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CATALATIC ACTIVITY OF LACTOPEROXIDASE IN THE PRESENCE OF SCN^-

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Summary: Lactoperoxidase catalyzed the catalatic decomposition of H_2O_2 in the presence of SCN^-. The pH optimum for O_2 evolution was 8.5, while the enzyme activity as disclosed by the rate of H_2O_2 disappearance was optimal at 4.5. Since the catalatic activity of lactoperoxidase was SCN^- dependent, and no O_2 was evolved, when H_2O_2 was added to $OSCN^-$ in the absence of lactoperoxidase, an enzyme-OSCN complex may be assumed to be an intermediate in the catalatic activity of lactoperoxidase.

Wilson and Harris (1) studied the chemical oxidation of SCN^- by H_2O_2, and found sulfate, cyanate, ammonia, and carbonate as final products. They postulated the following reaction mechanisms:

$$H_2O_2 + SCN^- \longrightarrow HOSCN + OH^- \quad (1)$$

$$HOSCN + H_2O_2 \longrightarrow HOOSCN + H_2O \quad (2)$$

$$HOOSCN + H_2O_2 \longrightarrow H_2SO_3 + HOCN \quad (3)$$

$$HOCN + 2H_2O \longrightarrow HCO_3^- + NH_4^+ \quad (4)$$

$$H_2SO_3 + H_2O_2 \longrightarrow H_2SO_4 + H_2O \quad (5)$$

with reaction 1 as the rate-determining step. It is this reaction, which can be catalyzed by lactoperoxidase (EC 1.11.1.7) and then gives $OSCN^-$ at neutral pH (2,3). Hogg and Jago (4) have suggested that reaction 2 also might be catalyzed by lactoperoxidase, since the enzymic oxidation of SCN^- by H_2O_2 yields the same final products as the chemical oxidation. The existence of HOOSCN as a product of the enzymic oxidation has, however, not been confirmed. In the present communication another product, O_2, which has not been postulated, was shown to be formed in the lactoperoxidase-catalyzed reaction between SCN^- and H_2O_2.

MATERIAL AND METHODS

Measurement of H_2O_2. A sample (40 µl) containing H_2O_2 was added to 2 ml of a reagent containing 50 mM phosphate buffer pH 6.5, 100 µg horse radish peroxi-

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dase, and 0.1 mM 2,2'-azino-di (3-ethylbenzthiazoline-6-sulfonic acid). The absorbance at 412 nm was read 2 min after the addition of the sample. The concentration of H_2O_2 in a standard solution was calculated assuming an extinction coefficient of H_2O_2 of 43.2 M^{-1} cm^{-1} at 240 nm.

Measurement of O_2. The O_2 evolution in reaction mixtures was followed polarographically with a Clark-type electrode (Oxygen monitor, model 53; Yellow Springs Instruments Co.) in an anaerobic box with an atmosphere of 10% H_2 in N_2. The instrument was calibrated by adding 20 µl of 1% catalase solution to 3 ml 50 mM phosphate buffer, pH 7.3, containing various amounts of H_2O_2. The response of the instrument was linear up to 1 mM O_2.

Measurement of $OSCN^-$. The concentration of $OSCN^-$ was determined by its reaction with 2-nitro-5-thiobenzoic acid (5). All products formed in a reaction mixture containing lactoperoxidase, SCN^- and H_2O_2, which oxidized the reagent, were considered to be $OSCN^-$ even if other possible reaction products such as HOOSCN may have contributed to the oxidation (4,6). The reagent was prepared by reducing 1 mM solution of 5,5-dithio-bis(2-nitrobenzoic acid) into 2-nitro-5-thiobenzoic acid with borohydride in the anaerobic box. This reagent was stable in the box. The concentration of 2-nitro-5-thiobenzoic acid was calculated assuming an extinction coefficient of 14 139 $M^{-1}cm^{-1}$ at 412 nm (7).

