Thiocyanate, a plausible physiological electron donor of gastric peroxidase

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Gastric peroxidase (GPO) was purified to apparent homogeneity to characterize its major physiological electron donor. The enzyme (RZ = 0.7), with a subunit molecular mass of 50 kDa, is a glycoprotein, with a relative abundance of aspartic and glutamic acid over arginine and lysine. It has a Soret maximum at 412 nm, which is shifted to 426 nm by H_2O_2 due to formation of compound II. Although the physiological electron donors I^-, Br^- and SCN^-, but not Cl^-, are oxidized by GPO optimally at acid pH, only I^- and SCN^- are oxidized appreciably at physiological pH. Considering that the I^- concentration in stomach is less than 1 μM, whereas the SCN^- concentration is about 250 μM, SCN^- may act as a major electron donor for GPO. Moreover, SCN^- oxidation remains unaltered in the presence of physiological concentrations of other halides. The second-order rate constant for the reaction of GPO with H_2O_2 (k_1) and compound I with SCN^- (k_2) at pH 7 was found to be $8 \times 10^7\ M^{-1} \cdot s^{-1}$ and $2 \times 10^5\ M^{-1} \cdot s^{-1}$ respectively. GPO has significant pseudocatalase activity also in the presence of I^- or Br^-, but it is blocked by SCN^-. The SCN^- oxidation product $OSCN^-$ may be reduced back to SCN^- by cellular GSH, and GSSG may be reduced back to GSH by glutathione reductase and NADPH. In a system reconstituted with pure glutathione reductase, NADPH, GSH, SCN^- and H_2O_2, GPO-catalysed SCN^- oxidation could be coupled to NADPH oxidation. This system where GPO utilizes SCN^- as the major physiological electron donor may operate efficiently to scavenge intracellular H_2O_2.

INTRODUCTION

Peroxidase utilizes intracellular H_2O_2 to oxidize a physiological electron donor for specialized cellular function [1,2]. Thyroid peroxidase catalyses thyroid-hormone formation through oxidation of I^- [3], whereas eosinophil and neutrophil peroxidases are involved in phagocytosis, using SCN^- and Cl^- as electron donor respectively [4–6]. The enzyme in milk, saliva and tears exerts antibacterial action through oxidation of SCN^- also [7,8]. We have reported the presence of a peroxidase in mouse and rat gastric mucosa [9,10] and characterised the latter to be different from thyroid and other extra-thyroid peroxidases [11]. This gastric peroxidase (GPO) is an endogenous protein of the gastric mucosa, modulated by glucocorticoids [10], and is not contributed by invading eosinophils, as is the case in the intestine [12]. It is mainly localized in the parietal cells of the stomach, although some activity is present in other cells also [13]. The enzyme is irreversibly inactivated by mercaptomethylimidazole (MMI), a potent inducer of gastric acid secretion [14,15]. From the correlation of the inhibition of GPO activity by MMI with the increase in gastric acid secretion [14], we postulated the possible involvement of the peroxidase–H_2O_2 system in MMI-induced acid secretion. In stress conditions, inactivation of GPO in concert with activation of superoxide dismutase causes gastric ulceration by oxidative damage through the generation of reactive oxygen species [16].

Animal peroxidases oxidize halides or pseudohalides by H_2O_2 to produce hypohalous acid oxidants:

$$H_2O_2 + X^- + H^+ \rightarrow HOX + H_2O$$

where $X^- = Cl^-/Br^-/I^-/SCN^-$ and HOX is the corresponding hypohalous acid. Although the physiological electron donors of several animal peroxidases have been characterized [3,5,6,8,17], we still do not know which electron donor is utilized by GPO. In this paper, we present further purification and characterization of GPO to identify its major electron donor, its kinetics of oxidation, and to understand how the enzyme system may operate *in vivo* for efficient elimination of endogenous H_2O_2.

