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PEROXIDATION OF PHOSPHOLIPIDS PROMOTED BY MYELOPEROXIDASE

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Myeloperoxidase was found to promote peroxidation of phospholipids under acidic conditions in the presence of hydrogen peroxide and iodide ions. The peroxidation was markedly enhanced by pyrophosphate-chelated ferric iron and was inhibited by desferrioxamine and superoxide dismutase. This observation adds lipid peroxidation to the oxidative damage caused by myeloperoxidase, which is a phagocytic cell enzyme involved in phagocyte-mediated cell destruction.

KEY WORDS: Myeloperoxidase, lipid peroxidation, superoxide dismutase, hypoiodite-mediated peroxidation, SOD-inhibited peroxidation.

INTRODUCTION

Phagocytic leucocytes contain a hypohalite-producing enzyme, myeloperoxidase, one of the functions of which is to modulate the inflammatory response and to destroy tumor cells and bacteria (review[1]). The ability of hypohalite ions to oxidize thiol and thioether compounds has been suggested as an explanation of the microbicidal activity of myeloperoxidase.[2] Myeloperoxidase-induced tissue destruction has been suggested in rheumatoid arthritis and it was recently found that thiol-containing antiarthritic drugs were scavengers of hypochlorite and inhibitors of its formation by leukocyte myeloperoxidase.[3] Lipid peroxidation is a commonly recognized mechanism of cell destruction but has so far not been considered to be a component of the myeloperoxidase-mediated injury. The present study has shown that myeloperoxidase-derived products are capable of initiating peroxidation of phospholipids in the presence of iodide and chelated iron.

MATERIALS AND METHODS

Myeloperoxidase (MPO) from human leukocytes was purchased from the Green Cross Corporation, Osaka, Japan. This lyophilized preparation (GCC-2200 lot EH001KY) contained 200 units per ml after reconstitution. Bovine superoxide dismutase (SOD) 6000 U/mg, was purchased from Grünenthal, West Germany. A 50 mM potassium phosphate buffer was used, the pH was 6.0 unless otherwise specified. Pyrophosphate-iron chelates were prepared in stock solution containing 3 mM $FeCl_3$ and 40 mM potassium pyrophosphate in water. Human albumin (lot

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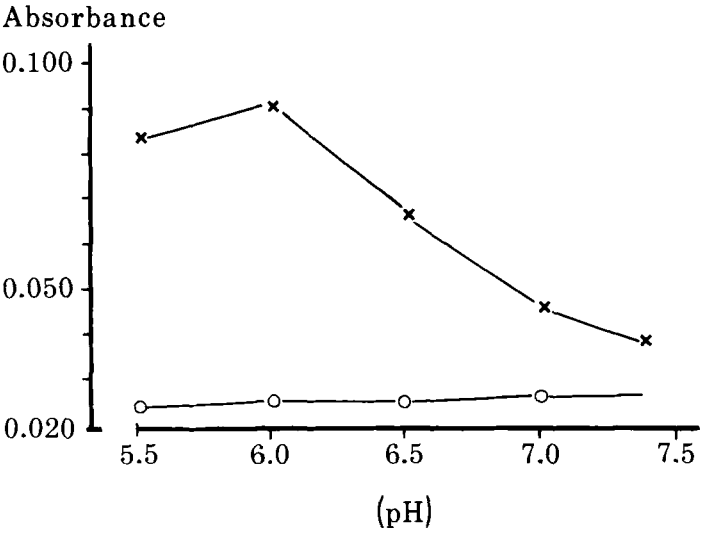

FIGURE 1 Effect of pH on myeloperoxidase-induced lipid peroxidation. (×) with myeloperoxidase. (○) without myeloperoxidase.

63130-51) was purchased from KABI, Stockholm, Sweden. Copper-histidine chelates were prepared to a final concentration of 0.1 mM $CuCl_2$ and 10 mM histidine in water.

Preparation of inactive SOD

SOD, 5 mg/ml in water, was brought to pH 11.8 by addition of NaOH. Hydrogen peroxide was added to a final concentration of 10 mM. After 3 min of incubation at room temperature, the pH was lowered to 7.5 with HCl, whereafter the mixture was dialyzed for 48 h to remove excess hydrogen peroxide. The inactivated SOD had residual activity of 120 U/mg.

