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The SbcCD complex of *Deinococcus radiodurans* contributes to radioresistance and DNA strand break repair *in vivo* and exhibits Mre11–Rad50 type activity in vitro

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ABSTRACT

Deinococcus radiodurans lacks a homologue of the *recB* and *recC* genes, and the *sbcA/B* genes, of *Escherichia coli*. Thus, DNA strand break repair in *Deinococcus* proceeds by pathways that do not utilize these proteins. Unlike *E. coli*, the absence of *recBC* and *sbcA/sbcB*, and presence of only *sbcC* and *sbcD* in *Deinococcus*, indicates an enigmatic role of SbcCD in this bacterium. Studies on *sbcCD* mutation in *Deinococcus* showed nearly a 100-fold increase in gamma radiation sensitivity as compared to wild type. The mutant showed a higher rate of *in vivo* DNA degradation during the post-irradiation recovery period that corresponds to the RecA-dependent DSB repair phase. These cells showed a typical NotI pattern of DNA reassembly during the early phase of DSB repair, but were defective for the subsequent RecA-dependent phase II of DSB repair. Hydrogen peroxide had no effect on cell survival of the mutant. While its tolerance to higher doses of UVC and mitomycin C was significantly decreased as compared to wild type. Purified recombinant SbcCD proteins showed single-stranded endonuclease and $3' \rightarrow 5'$ double-stranded DNA exonuclease activities similar to that of the Mre11–Rad50 complex, which is required for DNA strand break repair in higher organisms. These results suggested that the Mre11–Rad50 type nuclease activity of SbcCD proteins contributes to the radiation resistance of *D. radiodurans* perhaps by promoting the RecA-dependent DSB repair required for polyploid genome maturation.

1. Introduction

The RecBC recombination pathway has been shown to contribute to the DNA double-strand (DSB) break repair mechanism of *Escherichia coli* [1,2]. Experimental evidence on the survival of *recBC* mutants of *E. coli* showed that these mutants fail to form colonies unless they acquired mutations in *sbcA*/*sbcB* and *sbcC*/*sbcD* [3,4]. It was demonstrated that *sbcC* and *sbcD* play an important role in homologous recombination in *E. coli* [5]. Further, it is known that DNA sequences containing long DNA palindromes are difficult to maintain in wild-type strains of *E. coli* [6] and mutation in *sbcC* or *sbcD*, is required to overcome this problem [7,8]. The problem of long inverted repeats has also been observed in other bacteria [9,10], yeast [11], human [12] and transgenic mice [13]. This indicates the existence of a universal mechanism to deal with abnormally folded DNA structures during DNA metabolism and a similar role of Mre11–Rad50 complex in such a function was suggested. Biochemical characterization of the SbcCD complex from *E. coli* has shown that this complex can cleave a hairpin structure *in vivo* and *in vitro*, very similar to the Mre11–Rad50 complex of eukaryotes [14,15].

Deinococcus radiodurans R1 exhibits extraordinary tolerance to abiotic stresses including high doses of ionizing and non-ionizing radiations [16,17], except near UV [18]. Unlike *E. coli*, the high tolerance to DNA strand breaks in *D. radiodurans* is supported by a RecA-mediated homologous recombination process [19] that occurs in absence of RecBC proteins. Although *Deinococcus* lacks the *recBC*, *sbcA* and *sbcB* genes, it contains the canonical CHI_{EC} sequence (739 times in all four genomes versus 1008 times in *E. coli*), an aberrant RecD protein (classified as a solo-RecD type helicase) [20] and a functional SbcCD [21]. Further, transgenic *Deinococcus* cells expressing RecBC [22] and SbcB [23] from *E. coli* show hypersensitivity to γ-radiation and impairment of DSB repair. In addition, the genome of this bacterium is GC rich and contains a large number of inverted repeats and transposable elements. This data suggest that *sbcB* and *sbcC*/*sbcD* genes function differently in *E. coli* and *Deinococcus*. Here we report the characterization of recombinant deinococcal–SbcCD for its roles in radiation resistance and DSB repair and provide a possible mechanism for how these proteins function in this bacterium. The SbcCD proteins showed Mre11–Rad50 (MR) type activities such as ATP-dependent double-strand DNA $3' \rightarrow 5'$ exonuclease and a hairpin endonuclease activities. The *sbcCD* deletion mutant showed nearly normal

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reassembly of a fragmented genome, as monitored by NotI restriction pattern, during the initial phase of DSB repair. These cells, however, showed defective RecA-dependent DSB repair and had a faster *in vivo* DNA degradation during the later phase of DSB repair. These results suggest possible mechanisms by which SbcCD complex contributes to radiation tolerance and DSB repair in *D. radiodurans*.

