

Effect of Myeloperoxidase/Hydrogen Peroxide/Halide System on Thermal Gelation of Soluble Collagen[†]

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Abstract—Effect of myeloperoxidase (MPO)/H₂O₂/halide system on thermal gelation of soluble collagen was examined to test the ability of the system to alter the structure of this structural protein using MPO prepared from human polymorphonuclear leukocytes. The gelation that occurred at 37°C was markedly inhibited when collagen treated with MPO, H₂O₂ and halides. The inhibition required the three components. All the halides except F⁻ were effective as cofactors. The inhibition was prevented by ¹O₂ scavengers such as histidine and diazobicyclo(2,2,2)octane. With these findings it is confirmed that the inhibition of collagen gelation was caused by MPO-mediated peroxidation. The nature of structural alterations by the system that resulted in the inhibition of gelation was not explored in detail. But collagen when digested with collagenase did not gel properly and collagen treated with the MPO system was found to be cleaved on chromatographic analysis. Thus, degradation may be one of the changes in collagen structure caused by the MPO system.

The results obtained indicate that MPO/H₂O₂/halide system was able to cause structural alteration of collagen. The degradative effect of the MPO system on collagen structure was discussed as a possible mechanism of tissue damage in inflammatory conditions.

Key words: *Collagen, Myeloperoxidase, Polymorphonuclear leukocytes*

INTRODUCTION

In inflammatory response, polymorphonuclear leukocytes (PMNs) play important roles in tissue injury. Upon contact with bacteria, immune complexes or complements, PMNs are activated and undergo sequential events which are linked to secretion of various toxic components into extracellular space (Weissmann 1979; Weissmann *et al.* 1980). With regard to mechanisms of tissue damage, enzymatic processes mediated by the released granular proteases have been elucidated, where cathepsins, collagenase and elastase are involved (Barrett 1978; Ignaro 1974; Weissmann *et al.* 1980). But reactive oxygen products sec-

reted together with the proteases have also been implicated. The implication of the oxygen products as mediators in tissue damage are supported from their actions to cause depolymerization of hyaluronic acid (McCord 1974; Greenwald and Moy 1980; Lee *et al.* 1985), degradation of collagen (Chung *et al.* 1984 & 1985; Greenwald and Moy 1979) and destruction of proteoglycans (Greenwald *et al.* 1976). Thus, at present, the oxygen-dependent mechanism has received considerable attention in PMN-mediated tissue injury (Fantone and Ward 1982).

But in many studies showing destructive actions of the reactive oxygen products to biomolecules including hyaluronic acid and collagen, the interaction between O₂⁻ and H₂O₂ to produce OH· in the presence of metal ions, particularly iron (so called Haber-Weiss reaction) has been suggested as an underlying mechanism to explain their destructive

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actions (Fridovich 1978; McCord 1974; Greenwald and Moy 1979 & 1980). Despite the many *in vitro* studies showing this mechanism, however, Winterbourn (1981) observed that the Haber-Weiss reaction was hardly detectable in the body fluids such as lymph and synovial fluid unless extra iron salt was added, suggesting that body fluids have little capacity to catalyze $\text{OH}\cdot$ production from $\text{O}_2\cdot$ and H_2O_2 via this mechanism. Therefore, in oxygen-dependent tissue damage by PMNs *in vivo*, the contribution of the Haber-Weiss reaction may not be expected.

But in addition to the Haber-Weiss reaction, the activated PMNs have another reaction with which the oxygen products can be activated. This reaction is mediated by a granular enzyme, myeloperoxidase (MPO) using H_2O_2 as a substrate and halides as cofactors. Although the MPO/ H_2O_2 /halide system primarily serves as a microbicidal mechanism against phagocytized bacteria (Barbior 1978; Klebanoff 1980), the activated PMNs can exert extracellular toxicities through the MPO-mediated peroxidation, for example, lysis of mammalian cells (Clark and Klebanoff 1977), killing of hyphal forms of fungal pathogens (Diamond *et al.* 1980), and inactivation of enzymes (Jin *et al.* 1986) and serum factors such as α_1 -protease inhibitor (Clark *et al.* 1981). In the toxicities observed, the actions of oxygen products are primarily mediated by the MPO system rather than the Haber-Weiss reaction. Therefore, at sites of inflammation where PMNs are massively infiltrated, destruction of extracellular major components is expected to be mediated by the oxidative action of the MPO system. Then, the system may serve as an alternative oxygen dependent mechanism that can contribute to PMN-induced tissue injury in inflammation.

In order to test this possibility, the present study was performed using MPO prepared from human PMNs in order to test the ability of the MPO/ H_2O_2 /halide system to alter the structure of a major component of extracellular matrix, collagen. The effect of the MPO system on the structure of collagen was evaluated by observing the changes in thermal gelation of soluble collagen which is regarded as an *in vitro* model of fibrillogenesis of this protein (Woodhead-Galloway 1980). In the present paper, it was observed that the MPO system markedly affected the gelation of collagen, suggesting MPO-mediated peroxidation can cause alterations in the structural integrity of this protein including degradation.

MATERIALS AND METHODS

Collagenase and hydroxyproline were obtained from Sigma Chemical Co., sodium azide from Merck; sodium benzoate and sodium formate from Fisher Scientific Co.; mannitol from Hayashi Pure Chem.; hydrogen peroxide from Shinyo Chemical Co.; 1,4-diazobicyclo(2,2,2)octane (DABCO) from Aldrich Chem. Co.; glucose oxidase from Worthington and other chemicals were of analytical reagent grade.

1. Preparation of human leukocytes

Leukocytes were prepared by the method of Baugh and Travis (1976). Blood from different donors was mixed with 1/4 volume of a solution containing 0.025 M citric acid, 0.05 M sodium citrate and 0.075 M glucose, and centrifuged at 1,000 g for 15 min. Buffy coat was collected and mixed with an equal volume of 0.15 M KCl and centrifuged at 1,000 g for 10 min. The resulting pellet was washed with cold distilled water for 1 min to lyse contaminating erythrocytes and then 1.5 M KCl was added to bring the suspension to isotonic strength. The cells were recentrifuged and the above lysis procedure was followed until no erythrocyte remained. Approximately 6×10^9 cells was obtained from 1 liter of blood by this procedure.

2. Preparation of leukocyte granules and granule extract

The leukocyte preparation was used to isolate the leukocyte granules, from which extract was prepared according to the methods described by Baugh and Travis (1976).

3. Partial purification of human leukocyte MPO

MPO-rich fraction was obtained from the granule extract according to the procedures described by Matheson and Travis (1981). The extract was dialyzed against 0.05 M Tris-HCl, 0.1 M NaCl and 0.001 M dithiothreitol, pH 8.0 at 4°C overnight. The precipitate was removed by centrifugation at 2,000 g for 10 min. The green supernatant which contained MPO was lyophilized and dissolved in 10 ml of the above buffer solution. The concentrated solution after extensive dialysis against 0.05 M sodium phosphate, pH 6.1, was stored at -20°C and used as the enzyme preparation. One unit of MPO activity was defined as a change in absorbance of 1.0 per min at 510 nm (Matheson and Travis 1981).

