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The extended catalysis of glutathione transferase

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ABSTRACT

Glutathione transferase reaches 0.5–0.8 mM concentration in the cell so it works in vivo under the unusual conditions of, [S] ≪ [E]. As glutathione transferase lowers the pK_a of glutathione (GSH) bound to the active site, it increases the cytosolic concentration of deprotonated GSH about five times and speeds its conjugation with toxic compounds that are non-typical substrates of this enzyme. This acceleration becomes more efficient in case of GSH depletion and/or cell acidification. Interestingly, the enzymatic conjugation of GSH to these toxic compounds does not require the assumption of a substrate–enzyme complex; it can be explained by a simple bimolecular collision between enzyme and substrate. Even with typical substrates, the astonishing concentration of glutathione transferase present in hepatocytes, causes an unusual "inverted" kinetics whereby the classical trends of *v* versus E and *v* versus S are reversed.

1. Introduction

Glutathione transferases (GSTs) are a superfamily of detoxifying enzymes widely distributed in animals, plants and microorganisms [1,2]. Seven gene-independent cytosolic GST classes have been identified in mammals, *i.e.* Alpha, Mu, Pi, Omega, Sigma, Theta and Zeta [2]. A prominent role of these enzymes is to promote the conjugation of glutathione (GSH) to a variety of hydrophobic compounds with an electrophilic centre [1,2] but several additional functions have been discovered [2]. The Zeta and Omega class GSTs exhibit also a thiol transferase activity reminiscent of thioredoxin and glutaredoxin activities [3,4]. Interestingly, in some cells like rat hepatocytes GSTs account for 5–8% of all soluble proteins so their total cytosolic concentration is about 0.5–0.8 mM [5]. There does not appear to be any reason why the levels of this enzyme need to be so high since it may be calculated, based on the urinary output of glutathione conjugates, that in the liver the intracellular flux of toxic GST substrates is below 0.1 μM/min and that a single molecule of enzyme performs only one catalytic cycle every 5 days [5]. Glutathione transferase represents one of the few enzymes that work in vivo at [S] ≪ [E] in addition to some non-regulatory enzymes involved in glycolytic metabolism. The present study investigates this peculiar kinetic scenario and proposes a possible functional role arising from the high concentration of the enzyme. GSH, which saturates in vivo GSTs (K_m = 0.1–0.4 mM), is the most abundant thiol in the cell, reaching 8–10 mM in hepatocytes [6]. Its role is the maintenance of a proper redox status inside the cell and the elimination of dangerous compounds like alkylating compounds, disulfides and peroxides. Some of these reactions are catalyzed by specific enzymes (e.g. glutathione transferase, glutaredoxin and glutathione peroxidase), but many of them probably occur spontaneously inside the cells. However, only the deprotonated form of GSH is reactive towards these compounds. Since the pK_a of GSH is about 9.0, only 1% of the free GSH (0.08–0.1 mM) is present as the thiolate species at physiological pH values so the un-catalyzed reactions may be quite slow in vivo. However, glutathione transferase is able to lower the pK_a of the

Abbreviations: GSH, glutathione; GST, glutathione transferase; DTNB, 2,2′-dithionitrobenzoic acid; TNB^-, 2-thionitrobenzoate; BSA, bovine serum albumin; IAA, iodoacetic acid; DTOH, dithiodiethanol; CDNB, 1-choro-2,4-dinitrobenzene

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sulfydryl group of GSH from 9.0 to 6.2–6.6 [7] and is expressed at millimolar levels. We demonstrate here that this enzyme acts as a chemical machine that increases the effective concentration of the reactive GSH thiolate and speeds its interaction with many compounds even if they are non-typical and poor substrates of GSTs ($K_m \gg 10^{-2}$ M). Surprisingly, we found that in this extended catalytic role, the assumption of a classical enzyme–substrate complex is unnecessary.

2. Materials and methods

2.1. Enzyme purification

Human GSTA1-1 and GSTM2-2 were expressed in *Escherichia coli* and purified as reported previously [8].

2.2. Reaction with disulfides

Reactions of GSH with cystamine, L-cystine and dithiodiethanol (DTOH) were carried out at 25 °C in 0.1 M potassium phosphate buffer, pH 6.0 or 7.1. The GSH concentration ranged from 1 mM to 10 mM while the disulfide concentration was varied from 0.1 to 2 mM. The reduction process by GSH was followed spectrophotometrically at 340 nm by including 0.2 mM NADH and 10 U of glutathione reductase in the incubation mixture. The effect of hepatic GSTs (Alpha and Mu class enzymes) on these reactions was evaluated by adding 0.23 mM of GSTA1-1 and 0.27 mM GSTM2-2 (about 10 mg/ml) to the standard incubation mixture. Non-specific kinetic effects due to the presence of large amounts of protein were evaluated by replacing the GST with 10 mg/ml of bovine serum albumin (BSA).

