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Human glutathione transferase catalysis of the formation of *S*-nitrosoglutathione from organic nitrites plus glutathione

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Abstract The kinetics of spontaneous and human glutathione transferase catalysed formation of *S*-nitrosoglutathione(GSNO) from glutathione(GSH) and *n*-butyl- or amyl nitrite have been studied. At physiological pH and temperature, k_2 values of 22.3 and 21.0 $M^{-1} \cdot min^{-1}$ were obtained for *n*-butyl- and amyl nitrites, respectively. Rate enhancements, $(k_{cat}/K_m \times k_2) \times 10^{-4}$, due to purified human GSH transferases A1–1, A2–2 and M1a-1a were, respectively, 7.00, 2.94 and 10.6 for *n*-butyl nitrite and 121, 3.92 and 34.5 for amyl nitrite. GSH transferase P1–1 showed no detectable catalysis of the formation of GSNO. The data suggest that the presence of GSTs A1–1, A2–2 or M1–1 contribute substantially to intracellular metabolism of alkyl nitrites to GSNO. The results may be significant with regard to the immunotoxicity of alkyl nitrites.

Key words: *S*-Nitrosoglutathione; Amyl nitrite; Butyl nitrite; Glutathione; Glutathione transferase; Human; AIDS

1. Introduction

In recent years amyl and *n*-butyl nitrite have been commonly used as recreational drugs by homosexuals [1]. Such alkyl nitrites react readily with thiols to form *S*-nitrosothiols [2,3]. *S*-Nitrosothiols display many biological effects (smooth muscle relaxation, vasodilation, platelet deactivation and NMDA-associated neurotransmission) which are commonly associated with nitric oxide [4–7]. Like nitric oxide, *S*-nitrosothiols may also cause cytotoxicity e.g. in hepatocytes [8] and cytostasis in activated T-lymphocytes [9]. Toxic effects are thought to be due to *S*-nitrosation of important protein thiols (e.g. in the active site of glyceraldehyde-3-phosphate dehydrogenase) or the formation of nitric oxide complexes of mitochondrial iron–sulphur respiratory enzymes.

The toxicity of *n*-butyl nitrite to rat hepatocytes was shown to be mediated by GSNO, and its formation was catalysed by the cytosolic fraction [8]. Such a reaction is likely to be a novel property of GSH transferases(EC. 2.5.1.18). Hence, we have examined the activity of the major human GSH transferase homodimers (A1–1, A2–2, M1–1 and P1–1 [10]) towards amyl- and *n*-butyl nitrite.

2. Materials and methods

Human GSH transferases were purified from liver (GSTs A1–1, A2–2 and M1a–1a) or kidney (GSTP1–1) soluble fractions by affinity chromatography on GSH-agarose followed by hydroxy-apatite chromatography and anion-exchange FPLC [11]. Aliquots were snap-frozen and stored at −20°C. Upon thawing, the amount of active enzyme was determined according to its activity towards 1-chloro-2,4-dinitrobenzene [12].

The formation of GSNO from GSH and alkyl nitrites was quantitated from the increase in A_{334} (ε: 870 $M^{-1} \cdot cm^{-1}$). Assays were carried out at 37°C in 50 mM KCl, 40 mM sodium phosphate, pH 7.40 containing 10 μM bathocuproine disulphonate as copper chelator to stabilize the nitrosothiol [13]. Stock solutions (60 mM) of amyl- and butyl nitrite were prepared in acetone and added to assay buffer containing 1.5 mM GSH in the range from 2–0.02 mM. After determination of the spontaneous rate of GSNO formation, GST was added and the catalytic rate, if any, measured. Assays were duplicated. Values of *Km* and V_m were determined by plotting s/v vs. s. Statistical analysis, obtained by fitting v, s pairs to a rectangular hyperbola, gave standard errors from 6 to 13%.

To determine if GSTP1–1 was inhibited by amyl- or butyl nitrites (or GSNO), assays of activity towards 1-chloro-2,4-dinitrobenzene were carried out [12] in the presence or absence of 1 mM alkyl nitrite.

To determine if GSTP1–1 catalysed the decomposition of GSNO, the enzyme was incubated with 1 mM synthetic GSNO [13] in assay buffer and monitored at 334 nm.

3. Results

At physiological pH and temperature we obtained values for k_2 of 21.0 and 22.3 $M^{-1} \cdot min^{-1}$ for the reaction of amyl- and *n*-butyl nitrite respectively with GSH. These values approximate those expected from data obtained at 25°C [2]. The reactions were stimulated by addition of purified GSH transferases A1–1, A2–2 or M1a–1a (see Table 1). The greatest V_{max} and rate enhancement were seen with GST A1–1 and amyl nitrite. With *n*-butyl nitrite, however, GSTM1a-1a was most effective. GSTA2-2 was less efficient but displayed the lowest K_m values.

GSTP1–1 showed no detectable catalysis of GSNO formation in the presence of alkyl nitrite and GSH (< 0.1% of that of the other GSTs). This was not due to inhibition of the enzyme (e.g. by nitrosation) or binding of GSNO, since activity towards 1-chloro-2,4-dinitrobenzene was unaffected. Furthermore, GSTP1–1 did not stimulate the decomposition of GSNO.