4. Preparation of skin collagen

Collagen was prepared from the skin obtained from 50 rats, weighing about 100 g, according to the procedure of Oegema *et al.* (1975). Collagen solution in 0.1 M acetic acid was lyophilized and stored at -20°C . Collagen, when used, was dissolved in 0.5 M acetic acid to give a concentration of approximately 2 mg/ml, dialyzed extensively against 0.005 M acetic acid at 4°C , and the resulting collagen was used in the experiments.

5. Treatment of collagen with MPO/H₂O₂/halide system and measurement of collagen gelation

Collagen was mixed to give a final concentration of about 80 μg OH-proline/ml in 4 ml of reaction medium containing 160 mM NaCl and 100 mM NaH₂PO₄, pH 6.1, in the presence of MPO, H₂O₂ and halides at concentrations as indicated in the figures and incubated for 4 h at 4°C . After the reaction mixture was degassed, 3 ml of aliquot was pipetted into a cuvette. The cuvette was then transferred to the temperature-controlled chamber of a Pye Unicam SP 1750 spectrophotometer and it was maintained at 37°C . Gelation was monitored by continuous absorbance recording at 400 nm. But when collagen was treated with MPO and H₂O₂ in the presence of iodide, absorbance was read at 500 nm to avoid interference by iodide at 400 nm. OH-proline was assayed by the method of Woessner (1961).

6. Gel-permeation chromatographic analysis of collagen

Collagen was treated with the MPO system as described above and then mixed with concentrated acetic acid to give a final concentration of 0.1 M. Two and half ml aliquot was taken and placed on a Sephadex G-200 column (2.5 X 18 cm) which was equilibrated with 100 mM NaCl in 0.1 M acetic acid. Elutes were collected in 2.5 ml fraction and assayed for OH-proline. The flow rate was 18 ml/h, and the chromatography was run at room temperature.

RESULTS

1. Inhibition of collagen gelation by MPO/H₂O₂/halide system

Fig. 1 shows gelation of collagen that was not treated with the MPO system. When the collagen solution was warmed to 37°C , gelation indicated by the increase in absorbance at 400 nm was started after about 10 min of lag period.

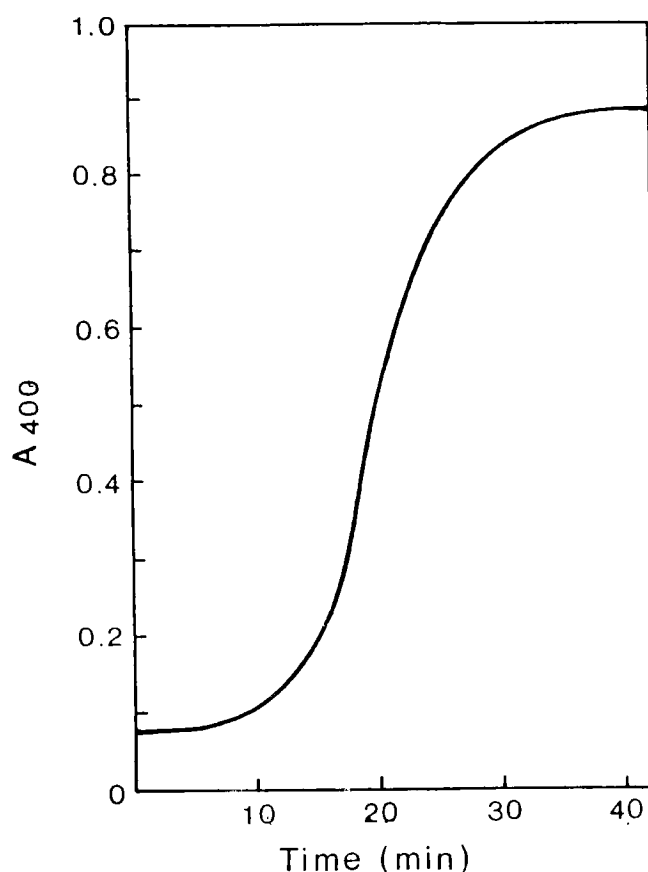


Fig. 1. Collagen gelation. Medium condition: collagen 84 μg OH-proline/ml, 160 mM NaCl and 100 mM NaH₂PO₄, pH 6.1.

When collagen was incubated with MPO and H₂O₂ in the presence of Cl^- at 4°C for 4 h, its gelation was markedly altered. As shown in Fig. 2, two changes were observed; shortening of the period required for initiation of gelation (lag phase) and decrease of the final absorbance to attain (maximal turbidity). The extent of the changes in the gelation was increased with increasing concentration of H₂O₂. But when H₂O₂ concentration exceeded 0.5 mM, the effect of the MPO system was a little decreased (Fig. 2, Curve e). That may be due to inactivation of MPO by excess H₂O₂ (Matheson and Travis, 1981). That was further supported by the observation that the concentration of H₂O₂ to show maximal effect on the gelation was lowered with smaller amount of MPO (data not shown). Either MPO (Fig. 2, Curve a) or H₂O₂ (data not shown) alone did not show any changes in the gelation within the concentration ranges used. But at concentration of H₂O₂ higher than 2 mM, H₂O₂ by itself caused a little changes in gelation (data not shown). But the effect was almost negligible when compared to that with lower concentrations of H₂O₂ in the presence of MPO.