Preparation of Phospholipid Liposomes

Folch fraction III from bovine brain (Sigma Chemical Co, St Louis, Mo, USA) stored at −20°C as a 10% solution in chloroform, containing 140 mM phospholipid phosphate, was used. According to the manufacturer the preparation contained 80–85% phosphatidyl serine. A 100 μl sample of the chloroform solution was dried under argon, after which 1 ml of ice-cold buffer was added and sonication was performed under argon for about 15 s. The liposomes were then kept ice-cold under argon.

Peroxidation of Phospholipids

The basic incubation mixture consisted of 2 units of MPO, 0.1 mM H_2O_2, 10 μM KI, 30 + 400 μM Fe^{3+}-pyrophosphate complex and 10 μl of phospholipid suspension in 1 ml of buffer at 37°C. The reaction was started by addition of the H_2O_2. Care was taken to keep the reaction mixture in the dark, since preliminary experiments had shown that exposure to sunlight increased the concentration of thiobarbituric acid

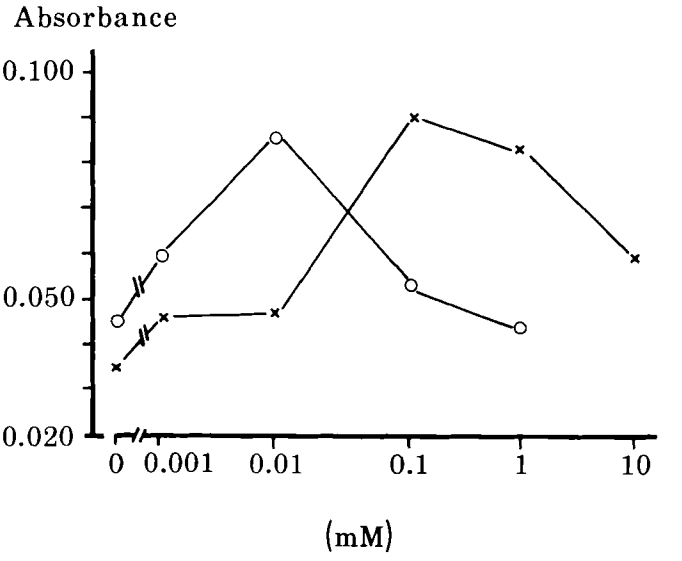

FIGURE 2 Effects of KI (○) and H_2O_2 (×) on myeloperoxidase-induced lipid peroxidation.

reactive substances (TBARS) at least three times that ever seen under optimal conditions, even in the absence of H_2O_2. After a 15 min incubation, TBARS were assayed as described elsewhere.[4] The results are expressed as absorbance values measured at 532 nm. Each result is the mean of at least two different experiments. The standard deviation was never more than 10%.

Enzyme Activity Measurements

The influence of 500 U/ml of SOD or 83 μm desferrioxamine on myeloperoxidase was determined with the guaiacol method.[5] SOD activity was assayed as described by Marklund and Marklund.[6]

RESULTS

The effects of pH, and of different concentrations of H_2O_2 and I^- are illustrated in Figures 1–2. All these variables showed an activity maximum, and the conditions giving maximal activities were chosen for the subsequent studies. The MPO activity was studied by adding 0.4, 2 and 10 units to the normal reaction mixture. The resulting absorbance values were 0.097, 0.125 and 0.075 respectively, indicating an activity maximum for 2 units. The time dependence is illustrated in Figure 3. As seen in Table I, the maximal expression of TBARS required the presence of pyrophosphate-chelated iron. About 60% of the maximal TBARS were formed in the absence of added Fe^{3+}-pyrophosphate. However, no TBARS were formed in the presence of the iron chelator desferrioxamine, which may indicate that iron contaminating the reaction mixture could have catalyzed the peroxidation reaction. Substitution of a Fe^{3+}-EDTA chelate for Fe^{3+}-pyrophosphate also resulted in no expression of TBARS,