MATERIALS AND METHODS

Chemicals

SDS, sodium deoxycholate, Cetab (cetyltrimethylammonium bromide), α-methyl mannoside, NADPH, GSH and glutathione reductase (from yeast) were purchased from Sigma (St. Louis, MO, U.S.A.). Sephadex G-150 and concanavalin A–Sepharose were obtained from Pharmacia, Uppsala, Sweden. Other reagents used were of analytical grade.

Purification of GPO

GPO was purified from the glandular part of the fundic stomach (100 g) of 90 Sprague–Dawley rats (220 g) as described previously [11] with some modifications. The crude mitochondrial fraction (12000 *g* pellet) was prepared by homogenizing the tissues in 50 mM sodium phosphate buffer, pH 7.2. The enzyme was enriched in the membrane fraction by initial extraction with 0.5% sodium deoxycholate and homogenization of the pellet in 100 mM Tris/HCl buffer, pH 10, followed by centrifugation at 105000 *g* for 1 h. The pellet was suspended in 50 mM sodium phosphate buffer, pH 7.2, containing 0.2% Cetab, frozen for 40 h, thawed, and then NH_4Cl was added to a final concentration of 1.2 M. After stirring for 4 h, the mixture was centrifuged at 140000 *g* for 1 h to give the soluble enzyme. It was concentrated to 3 ml by Amicon ultrafiltration (UM-20 filter) and applied to a Sephadex G-150 column (1.6 cm × 75 cm) previously equilibrated with 50 mM sodium phosphate buffer, pH 7.2, containing 1.2 M NH_4Cl. Elution was carried out with the same buffer. The enzyme eluted (peak 2, [11]) was finally applied to a concanavalin A–Sepharose column (0.5 cm × 1 cm), equilibrated with the same

Abbreviations used: GPO, gastric peroxidase; MMI, mercaptomethylimidazole; TNB, thionitrobenzoic acid.

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buffer. After washing with the same buffer until A_{280} reached near zero, the enzyme was eluted with the same buffer containing 0.25 M α-methyl mannoside (Figure 1a). The fractions (0.8 ml each) of tubes 51–55 were collected to obtain the pure enzyme. The purity was checked by SDS/PAGE [18], and its RZ value was determined from the A_{412}/A_{278} ratio.

Amino acid analysis

Dialysed and freeze-dried GPO (50 μg) was hydrolysed with 6 M HCl for 24 h (110 °C) before analysis. The amino acid analysis was done in duplicate using a LKB 4151 Alpha Plus analyser, calibrated with a standard mixture of amino acids. The detection limit was abut 0.2 nM for each amino acid.

Enzyme activity and spectral studies

Peroxidase activity was assayed with I^- as electron donor [11]. All activity assays and spectral studies were carried out at 25±1 °C in a Shimadzu UV-2201 spectrophotometer.

Thiocyanate oxidation determined by thionitrobenzoic acid (TNB) assay

The initial rate of oxidation of SCN^- was determined by measuring the rate of oxidation of TNB at 412 nm by $OSCN^-$ generated in the peroxidation reaction [19]. SCN^- was replaced by other halides when required [5]. TNB was prepared and its appropriate absorption coefficient at different pH values was used as described previously [20].

Determination of velocity constants of GPO

As the formation of compound II during the oxidation of SCN^- has not been observed, it seems likely that this donor is oxidized by a single-step two-electron transfer to compound I [21]. So, for the GPO/SCN^-/H_2O_2 system, the classical mechanism may be written as:

$$E + H_2O_2 \xrightarrow{k_1} EO + H_2O \quad (1)$$

$$EO + SCN^- \xrightarrow{k_2} E + OSCN^- \quad (2)$$

where E is the free enzyme, EO is compound I, and k_1 and k_2 are the bimolecular rate constants for the formation of compound I and $OSCN^-$ respectively. Applying the steady-state assumption with respect to compound I, we obtain

$$v = \frac{[H_2O_2][E_0][SCN^-]}{\frac{[H_2O_2]}{k_2} + \frac{[SCN^-]}{k_1}} \quad (3)$$