2.3. Reaction with alkylating compounds

Reactions of GSH with iodoacetate were carried out at 25 °C in 0.1 M potassium phosphate buffer, pH 6.0 or 7.1. The GSH concentration ranged from 1 to 10 mM while the concentration of the alkylating compounds was varied from 0.5 to 5 mM. Kinetics of the alkylation reaction by GSH were followed at fixed times on the basis of GSH disappearance. In a typical experiment, 0.02 ml aliquots of the incubation mixture were reacted with 0.1 mM 2,2′-dithionitrobenzoic acid (DTNB) in 1 ml (final volume) of 0.1 M potassium phosphate buffer, pH 8.0. The amount of GSH was evaluated on the basis of an extinction coefficient of the 2-thionitrobenzoate (TNB^-) of 13 600 M^{-1} cm^{-1} at 412 nm. The effect of the hepatic GSTs on these reactions was evaluated by adding 0.23 mM of GSTA1-1 and 0.27 mM GSTM2-2 (about 10 mg/ml) to the standard incubation mixture. Non-specific kinetic effects due to the use of high amounts of protein were evaluated by replacing the GST with 10 mg/ml of BSA.

2.4. Kinetic simulations

Simulations were carried out with the software package COPASI 4.4.27 [9]. The software simulates the time courses of Scheme 1 by means of numerical integration of the ordinary differential equations. The kinetics of the reaction of 1-chloro-2,4-dinitrobenzene (CDNB), with GSH catalyzed by human glutathione transferase P1-1 isoenzyme involves several intermediates [10]. However, we have used the simplified Michaelis–Menten steady-state model to simulate the overall dependence of the rate of product formation on either substrate (S) or enzyme (E) concentrations (Scheme 1)

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E\text{-}S \xrightarrow{k_3} E + P$$

Scheme 1.

Table 1
Effect of the hepatic GSTs on kinetics ($t_{1/2}$) of GSH with selected non-substrates.

	pH 7.1		
	GSH 10 mM $t_{1/2}$ (min)	GSH 10 mM + BSA 10 mg/ml $t_{1/2}$ (min)	GSH 10 mM + GST 0.5 mM $t_{1/2}$ (min)
Cystamine[a]	5.8	3.6	0.16
DTOH	8.0	5.3	0.16
Cystine	5.3	4.3	0.20
Iodoacetate	265	147	5.3
	GSH 1 mM	GSH 1 mM + BSA 10 mg/ml	GSH 1 mM + GST 0.5 mM
Cystamine	56	38	0.18
DTOH	83	55	0.15
Cystine	52	43	0.22
Iodoacetate	2500*	1400*	5.8
	pH 6.0		
	GSH 10 mM $t_{1/2}$ (min)	GSH 10 mM + BSA 10 mg/ml $t_{1/2}$ (min)	GSH 10 mM + GST 0.5 mM $t_{1/2}$ (min)
Cystamine	46	29	0.47
DTOH	69	43	0.55
Cystine	46	36	0.68
Iodoacetate	2500*	1500*	15
	GSH 1 mM	GSH 1 mM + BSA 10 mg/ml	GSH 1 mM + GST 0.5 mM
Cystamine	450*	288	0.51
DTOH	700*	400*	0.60
Cystine	450*	350*	0.74
Iodoacetate	26 000*	14 000*	16

[a] The experiments were performed at pH 6.0 and 7.1 (see Section 2). Values with the asterisk were not measured experimentally as these reactions were too slow. Instead the values were estimated by extrapolating from experiments performed at higher reagent concentrations or at higher pH values, assuming a simple bimolecular interaction.

In Scheme 1, E represents the enzyme in complex with GSH (K_m = 0.1 mM; the intracellular GSH concentration being up to 10 mM) and S the co-substrate having $K_m \approx 1$ mM. The following rate constants according to Scheme 1, $k_1 = 10^3$ M^{-1} s^{-1}; $k_2 = 1$ s^{-1}; $k_3 = 0.1$ s^{-1} were used. These rate constants are close to those for the overall reaction of the classical co-substrate CDNB, with GSH catalyzed by human GST P1-1 [10].

3. Results

3.1. Effect of GST on the reaction of GSH with selected non-typical substrates

Glutathione transferases from rat liver are able to conjugate GSH to many hydrophobic toxic compounds. Small and hydrophilic disulfides like cystine, cystamine and dithioethanol, and hydrophilic alkylating compounds like iodoacetate, are not known as typical substrates of this enzyme. As expected, we did not find any trace of activity with these compounds in the standard activity conditions, *i.e.* [S] = 1 mM, [GSH] = 1 mM and using high enzyme concentrations (up to 5 μM Alpha or Mu GSTs) (not shown). Actually, only the Omega class GST and a peculiar lens GST display a moderate thiol transferase activity with dithioethanol while the liver Alpha and Mu GSTs have been reported to be completely ineffective [4,11]. In the absence of specific enzymes all these hydrophilic compounds react with GSH spontaneously, following a pH-dependent bimolecular mechanism (not shown). At a pH value and GSH concentration similar to those found in the liver cytosol (pH 7.1, GSH 10 mM) these reactions are quite slow, showing apparent $t_{1/2}$ values ranging from 3 min to about 4 h (Table 1). When the same reactions were carried out in the presence of