IDENTIFICATION OF A MULTIPROTEIN DNA METABOLIC COMPLEX FROM A RADIORESISTANT BACTERIUM DEINOCOCCUS RADIODURANS

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A b s t r a c t

Deinococcus radiodurans R1 (DEIRA) a member of Deinococcae family can survive ~12 kGy dose of γ radiation which can generate about 200 double strands and 3000 single strand breaks per genome. An efficient DNA strand break repair, contributes maximum to the radioresistance of Deinococcus radiodurans. Distinction in DNA strand break repair from others seems to lie in the mechanism of action of DNA metabolic proteins, in DNA catabolic and anabolic processes, involved in DNA strand break repair. Here, we report the identification of a multiprotein complex comprised of 15 proteins, from Deinococcus radiodurans. The complex showed DNA ends processing and end joining activities. Biochemical and immunological characterization showed the presence of several DNA repair proteins including PprA, DNA polymerase and DNA ligase. Complex lacked RecA but has three uncharacterized phosphoproteins. The results suggest the presence of various DNA metabolic proteins required for DNA repair functions, in the form of an active multiprotein complex and the regulation of DNA degradation function by high energy phosphate.

Introduction

Deinococcus radiodurans R1 can withstand the lethal and mutagenic effects of DNA damaging agents, including ionizing radiation, far-UV and desiccation (1, 2). Although the biochemical and physiological basis of Deinococci’s extreme radiotolerance has not been adequately explained, it is clear that irradiated cells are not passively protected from damaging effects of the incident radiation instead; they suffer massive DNA damage following irradiation (3). The comparison of its genome sequences with E. coli, a radiosensitive bacterium and transcriptome analysis of cells recovering from ionizing radiation stress have indicated that, the functional modulation of proteins involved in DNA recombination/repair would be more important than
mere presence, in regulating the extraordinary stress tolerance of this bacterium (4,5). To understand how these proteins function in vivo, the possible existence of different repair proteins in a multiprotein complex form and the functionality of these proteins in a functional module, were studied.

From the cell free extract of *Deinococcus radiodurans* R1, the multiprotein complexes were isolated on molecular sieve column chromatography and characterized on the basis of DNA metabolic activities. A fraction consisting of nearly 15 different size proteins, showed some interesting DNA metabolic activities such as metal independent topoisomerase, an uncharacterized ATP sensitive ends processing nuclease, DNA polymerase and ATP stimulated DNA ligase activities. The multiprotein complex nature of this particular fraction was ascertained, using chemical cross-linking, gradient centrifugation and repeated filtration through gel filtration columns. Immunoblotting experiments showed the presence of PprA (suspected homologue of Ku proteins), single stranded DNA binding protein, protein kinase activity and absence of RecA. Existence of several DNA metabolic proteins together in multiprotein complex would make them to work with greater efficiency and would further explain the mechanism of efficient DSB repair in this super bug.

**Methodology**

Molecular sieve column chromatography of Sephacryl S-300 (Pharmacia, Sweden) was performed, to isolate the multiprotein complexes from the total cell free extract of the *Deinococcus radiodurans*. Protein contents of the different fractions were measured at 280nm. Six different peak fractions were obtained based on their protein content. These peak fractions when analyzed on the SDS-PAGE, showed different patterns of proteins profile (Fig. 1A), indicating the distinct fractionation of all the proteins based on their molecular size. Activity screening of peak fractions showed the presence of metal-independent DNA relaxation of supercoiled plasmid DNA by peak II fraction (Fig. 1B). In the presence of Mg$^{2+}$, these fractions showed either reckless DNA degradation irrespective of DNA topology or no activity on DNA substrate (data not shown). This indicated that peak II fraction (hereafter referred to as Peak-II) contains topoisomerase (s) type proteins which have shown the important enzymes in DNA repair function, along with other proteins.