where v is the initial rate of generation of $OSCN^-$ and $[E_0]$ is initial enzyme concentration. $[H_2O_2]$ and $[SCN^-]$ are the steady-state concentrations of H_2O_2 and SCN^- respectively. The above equation [22] is valid under the conditions of $[H_2O_2]$ 0.2 mM, $[SCN^-]$ 4 mM and pH 7.0. Rearranging equation (3) gives:

$$\frac{v}{[E_0]} = \frac{k_2[SCN^-][H_2O_2]}{\frac{k_2}{k_1}[SCN^-] + [H_2O_2]} = \frac{B_1[H_2O_2]}{B_2 + [H_2O_2]} \quad (4)$$

here B_1 and B_2 are constants for a fixed concentration of SCN^-.

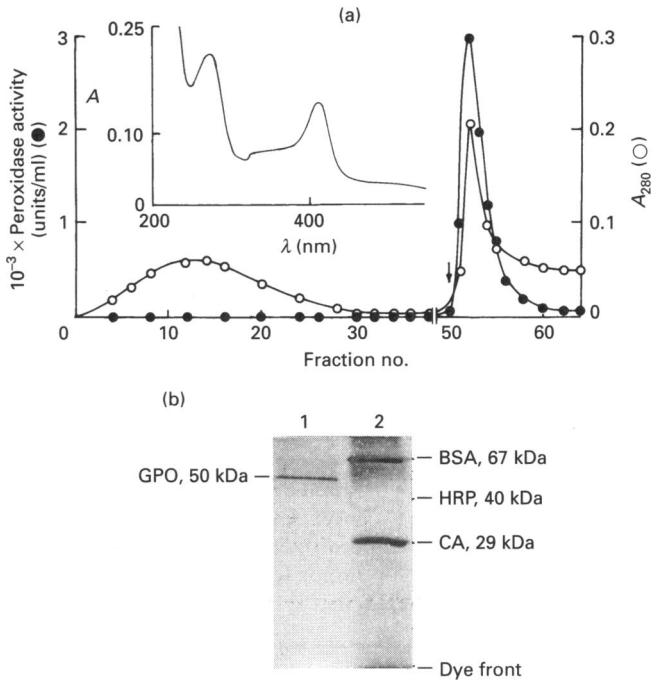


Figure 1 Affinity chromatography on concanavalin A–Sepharose and SDS/PAGE pattern of GPO

(**a**) Elution pattern of GPO from concanavalin A–Sepharose: ○, A_{280}; ●, enzyme activity with I^- as electron donor. An increase of 1 A_{353} unit/min was defined as 1 unit. The assay system contained, in a final volume of 3 ml: 50 mM sodium acetate buffer, pH 5.5, 1.7 mM KI, the enzyme preparation, and 0.27 mM H_2O_2 added last to start the reaction. The arrow indicates the start of elution of peroxidase with eluting buffer containing 0.25 M α-methyl mannoside and 1.2 M NH_4Cl in 50 mM sodium phosphate buffer, pH 7.2. The inset shows the absorption spectrum of 1.5 μM purified GPO as obtained from concanavalin A–Sepharose in eluting buffer, pH 7.2. (**b**) SDS/PAGE (12% gel) of the enzyme, with silver staining. Lane 1 shows the pure enzyme (1.5 μg) and lane 2 the standards (HRP, horseradish peroxidase; CA, carbonic anhydrase).

A plot of $v/[E_0]$ against $[H_2O_2]$ should yield a rectangular hyperbola, where B_1 is $K_{cat.}$, the maximum molar catalytic activity for a fixed concentration of SCN^-, and B_2 is related to the Michaelis parameter K_m, the H_2O_2 concentration required to obtain half-maximal velocity [23]. k_1 and k_2 could be calculated from the experimentally determined B_1 and B_2 values as shown in the Results section.

Pseudocatalase activity as measured by O_2 evolution

Peroxidase-catalysed O_2 evolution (pseudocatalase activity) in the presence of an electron donor was measured in a Gilson oxygraph fitted with a Clark-type oxygen electrode [24].

