Effect of N^+ beam exposure on the activities of Mn-SOD and catalase in Deinococcus radiodurans

SONG Dao-Jun*, CHEN Ruo-Lei, WU Li-Fang, LI Hong, YAO Jianming, SHAO Chun-Lin, WU Li-Jun, YU Zeng-Liang

(*Center of Fengle Ion Beam Bioengineering, Institute of Plasma Physics, the Chinese Academy of Sciences, Hefei 230031*)

Abstract Though the radiation-resistant bacteria Deinococcus radiodurans (D. radiodurans) have a high resistance to the lethal and mutagenic effects of many DNA-damaging agents, the mechanisms involved in the response of these bacteria to oxidative stress are poorly understood. In this report, the superoxide dismutase (SOD) and catalase (CAT) activities produced in bacteria (D.radiodurans AS1.633) and their change caused by 20 keV N^+ beam exposure were examined. Results showed that the activities of the enzymes were increased in the case of N^+ beam exposure from 8×10^{14} ions/cm^2 to 6×10^{15} ions/cm^2. In addition, the treatment of H_2O_2 and [$CHCl_3$+CH_3CH_2OH] and the measurement of absorption spectrum showed that the increase of whole SOD activity resulted from inducible activities of Mn-SOD in (a sub-type) D.radiodurans AS1.633. These results suggested that these bacteria possess inducible defense mechanisms against the deleterious effects of oxidization.

Keywords N^+ beam exposure, Deinococcus radiodurans AS1.633, Superoxide dismutase, Catalase

CLC numbers Q813.5, Q814, Q691.5

1 INTRODUCTION

Bacteria Deinococcus, formerly a part of Micrococcus[1], are the types of a small number of exceptionally radiation-resistant, red-pigmented cocci considered to comprise one of the eight major groups of the enbacteria. These bacteria share high resistance to both the lethal and mutagenic effects of ionizing and ultraviolet (UV) radiation. Most of the studies on the radiation resistant mechanisms of D.radiodurans mainly focused on D.radiodurans R1[2~5]. Extensive investigation on radiation-sensitive mutants has found that the unusually high radiation resistance of D.radiodurans is attributed to their efficient DNA repair system[6,7]. Two mechanisms for the repair of damaged DNA by ionizing and UV light radiation in D.radiodurans have been described: excision repair and recombination repair[8,9]. Other protective mechanisms may also be involved. Since many effects of ionizing radiation are oxygen-enhanced[10], reactive factors, including hydrogen peroxide and oxygen free radicals, they play an important role in radiation toxicity.

Supported by the National Natural Science Foundation of China (No. 19605005)

Manuscript received date: 2000-05-07 *E-mail: zlyu@mail.ipp.ac.cn or cdhu@mail.ipp.ac.cn

Some reduced molecules, such as superoxide radicals (O_2^-), H_2O_2 and hydroxyl radicals (•OH), are produced during normal cellular respiration[10], and can also be generated by ionizing radiation[11~14]. Biological damage caused by them includes oxidization of membrane fatty acids (resulting in lipid peroxidation), oxidization of proteins, and DNA damage[10]. D.radiodurans have developed defense mechanisms to prevent and repair the oxidative damages. Enzymatic defenses, including SOD, CAT and peroxidase, exert an important effect on the cell's defensive repertoire against reactive oxygen species[15]. It possesses relatively high levels of SOD and CAT activities[16]. However, Antioxidant enzyme responses to ion beam exposure in these bacteria are as yet poorly understood.