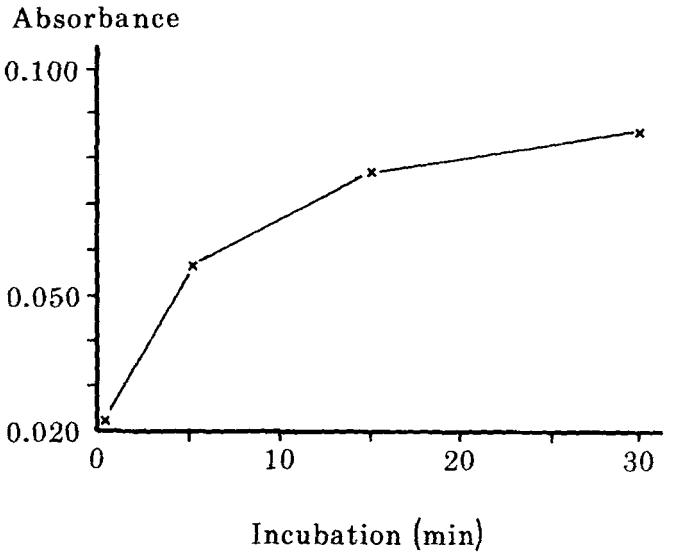

FIGURE 3 Increase in lipid peroxidation with incubation time.

probably due to chelation of contaminating iron ions by the excess EDTA. Desferrioxamine did not interfere with the TBARS assay.

When iodide ions were replaced by chloride in the form of NaCl (1–100 mM), no TBARS were formed. Preliminary experiments on the effect of pH, in which the pH of the phosphate buffer was adjusted with HCl, also indicated that chloride ions may inhibit iodide-catalyzed TBARS formation.

The peroxidation reaction was inhibited by bovine SOD in a dose-dependent fashion, as illustrated in Figure 4. Hydrogen peroxide-inactivated SOD also inhibited the reaction. Inactivated SOD was only about 10% as effective as active SOD. Addition of SOD (50 μg/ml) after the peroxidation reaction, but before the TBARS assay, reduced the absorbance values for active and inactivated SOD to 84 and 77% respectively. A Cu^{2+}-histidine chelate, 10 nM to 10 μM with respect to $Cu,^{2+}$ had no effect on the peroxidation reaction. Human albumin, 0.5, 5 and 50 μg/ml, inhibited the peroxidation reaction by 21, 44 and 72% respectively, which is very similar to the inhibition by inactivated SOD.

The MPO activity was not significantly altered by the presence of SOD, while 83 μM desferrioxamine reduced the MPO activity by 37%.

DISCUSSION

The results of this study show that myeloperoxidase is capable of promoting lipid peroxidation in the presence of hydrogen peroxide, iodide and chelated ferric iron. The peroxidation was markedly enhanced by addition of pyrophosphate-bound Fe^{3+}. Desferrioxamine inhibited the expression of TBARS completely. This inhibition may have been partly due to the slight inactivation of MPO by desferrioxamine, but most of it was probably attributable to chelation of ferric iron. Earlier studies have shown

TABLE I
Effect of various reagents on myeloperoxidase-induced phospholipid peroxidation

Reagents	Absorbance	% TBARS
None	0.033	0
I	0.031	0
FePPi	0.027	−10
MPO	0.025	−14
H_2O_2	0.033	0
MPO, FePPi, I, H_2O_2 (Complete)	0.090	100
Complete − I	0.045	21
Complete − FePPi	0.067	60
Complete − MPO	0.027	−10
Complete − H_2O_2	0.033	2
Complete + desferrioxamine		
15 μM	0.044	21
30 μM	0.035	6
30 μM‡	0.099	115
150 μM	0.030	0
Complete + FeEDTA	0.033	2
Complete − I + 100 mM NaCl	0.018	−12
Complete − I + 10 mM NaCl	0.021	−8
Complete − I + 1 mM NaCl	0.029	5

Note: FePPi = 30 + 400 μM Fe^{3+}-pyrophosphate, H_2O_2 = 0.1 mM H_2O_2, I = 10 μM KI, FeEDTA = 400 μM EDTA instead of pyrophosphate, MPO = 2 units of myeloperoxidase sample.

‡Added after the incubation.