Measurement of SCN^-, GSH content and glutathione reductase activity in rat stomach

The supernatant obtained after deproteinization of tissue homogenate with 5% trichloroacetic acid was used for SCN^- determination [25]. GSH (acid-soluble thiol) content was determined by the method of Sedlak and Lindsay [26]. Glutathione reductase activity of the cell-free supernatant of stomach homogenate was measured by a standard procedure [27].

Table 1 Amino acid composition of rat GPO and its comparison with other peroxidases

The mean value of two sets of experiments in duplicate was given for GPO: residues per molecule of enzyme were calculated on the basis of a molecular mass of 50 kDa for GPO for comparison. ND, not determined. The numbers in brackets indicates the references from which the data were taken.

	Rat gastric peroxidase		Pig intestinal peroxidase [28]		Bovine lactoperoxidase [27]	
Amino acid	(residues/ molecule)	(%)	(residues/ molecule)	(%)	(residues/ molecule)	(%)
Lysine	25	6.54	21	5.4	34	5.6
Histidine	6	1.67	7	1.8	14	2.3
Arginine	17	4.49	38	9.8	37	6.1
Tryptophan	ND	ND	7	1.8	15	2.5
Aspartic acid	41	10.73	41	10.6	71	11.6
Threonine	23	6.02	20	5.2	32	5.2
Serine	24	6.20	23	6.0	33	5.4
Glutamic acid	42	10.99	29	7.5	61	10.0
Proline	17	4.49	28	7.3	42	6.9
Glycine	44	11.52	25	6.5	40	6.5
Alanine	47	12.45	30	7.8	37	6.1
Half-cystine	ND	ND	10	2.6	16	2.6
Valine	24	6.40	19	4.9	28	4.6
Methionine	4	1.06	5	1.3	11	1.8
Isoleucine	15	3.92	17	4.4	27	4.4
Leucine	28	7.39	38	9.8	68	11.1
Tyrosine	8	2.21	8	2.1	15	2.5
Phenylalanine	12	3.28	20	5.2	30	4.9

SCN^- oxidation by GPO coupled with NADPH oxidation by glutathione reductase

SCN^- oxidation by GPO was measured by coupling NADPH consumption at 340 nm by purified glutathione reductase from yeast. The details of the reaction mixture are described in the legend of Table 2. The rate of NADPH oxidation was measured for 3 min as the decrease in A_{340} (ϵ_{340} = 6.3 $mM^{-1} \cdot cm^{-1}$) at an interval of 15 s.

RESULTS

Characterization of GPO

GPO purified and characterized previously [11] yielded a lower RZ value (0.33), probably due to the presence of some contaminant. The RZ value was improved by introducing affinity chromatography on concanavalin A–Sepharose (Figure 1a) to give a more purified enzyme, of specific activity of 40000 units/mg of protein (I_3^- assay) and RZ value 0.7 (Figure 1a, inset). By gel-permeation studies, its native molecular mass was estimated to be 47 kDa [11]. In SDS/PAGE (slab gel), the enzyme showed a single band on silver staining (Figure 1b), with an apparent subunit molecular mass of 50 kDa. GPO is thus a monomeric protein. The binding of peroxidase to concanavalin A–Sepharose and its elution with α-methyl mannoside suggest that it is a glycoprotein. Its amino acid composition is shown in Table 1, for comparison with that of bovine lactoperoxidase [28] and pig intestinal peroxidase [29]. GPO has no catalytically active thiol group, as evidenced by its insensitivity to some specific thiol-group reagents such as 5,5′-dithiobis-(2-nitrobenzoic acid), *N*-ethylmaleimide, methylmethanethiosulphonate or *p*-chloromercuribenzoate, and vicinal thiol-group blockers, diamide or sodium arsenite/2,3-dimercaptopropanol. However, thiol-reagent-reactive thiol group(s), if any, was not determined by the Ellman reaction due to insufficient amount of enzyme being available. Native GPO shows Soret absorption at 412 nm, which is shifted to 426 nm by H_2O_2 due to formation of compound II through an isosbestic point at 420 nm (results not shown). The enzyme shows visible peaks at 496, 600 and 634 nm. Due to the limited amount of enzyme, visible peaks for compound II were not characterized.

