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Concentration-Dependent Paradoxical Actions of Myeloperoxidase for DNA : Cleavage and Shielding of DNA

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Novel functions of intranuclear myeloperoxidase (MPO), cleaving and shielding of DNA, have been proposed in this study. We investigated MPO-DNA interaction by using agarose or polyacrylamide gel electrophoresis, and firstly found paradoxical duplex effects of MPO on DNA molecules. Nucleotide sequence analysis and footprinting results showed that the cleaving and shielding of DNA by MPO is significantly dependent upon the concentration of the enzyme. At the high MPO concentration, intranuclear MPO protects DNA damage from oxygen radicals produced during myeloid cell maturation and its function is due to DNA binding ability of the enzyme. On the contrary, the considerable decline of MPO concentration clearly induces the DNA cleavage by the enzyme. When the cells can't satisfactorily function as immunocyte under the condition of decreasing MPO concentration, therefore, MPO may actively lead to the death of cells

KEY WORDS: Myeloperoxidase/ DNA cleavage/ DNA binding/

INTRODUCTION

Myeloperoxidase (MPO; EC 1.11.1.7) localizes in azurophil granules cytoplasmic structures of phagocytic cells. The enzyme plays an important role in the oxygen- and halogen-dependent microbicidal system of leucocytes.[1] During phagocytosis, MPO promotes peroxidation of Cl^- to HOCl at the expense of H_2O_2 formed by the membrane-bound NAD(P)H oxidase[2-4] during the respiratory burst. The product can cause rapid oxidative degradation of a wide variety of biological substances.[5] Recently, it has been reported that the nuclei of different cell types such as human granulocytes and myeloid leukemia involve MPO.[6,7] Huberman's group has proposed that intranuclear MPO may help to protect DNA against damage resulting from oxygen radicals produced during myeloid cell maturation and function.[7] There is great interest in the relation between intranuclear and intragranular functions of the enzyme.

In this study, MPO-DNA interaction was investigated by using agarose or polyacrylamide gel electrophoresis, and we found that the enzyme significantly contributes to not only protecting of DNA but also cleavage of DNA, depending upon the concentration of MPO. Biological implication for these phenomena has been considered on the basis of the experimental results.

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EXPERIMENTAL PROCEDURES

Enzymes and Chemicals. Myeloperoxidase, prepared from normal human leukocytes as described previously,[8] was offered by Dr. H. Iwamoto (Kyoto University). Methidiumpropyl-EDTA (MPE) was a kind gift of Prof. Peter B. Dervan (California Institute of Technology). SalI and DraII restriction endonucleases were obtained from Takara Shuzo (Kyoto, Japan). All other chemicals used were of commercial reagent grade.

DNA Cleavage Reaction. The reactions were carried out by incubating a mixture (total volume, 20 μl) of pBR322 DNA (20 μg/ml) and MPO in a solution of 50 mM sodium phosphate buffer (pH 7.5) at 37°C for 20 min. The cleavage reactions were initiated by addition of NADH (1 mM) and H_2O_2 (3 mM), and allowed to proceed at 37°C for 60 min. The reactions were terminated by the addition of cold ethanol (60 μl) and 3M sodium acetate (2 μl) and then the samples were immediately chilled at −70°C. Each lyophilized sample was dissolved in 20 μl of loading buffer containing 0.05% bromophenol blue and 10% glycerol, and heated at 75°C for 1 min before electrophoresis. The electrophoresis was performed by using 1% agarose gel containing ethidium bromide (0.5 μg/ml).

Gel Mobility Shift Assay. Mobility shift assay was carried out by combining the 5'-end labeled 128-base pairs DNA fragment (pBR322 SalI-DraII DNA), sonicated calf thymus DNA (20 μg/ml), and MPO in a solution of 4 mM Tris-HCl (pH 7.5), 12 mM Hepes-NaOH (pH 7.5), 60 mM KCl, 0.5 mM EDTA, 0.5 mM dithiothreitol and 12% glycerol, and then the mixture (total volume, 10 μl) was incubated at 37°C for 20 min. Protein-DNA complexes were resolved from free DNA by electrophoresis on 4% polyacrylamide gel with running buffer containing 90 mM Tris-borate (pH 8.0) and 2.5 mM EDTA at 250 V.

Nucleotide Sequence Analysis and MPE-Fe(II) Footprinting. Nucleotide sequence reactions were carried out by combining the 5'-end labeled 128-base pairs DNA fragment (pBR322 SalI-DraII DNA), sonicated calf thymus DNA (20 μg/ml), and MPO in a solution of 50 mM sodium phosphate buffer (pH 7.5), and then the mixture (total volume, 20 μl) was incubated at 37°C for 20 min. The reactions were initiated by addition of NADH (5 mM) and H_2O_2 (10 mM), and allowed to proceed at 37°C for 120 min. Cold ethanol was added to the sample solutions to stop the reaction. The MPE-Fe(II) footprinting reactions were performed by combining a mixture (total volume, 20 μl) of the 5'-end labeled 128-base pairs DNA fragment (pBR322 SalI-DraII DNA), sonicated calf thymus DNA (20 μg/ml), and MPO in a solution of 20 mM Tris-HCl buffer (pH 7.5), and then by incubating at 37°C for 20 min. The reactions were started by addition of MPE-Fe(II) reagent (20 μM) and dithiothreitol (2 mM), and then allowed to proceed at 37°C for 10 min. In order to stop the reaction, cold ethanol was added to the sample solutions. Electrophoresis was performed in a 10% polyacrylamide/7 M urea slab gel at 2000 V for 2 hr. DNA sequencing (G+A) was carried out by the Maxam-Gilbert method.[9] The amount of radioactivity of each samples subjected to electrophoresis was approximately 10,000 cpm.

