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Hypochlorous acid is a potent inhibitor of GST P1-1

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Abstract

Glutathione S-transferase is a phase II detoxification enzyme that can be inactivated by H_2O_2 . During oxidative stress various other reactive oxygen species are generated that are more reactive than the relatively stable H_2O_2 . Hypochlorous acid (HOCl) is a powerful oxidant which is highly reactive towards a range of biological substrates. We studied the influence of HOCl on the activity of GST P1-1. HOCl inhibits purified glutathione S-transferase P1-1 in a concentration dependent manner with an IC_{50} -value of $0.6 \mu\text{M}$, which is more than 1000 times as low as IC_{50} reported for H_2O_2 . HOCl lowered the V_{max} value, but did not affect the K_m for CDNB. Our results show that HOCl is a potent, non-competitive inhibitor of GST P1-1. The relevance of this effect is discussed. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Hypochlorous acid; Glutathione S-transferase P1-1; Inhibition; Human

1. Introduction

Glutathione S-transferases are a superfamily of phase II detoxification enzymes, which can catalyse the conjugation of glutathione with various electrophiles. The superfamily consists of at least five gene families of which four (alpha, mu, pi and theta) encode the cytosolic GSTs, whereas the fifth encodes a microsomal form of the enzyme [1–3]. In humans the pi-isoform of the enzyme occurs mostly in red

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blood cells and placenta [4] and has been shown to be especially vulnerable to oxidative stress [5,6]. It is known that GST P1-1 is inactivated in human erythrocytes by H_2O_2 [7].

The inhibition of GST by oxidative stress seems to be specific for GST P1-1. It is shown that GST P1-1 can be inactivated by H_2O_2 , whereas GST M is not sensitive for inactivation with H_2O_2 . In fact, GST M can even be activated by active oxygen species [8]. During oxidative stress various other reactive oxygen species are generated that are more reactive than the relatively stable H_2O_2 . Hypochlorous acid (HOCl) is a powerful oxidant generated by the neutrophil enzyme myeloperoxidase from H_2O_2 and chloride ions and it plays a role in the bactericidal function. It is highly reactive toward a range of biological substrates [9,10]. In the present study, the effect of HOCl on GST P1-1 activity is examined.

2. Materials and methods

2.1. Chemicals

1-Chloro-2,4-dinitrobenzene (CDNB), lipoic acid, sodium hypochlorous acid and glutathione S-transferase P1-1 (from human placenta) were obtained from Sigma, St. Louis, USA. Reduced glutathione (GSH) was obtained from ICN Biomedicals Inc., Costa Mesa, USA and hydrogen peroxide (H_2O_2) from Merck, Darmstadt, Germany. All other chemicals were of analytical grade purity.

2.2. Assay of glutathione S-transferase activity

GST activity was measured as described by Mannervik and Guthenberg [11] with slight modifications. In short, the reaction of 1 mM CDNB with 1 mM GSH in the presence or absence of GST was monitored spectrophotometrically by recording the increase in absorbance at 340 nm. Measurements were performed at pH 6.5 to reduce the spontaneous reaction for the formation of the conjugate of GSH and CDNB.

2.3. Incubations

2.3.1. Purified enzyme

Effects of various concentrations of HOCl (final concentration 0.2–1 μM) on activity of purified GST P1-1 (0.02 U/ml) were determined. The GST enzyme was incubated in 100 mM potassium phosphate pH 6.5 or 7.4 (37 °C) with or without lipoic acid. After preincubation (1 min), HOCl was added and the reaction was allowed to proceed for 30 s before the activity of GST P1-1 was determined. The effect of H_2O_2 on the activity of purified GST P1-1 was determined in the same way.

To study the inhibitory mechanism of HOCl on GST P1-1, substrate concentrations (CDNB or GSH) were varied. When CDNB was varied, the GSH concentra-

tion was kept at 1 mM and vice versa. In these experiments the concentration of HOCl (0.75 μM) was constant.

2.4. Lysate of human erythrocytes

Effects of various concentrations HOCl (final concentration 0–75 μM) on GST activity in the lysate of human erythrocytes were also determined with the method described above. The lysate was prepared by mixing 1 ml frozen blood from a male volunteer with 1 ml cold water and 2 ml cold 100 mM potassium phosphate pH 7.4. This solution was mixed vigorously and centrifuged for 10 min at 13 000 rpm at 4 °C. The supernatant was diluted 45 times. The incubation of the lysate with HOCl was performed at pH 7.4, and the activity of GST was determined with GSH and CDNB as substrate after the pH was adjusted to pH 6.5.

Additionally, the reversibility of GST P1-1 inactivation by HOCl was studied. The GST was treated with 1 μM HOCl at 37 °C at pH 7.4. After 30 s, 1 mM GSH was added and the mixture was incubated again for 5 min at 37 °C. Subsequently, the GST activity was measured. This activity was compared with the GST activity observed when GSH was added after the incubation of 5 min, just before the addition of CDNB.

3. Results

As shown in Fig. 1, the glutathione S-transferase activity was inhibited by HOCl in a concentration dependent manner. The concentrations of HOCl giving 50% inhibition (IC_{50}) of GST P1-1 activity were 0.6 μM for both pHs. H_2O_2 in a concentration of 1 mM inhibited the GST P1-1 activity only 15%. In the lysate of human erythrocytes (pH 7.4), the IC_{50} value of HOCl was $44 \pm 4 \mu\text{M}$. A HOCl scavenger such as lipoic acid [12] can prevent the effect of HOCl on GST P1-1 (Fig.