Oxidation of physiological electron donors by GPO

GPO can oxidize I^-, Br^- and SCN^-, but not Cl^- (Figure 2). I^- is oxidized at a much higher rate than Br^- and SCN^- at their optimum pH values of 5.0–5.5, 5.0 and 5.5–6.25 respectively. Near physiological pH, Br^- is not oxidized at all, whereas I^- oxidation is only 15% of that at the optimum pH and SCN^- oxidation is about 50%. From Lineweaver–Burk plots (not shown), the apparent K_m for I^-, Br^- and SCN^- at optimum pH was found to be 0.7 mM, 2.2 mM and 0.4 mM respectively.

SCN^- oxidation at physiological pH

Except in thyroid gland, I^- concentration in other tissues is too low to be the physiological electron donor *in vivo* [30], and both Br^- and Cl^- are not appreciably oxidized by GPO at physiological pH. On the other hand, SCN^- is considerably oxidized by GPO at neutral pH in a concentration-dependent manner, showing saturation kinetics (results not shown). The apparent K_m value for SCN^- was found to be 0.4 mM from the Lineweaver–Burk plot (not shown). SCN^- oxidation was also studied as a function of H_2O_2 concentration. The apparent K_m value for H_2O_2 was calculated to be 12 μM from the Lineweaver–Burk plot (not shown). When the data were replotted as $v/[E_o]$ versus $[H_2O_2]$ (where $[E_o]$ = 1 nM), it yielded a rectangular hyperbola (not

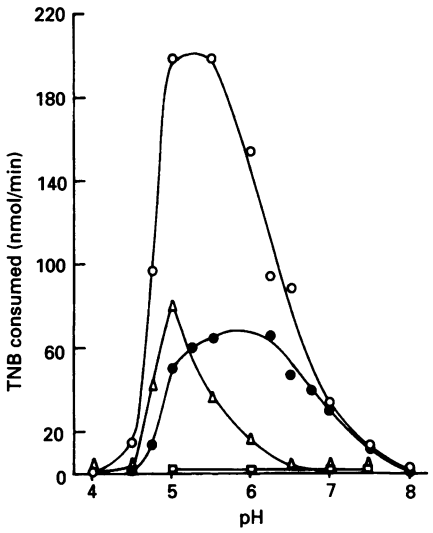


Figure 2 pH-dependent peroxidation of I^-, Br^- and SCN^- by GPO

The reaction mixture contained 50 mM sodium citrate buffer (pH 4.0–5.75) or sodium phosphate buffer (pH 6.0–8.0), 0.1 mM TNB, 1 nM GPO and electron donor. Reaction was started with 0.2 mM H_2O_2. ○, 4 mM KI; △, 4 mM KBr; ●, 4 mM KSCN; □, 150 mM KCl.

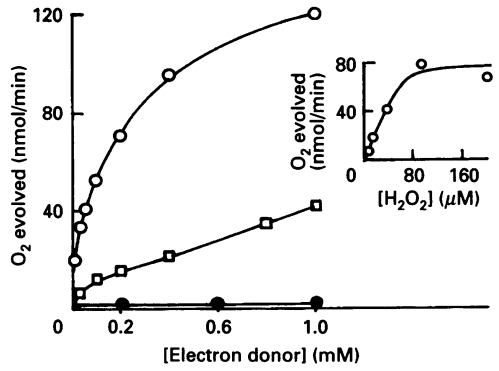


Figure 3 Effect of various concentrations of I^-, Br^- and SCN^- on GPO-catalysed pseudocatalase activity

The reaction mixture contained 50 mM sodium phosphate buffer, pH 7.0, 7 nM GPO, electron donors as indicated and 0.1 mM H_2O_2: ○, I^-; □, Br^-, ●, SCN^-. The inset shows the effect of H_2O_2 concentration on I^--dependent pseudocatalase activity. The reaction mixture contained the same components as described above, except that 0.2 mM I^- was used.

