All starting materials and solvents used in this work were reagent grade in generally 98% pure. Organic azides were synthesized as mention in Hunaiti *et al.* (1988).

To prepare the enzymes, twenty grams of each liver were cut into small pieces and separately homogenized with 30 ml of 0.1 M potassium phosphate buffer, pH 6.5, using a Wraing blender for 1 min. All further steps were carried out at 4 ^{o}C. The homogenates were centrifuged at 37,000 g for 30 min and the resulting supernatants filtered through glass wool to remove floating lipids. The supernatants were applied to a small GSH-agarose affinity column and the glutathione S-transferases purified essentially as described by Hunaiti & Sarhan (1987).

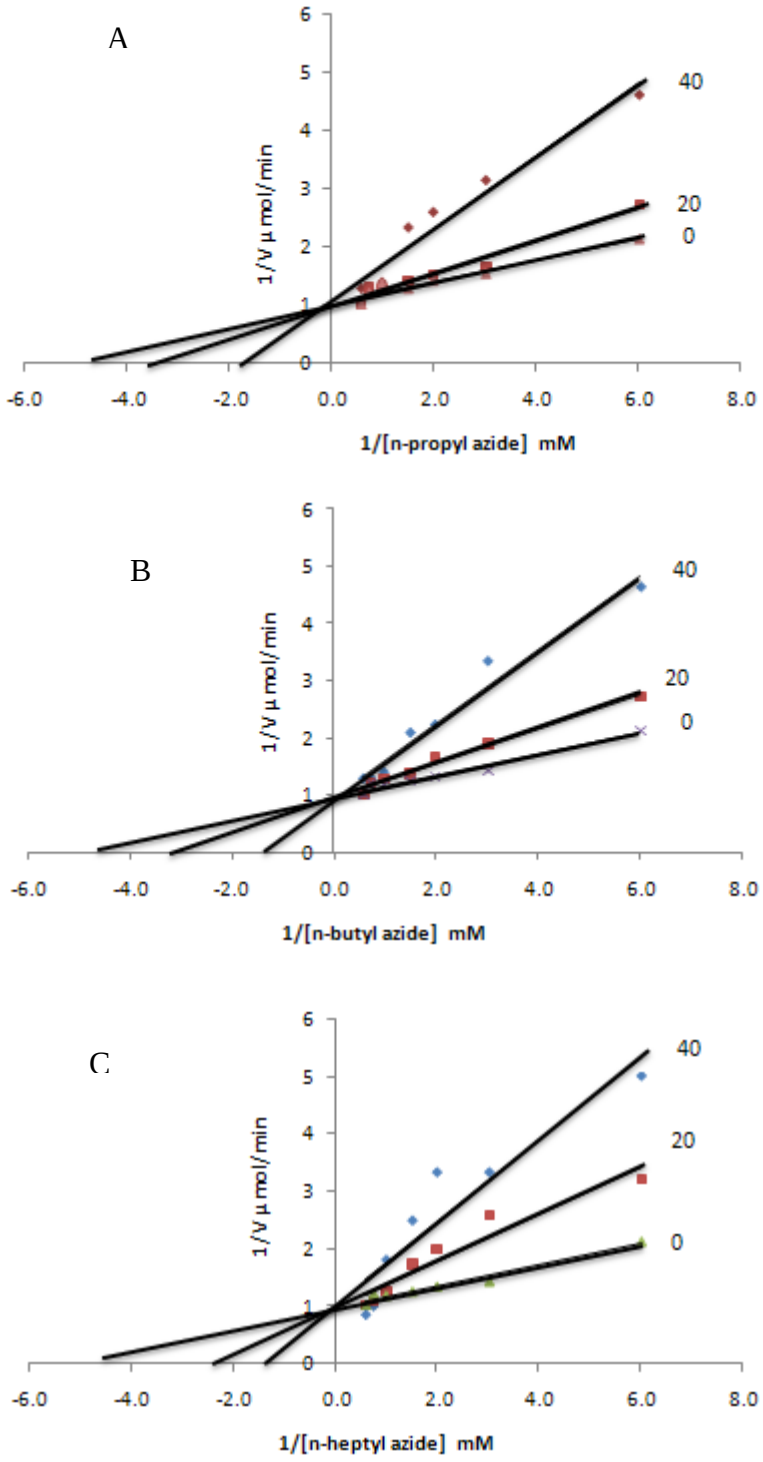
The activity of Glutathione S-transferase was assayed in a reaction mixture containing 100 mM phosphate buffer, pH 6.5, 1 mM 1- Chloro-2,4- dinitrobenzene (CDNB) and purified enzyme equivalent to 40 µg of purified enzyme. The reaction was initiated by addding 1 mM reduced glutathione (GSH) and formation of S-(2,4-dinitrophenyl) glutathione (DNP-GSH) was monitored as an increase in absorbance at 340 nm as described previously (Habig *et al.* 1974). Protein levels were determined spectrophotometrically at 600 nm using bovine serum albumin (BSA) as a protein standard (Lowry *et al.*, 1951).

In order to determine the effect of the organic azides on GST, two fixed concentrations of each organic azide (20 and 40 mM) were used as inhibitors. The samples were incubated for 2 min at room temperature in separate cuvettes containg 100 mM potassium phosphate buffer, pH 6.5, 1 mM GSH, and purified enzyme (40µg) in a total volume of 3 ml. Following a preincubation period in the presence of inhibitors for 2 min at 25 ^{o}C, CDNB was used as a substrate at seven different concentrations (1.66, 1.33, 1.0, 0.66, 0.50, 0.33, and 0.166 mm). The spectrophotometric assay was performed as described above and corrections made for dilution. For each inhibitor concentration, linear relations were produced between 1/V and 1/[S] values (Lineweaver & Burk 1934). *Ki* constants and inhibition types were estimated from these plots.

Table 1: *Ki* constants, and inhibition type of n-propyl, n-butyl and n-heptyl azides for camel hepatic GST

Inhibitor	I (mM)	*Ki* constant (mM)	Mean *Ki* constant (mM) ± SD	Inhibition type
n-propyl azide	20	0.234	0.419 ± 0.068	competitive
	40	0.604		
n-butyl azide	20	0.316	0.501 ± 0.068	competitive
	40	0.686		
n-heptyl azide	20	0.427	0.563 ± 0.036	competitive
	40	0.698		

Figure 1: Lineweaver-Burke plots showing the concentration-dependent inhibition of GST activity by: (A) n-propyl azide; (B) n-butyl azide; and (C) n-heptyl azide. Experiments were performed in duplicate.



Results

N-probyl, n-butyl, and n-heptyl azides were found to be inhibitors of GST. Estimates for the *Ki* constants are given in Table 1. They were estimated using the Lineweaver-Burk plots shown in Figure 1.

Discussion

We previously demonstrated multiple forms of GST enzymes in camel tissues, and showed that they have structural and functional similarities to the drug-metabolizing enzymes from other mammalian species (Hunaiti *et al.* 1988, Hunaiti & Sarhan 1987). In the present study, we report evidence of the inhibitory potential of three different organic azides on camel hepatic glutathione S-transferase activity in a concentration-dependent pattern. The enzyme was severely inhibited by these organic azides at relatively low concentrations. The results also showed a relationship of chain length to inhibitory potency. The present study complements our previous reports on the presence of multiple forms of drug-metabolizing enzymes in camel tissues, which are capable of metabolizing a wide variety of compounds and contribute to the detoxification ability of camels.

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