**Results and Discussion**

**Fractionation of the total cellular protein of Deinococcus radiodurans**

Total cellular proteins of the *Deinococcus* were extracted from the stationary phase cells and fractionated through the Sephacryl S 300 Molecular sieve column chromatography. All the fractions obtained were measured for their protein content at 280nm. Six different peak fractions were obtained based on their protein content. These peak fractions when analyzed on the SDS-PAGE, showed different patterns of proteins profile (Fig. 1A), indicating the distinct fractionation of all the proteins based on their molecular size. Activity screening of peak fractions showed the presence of metal-independent DNA relaxation of supercoiled plasmid DNA by peak II fraction (Fig. 1B). In the presence of Mg$^{2+}$, these fractions showed either reckless DNA degradation irrespective of DNA topology or no activity on DNA substrate (data not shown). This indicated that peak II fraction (hereafter referred to as Peak-II) contains topoisomerase (s) type proteins which have shown the important enzymes in DNA repair function, along with other proteins.

**Proteins of peak II fraction exit in a multiprotein complex form**

The coexistence of all the proteins, in the peak II fraction was ascertained, by glutaraldehyde cross-linking (Fig. 2A),
Fig. 1: Size fractionation and metal independent DNA metabolic activity assay of soluble proteins isolated from stationary phase cells of Deinococcus radiodurans R1. (A) SDS-PAGE analysis of peak fractions (I-V) of Sephacryl S-300 column chromatography. (B) Covalently closed circular plasmid DNA was incubated with different peak fraction in absence of divalent metal ions and product were analyzed on 1% agarose gel.

Fig. 2: Peak II proteins characterization for their presence of these proteins in complex form. Peak II proteins were treated with glutaraldehyde (A), refiltered through second Sephacryl S-300 (B) and sucrose density gradient centrifugation (C) and analyzed on SDS-PAGE.
Protein complex exhibits DNA end processing and ligase activities

In the presence of divalent metal ions, the complex showed nuclease activity. This activity was qualitatively higher with Mn\(^{2+}\) than equimolar concentration of Mg\(^{2+}\) ion (data not shown). Further nuclease activity was distinguished for its exo- and/or endo- nature on linear DNA substrate labelled on both 3' (Fig. 3A) and 5' (Fig. 3B) ends, separately. Results indicated that protein complex removes 5' radiolabel much faster than label at 3' end of double stranded DNA (Fig 3B), suggesting that complex has 5'→3' end processing activity which is an important function in recombination repair of DNA double strand breaks. The importance of 3'→5' recombinogenic ends in DSB repair and radiation tolerance phenotype of Deinococcus has been demonstrated (17). Multiprotein complex showed DNA end joining activity in presence of Mg\(^{2+}\) and exogenously added ATP (Fig. 3C). This suggested the association of DNA end processing and ends joining activity in multiprotein complex.
Multiprotein complex contains known DNA repair proteins

In addition to various DNA metabolic activities integral to DNA strand break repair, the complex also showed the presence of SSB, PprA and phosphoproteins while showing lack of RecA. Among the proteins present in complex, SSB is known as a DNA recombination repair protein (18) while PprA, a unique protein in Deinococcus that plays an important role in DNA damage repair function (19).

Thus this study suggested that Deinococcus radiodurans, a well known extraordinary radiation resistant organism, outperforms other organisms in DNA strand break repair, possibly by allowing the strong interaction of several DNA repair proteins together in a functional module.

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References

About the Authors

Ms Swathi Kota, M.Sc. Biochemistry from IARI, New Delhi, joined Molecular Biology Division, BARC after graduating from 46th batch of Biology-Radiobiology BARC Training School batch in 2002. Since then she has been actively involved in understanding the molecular mechanism underlying the extreme radioresistance of Deinococcus radiodurans. She has been a recipient of Homi Bhabha Meritorious Award for OCES-46th batch (Bioscience Group).

Dr Hari S Misra joined Bhabha Atomic Research Centre in 1990. Since then he has been working on molecular genetics of bacterial response to abiotic stress and the development of insect resistant transgenic plants. Dr Misra’s group is currently working on the elucidation of molecular mechanisms underlying the radiation response to biological system by employing the advanced molecular biology tools. His group reports for the first time, the involvement of a signal transduction component in DNA strand break repair in bacteria. Dr. Misra is the recipient of INS Medal 2004 and is an elected Fellow of the Maharashtra Academy of Sciences, 2003.