shown) having two parameters, B_1 ($K_{cat.}$) and B_2 (K_m), as described in the Materials and methods section.
Now

$$\frac{B_1}{B_2} = \frac{k_2[SCN^-]}{\frac{k_2}{k_1}[SCN^-]} = k_1 \quad (5)$$

and

$$\frac{B_1}{[SCN^-]} = k_2 \quad (6)$$

B_1 and B_2 were determined by computer best fit, using the experimental values of $[E_o]$, $[H_2O_2]$ and v. The second-order rate constant, k_1, for the reaction of GPO with H_2O_2 was calculated to be $8 \times 10^7\ M^{-1} \cdot s^{-1}$, and that, of k_2, for the reaction of compound I with SCN^- to be $2 \times 10^5\ M^{-1} \cdot s^{-1}$, values comparable with those of other peroxidases [22,29]. The rate of SCN^- oxidation remains unaltered in presence of physiological concentration of other electron donors such as I^- as high as 2 μM, Br^- (20 μM) or Cl^- (150 mM), either separately or in combination (results not shown).

Other catalytic activity of GPO

GPO does not have catalatic activity to decompose H_2O_2 to O_2 and H_2O. In contrast, it showed pseudocatalase activity to evolve O_2 from H_2O_2 in the presence of I^- or Br^-, but not in the presence of SCN^-. This pseudocatalase activity is dependent on I^- or Br^- concentration (Figure 3). With I^- concentration greater than 1 mM, the activity decreases, and becomes insignificant at 20 mM (results not shown). Br^- up to 1 mM is less effective than I^-. This effect increases with the increase in concentration up to 20 mM, where the Br^--dependent rate is similar to that with 1 mM I^- (results not shown). The pseudocatalase activity in the presence of I^- is also dependent on H_2O_2 concentration (Figure 3 inset). It has a sharp optimum at pH 7.0 in the presence of I^- and a broad optimum at pH 5–6 in the presence of Br^- (Figure 4). No significant activity was evident with SCN^- at pH 5–9. The pseudocatalase activity in the presence of I^- is blocked by SCN^- in a concentration-dependent manner (Figure 4 inset), due to competition of SCN^- with I^- for oxidation, or its chemical reaction with I^+, or both.

SCN^- oxidation by GPO is coupled with NADPH oxidation by glutathione reductase

The major product of SCN^- oxidation at neutral pH is hypothiocyanite, $OSCN^-$ [31], which is reactive mainly with thiol compounds [31,32]. Rat gastric mucosa contains a high level of GSH [33]. We have estimated SCN^- and GSH contents to be 232.6 ± 5 nmol/g of stomach and 1.7 ± 0.1 μmol/g of stomach respectively. Due to thiol reactivity, $OSCN^-$ may oxidize GSH to GSSG, with concomitant reduction to SCN^-. GSSG thus formed may be reduced back to GSH by glutathione reductase in the presence of NADPH. To test this hypothesis, we have reconstituted a system *in vitro* using GPO, H_2O_2, SCN^-, GSH, glutathione reductase and NADPH to see whether SCN^- oxidation could be coupled with NADPH oxidation. Although rat gastric mucosa contains glutathione reductase (2.3 ± 0.3 units/g of stomach), enriched in the cytosolic fraction, this crude preparation could not be used because of the interference with the assay system by other components in it. The problem was avoided by using pure glutathione reductase from yeast. The result (not shown) indicated that peroxidation of SCN^- is coupled to NADPH consumption (oxidation) and is dependent on the concentration of GPO. The slightly decreased initial rate observed was due to the limiting concentration of GSSG generated within the system, and this was overcome after 45 s. When either GPO or H_2O_2 was omitted (systems 2 and 3 in Table 2) from the reaction mixture, NADPH consumption falls to an insignificant level. GPO is also unable to oxidize NADPH by H_2O_2 (system 4). Although in the absence of GSH and glutathione reductase $OSCN^-$ may react with NADPH slowly (system 5), this reactivity is completely blocked when GSH was supplied to the assay system (system 6). In the absence of SCN^-, a slow rate of oxidation of NADPH (system 7) occurs, probably due to some $OSCN^-$-independent GSH oxidation.

