[Short Communication]

Gel-electrophoretic characterization of DNA damaged by oxidation with hydrogen peroxide and iron ions

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INTRODUCTION

In aerobic organisms, cellular metabolism generates reactive oxygen species, i. e. superoxide radicals, hydrogen peroxide, singlet oxygen and hydroxyl radicals.^{1,2)} Recent experiments indicate that these reactive oxygen species cause damages or modifications of biological molecules and implicated in the etiology of many human diseases including cancer.³⁻⁵⁾ The formation of hydroxyl radicals is due to participation of hydrogen peroxide with trace metals like copper or iron.^{5,6)} They bring about chemical base modifications, single- and double-strand breaks and conformational changes in DNA.⁷⁻¹⁰⁾ The strand breaks in DNA have been conventionally detected mainly by alkaline sucrose-gradient centrifugation.^{5,11)} However, gel electrophoretic analysis may offer a reliable simple means for characterization of damaged DNA. In the present studies, therefore, agarose-gel electrophoretic analyses were made for authentic DNA which was *in vitro* damaged by oxidation with hydrogen peroxide and iron ions.

MATERIALS AND METHODS

DNA and reagents: Bacteriophage λ DNA and λ DNA / *Hind* III fragments were purchased from Takara-shuzo Co. Agarose was from Bio-Rad. Other chemical reagents were from Wako Chemical Co. The solutions were prepared freshly before use

in each experiment.

Application of oxidative damages to DNA: Assay tubes containing the mixture of 3.4 μ g λ DNA, 5 mM hydrogen peroxide, 0.5 to 1 mM ferrous ammonium sulfate or ferric chloride, and 0.5 mM EDTA in a final volume of 20 μ l of 5 mM Tris-HCl buffer at pH 8.0 were incubated at room temperature (20-25°C) for 60 minutes. λ DNA was also incubated with hydrogen peroxide and with ferrous or ferric ions separately. After incubation, glycerol and bromophenol blue were added to the mixture, which was immediately subjected to the electrophoretic analysis.

Electrophoretic analysis: The submarine agarose-gel electrophoresis apparatus model AE-6100, ATTO Co. was used. Electrophoresis was conducted by the standard methods with agarose-gel of 0.7% in concentration and 3 mm in thickness in Tris-acetic acid-EDTA buffer at pH 8.3 and at 60 V for 90 minutes. λ DNA/*Hind* III fragments were used as DNA size markers. The patterns were detected by ethidium bromide staining, followed by UV illumination at 302 nm.

RESULTS AND DISCUSSION

The results of the electrophoretic analyses are shown in Fig. 1. The λ DNA specimens incubated separately with hydrogen peroxide at 5 mM showed a single band which corresponded to that of untreated λ DNA. The specimens incubated separately with

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Fig. 1. Gel-electrophoretic patterns of λ DNA specimens incubated with hydrogen peroxide and ferrous ions.

Lane 1; Untreated λ DNA, Lane 2; λ DNA incubated with 5 mM hydrogen peroxide, Lane 3; λ DNA incubated with 0.5 mM ferrous ions, Lane 4; λ DNA incubated with 1mM ferrous ions, Lane 5; λ DNA incubated with 5 mM hydrogen peroxide and 0.5 mM ferrous ions, Lane 6; λ DNA incubated with 5 mM hydrogen peroxide and 1 mM ferrous ions, Lane 7; λ DNA/*Hind* III fragments (Six fragments are of 23130, 9416, 6557, 4361, 2322 and 2027 base pairs, respectively).

ferrous ions at 0.5 and at 1 mM showed the characteristic electrophoretic patterns tailing and remaining a part of the component at the original position. This is possibly caused by the interaction of DNA with ferrous ions, which might mask the electric charge of DNA. These findings indicate that neither hydrogen peroxide nor ferrous ions bring about strand breaks in DNA solely.

A remarkable change in the electrophoretic pattern was detected in DNA specimens incubated with both hydrogen peroxide and ferrous ions. As shown in Fig. 1, the pattern had no distinct band and appeared as a smear zone of DNA fragments. As compared with the electrophoretic mobilities of DNA/*Hind* III fragments (DNA size markers), the length of the fragments seemed to range from about

Fig. 2. Gel-electrophoretic patterns of λ DNA incubated with hydrogen peroxide and ferric ions.

Lane 1; λ DNA incubated with 0.5 mM ferric ions, Lane 2; λ DNA incubated with 1mM ferric ions, Lane 3; λ DNA incubated 5 mM hydrogen peroxide and 0.5 mM ferric ions, Lane 4; λ DNA incubated with 5 mM hydrogen peroxide and 1 mM ferric ions, Lane 5; λ DNA/*Hind* III fragments (the same as in Fig. 1).

20,000 to 2,000 base pairs.

DNA specimen was also incubated with hydrogen peroxide and ferric ions. It was found that the electrophoretic pattern of λ DNA after the incubation showed almost the same change as λ DNA incubated with hydrogen peroxide with ferrous ions (Fig. 2). In λ DNA specimens incubated with hydrogen peroxide at 5 mM and ferric ions at 3 mM for 120 minutes, no distinct band was observed on the electrophoretic pattern.

It has been documented that the combination of ferrous or ferric ion with hydrogen peroxide is a good system for hydroxyl radical generation.¹⁰⁻¹²⁾ Accordingly, the changes in the electrophoretic pattern described above indicate that hydroxyl radicals cause λ DNA double-strand breaks and generate

varying lengths of fragments ranging approximately one half to one twentieth of the original molecules.

It has been shown that when cells are exposed to oxidative stress, DNA damage occurs.^{5,7,10} The damage includes various oxidative base modifications, strand breaks, and consequent conformational changes in DNA. These changes, however, have not been fully characterized by electrophoretic analyses. To our knowledge, no electrophoretic pattern has been presented for DNA exposed to hydroxyl radicals. It has been emphasized that the molecular mechanism causing strand breaks of DNA in cells includes the activation of cellular nuclease or direct reactions of hydroxyl radicals with DNA.² The concentration of hydrogen peroxide in the present *in vitro* experiments is noticed to be extremely higher than that of *in vivo* concentrations. Studies *in vivo* are wanted essentially for deeper understanding on the damages in DNA which are caused by hydroxyl radicals in aerobic organisms.

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