Preparation of a lactoperoxidase-free $OSCN^-$ solution. A stirred 10-ml ultrafiltration cell (Amicon Corp.) fitted with a Diaflo membrane (PM30) contained 9 ml of 50 mM phosphate buffer, pH 7.3, 1 mM KSCN and 100 µg of lactoperoxidase. H_2O_2 was added to give a final concentration of 1 mM; 5 min later the solution was filtered to remove lactoperoxidase. The concentration of $OSCN^-$ in the filtered solution was around 400 µM.

Buffer solutions. The following buffers were used: citric acid-sodium citrate pH 3.0-4.0; acetic acid-sodium acetate pH 4.0-5.5; K_2HPO-KH_2PO pH 6.5-7.5; 3-N-morpholinopropane sulfonic acid-KOH pH 6.0-7.0; tris(hydroxymethyl) aminomethane-HCl pH 7.5-8.5; glycine-KOH pH 8.5-10.0.

Chemicals. Lactoperoxidase, horse radish peroxidase and 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) were from Boehringer Mannheim GmbH. Catalase from bovine liver (C10) and 5,5'-dithio-bis(2-nitrobenzoic acid) were from Sigma Chemical Co. H_2O_2 (Perhydrol) was from E. Merck AG.

RESULTS

Significant amount of O_2 was evolved in a reaction mixture at 28 C containing 1 mM H_2O_2, 1 mM KSCN, lactoperoxidase (10 µg/ml), and 50 mM buffer (Fig. 1). The highest amount of O_2 was formed at pH 8.5. No or insignificant amounts of O_2 were formed by omitting SCN^-, H_2O_2 or lactoperoxidase from the mixture. Lactoperoxidase heated in a boiling water bath for 5 min did not catalyze the reaction. Similar amounts of O_2 were formed, whether the reaction was initiated by adding SCN^-, H_2O_2, or lactoperoxidase. The amount of O_2 increased with increasing concentration of H_2O_2 in the reaction mixtures (Fig. 2). Different types of buffers or addition of 0.15 M NaCl to the reaction mixtures did not influence the amount of O_2 evolved. O_2 was also formed in the presence of I^- or Br^- with pH optima of 8.0 and 4.5, respectively (Fig. 1).