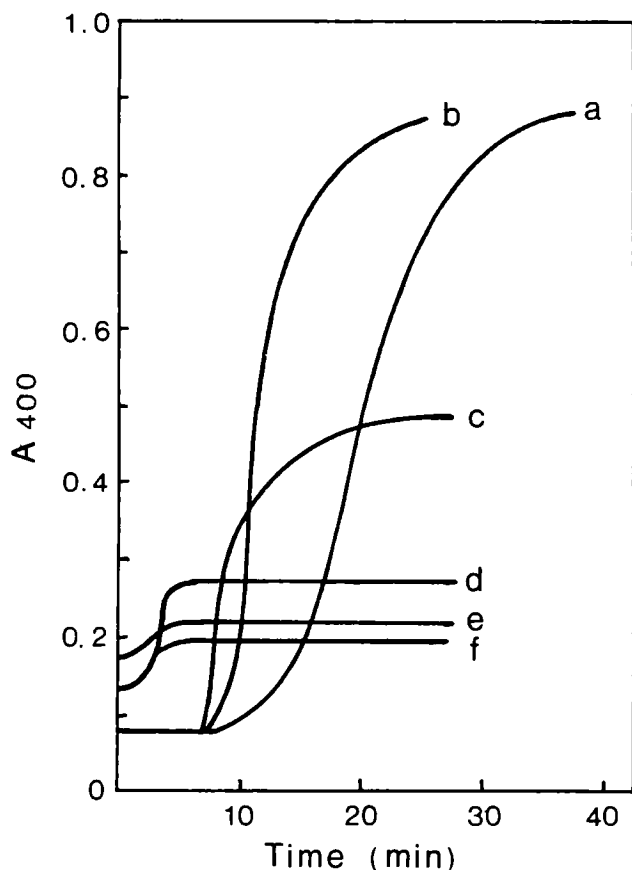


Fig. 2. Effects of H_2O_2 concentration in the MPO system on collagen gelation. Collagen ($84 \mu\text{g}$ OH-proline/ml) was incubated at 4°C for 4 h in 4 ml of reaction mixture containing 0.2 units MPO, 160 mM NaCl and 100 mM NaH_2PO_4 , pH 6.1 in the presence of varying concentrations of H_2O_2 . After incubation the reaction mixture transferred to the cuvette was warmed to 37°C to initiate gelation. The gelation was monitored by continuous absorbance recording at 400 nm. Curve a; 0 mM H_2O_2 and b, c, d, e and f; 0.063, 0.125, 0.25, 1 and 0.5 mM H_2O_2 , respectively.

2. Requirement for MPO, H_2O_2 and halide in the alteration of collagen gelation

In the following experiments, it was further tested whether the observed effect of the MPO system was due to MPO-mediated peroxidation in the presence of halides as a cofactor.

In Fig. 3, NaN_3 , an inhibitor of MPO, or catalase showed complete inhibition of the observed alterations of collagen gelation caused by MPO, H_2O_2 and Cl^- . This indicates that inhibition of the enzyme or removal of H_2O_2 abolished the peroxidative effect on the collagen gelation. Essential requirement of halides was shown in Fig. 5 where no changes were observed when each halide was omitted from the complete MPO system. In the

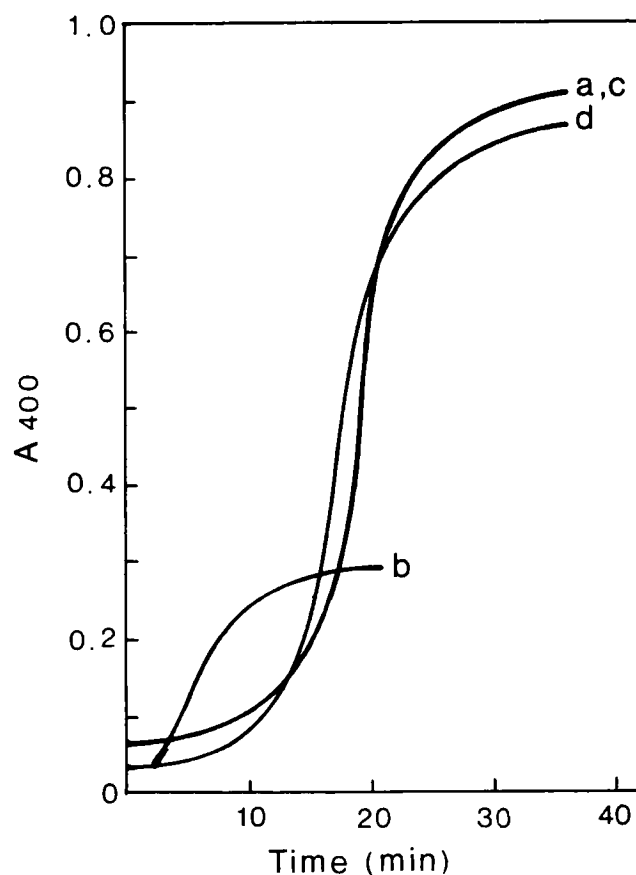


Fig. 3. Effects of catalase and NaN_3 on the inhibition of collagen gelation by the MPO system. Collagen was incubated with 0.053 units MPO, 0.25 mM H_2O_2 , 160 mM NaCl and 100 mM NaH_2PO_4 , pH 6.1 in the presence of $10 \mu\text{g}/\text{ml}$ catalase or 0.5 mM NaN_3 . Other conditions were the same as in Fig. 1. Curve a; no MPO and H_2O_2 , b; no addition, c; catalase and d; NaN_3 .

presence of MPO and Cl^- , H_2O_2 can be substituted by glucose oxidase system (Fig. 4). The degree of alteration in the gelation was dependent on the ability of the substrates to produce H_2O_2 in this enzyme system; among the substrates D-glucose which is a natural substrate was most effective while L-glucose which can not serve as a substrate showed no action. All these findings indicate that the observed alteration of collagen gelation was due to peroxidative action of MPO, H_2O_2 and halides.

3. Comparison of effectiveness of halides as a cofactor

As shown in the previous experiments, MPO-catalyzed peroxidation required halides as a cofactor. Except F^- (data not shown) all halides were shown to be effective.

Effect of Cl^- was shown in Fig. 5. With increasing concentration of this anion up to 250 mM, inhibition of gelation was increased. The potency of

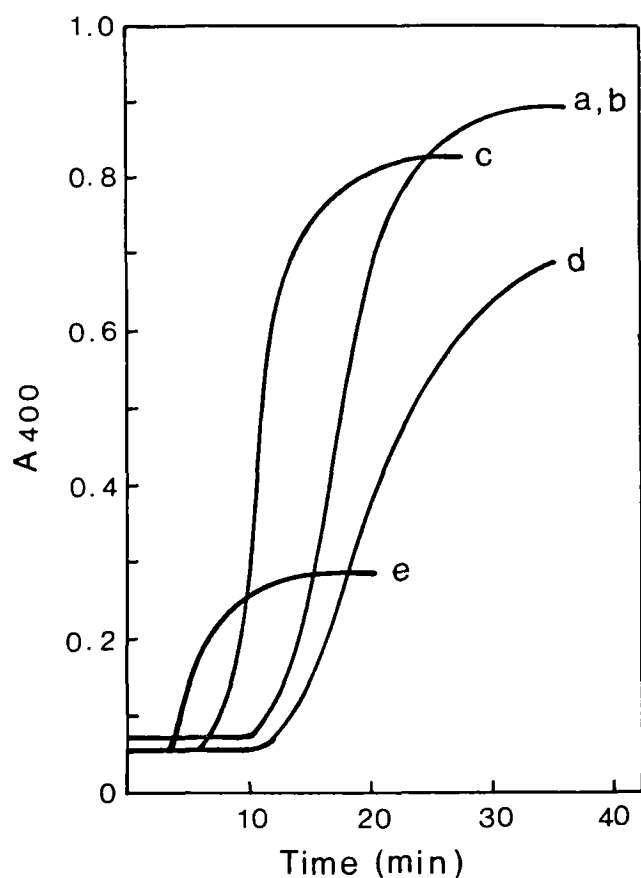


Fig. 4. Effect of glucose oxidase system on the collagen gelation in the MPO system. Collagen (84 μ g OH-proline/ml) was incubated at 4°C for 4 h in 4 ml of reaction mixture containing 0.053 units MPO, 160 mM NaCl, 2.5 μ g glucose oxidase and 100 mM NaH₂PO₄, pH 6.1 in the presence of various substrates. After incubation the reaction mixture transferred to the cuvette was warmed at 37°C to initiate gelation. The generation was monitored by continuous absorbance recording at 400 nm. Curve a; no substrate, b; 0.5 mM L-glucose, c; 0.5 mM deoxyglucose, d; 0.5 mM methylglucose and e; 0.5 mM D-glucose.

