Iron(II)-mimosine Catalyzed Cleavage of DNA

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Supercoiled plasmid DNA was treated *in vitro* with H_2O_2, DTT and either Fe (II), Fe (II)-EDTA or Fe (II)-mimosine. The rate of DNA break formation was followed by the conversion of the supercoiled form into relaxed-circular and linear forms. In the concentration interval of 0−4 μM Fe (II), Fe (II)-EDTA slowed-down the formation of DNA breaks, while Fe (II)-mimosine enhanced the rate of break formation up to several times. A conclusion is drawn that this enhancement is due to the increased affinity of the Fe (II)-mimosine complex to DNA.

Introduction

Mimosine, β-[N-(3-hydroxy-4-pyridone)]-α-aminopropionic acid, is a toxic nonprotein amino acid, derived from *Mimosa* and *Leucaena* plants. The chemical structure of mimosine is based on ketohydroxy metal-chelating site on a pyridine ring with an amino acid side-chain substituent, which indicates that a part of the toxicity of mimosine may be a result of metal complexing. It is widely used for synchronization of mammalian cells at the G1/S phase boundary of the cell cycle. That is why a considerable effort has been made to elucidate its mechanism of action. It has been reported that mimosine reversibly inhibits DNA synthesis and arrests the cells at checkpoints in G1, S, and G2/M phases (Kalejta and Hamlin, 1997; Tsvetkov *et al.*, 1997; Alpan and Pardee, 1996; Krude, 1999; Ji *et al.*, 1997). Cells treated with mimosine undergo changes in their chromatin organization (Vogt, 1991). It has clastogenic effect and can cause apoptosis (Jha *et al.*, 1995). These data imply that treatment of mammalian cells with mimosine induces chromosomal damage. The effects of mimosine on DNA synthesis and cell cycle progression are similar to those of ionizing radiation (Tsvetkov *et al.*, 1997; Mikhailov *et al.*, 2000). γ-Radiation causes a plethora of damages in DNA, most prominent of them being DNA breaks, which were identified as the primary signal for its biological effect (Lallev *et al.*, 1993; Kunnev *et al.*, 1997; Goodhead, 1994). The introduction of DNA breaks is independent of the metabolic activity of DNA and is a result of reactive oxygen species produced by radiolysis of water.

In the present communication we have explored the possibility that mimosine generates breaks in DNA on the basis of its ability to form complexes with transition metals thus causing oxidative damage of DNA by Fenton-like reactions.

Materials and Methods

Isolation and electrophoresis of DNA

Plasmid DNA – pBlueskript II (KS+) – was isolated by the alkaline lysis method (Sambrook *et al.*, 1989). DNA concentration was determined by reading the optical density at 260 nm. Agarose gel electrophoresis was performed in 1% agarose in 0.1 M Tris-acetic acid, 0.4 mM EDTA, pH 8 and the gels were stained with ethidium bromide.

Cleavage of DNA

The experiment was carried out with 200 ng pBlueskript II (KS+) DNA. The reaction buffer contained 10 mM Tris-HCI, pH 7.4 and 50 mM NaCI. A solution of 5 mM mimosine or EDTA and 5 mM metal ion was prepared and diluted to the desired concentration immediately before use. Dithiothreitol (DTT) and H_2O_2 were always added last to final concentration 0.5 mM and 2 mM, respectively. The final volume of the reactions was 10 μl. The reactions were carried out for 60 min at room temperature and immediately analyzed by agarose gel electrophoresis. The gels were visualized on a UVP's video capturing system GDS 7600, and analyzed by Gel Pro Analyzer version 3 software for Windows, Media Cybernetics, L. P.

Results and Discussion

By using alkaline single cell gel electrophoresis and nucleoid sedimentation analysis we have

shown that mimosine introduces breaks into DNA *in vivo*. The break formation was not connected with the process of DNA synthesis, since DNA breaks accumulated in quiescent, not stimulated human peripheral blood lymphocytes that represent 100% G0/G1 cell population (Mikhailov *et al.*, 2000). This indicated that the cleavage of DNA might be a result of either direct chemical attack or mimosine-assisted chemical attack on DNA. To check whether mimosine directly attacked the DNA molecules, supercoiled plasmid was incubated with mimosine for 1 hour and subjected to agarose gel electrophoresis. The results showed that mimosine alone did not inflict any damage on DNA *in vitro* since there were no differences in the electrophoretic mobility of the supercoiled plasmid. Thus, the possibility remained that the break formation was due to an mimosine-assisted chemical attack on DNA.