RESULTS AND DISCUSSION

Figure 1 shows typical gel electrophoretic patterns of covalently closed-circular (form I) pBR 322 DNA treated with the MPO-NADH-H_2O_2 system (lanes 2–7). In compared with control (lane 1), the MPO-NADH-H_2O_2 system significantly caused single- and double-strand breaks of DNA to form nicked circular (form II) and linear (form III) duplex. In lanes 6 and 7, despite careful experiment, a part of DNA seems to be lost due to physical adsorption by excess amount of the protein during ethanol precipitation step. Table 1 summarizes the quantitative relationship between MPO concentration and DNA cleaving activity. The DNA cleavage activity was estimated by microdensitometer. Of special interest is different duplicate effect of MPO on DNA, namely the induction of DNA breakage

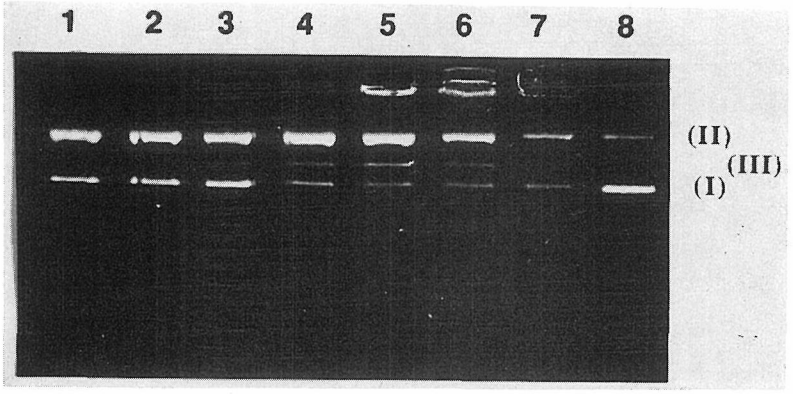

Fig. 1. Agarose (1%) gel electrophoretic patterns of ethidium bromide stained pBR 322 DNA after treatment with the MPO-NADH-H_2O_2 system. The samples contained the following concentration of MPO: lane 1, none; lane 2, 1 nM; lane 3, 10 nM; lane 4, 50 nM; lane 5, 100 nM; lane 6, 200 nM; and lane 7, 300 nM. Lane 8 shows intact DNA alone. The positions of forms I-III DNAs were indicated.

Table 1. Dependency of MPO concentration for DNA cleavage by MPO-NADH-H_2O_2 system

MPO concentration (nM)	DNA cleavage activity (%)		
	form I	form II	form III
blank (intact DNA)	79.1	20.9	0.0
0	31.8	67.1	1.1
1	32.1	66.5	1.4
10	37.2	58.6	4.2
50	14.8	77.2	7.9
100	9.9	78.0	12.1
200	20.0	73.2	6.8
300	38.1	57.9	4.0

at low concentration of MPO and the depression of DNA strand scission at high concentration of the enzyme. Typical inhibitors of MPO, sodium azide and deferoxamine,[10] strongly suppressed the DNA cleavage reaction by MPO. Evidently, fully desalted sample of the enzyme possessed reproducible DNA breakage activity (data not shown).

In order to confirm MPO interaction with DNA, we performed mobility shift DNA binding assay using gel electrophoresis. Figure 2 shows the dependency of MPO concentration for DNA binding of MPO. The shielding of DNA by MPO was evidently observed at high concentration (300 nM) of MPO. The result is correspond with the following DNA footprinting result for MPO by using MPE.

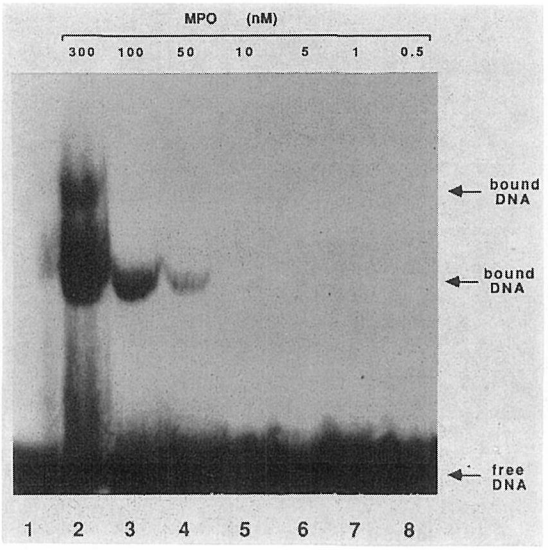

Fig. 2. Autoradiograms of DNA binding of MPO by gel mobility shift assay. The samples with radioactive DNA fragment (128-bp) contained the following concentration of MPO: lane 1, none; lane 2, 300 nM; lane 3, 100 nM; lane 4, 50 nM; lane 5, 10 nM; lane 6, 5 nM; lane 7, 1 nM; and lane 8, 0.5 nM. The arrows indicate protein-DNA complex or free DNA.