Fig. 1. Inactivation of isolated GST P1-1 by HOCl. The inactivation was performed at pH 7.4 (dashed line, ●) or pH 6.5 (solid line, ○). The IC_{50} values of HOCl are 0.56 μM and 0.61 μM at pH 6.5 and 7.4, respectively. The concentration of the enzyme was 0.017 U/ml. Each point denotes the means ($\pm$ S.E.M.) of three experiments.

Fig. 2. Protection by lipoic acid against the inhibition of GST P1-1 by HOCl. The concentration of enzyme and HOCl were respectively 0.034 U/ml and 1.8 μ M. Each point denotes the means ($\pm$ S.E.M.) of four measurements.

2). The inhibition of GST P1-1 by HOCl was not reversible by addition of GSH to the HOCl inactivated enzyme (data not shown).

To obtain information on the nature of the inhibition of GST P1-1 by HOCl, GST activity of the purified enzyme with or without pre-treatment of HOCl (0.75 μ M), was measured with variable concentrations of either CDNB or GSH. GST shows characteristic Michaelis Menten behaviour towards both substrates. The Lineweaver–Burk plot of the substrate CDNB is depicted in Fig. 3. As shown also in this figure, HOCl lowered the $V_{\max}$, but did not affect the K_m for either CDNB or GSH. This indicates that the GST P1-1 enzyme is non-competitively inhibited by HOCl.

Fig. 3. Lineweaver–Burk plot showing non-competitive inhibition of human placenta GST P1-1 isoenzyme towards CDNB by 0.75 μ M HOCl. The K_m and $V_{\max}$ of the enzyme for CDNB (●) are respectively 1.2 mM and 26 μ mol/min mg. After HOCl treatment (○) these values are 1.1 mM and 11 μ mol/min mg. The concentration enzyme used was 0.017 U/ml. Each points denotes the means of four measurements.

4. Discussion

GSTs can conjugate numerous electrophilic compounds, many of which are toxic, to GSH. In most cases, this conjugation leads to detoxification of the compounds [13]. The catalytic activity of the enzyme can be altered by different mechanisms. It is known that GST P1-1 can be inhibited by H_2O_2 [7].

This study confirmed the inhibition of GST P1-1 by H_2O_2 . However, a relatively high concentration of H_2O_2 (1 mM) inhibited the enzyme 15%. The potency of HOCl to inhibit the enzyme is much higher. When HOCl was added to a solution of isolated GST P1-1, the IC_{50} -value was 0.6 μM (Fig. 1). Higher potency of HOCl compared with H_2O_2 was also found for other important biological effects such as induction of apoptosis via caspase 3 [14]. For induction of apoptosis, the activity of HOCl exceeded even that of peroxyntirite [14].

It was shown that the inactivation of GST by HOCl is non-competitive and not reversible by GSH. The HOCl scavenger lipoic acid can protect against the inactivation of GST by HOCl. Lipoic acid contains an intramolecular S-S bridge in a ring in which some strain exists. By scavenging HOCl the lipoic acid is converted into a sulfoxide [12].

Human GST contains 4 cysteine residues at positions 14, 47, 101 and 169. Disulphide formation between cysteine residues at positions 47 and 101 was found to be critical for the inactivation of GST P1-1. This results in steric hindrance which is the most probable cause for the inactivation [15]. It is known that HOCl also shows a high reactivity towards thiol groups [12,16,17]. Probably HOCl inhibits the GST P1-1 activity by oxidation of a cystein residue.

In erythrocytes GST P1-1 is the isoenzyme with the highest activity with respect to CDNB [18]. To inhibit the activity of GST in lysate of human erythrocytes, a much higher concentration of HOCl was needed compared with purified GST P1-1 (IC_{50} -values were 44 and 0.6 μM , respectively). The higher IC_{50} value in the lysate can be explained by the presence of GSH and thiol containing proteins that also can react with HOCl.

One of the major targets of HOCl in vivo is α_1 -antiproteinase (α_1 -AP). The HOCl mediated oxidation of this protein is an essential step in the etiology of lung emphysema [12]. A competition experiment, where α_1 -AP and GST P1-1 are mixed, revealed that HOCl first inactivates GST P1-1 before reacting with α_1 -AP (not published). This indicates that the inactivation of GST P1-1 by HOCl is of physiological relevance.

Besides the transferase activity of GST P1-1, another function has been reported recently. As a guardian of Jun N-terminal Kinase (JNK) in normally growing cells, GST P1-1 may serve as a sensor of intramolecular changes in redox potential that are elicited by various forms of stress [19]. Phosphorylation of Jun by JNK has been implicated in changes in the cell cycle, DNA repair or apoptosis [18]. It is tempting to speculate that HOCl is one of the important triggers in this system since HOCl is a much more potent inhibitor of GST P1-1 than the other stressors (i.e. H_2O_2) studied thus far. Together with the proclaimed role of HOCl in activation of caspase 3 [14], elevation of p53 levels [20], induction of the expression

of apurinic endonuclease [21] and the activation of NF- κ B [22], this points toward a pivotal function of HOCl in (patho-)physiology.

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