As we know, study of the interaction between low energy ions and solid material was started about thirty years ago. Since then science of ion implantation materials has been quickly developed. However, the biological effect of low energy ion exposure was ignored for a long time because of its very low energy. Until the middle of 1980's, the biological effect of ion beam exposure on seeds was observed in our laboratory[17]. program for rice of mutation breeding by low energy ions beam then was performed in Institute of Plasma Physics, in collaboration with China Institute of Rice. Through exploring the mutagenic effect for years, it has been found that this approach can induce higher mutation rate and wider mutational spectrum with higher survival rate for crop breeding. Since then the ion implantation, as a new source for mutation, has been widely used in improving crops in agriculture. Up to now, there have been more than one hundred groups taking part in this project in China[18]. In 1989, Yu Z. L. observed that the cellular walls could be etched by ion beam, and suggested studying the action of low energy ion on cells and tried to find its application in bioengineering. As a result, Gus gene and CAT gene were successfully transferred into the intact cells and ripe embryo of rice etched by ion beam, and the expression of the foreign genes were detected in these samples[19]. In the middle of 1990's, Yu Z. L. and his collaborators measured the mass deposition effect of slowed-down implantation ions. For example, after nitrogen ions of 30 keV were implanted into CH_3COONa, a new chemical group cyano-group ($-C\equiv N$) was detected out. Furthermore, a number of amino groups ($-NH_2$) were formed when the irradiated samples were dissolved in water[20].

In fact, the action mechanism of ion beam exposure in some areas is different from that of traditional irradiation. For example, implanted ions in cells cause energy exchanging, ions sputtering and slowed-down ions depositing. However, the biological effect of low energy ions was only recognized for more than ten years. It is obvious that the basic mechanisms of exposure effects have been poorly understood. Therefore, it is necessary to study the biological effects induced by low energy ions. In this study, we examined the adaptive response of D.radiodurans to N^+ beam exposure, and found that it could stimulate the increase of activities of SOD and CAT, and Mn-SOD of D.radiodurans AS1.633.

2 MATERIALAS AND METHODS

2.1 Strains, media, and growth condition

D.radiodurans AS1.633 were obtained from Institute of Microbiology, the Chinese Academy of Sciences. They were grown at 32°C with shaking (200r/min) in TGY medium[18], which consisted of 10 g of tryptone, 5 g of yeast extracts, and 1.0 g of glucose per litre. Exponentially proliferating cultures were used for all experiments.

2.2 N^+ implantation and incubation

N^+ beam exposure was performed in the following way: cells harvested were suspended in 50 mmol phosphate buffer (pH7.0) containing 0.1 mmol/L $MgSO_4$, and were washed three times with phosphate buffer and re-suspended in the same buffer. 50μL suspension (approx. 3×10^8CFU/mL) of bacteria, which had been cultured in the exponential growth phase for about 18 h, were spread on four glass plates (2cm×3cm). Then the plates were put into vacuum (10^{-5} Pa) target chamber (designed for studying the survival rate of the living cells), and implanted by 20 keV N^+ beam after dry in air (approx. 5 min). The Nitrogen ions were produced by the ASIPP's ion beam bioengineering instrument, and the pulse implantation technique was used with pulse time of 5 s and interval time of 30 s. The beam of each pulse to the sample was 10^{14} ions/cm^2, and the sample in chamber of vacuum without ion implantation was used as control.

Immediately after implantation, the samples in the presence or absence of 100 μg/mL chloramphenicol were propagated at 32°C with shaking at 200 r/min for 18 hr.

2.3 Preparation of cell extracts

Cells harvested were washed twice with 50mM phosphate buffer (pH7.0) and suspended in the same buffer containing 150μM/mL chloramphenicol (to prevent added protein synthesis). Cell suspensions were then sonicated (using JY-92II sonicator) at 4°C (30 s pulse on, 20 s pulse off) for a total of 20 min on ice. The homogenate was clarified by centrifugation (6,000 g, 4°C, 30 min), the sediment was discarded and the cell extracts solution was stored at 4°C for use.

2.4 Enzyme activity assays

The SOD activity was assayed by the method of Beauchamp and Fridovich[21]. In this method, photochemically-generated superoxide radicals react with nitroblue tetrazolium (NBT), which produce insoluble purple-blue formazan. Since SOD can compete with NBT in scavenging superoxide radicals, and inhibit the colour formation, this reaction can be used to quantitative SOD activity in a sample. The reaction mixture contained 56 μmol/L NBT, 10 mmol/L methionine, 1.2 μmol/L riboflavin, 20 μ,mol/L sodium cyanide, 0.1 mmol/L EDTA, 50 mmol/L potassium phosphate (pH 7.8) in a total volume of 5.0 mL. The reaction mixture was illuminated at 26°C with a 40 W fluorescent

lamp; the colour formation due to NBT reduction was quantitated by determination of A_{560}. Inhibition of this reaction by SOD in a sample was measured by plotting A_{560} against the quantity of the sample. Under the experiment conditions, 1 U of SOD activity was defined as the amount causing 50% inhibition of the rate of NBT reduction. Catalase was determined by measuring the decomposition rate of hydrogen peroxide at 240 nm[21]. 1 U is defined as 1 μmol of H_2O_2 hydrolyzed/min.