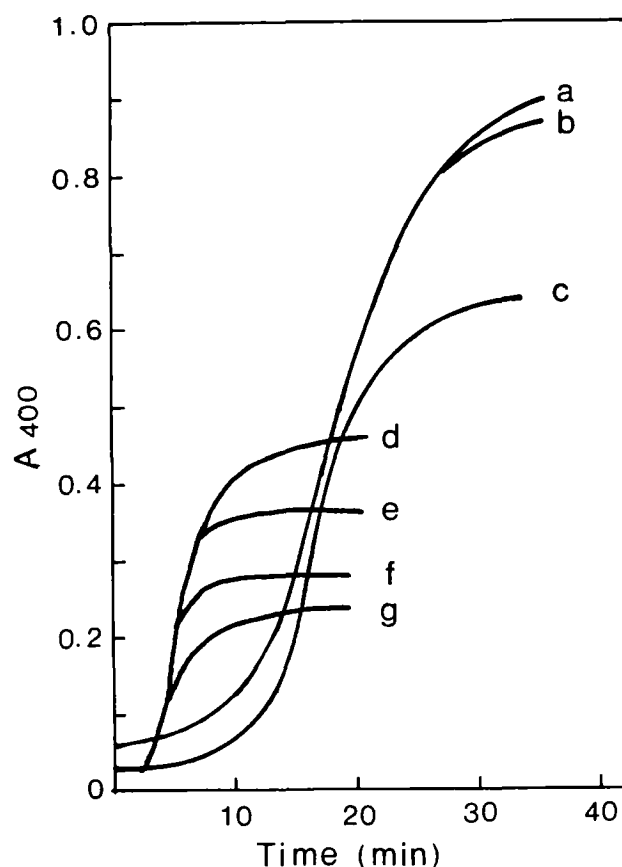


Fig. 5. Effects of chloride concentration in the MPO system on collagen gelation. Collagen was incubated with 0.053 units MPO and 0.25 mM H₂O₂ in the presence of varying concentrations of NaCl. Curve a; no MPO and H₂O₂ and b, c, d, e, f and g; 0, 50, 80, 100, 160 and 250 mM NaCl, respectively. Other conditions were the same as in Fig. 1.

Br⁻ was a little higher than that of Cl⁻ but its effect was levelled off at about 100 mM (Fig. 6). In Fig. 7, effect of iodide was shown. I⁻ was effective at much lower concentration ranges; at 50 mM, its effect attained the maximum.

4. Singlet oxygen involvement in the MPO system

To determine what reactive oxygen species was involved in the inhibition of collagen gelation by the MPO-mediated peroxidation, effects of various scavengers for suggested oxygen species, ¹O₂ or OH· were observed.

In the quencher studies, several OH· scaven-

gers such as benzoate, formate and methanol at 5 mM did not give any significant effect on the altered gelation by the MPO system (Fig. 8) while histidine and DABCO which are known as scavengers for ¹O₂ (Klebanoff *et al.* 1976) were able to restore considerably, but partially the altered gelation to the control level (Fig. 9). The results suggesting the ¹O₂ involvement accord with many reports implying ¹O₂ as a final toxic mediator in the MPO system. But the effects of ¹O₂ scavengers in the present experiment (Fig. 9) was not complete. Because of their own inhibitory effect on the collagen gelation, however, it was difficult to see the effect of scavengers at higher concentrations.

5. Evidence for collagen degradation by MPO system

Of the two observed alterations of collagen gelation, the decrease in maximal turbidity may reflect the degradation of collagen. This interpretation was

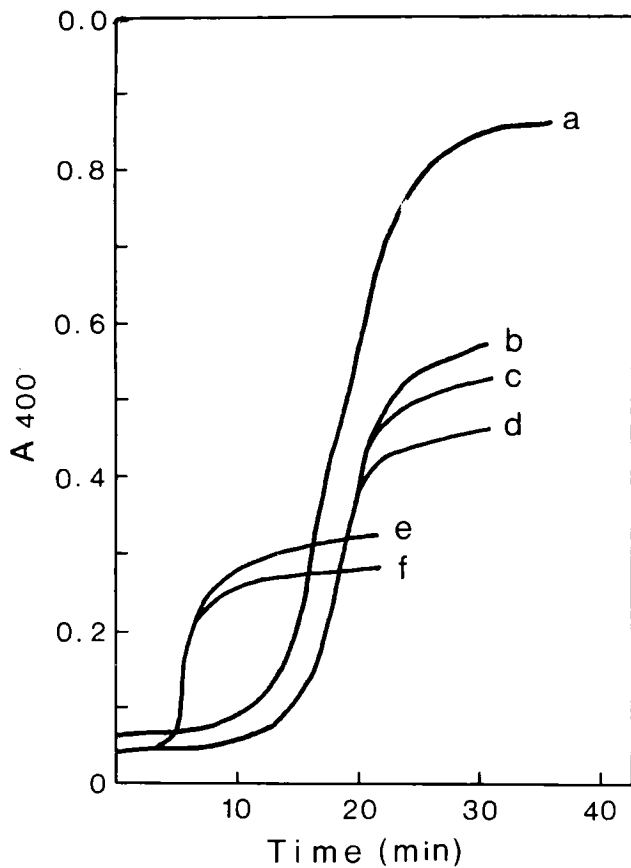


Fig. 6. Effect of bromide concentration in the MPO system on collagen gelation. Collagen was incubated with 0.053 units MPO and 0.25 mM H_2O_2 in the presence of varying concentrations of NaBr. Curve a; no MPO and H_2O_2 , b; 10 mM, c; 25 mM, d; 50 mM, e; 80 mM and f; 100, 160 and 200 mM NaBr. Other conditions were the same as in Fig. 1.