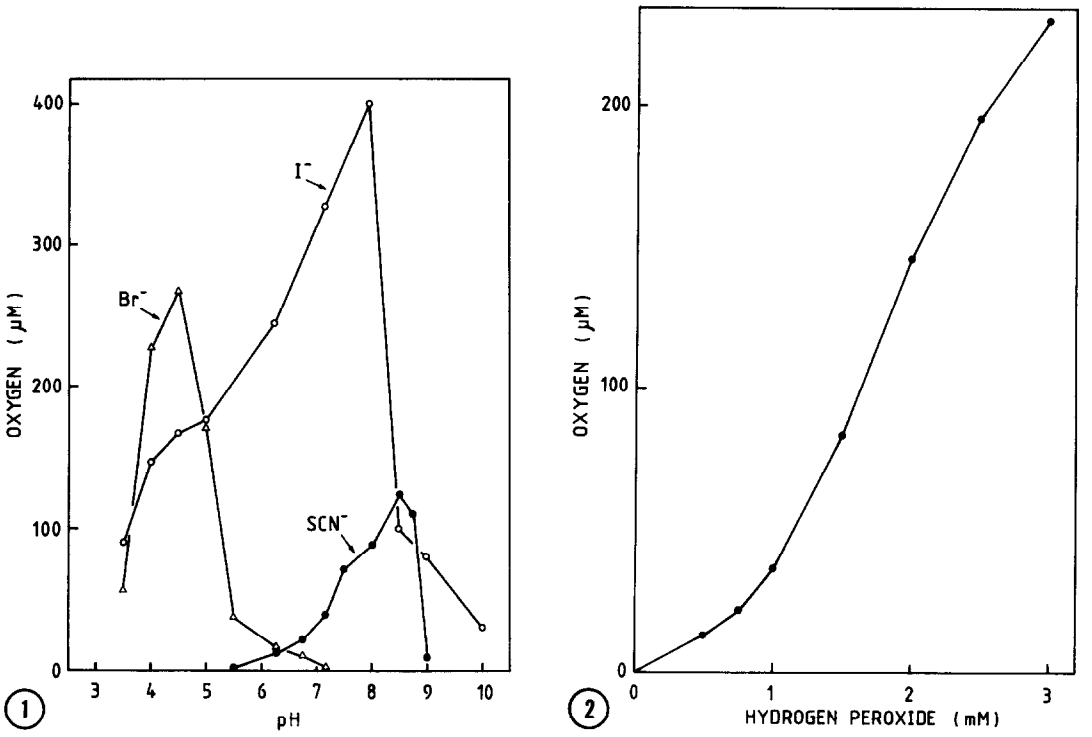

Fig. 1. O_2 concentration in reaction mixtures containing 1 mM H_2O_2, lactoperoxidase (10 μg/ml), and 50 mM of various buffers at 28 C. The reactions were initiated by the addition of KSCN, KBr or KI to give a final concentration of 1 mM. The response of the oxygen electrode usually reached a steady maximum reading 3 min after this addition.

Fig. 2. O_2 concentration in reaction mixtures containing 1 mM KSCN, lactoperoxidase (10 μg/ml) and 50 mM phosphate buffer pH 7.3 at 28 C. The reactions were initiated by the addition of H_2O_2.

The rate of the lactoperoxidase-catalyzed reaction in the presence of SCN^-, I^-, or Br^-, as disclosed by the rate of H_2O_2 disappearance, was most rapid in the presence of I^- and slowest in the presence of Br^- (Fig. 3). The pH optimum was 4.5 in the presence of SCN^- or Br^-, and 6.8 in the presence of I^- (Fig. 3). $OSCN^-$ was most rapidly formed at pH 4.0 (Fig. 4).

No O_2 was evolved, when 1 mM H_2O_2 was added to a lactoperoxidase-free solution containing 350 μM $OSCN^-$. When a commercial sodium hypochlorite solution was diluted in the same buffer to give 350 μM OCl^- as disclosed by reaction with 2-nitro-5-thiobenzoic acid, 345 μM O_2 evolved on addition of 1 mM H_2O_2. Addition of 1 mM H_2O_2 to 700 μM OCl^- gave 640 μM O_2.