2.5 Distinction of SOD isoenzymes types

Different types SOD iso-enzymes can be inhibited by different inhibitors, and CuZe-SOD activity is inhibited in the presence of H_2O_2, Mn-SOD activity is inhibited by $CHCl_3$+CH_3CH_2OH, (1:1.5 V/V), and Fe-SOD by H_2O_2 or ($CHCl_3$+CH_3CH_2OH), so they can be identified[23]. In this test, the CuZe-SOD, Mn-SOD and Fe-SOD were distinguished from SOD iso-enzymes by adding 20 mmol/L H_2O_2 and ($CHCl_3$+CH_3CH_2OH) containing 0.1 mmol/L EDTA and 50mmol/L potassium phosphate (pH7.8) in cell extracts solution for reaction 60 min[22], respectively.

3 RESULTS

3.1 Effect of N^+ beam exposure on the activities of SOD and CAT in D. radiodurans AS1.633

The activity curves of SOD and CAT of D.radiodurans AS1.633 was showed in Fig.1. Their activities kept at a relatively stable level when the N^+ beam exposure dose was less than 8×10^{14}ions/cm^2. They increased gradually and reached maximum within the dose range from 8×10^{14} ions/cm^2 to 6×10^{15} ions/cm^2(SOD) and 5×10^{15} ions/cm^2(CAT), then decreased when doses were more. Generally, when dose was less than 7×10^{15} ions/cm^2, activities of the two enzymes were improved by N^+ beam exposure as compared with control.

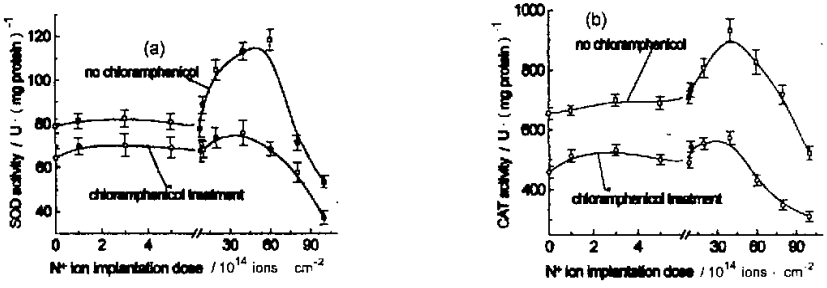

Fig.1 Effect of N^+ beam exposure on SOD and CAT activities of D.radiodurans AS1.633

Compared with cells that hadn't been implanted (control), N^+ beam exposure in absence of chloramphenical induced the activity increase of SOD and CAT in exponentially

growing D.radioduransAS1.633. However, cells treated in the presence of chloramphenical increase slightly these enzyme activities within the dose range from 8×10^{14} ions/cm^2 to 6×10^{15} ions/cm^2. This result indicated that de novo protein synthesis was required for adaptation of D.radiodurans to oxidative stress by N^+ beam.

3.2 Effect of H_2O_2 addition on SOD activity of Deinococcus radiodurans

After H_2O_2 treatment, the change trend of SOD activity (see Fig.2a) was similar to that in Fig.1. However, when exposure dose was less than 8×10^{14} ions/cm^2, SOD activity was inhibited seriously by H_2O_2, that was about one-sixth as many as control. In this examination, CuZn-SOD and Fe-SOD lost their most activities, and Mn-SOD kept its more than 90% activity in the presence of 20 mmol/LH_2O_2. In the light of the different responses of the different types of SOD iso-enzymes to H_2O_2[21,22], we could deduced that N^+ beam exposure induce increase activity of Mn-SOD, and this confirmed that increase activity of SOD in D.radiodurans resulted from inducement of N^+ beam exposure.