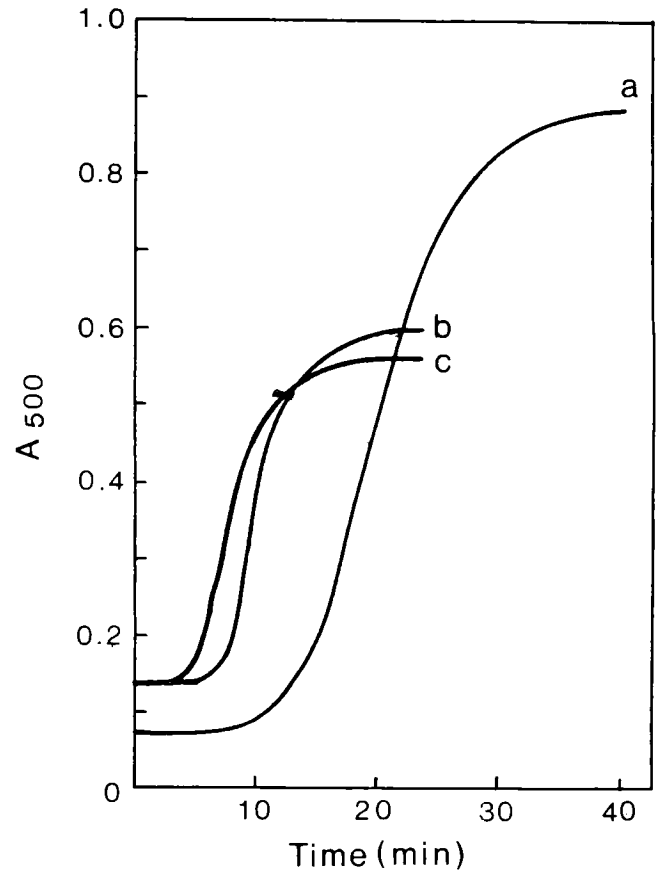


Fig. 7. Effect of iodide concentration in the MPO system on collagen gelation. Collagen was incubated with 0.053 units MPO and 0.25 mM H_2O_2 in the presence of varying concentrations of NaI. Curve a; no MPO and H_2O_2 , b; 50 mM and c; 250 mM NaI. Other conditions were the same as in Fig. 1.

based upon the fact that collagen when treated with collagenase showed the decrease in maximal turbidity with no change of lag phase (Fig. 10). The degree of decrease in the maximal turbidity was increased with increasing dose of collagenase (Fig. 10). In support of this, chromatographic analysis (Sephadex G-200) of the collagen treated with the MPO system showed broadening and tailing the elution profile of OH-proline (Fig. 11). The reason for the shortening of lag phase was not explored. Possibly it may be attributed to destruction of proteoglycans contaminated in the collagen preparation used in this study since proteoglycans were reported to have an effect to delay the initiation of collagen gelation (Oegema *et al.* 1975). In a separate assay, uronic acid was detected from the collagen preparation ($10.3 \mu\text{g}$ uronic acid/ $100 \mu\text{g}$ OH-proline). Uronic acid was determined according to the method of Bitter and Muir (1962).

DISCUSSION

Wood and Keech (1960) showed that as soluble collagen is warmed, it becomes turbid and forms gel where collagen molecules precipitate into fibrils. This thermal gelation is a physico-chemical property of soluble collagen which may represent an *in vitro* model of fibrillogenesis of this structural protein (Woodhead-Galloway 1980) since fibers in the resulting gel demonstrate normal banding as seen *in vivo*. In the nucleation phase of gelation, collagen molecules aggregate with no major change in absorbance, but in the growth phase, there is a sigmoidal increase in absorbance (Fig. 1). Thermal gelation requires structural integrity of collagen because it does not gel properly when the protein was degraded enzymatically (Fig. 10) or its amino acid side chains participating in noncovalent aggregation during nucleation are modified (Venkatasubramanian and Joseph 1977). In the present study,

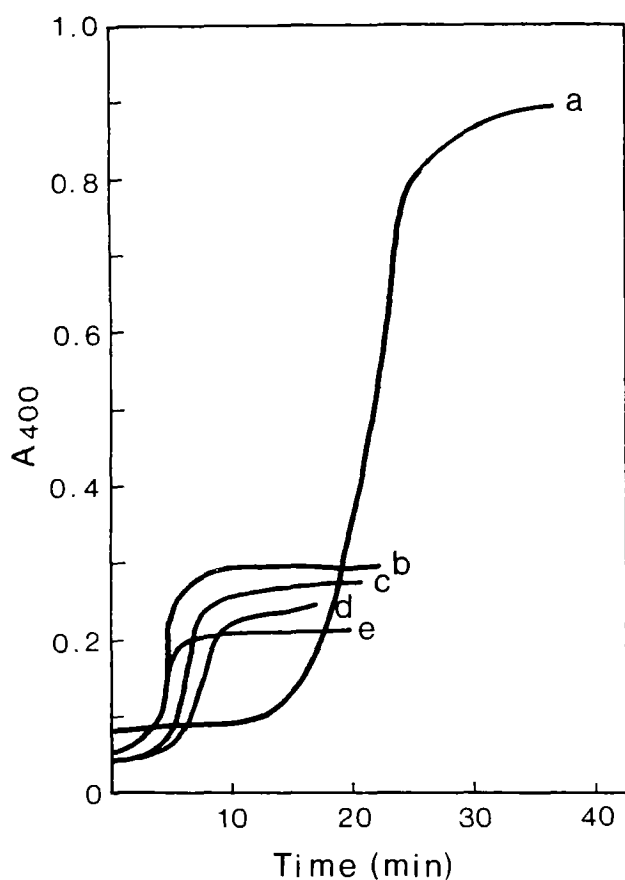


Fig. 8. Effect of hydroxyl radical quenchers on the inhibition of collagen gelation by the MPO system. Collagen gelation was measured in the presence of hydroxyl radical quenchers under the same experimental condition as in Fig. 2. Curve a; no MPO and H₂O₂, b; 5 mM benzoate, c; no addition, d; 5 mM formate and e; 5 mM methanol.

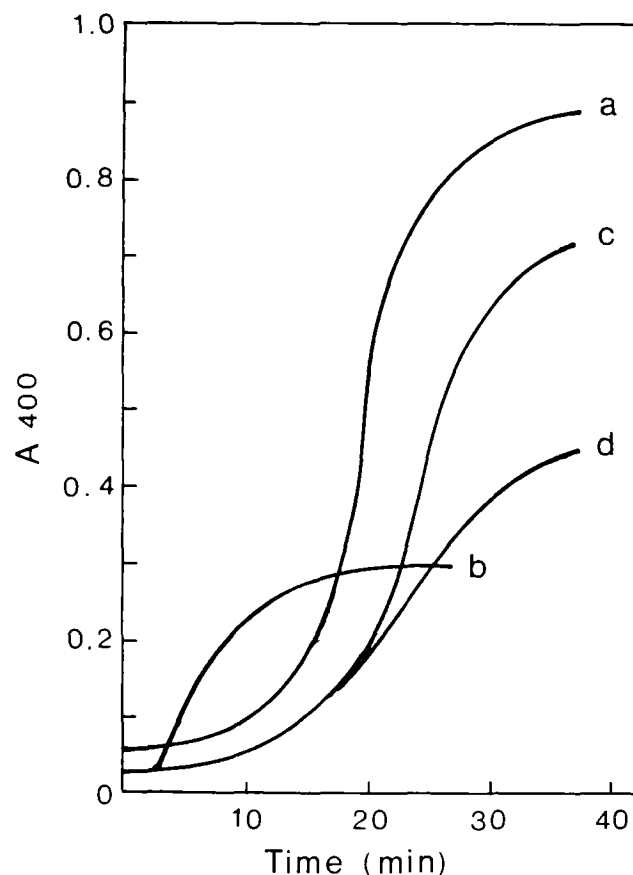


Fig. 9. Effect of singlet oxygen quenchers on the inhibition of collagen gelation. Collagen gelation was measured in the presence of singlet oxygen quenchers under the same experimental condition as in Fig. 2. Curve a; no MPO and H₂O₂, b; no addition, c; 1 mM histidine and d; 5 mM DABCO.