Attack by reactive oxygen species (ROS) is considered as a major source of spontaneous damage to DNA. 1% or more of the molecular oxygen passing through the respiratory chain in mitochondria may be released and undergo consecutive univalent reductions to water producing the intermediate superoxide radicals and hydrogen peroxide. They do not react directly with DNA but take part in the Fenton reactions with transition metal ions as catalyst to produce the very reactive hydroxyl radical, which cleaves DNA. That is why we decided to check whether mimosine would influence the Fe (II)/H_2O_2 driven cleavage of DNA *in vitro*. To this end, supercoiled plasmid was incubated in a buffer containing H_2O_2, DTT and EDTA or mimosine and different concentrations of Fe (II). Cleavage efficiency was determined by

following the conversion of the supercoiled plasmid (form I) to relaxed-circular form (form II) and linear form (form III) and was expressed in arbitrary units as the ratio between the total amount of the plasmid and the amount of the supercoiled form (Table I). When this arbitrary cleavage capability was plotted against Fe (II) concentration, clear differences showed up between the rate of break formation by Fe (II)-EDTA, Fe (II)-mimosine and Fe (II) alone (Fig. 1). Fe (II) did not show any significant cleavage capability in concentrations up to 1 µM. After this threshold value, it begins to cleave the plasmid efficiently and at 4 µM concentration only about 14% remained supercoiled. On the other hand, Fe (II)–EDTA did not cleave significantly the supercoiled plasmid in the concentration range 0–4 µM Fe (II)–EDTA and even at 4 µM concentration between 55% and 60% remained supercoiled. Finally, Fe (II)-mimosine, cleaved the supercoiled plasmid much more efficiently than Fe-EDTA and even more efficiently than Fe (II) alone.
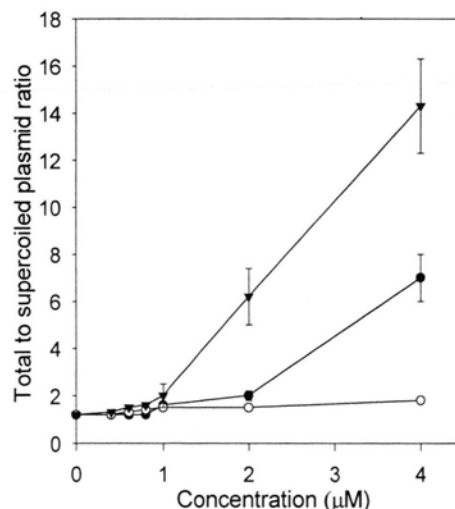

Fig. 1. DNA cutting capacity *in vitro* of Fe (II)-EDTA (○), Fe (II) (●) and Fe (II)-mimosine (▼). pBlueskript II (KS+) DNA was treated with increasing concentrations of Fe (II), Fe (II)-EDTA and Fe (II)-mimosine in the presence of H_2O_2 and DTT at room temperature for 60 min. DNA was run on 1% agarose gel, stained with ethidium bromide and analyzed. The ratio of the total plasmid to supercoiled plasmid was used to express the DNA cutting capacity of the agents as a function of Fe (II) concentration. Figures are means of three independent experiments and vertical bars show standard deviations from the mean.

Table I. Percentage of the supercoiled form of the plasmid after treatment with different concentrations of Fe (II), Fe (II)-EDTA and Fe (II)-mimosine for 1 hour. Figures are means of three independent experiments. Standard deviations of the means are shown.

Fe (II) concentration (µM)	Fe (II)	Fe (II)-EDTA	Fe (II)-minosine
0	85±4	85±4	85±4
0.4	83±6	77±7	77±5
0.6	82±7	72±5	73±5
0.8	80±5	72±5	66±6
1.0	62±4	67±6	34±5
2.0	50±5	66±5	17±3
4.0	14±2	56±6	7±2

The experiments described here show that mimosine can enhance the cleavage reaction of DNA by Fe (II) and oxidative species *in vitro* and suggest that this could be the mechanism of mimosine-assisted DNA cleavage of DNA *in vivo*. One possible explanation of the enhanced cleavage of DNA by mimosine could be that mimosine is strong iron chelating agent and due to the presence of pyridine ring in the mimosine molecule,

the mimosine-Fe (II) complex has greater affinity towards DNA than EDTA-Fe (II) and the Fe ions. This would bring about higher free radical concentration and hence higher cutting capacity in the vicinity of DNA.

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