4. Discussion

The reaction of alkyl nitrites with thiols to produce *S*-nitrosothiols is well known [1], and catalysis by GSH transferases of the reaction with GSH is to be expected. The data presented suggest that the catalysis of GSNO formation from *n*-butyl nitrite in rat hepatocyte cytosol, as seen by Meloche and O'Brien [8] was due to GSH transferases.

While the catalysis of GSNO formation by GSH transferases A1–1, A2–2 and M1–1 was expected, the failure of GSH trans-

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Abbreviations: GSH, reduced glutathione; GSNO, *S*-nitrosoglutathione.

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Table 1
Kinetic data for spontaneous and GST-catalysed formation of GSNO from GSH and amyl- or butyl nitrite

GST	K_m (mM)		V_m (μmol/min/mg)		$K_{cat}/K_m/k_2$* ($\times 10^{-4}$)	
	amyl-	butyl-	amyl-	butyl-	amyl-	butyl-
A1–1	1.40	1.34	1370	83.8	116	7.0
A2 2	0.39	0.23	12.3	6.1	3.7	3.0
M1a–1a	1.78	1.58	494	149	33.0	10.6

*Values for k_2 were 21.0 and 22.3 $M^{-1}\cdot min^{-1}$ for amyl- and butyl nitrite, respectively.

ferase P1–1 to carry out this reaction is curious and, so far, unexplained. GSNO has been shown to inhibit rat GSH transferase activity [14] but this was not the cause of lack of GSTP1–1 activity in GSNO synthesis. Furthermore, GSTP1–1 catalysed neither the decomposition of GSNO, nor the formation of *S*-alkyl glutathione from alkyl nitrite (data not shown).

The inhibition of T-lymphocyte proliferation by GSNO was studied by adding 10 μM GSNO to the medium [9]. Since no mechanism for uptake of GSNO has been described, it remains unclear whether GSNO acted at the cell surface or intracellularly. Amyl- and butyl nitrites, on the other hand, are likely to enter cells by free diffusion, and lead to rapid intracellular GSNO formation as seen with rat hepatocytes in vitro [8]. Extracellular *S*-nitrosothiol formation is likely to be limited due to the low concentration of low molecular weight thiols in extracellular fluid [15]. Using the data in Table 1, the contribution of GSH transferases to intracellular GSNO formation may be estimated. Table 2 shows the estimated initial rates of formation of GSNO, spontaneous and GSH transferase-catalysed, in a 'typical' human hepatocyte containing 1mg/ml GSTA1–1 [16] or a peripheral blood mononuclear leucocyte containing 0.1 mg/ml GSTM1–1 [17] assuming that each contains 5 mM GSH and the concentration of alkyl nitrite achieved is 10 μM. The analysis shows that GSH transferase catalysis is of far greater significance than the spontaneous reaction. The estimated rate of hepatocyte GSNO synthesis from 10 μM alkyl nitrite is so high that most of the reaction would occur in seconds (less than 1 s for amyl nitrite). Therefore, amounts of these alkyl nitrites passing the liver unaltered should be very low. Nevertheless considerable extrahepatic GSNO synthesis is to be expected following inhalation of alkyl nitrite in tissues and blood cells. The bulk of the leucocyte GSNO synthesis should occur in approximately 10 s.

GSTM1–1 is expressed in peripheral blood mononuclear leucocytes [17], but has a null allele which, when homozygous (as in about 50% of the population), gives a null phenotype [18]. If GSTM1 is expressed in T-lymphocytes, they might be more sensitive to effects of alkyl nitrites than T-lymphocytes which are GSTM1 null, and could perhaps affect the development of AIDS. However, much may depend on the cellular distribution of the alpha class GSTs, and the activity of other minor GSTs (M2–2, M3–3), not yet studied in this regard, which might offset a lack of GSTM1–1.

Since prior GSH depletion protected hepatocytes from butyl nitrite toxicity [8], it seems possible that repeated administration of these drugs might select for GSH-depleted populations of cells which are particularly sensitive to GSNO. Perhaps such an effect contributes to the increased population of GSH-depleted T-cells observed in AIDS [19].

Table 2
Estimates of rates of spontaneous and GST-catalysed formation of GSNO in hepatocytes and peripheral blood mononuclear leucocyte

Alkyl nitrite	Initial rate of GSNO synthesis (μM/s)		
	Spontaneous	Hepatocyte[a]	Lymphocyte[b]
Amyl nitrite 10 μM	0.018	166 (9194)[c]	4.6 (256)
Butyl nitrite 10 μM	0.019	10 (542)	1.6 (84)

[a]Calculated using data from Table 1 and assuming 5 mM GSH and 1.0 mg/ml GST A1–1 [16].
[b]Calculated using data from Table 1 and assuming 5 mM GSH and 0.1 mg/ml GST M1–1 [17].
[c]Fold increase over spontaneous rate in parenthesis.

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