it was observed that collagen when treated with MPO system was markedly affected in its gelation pattern (Fig. 2). It is indicated that the peroxidative action of the system is capable of modifying biochemical properties of this protein, leading to altering the normal behavior of this protein. Degradation will definitely be one of the changes in the structure of collagen molecules that influence the gelation (Fig. 10 and 11).

The MPO preparation from human leukocytes used in the present study was not a pure form but partially purified, which may be contaminated with other granular components including several proteases (Engelbrecht 1982). Therefore, caution should be used before concluding that the observed effect on the gelation in the present experiments has indeed been mediated by the peroxidative action of the MPO system. In other words, the altered gelation was possibly due to the effect of the contaminated proteolytic enzymes. But this

possibility was eliminated by the following experiments: the MPO preparation alone did not show any effect on the gelation (Fig. 2); omission of any of the three components failed to affect the gelation (Fig. 2 and 5) and the altered gelation by the complete system was completely inhibited by either a MPO inhibitor, azide or catalase decomposing H₂O₂ (Fig. 3). Further, the observed effect of the MPO system was also inhibited by histidine and DABCO, scavengers for ¹O₂ which has been reported to be a mediator in the reaction of the MPO system (Allen 1975; Jin *et al.* 1986; Chung and Kim 1984; Krebanoff *et al.* 1976). All these findings clearly support that the alteration of the gelation was due to the peroxidative action carried out by the three components, MPO, H₂O₂ and halides.

All halides used except F⁻ were effective and the potency of the halides as a cofactor was almost similar in the present experiments. But in other studies showing toxicity of the MPO system, I⁻ was much more potent than Cl⁻ and Br⁻ (Klebanoff

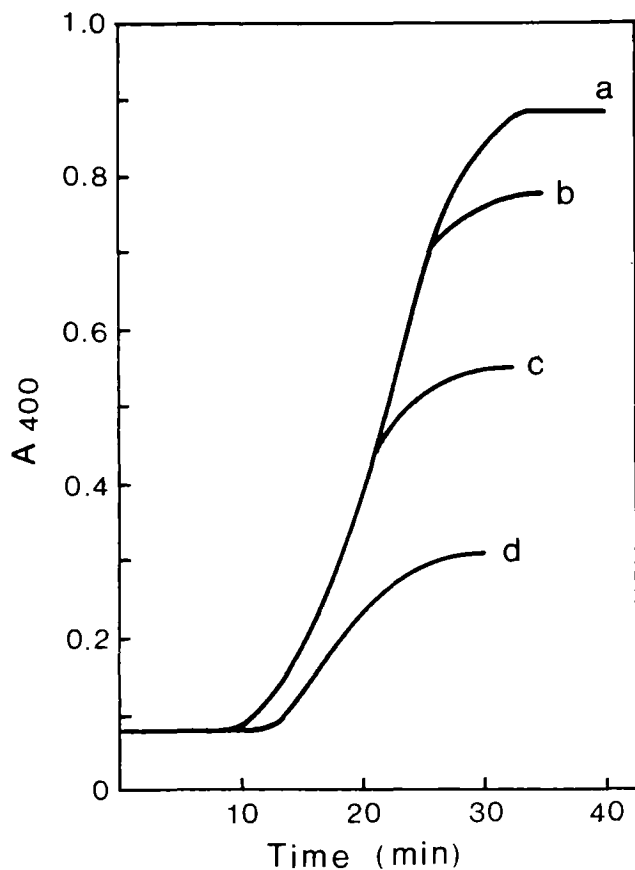


Fig. 10. Gelation of collagenase-treated collagen. Collagen (80 μ g OH-proline/ml) was incubated with varying concentrations of collagenase at 37°C for 5 h in 4 ml medium containing 0.36 mM CaCl₂, 160 mM NaCl and 100 mM Tris-HCl, pH 7.5 and then gelation was started as described in Fig. 1. Curve a; 0 and b, c, and d; 15, 25 and 40 μ g of collagenase, respectively.

1968; Matheson *et al.* 1979). In physiological condition, however, Cl⁻ may be a natural cofactor in the MPO-mediated reaction, since the MPO system was fully active in its concentration ranges in the body fluid (Fig. 5).

The alteration of gelation reflects the changes in structural integrity of this protein but the precise nature of the changes to influence the gelation was not clearly understood in molecular level. Foote (1968), in his study of the action of ¹O₂ on free amino acids, observed that only methionine, tyrosine, histidine, tryptophan and cystine were affected by ¹O₂. Venkatasubramania and Joseph (1977) showed the loss of methionine, tyrosine and histidine in the collagen molecule treated with chemically generated ¹O₂. In view of the evidence for ¹O₂ involvement in the MPO system of the present study and other reports (Allen 1975; Jin *et al.* 1986), those amino acid residues in the collagen can be the susceptible sites that are destroyed by the MPO system. In this study it was further shown that the MPO system caused degradation of the collagen (Fig. 11). Possibly the modification or loss of some of the amino acids may result in the cleavage of collagen.

Based upon the *in vitro* action for the MPO system of degrading collagen, it seems hard to assess its contribution to tissue damage in inflamed sites *in vivo*. But information obtained from PMN functions support the potential roles of the MPO system in the PMN-induced tissue injury. Firstly, as mentioned before, the toxicity of the oxygen products