3.3 Effect of ($CHCl_3$+CH_3H_2OH) treatment on SOD activity of D. radiodurans

After the cellular extracts solution was treated with ($CHCl_3$+CH_3CH_2OH), SOD activity was inhibited substantially and its decrease was more serious when exposure dose was more than 8×10^{14} ions/cm^2. Residual SOD activity was showed in Fig.2b. In this study, SOD activity was inhibited fully by the treatment of [$CHCl_3$+CH_3CH_2OH], and more seriously when exposure dose was more than 8×10^{14} ions/cm^2. According to the principle, which Mn-SOD and Fe-SOD lost their activities in the order of Mn-SOD>Fe-SOD, and CuZn-SOD was affected by ($CHCl_3$+CH_3CH_2OH) little, different types of SOD iso-enzymes against [$CHCl_3$+CH_3CH_2OH] were different[21,22], we drew a conclusion that ions beam exposure could stimulate and induced increase of Mn-SOD activity of D.radiodurans AS1.633.

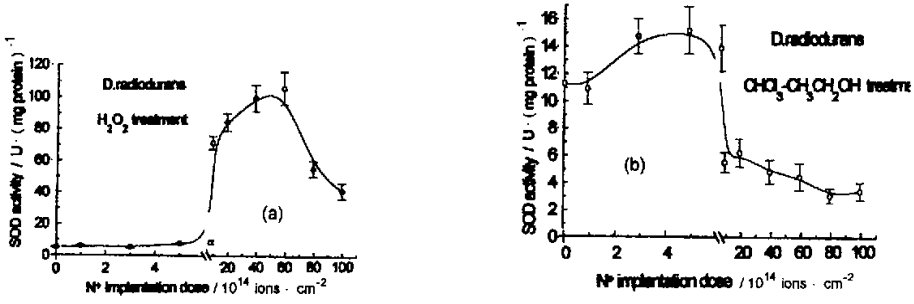

Fig.2 Effect of H_2O_2 (a) and [$CHCl_3$+CH_3CH_2OH] (b) on the activities of SOD iso-enzymes of D.radiodurans AS1.633

3.4 The Vis-UV absorption spetrum of SOD treated with H_2O_2

The Vis-UV absorption spectrum of SOD extract solution treated with 20 mmol/L H_2O_2 was showed in Fig.3. This absorption spectrum confirmed that ions beam exposure induced the increase of Mn-SOD activity. The two absorption peaks of Fig.3 were the characteristic absorption of Mn-SOD in 475nm vis-light and 280 nm UV-light[23]. The reason for this is that the high content of Tyrosine of Mn-SOD led to its maximum absorption at 280 nm UV, and Mn^{3+} was the main reason for the maximum absorption at 475 nm Vis[24].

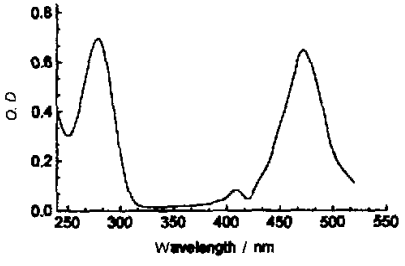

Fig.3 The absorption spectrum of SOD treated with H_2O_2

4 DISCUSSION

SOD, CAT and peroxidase(POD) may play an important part in the defense against oxidative stress, and they were called protective enzymes. Our results indicate that D.radiodurans AS1.633 responds to oxidative stress of ion beam exposure by inducing increase of protective enzyme activity, especially Mn-SOD activity.

Some investigations have showed that Mn-SOD plays a more important role in radiation-resistance than Fe-SOD and CuZn-SOD[25,26]. These findings were of importance to understand mechanisms involved in the response of D.radiodurans AS1.633 to oxidative stress of ion beam exposure. It was reported that energy deposition, momentum transfer, mass deposition and electric charge neutralization and exchange characterized the action of low energy ion to organisms. Therefore, the response of antioxidant enzyme to ion beam exposure has close relation to the four action types mentioned above, we conclude that the effect of mass deposition and electric charge was responsible for the increase of Mn-SOD and CAT activities.

Acknowledgement

The authors would like to acknowledge Wang J, Wang X, for their help. We are also grateful to Dr Han J for valuable discussion and Yu Li-Xiang and Liu Xin-Hai for help in the experiment of ion implantation.

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