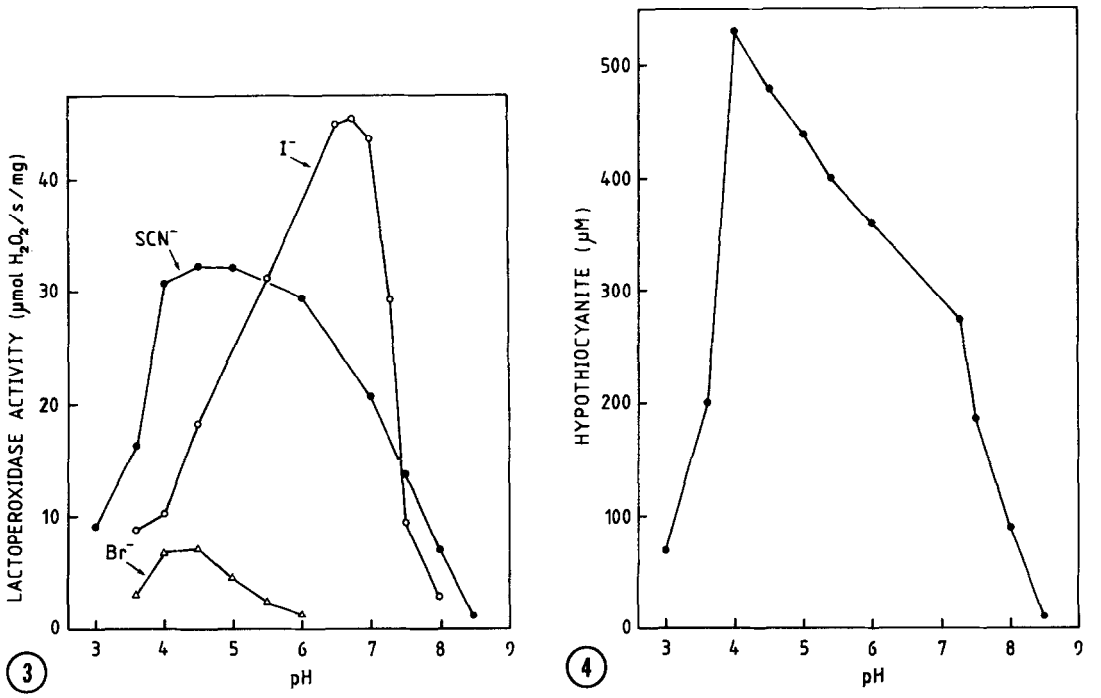

Fig. 3. Lactoperoxidase activity as disclosed by the rate of H_2O_2 disappearance from reaction mixtures containing 1 mM H_2O_2, 1 mM of KSCN, KBr or KI, and 50 mM of various buffers at 28 C. The reactions were initiated by the addition of lactoperoxidase to give a final concentration of 2 μg of enzyme per ml. Samples for measurement of H_2O_2 were taken before and 10 s after the addition of the enzyme.

Fig. 4. The concentration of $OSCN^-$ in reaction mixtures containing 1 mM H_2O_2, 1 mM KSCN and 50 mM of various buffers at 28 C. The reactions were initiated by the addition of lactoperoxidase to give a final concentration of 2 μg of enzyme per ml. Samples for measurement of $OSCN^-$ were taken before and 60 s after the addition of the enzyme.

DISCUSSION

Although it has been known for some time that chloroperoxidase (EC 1.11.1.10) displays significant catalatic activity (8), it is only recently that this activity of lactoperoxidase has attracted attention (9,10,11). It has been shown that O_2 evolution by a lactoperoxidase-Br^--H_2O_2 system has a pH optimum of 4.5 (9) and that O_2 is evolved at pH 7.0 in a lactoperoxidase-I^--H_2O_2 system (10). The present study confirmed these observations and further demonstrated that lactoperoxidase had catalatic activity also in the presence of SCN^- with a pH optimum of 8.5, and that the pH optimum of the catalatic activity was 8.0 in the presence of I^-.

When Piatt and O'Brien (9) found that the addition of H_2O_2 to chemically prepared OBr^- resulted in O_2 evolution, they suggested the following mechanisms for the catalatic activity of lactoperoxidase in the presence of Br^-:

$$H_2O_2 + Br^- \xrightarrow{\text{lactoperoxidase}} OBr^- + H_2O \quad (6)$$

$$OBr^- + H_2O_2 \longrightarrow O_2 + Br^- + H_2O \quad (7)$$

Similar mechanisms could not explain, however, the catalatic activity of lactoperoxidase in the presence of SCN^-, since no O_2 was formed, when $OSCN^-$ in the absence of lactoperoxidase was exposed to H_2O_2. Chloroperoxidase also has catalatic activity in the absence of halide, and this activity has been explained by assuming the formation of a three-oxygen intermediate in a reaction of Compound I of the peroxidase with the peroxide molecule (12). Such a mechanism is not applicable, however, to the catalatic activity of lactoperoxidase, since this activity was dependent on SCN^-. To explain the SCN^--dependent catalatic activity of lactoperoxidase one may assume the involvement of enzyme-OSCN complexes in analogy with the enzyme-hypohalite complexes predicted in peroxidase-catalyzed halogenations (13,14), and suggested in the catalatic activity of lactoperoxidase in the presence of I^- (10). The present demonstration of significant differences in catalatic activity at various pH and in the presence of Br^-, I^- or SCN^- will be helpful in the further elucidation of the catalytic mechanisms of lactoperoxidase.

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