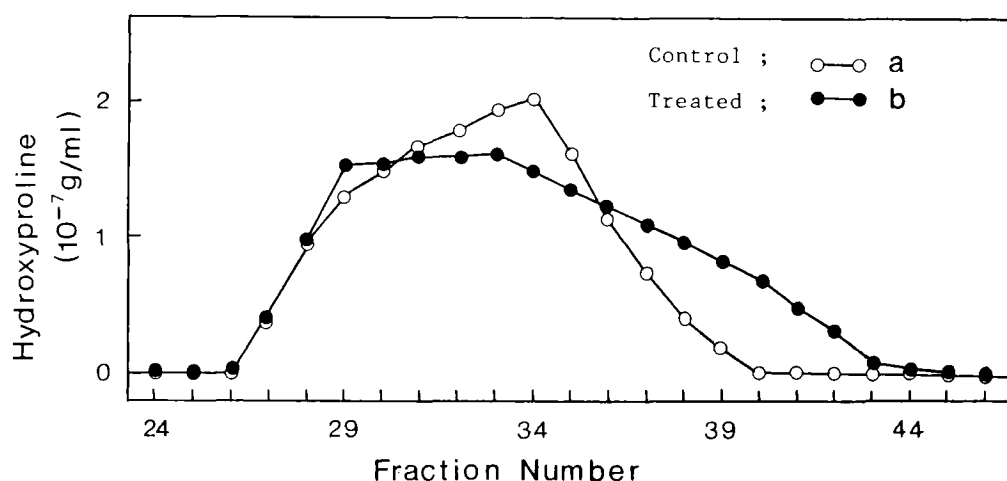


Fig. 11. Gel-permeation chromatographic analysis of collagen treated with the MPO system. Collagen (80 μ g OH-proline/ml) was treated with 0.053 units MPO, 0.25 mM H₂O₂, 160 mM NaCl and 100 mM NaH₂PO₄, pH 6.1 at 4°C for 3 h. After incubation, concentrated acetic acid was added to give final concentration 0.1 M and then 2.5 ml aliquot was added to the Sephadex G-200 column (2.5 x 18 cm) which was equilibrated with 100 mM NaCl and 0.1 M acetic acid. 2.5 ml fractions were assayed for OH-proline. Curve a; untreated and b; treated collagen.

generated from the activated PMNs depends principally on enzymatic action of MPO rather than the iron-catalyzed Haber-Weiss reaction (Clark and Klebanoff 1975; Clark and Szot 1981; Jin *et al.* 1986). Secondly, the MPO system effectively can function outside of the PMNs (Clark 1983). Concentration of MPO is quite high as it accounts for at least 5% of the dry weight of PMNs (Schultz and Kaminker 1962). It was also shown that substantial amount of extracellular MPO can be detected by enzymatic assay when PMNs are subject to the stimuli (Bentwood and Henson 1980; Henson 1971) along with secretion of H_2O_2 (Root *et al.* 1975). Moreover, 80% of $O_2 \cdot$ generated from activated PMNs was found to dismutate to H_2O_2 (Root and Metcalf 1977). Accordingly, H_2O_2 will be a major oxygen product of activated PMNs and thus, be much available as a substrate. Thirdly, Cl^- is a most abundant anion in extracellular fluid and effective as a cofactor within the ranges of its physiological concentration (Fig. 5). This situation is quite different from the condition to which Haber-Weiss reaction is subject, *i.e.*, the low concentration of iron in the body fluid which limits the reaction to proceed *in vivo* (Winterbourn 1981). Thus, at sites of inflammation, MPO and concurrently released H_2O_2 may be localized in concentrations sufficiently enough to cause a substantial effect on extracellular collagen integrity.

Among the inflamed tissues, one in which collagen degradation is of critical importance will be joint cartilage. The destruction of articular cartilage in various arthritis particularly, rheumatoid origin is a basic pathological process that leads to irreversible articular dysfunction (Dingle 1979). As a mechanism of cartilage destruction, collagen degradation by enzymatic processes has been suggested. But if reactive oxygen products are also involved, they may be able to have destructive effects mainly through the MPO-mediated peroxidation.

REFERENCES

- Allen RC. The role of pH in the chemiluminescent response of the myeloperoxidase-halide- $HOOH$ antimicrobial system. *Biochem. Biophys. Res. Comm.* 1975, 63:684-691
- Babior BM. Oxygen-dependent microbial killing by phagocytes. *New Engl. J. Med.* 1978, 298:659-668
- Barrett AJ. The possible role of neutrophil proteinases in damage to articular cartilage. *Agents Actions* 1978, 8:11-18
- Baugh RJ, Travis J. Human leukocyte granule elastase: rapid isolation and characterization. *Biochem.* 1976, 15:836-841
- Bentwood BJ, Henson PM. The sequential release of granule constituents from human neutrophils. *J. Immunol.* 1980, 124:855-862
- Bitter T, Muir HM. A modified uronic acid carbazole reaction. *Analytical Biochem.* 1962, 4:330-334
- Clark RA. Extracellular effects of the myeloperoxidase-hydrogen peroxide-halide system in *Advances in Inflammation Research*, G. Weissmann, Ed. Raven Press, 1983, pp. 107-146
- Clark RA, Klebanoff S. Neutrophil-mediated tumor cell cytotoxicity: role of the peroxidase system. *J. Exp. Med.* 1975, 141:1442-1447
- Clark RA, Klebanoff SJ. Myeloperoxidase- H_2O_2 -halide system: cytotoxic effect on human blood leukocytes. *Blood* 1977, 50:65-70
- Chung MH, Kesner L, Chan PC. Degradation of articular cartilage by copper and hydrogen peroxide. *Agents Actions* 1984, 15:328-335
- Chung MH, Kim MS, Lee CS. Effect of Cu^{++} -catalyzed peroxidation on collagen gelation. *Korean J. Pharmacol.* 1983, 19:35-44
- Chung MH, Kim YS. Evidence for singlet oxygen involvement in cell-free myeloperoxidase/ H_2O_2 /Chloride system: exclusion of hydroxyl radical involvement. *Korean J. Pharmacol.* 1984, 20:1-11
- Clark RA, Stone PJ, Hag AE, Calore JD, Franzblau C. Myeloperoxidase-catalyzed inactivation of α_1 -protease inhibitor by human neutrophils. *J. Biol. Chem.* 1981, 256:3348-3353
- Clark RA, Szot S. The myeloperoxidase-hydrogen peroxide-halide system as effector of neutrophil-mediated tumor cell cytotoxicity. *J. Immunol.* 1981, 126:1295-1301
- Diamond RD, Clark RA, Handenschild CC. Damage to *Candida Albicans* hyphae and pseudohyphae by the myeloperoxidase system and oxidative products of neutrophil metabolism *in vitro*. *J. Clin. Invest.* 1980, 66:908-917
- Dingle JT. Recent studies on the control of joint damage: the contribution of Strangeways Research Laboratory. *Ann. Rheum. Dis.* 1979, 38:201-214
- Engelbrecht S, Pieper E, McCartney HW, Rautenberg W, Wenzel HR, Tschesche H. Separation of the human leukocyte enzymes: alanine aminopeptidase, cathepsin G, collagenase, elastase and myeloperoxidase. *Hoppe-Selyer's Z. Physiol. Chem.* 1982, 363:305-315
- Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am. J. Pathol.* 1982, 107:397-418
- Foot CS. Mechanism of photosensitized oxidation: there are several different types of photosensitized oxidation which may be important in biological systems.