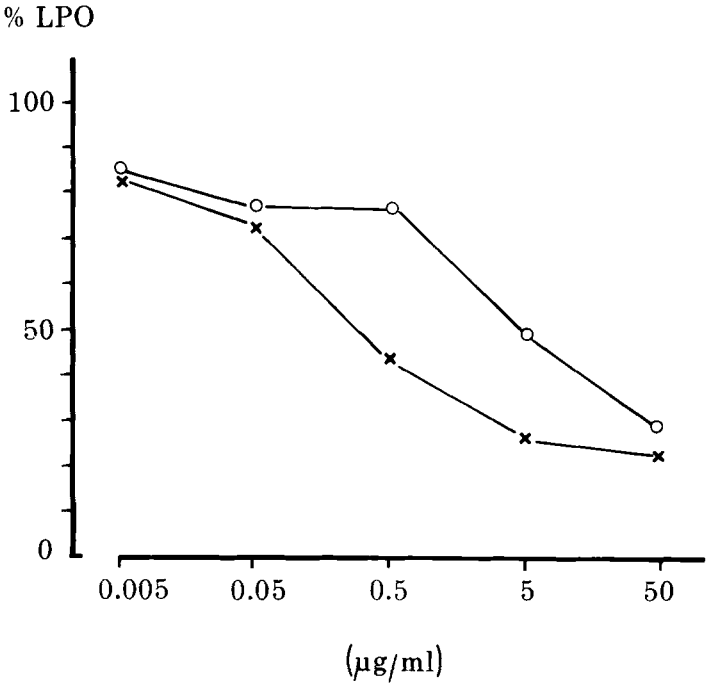

FIGURE 4 Inhibitory effects of active (×) and inactivated (○) superoxide dismutase on lipid peroxidation.

that a very similar enzyme, lactoperoxidase, can catalyze peroxidation of lipids.[7,8] The dose-response relations found for H_2O_2 and KI in the present study are similar to those found for lactoperoxidase,[7] and the optimal H_2O_2 concentration resembles that found by Andrews *et al.*[9] for MPO activity. These authors also found that Cl^- inhibited MPO and increased the K_M value for H_2O_2 dramatically, so that in the presence of 0.1 M NaCl and 0.1 mM H_2O_2 virtually no oxidants were produced, which may explain our negative results when I^- was replaced by Cl^-. Iodide is present *in vivo* in far lower concentrations than chloride, but may still be of physiological importance, as suggested by Weiss and LoBuglio since neutrophils are capable of deiodinating thyroid-hormones.

The lactoperoxidase-catalyzed peroxidation reported by Buege and Aust[7] differs from the peroxidation studies in the present investigation in several respects. Notably, with lactoperoxidase SOD had no effect and iron was not required. Furthermore EDTA had only a minor effect, while EDTA in the present study abolished the TBARS production.

Superoxide has been reported to be produced by MPO,[10] but several observations contradict the idea that superoxide is the initiator of lipid peroxidation. Many chelates between copper and amino acids are superoxide dismutators,[11,12] but in the present study the copperhistidine chelates did not affect TBARS expression, whereas SOD inhibited it strongly. Inactivated SOD was about 10% as potent as fully active SOD. This effect may have been due to the low residual activity, but it may also have been a nonspecific protein effect since human albumin was equally potent.

In a study of myeloperoxidase-mediated damage to liposomes, Sepe and Clark[13] concluded that the damage observed was not due to lipid peroxidation but rather to lipid halogenation. However, they also found that both active and inactive SOD inhibited the liposome damage, perhaps by interchelation and stabilization of the lipid bilayers rather than by enzymatic activity.[14] The inactivation of the TBARS expression by SOD is therefore puzzling. It is possible that SOD inhibits the propagation reaction by interfering with lipid peroxides rather than by inhibiting a superoxide-dependent initiation. SOD added after the peroxidation reaction did not interfere with the TBARS assay.

No precautions were taken in the present study to free the lipid preparation used from hydroperoxide contamination other than to keep it under argon, therefore it cannot be excluded that the initiation of lipid peroxidation was at least partly lipid hydroperoxide dependent. In a review of the role of iron in enzymatic lipid peroxidation Aust and Svingen[15] stated that ADP-Fe (and *e.g.* PP_i-Fe) promoted hydroperoxide independent peroxidation, while reduced EDTA-Fe was only an efficient promoter of hydroperoxide dependent peroxidation. Since no peroxidation was seen in presence of EDTA-Fe we conclude that myeloperoxidase is capable of initiating hydroperoxide independent lipid peroxidation.

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