Figure 3 demonstrates the nucleotide-sequence cleavage modes on the DNA fragment (128-base pairs) by MPO-NADH-H_2O_2 system (lanes 6&7), together with the DNA footprinting results for MPO by using MPE (lanes 3-5). The result also shows that the inhibition of DNA strand scission occurs under the condition of high MPO concentration, and that MPO shields the DNA molecules from the DNA cleaving reagent (lane 4). At the same concentration of the enzyme in which DNA was strongly cleaved (lane 5), the clear shielding of DNA molecules by MPO was observed in contrast with MPE control (lane 3). Similar inhibition of the DNA cleavage induced by MPE was not observed in the presence (300 nM) of another protein such as serum albumin. The ability of any protein to induce

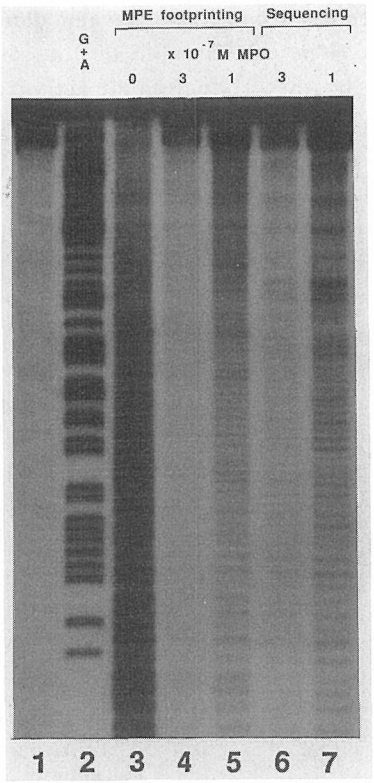

Fig. 3. Autoradiograms of a 10% polyacrylamide gel for the cleavage reactions by MPO-NADH-H_2O_2 system (lanes 6&7) and for the MPE-Fe(II) footprint of MPO (lanes 3–5). The samples contained the following concentration of MPO: lane 3, none; lanes 4&6, 300 nM; and lanes 5&7, 100 nM. Lane 1 shows intact DNA alone. Lane 2 exhibits DNA sequencing (G+A) by the Maxam-Gilbert method.

a footprinting depends on the strength of interaction and slowness of protein-DNA dissociation.

Herein, we firstly demonstrate important paradoxical function of MPO on DNA: the cleaving activity for DNA and the protection from DNA damage. Probably, the DNA shielding is attributable to DNA binding properties of the basic protein MPO, and the DNA breakage to active oxygen species produced by MPO enzyme system. It is of special importance to note that the paradoxical effect results from imbalance depend upon the concentration of MPO. Indeed, the present data indicate that at high concentration of MPO the enzyme shields DNA by its binding without active oxygen production from MPO enzyme system. Two distinct papers have been reported: MPO is present in the nuclei of human monomyelocytic cells[7] and MPO activity is not shown in the nucleus of human monocytes.[11] These observations appear to support our hypothesis: the different duplex actions of MPO depend upon the enzyme concentration, and MPO may

usually protect DNA molecules from active oxygen damage via shielding of DNA in the nucleus of the cells. The considerable decline of MPO concentration, may lead to the DNA cleavage by the production of active oxygen species. When the cells can't satisfactorily act as immunocyte by decreasing of MPO concentration, that is, the enzyme may positively induce the death of cells by DNA cleaving damage.

Many T cells bearing self-reactive receptors are selectively deleted during thymus development.[12] The phenomenon is very important for organisms to maintain its immunological mechanism. Several groups have reported that T cell receptor plays significant roles to lead to programmed cell death.[12–15] Furthermore, MacDonald et al. have described that self-reactive population *in vitro* includes at least some cells with a mature ($CD4^+CD8^-$) surface phenotype, and they proposed the mechanism of death—i.e., contact with self-antigen, influx of extracellular Ca^{2+}, and activation of endonuclease.[15] In phagocytic cells which actively play cell functions through immense oxygen metabolism, on the other hand, the decrease of intranuclear MPO concentration would elevate a probability of DNA mutation in nuclei. Under the condition of high probability of DNA mutation, one important role of the enzyme may be to guide actively these cells to death by its endonuclease-like activities. This difference of mechanisms of cell death appears to be associated with the evolution level of these cells as immunocytes.

Our present finding clearly indicates the concentration-dependent paradoxical action of MPO for DNA. The protection of DNA damage and the induction of cell death by direct shielding and cleaving of DNA respectively, seem to be biologically significant as the functions of intranuclear MPO.

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