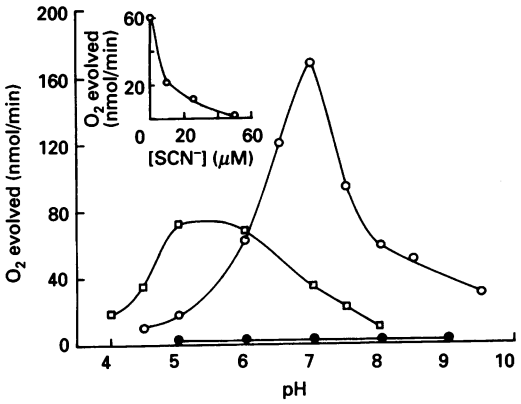


Figure 4 pH-dependent pseudocatalase activity of GPO

The reaction mixture contained 50 mM sodium citrate (pH 4.0–5.75), sodium phosphate (pH 6.0–8.0) or Tris/HCl (pH 8.25–9.0) buffer, 8 nM GPO, 1 mM I^- (○), Br^- (□) or SCN^- (●) and 100 μM H_2O_2. The inset shows the effect of SCN^- concentration on GPO-catalysed pseudocatalase activity. The reaction mixture contained 50 mM sodium phosphate buffer, pH 7.0, 5 nM GPO, 0.2 mM I^-, 0.1 mM H_2O_2 and SCN^- concentrations as indicated.

Table 2 Requirement of the components for GPO-catalysed SCN^- oxidation coupled to NADPH consumption

The reaction system when present contained 50 mM sodium phosphate buffer, pH 7.0, 0.15 mM NADPH, 1 mM GSH, 4 mM SCN^-, 2 nM GPO, 1 unit of glutathione reductase (GR) and 0.2 mM H_2O_2 to start the reaction.

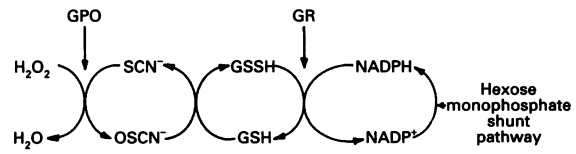
System	NADPH consumed (nmol/min)
(1) NADPH + GSH + SCN^- + GPO + GR + H_2O_2	15.8
(2) As (1), − GPO	1.2
(3) As (1), − H_2O_2	0.9
(4) NADPH + GPO + H_2O_2	0
(5) NADPH + SCN^- + GPO + H_2O_2	4.0
(6) NADPH + GSH + SCN^- + GPO + H_2O_2	0
(7) NADPH + GSH + GPO + GR + H_2O_2	3.0

DISCUSSION

The purified GPO is a 50 kDa monomeric glycoprotein with an RZ value of 0.7, close to that of other highly purified peroxidases [28,29,34,35]. It is a basic protein [11] similar to lactoperoxidase [28] and pig intestinal peroxidase [29]. It has no catalytically active thiol group. This is in contrast with our previous paper [11], where thiol blockers, *p*-hydroxymercuribenzoate or mersalyl apparently inhibited the enzyme activity by interacting with I^+ generated in the I^--oxidation assay. GPO resembles lactoperoxidase in having a Soret maximum at 412 nm, which is shifted to 426 nm with H_2O_2 due to formation of compound II. The formation of compound I could not be detected by conventional spectrophotometry, due to its very rapid one-electron reduction to compound II by an endogenous electron source, presumably the sugar moieties.