- Science 1968, 162:963-970
- Fridovich I.** The biology of oxygen radicals. *Science* 1978, 201:875-880
- Goldstein IM, Roos D, Kaplan HB, Weissman G.** Complement and immunoglobulins stimulate superoxide production by human leukocyte, independently of phagocytosis. *J. Clin. Invest.* 1975, 56:1155-1163
- Greenwald RA, Moy WW.** Inhibition of collagen gelation by action of the superoxide radical. *Arthritis and Rheumatism* 1979, 22:251-259
- Greenwald RA, Moy WW.** Effect of oxygen-derived free radicals on hyaluronic acid. *Arthritis and Rheumatism* 1980, 23:455-463
- Greenwald RA, Moy WW, Lazarus D.** Degradation of cartilage proteoglycans and collagen by superoxide radical (abstract). *Arthritis and Rheumatism* 1976, 9:799
- Henson PM.** The immunologic release of constituents from neutrophil leukocytes. I. The role of antibody and complement on nonphagocytosable surfaces or phagocytosable particles. *J. Immunol.* 1971, 107:1535-1546
- Jin CG, Chung MH, Yoon JS, Park CW.** The myeloperoxidase-hydrogen peroxide-halide system as an effector of polymorphonuclear leukocytes-mediated inactivation of isocitrate dehydrogenase. *Environ. Mutagens & Carcinogens* 1986, 6:35-48
- Ignarro LJ.** Regulation of lysosomal enzyme secretion: role in inflammation. *Agents and Actions* 1974, 4: 241-258
- Kellogg III. EW, Fridovich, I.** Liposome oxidation and erythrocyte lysis by enzymatically generated superoxide and hydrogen peroxide. *J. Biol. Chem.* 1977, 252: 6721-6728
- Klebanoff S.** Myeloperoxidase-halide-hydrogen peroxide antibacterial system. *J. Bacteriol.* 1968, 95:2131-2138
- Klebanoff SJ.** Oxygen metabolism and the toxic properties of phagocytes. *Ann. Int. Med.* 1980, 93:480-489
- Klebanoff SJ, Clark RA, Rosen H.** Myeloperoxidase-mediated cytotoxicity. *Cancer Enzymology* 1976, 12:267-285
- Lee JS, Chung MH, Lim JK, Park CW, Cha IJ.** Role of ascorbic acid in the depolymerization of hyaluronic acid by Fe^{++} and H_2O_2 . *Korean J. Pharmacol.* 1985, 21:62-67
- Matheson NR, Travis J.** Isolation and properties of human neutrophil myeloperoxidase. *Biochem.* 1981, 20:325-330
- Matheson NR, Wong PS, Travis J.** Enzymatic inactivation of human alpha-1-proteinase inhibitor by neutrophil myeloperoxidase. *Biochem. Biophys. Res. Comm.* 1979, 88:402-409
- McCord JM.** Free radical and inflammation: protection of synovial fluid by superoxide dismutase. *Science* 1974, 185:529-531
- Oegema TR, Laidlaw J, Hascall VC, Dziewiatkowski DD.** The effect of proteoglycans on the formation of fibrils from collagen solutions. *Arch. Biochem. Biophys.* 1975, 170:698-709
- Root RK, Metcalf JA.** H_2O_2 release from human granulocytes during phagocytosis: relationship to superoxide anion formation and cellular catabolism of H_2O_2 : studies with normal and cytochalasin B treated cells. *J. Clin. Invest.* 1977, 60:1266-1279
- Root RK, Metcalf J, Oshino N, Chance B.** H_2O_2 release from human granulocytes during phagocytosis. I. Documentation, quantitation and some regulatory factors. *J. Clin. Invest.* 1975, 55:945-955
- Schultz J, Kaminker K.** Myeloperoxidase of the leukocyte of normal human blood. I. Content and localization. *Arch. Biochem. Biophys.* 1962, 96:465-467
- Venkatasubramanian K, Joseph KT.** Action of singlet oxygen on collagen. *Indian J. Biochem. Biophys.* 1977, 14:217-220
- Weissmann G.** Mediators of tissue damage in rheumatoid arthritis: phagocytes as secretory organs of rheumatoid inflammation. *Triangle* 1979, 18:45-52
- Weissmann G, Smolen JE, Korchak HM.** Release of inflammatory mediators from stimulated neutrophils. *New Engl. J. Med.* 1980, 303:27-34
- Winderbourn CC.** Hydroxyl radical production in body fluid: roles of metal ions, ascorbate and superoxide. *Biochem. J.* 1981, 198:125-131
- Woessner JF.** The determination of hydroxy proline in tissue and protein samples containing small proportions of this imino acid. *Arch. Biochem. Biophys.* 1961, 93:440-447
- Wood GC, Keech MK.** The formation of fibrils from collagen solutions. The effect of experimental conditions: Kinetic and electron microscopic studies. *Biochem. J.* 1960, 75:588-597
- Woodhead-Galloway J.** Collagen: the Anatomy of a Protein, Edward Arnold Ltd., London 1980, pp. 18-19

= 국문초록 =

Myeloperoxidase/H₂O₂/Halide 산화계에 의한 용해성 Collagen의 열성 Gel 형성에 미치는 영향

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Myeloperoxidase (MPO)/H₂O₂/Halide 산화계(이하 MPO 산화계)가 collagen 구조에 변성을 초래할 수 있는지의 여부를 알아보기 위하여 이 단백질의 특성인 열성 gel형성 (thermal gelation)에 이 산화계가 미치는 영향을 관찰하였다. MPO는 인체 다형핵 백혈구에서, collagen은 흰쥐 피부에서 추출하여 사용하였다.

Collagen을 MPO, H₂O₂ 및 각종 halide로 구성된 MPO 산화계로 처리하였을 때, F⁻ 이외의 halide를 포함하는 산화계에 의해서 37°C에서 일어나는 gel형성이 현저히 억제되었다. MPO 산화계에서 상기 세 요소중 어느 한가지를 빼거나, 이 산화계에 MPO 억제제인 azide 혹은 catalase를 첨가하면, 이 산화계에 의한 gel 형성 억제효과는 완전히 소실되었다. 또한 이 억제현상은 ¹O₂ 제거제인 histidine 혹은 diazobicyclo(2,2,2)octane에 의해서도 상당히 감소되었다. collagen을 collagenase로 처리 하였을 때 gel형성이 일어나지 아니하였고, MPO 산화계로 처리된 collagen이 gel-permeation chromatography 상에서, 분해된 양상이 관찰된 결과로 미루어, gel 형성 억제를 초래하는 MPO 산화계에 의한 collagen 구조의 변화중의 하나가 이 단백질의 분해임을 알 수 있었다.

Collagen의 열성 gel형성은 이 단백질의 구조적 완전성을 요구한다는 점을 감안할 때 MPO/H₂O₂/Halide 산화계는 확실히 collagen 구조에 변성을 초래함을 알 수 있었다. 이와 같은 MPO 산화계의 작용이 염증에서 백혈구에 의한 조직 손상의 요인이 될 수 있다는 가능성에 대하여 고찰하였다.