GPO can oxidize I^-, Br^- and SCN^-, but not Cl^-. In serum and extracellular fluid, the concentrations of these ions are 100 mM Cl^-, 20–100 μM Br^-, 0.1 μM I^- and 20–120 μM SCN^- [36,37]. The stomach can concentrate I^- and SCN^- almost 3-fold compared with blood [30,38]. As the I^- concentration in extracellular fluid is very low (0.1 μM), it cannot exceed 1 μM within the stomach. However, the SCN^- concentration reaches a millimolar level, as the stomach can concentrate it [38]. As the I^- concentration is too low to be oxidized by GPO, it can be excluded as its physiological electron donor. Although Br^- is oxidized at acidic pH, the rate of oxidation at physiological pH is insignificant (Figure 2). Moreover, HOBr, the peroxidative product, is toxic for mammalian tissue [39,40]. For these reasons, Br^- seems ill-suited as the physiological donor for GPO. On the other hand, SCN^- is relatively abundant in stomach, and its peroxidative product, $OSCN^-$, is less cytotoxic [5]. At physiological pH, SCN^- is oxidized at 50% of the rate at optimum pH. Moreover, physiological concentrations of I^-, Br^- and Cl^- do not affect SCN^- oxidation. The apparent K_m of SCN^- (0.4 mM) is also close to its concentration (0.25 mM) in the stomach. Considering all these parameters, SCN^- appears to be the major physiological electron donor for GPO.

Apart from the peroxidase activity, GPO has a pseudocatalase activity only in the presence of I^- or Br^-. Oxidation of H_2O_2 to O_2 by I^- or Br^--oxidation products in the free or enzyme-bound state has been reported [24,41–43]. Although GPO optimally oxidizes I^- at acid pH, its pseudocatalase activity shows a sharp optimum at neutral pH. This anomalous behaviour is probably due to the fact that I^+ reduction by H_2O_2 is favoured at alkaline pH at low I^- concentration, where the rate of formation of the association complex (I^+ to I_2 and I^-_3) is low. In the presence of Br^-, the pH optima of both pseudocatalase activity and Br^- oxidation are in the acidic region. GPO does not show a pseudocatalase activity with SCN^- similar to that of lactoperoxidase and thyroid peroxidase [43], because of the low reactivity of $OSCN^-$ with H_2O_2 [24]. Inhibition of pseudocatalase activity by SCN^- (Figure 4, inset) may further suggest that GPO mainly behaves as a peroxidase in the presence of high concentration of endogenous SCN^-. However, the SCN^--oxidation product $OSCN^-$ cannot accumulate in the stomach, because it is reduced back to SCN^- by a high level of GSH [33], which is oxidized to GSSG. In order to operate an efficient scavenging system for endogenous H_2O_2, GSSG should be reduced back to GSH by cellular glutathione reductase in the presence of NADPH, and we have observed the presence of this enzyme in the cytosolic fraction of rat stomach. Oxidation of SCN^- by GPO is also coupled with NADPH oxidation in presence of glutathione reductase (GR). Based on these results, the coupled reactions shown in Scheme 1 could be suggested for continuous elimination of endogenous H_2O_2 by GPO. Stomach glutathione reductase can efficiently consume the oxidants, as long as the supply of NADPH from the hexose monophosphate shunt pathway is not limiting.



Scheme 1

GPO when irreversibly inactivated by MMI leads to increased gastric acid secretion [13,14]. As GPO is mainly located in the parietal cell [13], containing low catalase activity (U. Bandyopadhyay and R. K. Banerjee, unpublished work), its inactivation should lead to an increased intracellular level of H_2O_2. We speculate that this H_2O_2 may either activate the intracellular signal-transduction system of the parietal cell, or stimulate the adjacent mast cell for histamine release [1] for increased acid

secretion. Although this role of H_2O_2 remains to be established, this speculation is based on the observation that H_2O_2 can alter many cellular phenomena [44]. Under normal physiological conditions, endogenous H_2O_2 likely to be generated in the secretagogue-activated parietal cell [45] is scavenged by the GPO–SCN^- system to maintain the acid secretion at a controlled rate. Under stress conditions, when this H_2O_2 concentration reaches a critically high level due to stimulation of superoxide dismutase and inactivation of GPO, oxidative damage becomes predominant to cause gastric ulceration [16].

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