2. Materials and methods

2.1. Bacterial strains and materials

The ATCC13929 strain of *D. radiodurans* was a generous gift from Dr. M. Schafer, Germany [24]. The wild type and derivatives were grown aerobically in TGY (0.5% Bacto Tryptone, 0.3% Bacto Yeast Extract, 0.1% glucose) broth or on agar plates as required at 32 °C. Antibiotics such as chloramphenicol (5 μg/ml) and kanamycin (10 μg/ml) were added as required. The TGY agar plates containing *D. radiodurans* R1 and its derivatives were incubated at 32 °C for 48 h before colonies were counted. Shuttle expression vector pRADgro and its derivatives were maintained in *E. coli* strain HB101 as described [23]. Other recombinant techniques used as described [25].

2.2. Generation of sbcCD knockout mutant of D. radiodurans

The strategy used for making a genetic knockout derivative of *D. radiodurans* was described earlier [22]. In brief, 1 kb upstream and 1 kb downstream to the *sbcDC* operon were PCR amplified using forward primer 5′-CCGGGCCCCGATTTCTACGAGATTGATCTG-3′ and reverse primer 5′-GGAATTCTAGAAGCTGAAGCGACGATGTGA-3′, for the upstream fragment, and forward primer 5′-CGGGATCCGAGCAAGAGCATGGCGGGCAGCA-3′ and reverse primer 5′-GCTCTAGACGGCTTAGGGCGTACGTTGCA-3′ for the downstream fragment. The upstream fragment was cloned at ApaI and EcoRI sites in pNOKOUT [23] to yield pNokDup and the downstream fragment was cloned at BamHI and XbaI sites in pNOKDup to yield pNOKDC (Fig. S1). The recombinant plasmid was linearised with ScaI and transferred into *D. radiodurans*. The transformants were grown for several generations in TGY supplemented with Kan (8 μg/ml) to obtain a homozygous deletion mutant. Homozygous replacement of the *sbcDC* operon with an antibiotic marker was ascertained by scoring the loss of the 750 base pair junction sequence of the *sbcDC* operon. Clones showing the complete absence of the *sbcDC* operon were considered as homozygous deletion mutants and were used for further studies.

2.3. Construction of sbcD and sbcC expression plasmids

Genomic DNA of *Deinococcus* was isolated as published previously [26]. The ~2.77 kb *sbcC* gene was PCR amplified from the genomic DNA of *D. radiodurans* using gene-specific forward 5′-GGAATTCCATATGAAGCCGCTGCACCTCACCCTG-3′ and reverse 5′-CGGGATCCTTATCCGTCCACCCGAATCAC-3′ primers. Similarly, the ~1.29 kb *sbcD* gene was amplified from genomic DNA using gene-specific forward 5′-GGAATTCCATATGGAGATACTGGAAAACGA-3′ and reverse 5′-GGGATCCTACTGCGCGCCCTCCCGCGCCTG-3′ primers. The identity and correctness of these genes were ascertained by restriction analysis and partial nucleotide sequencing. The *sbcC* and *sbcD* genes were cloned at the NdeI and BamHI sites in pET28a$^+$. The recombinant plasmids, pETsbcC and pETsbcD (Fig. S2), respectively, were transformed into *E. coli* for expression of recombinant proteins.

2.4. The effect of DNA damaging agents on cell survival

Deinococcus cells were treated with different doses of UV and γ-radiations as described earlier [22]. Briefly, mutant and wild-type *D. radiodurans* were grown in TGY to late log phase at 32 °C. Cells were suspended in sterile phosphate buffered saline (PBS) and exposed to different doses of γ-radiation on ice, at a dose rate of 5.89 kGy/h (Gamma 5000, ^{60}Co, Board of Radiation and Isotopes Technology, DAE, India). Appropriate dilutions were plated on TGY agar plates and incubated at 32 °C. For UV irradiation, cells were prepared as described above and different dilutions were plated. Cells were exposed to different doses of UV radiation at 254 nm (UVC) and incubated at 32 °C. Mitomycin C (MMC) treatment was given as described [27]. Briefly, late log-phase cells were treated with MMC (20 μg/ml) and aliquots were withdrawn at regular intervals. Appropriate dilutions were plated on TGY agar supplemented with kanamycin (8 μg/ml) when required, and plates were incubated at 32 °C. Colony forming units were recorded after 48 h of incubation.

2.5. Protein purification and SbcCD activity characterization

Transgenic *E. coli* BL21 cells harboring pETsbcC and pETsbcD were induced with 200 μM IPTG and cells expressing recombinant protein were disrupted by sonication at 4 °C. Recombinant proteins were purified by nickel-affinity chromatography as described previously [28]. In brief, the clear supernatant was mixed with 500 μl of 50% Ni-NTA agarose slurry and hexahistidine-tagged recombinant protein was eluted from the matrix with elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM Imidazole, pH 8.0). The fractions were analysed on SDS–PAGE, and those containing proteins with highest purity were dialyzed overnight in buffer (20 mM Tris–HCl pH 7.6, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM PMSF, 1 mM DTT). The partially purified proteins were further purified with a heparin–sepharose affinity column to near homogeneity and dialyzed in buffer containing 20 mM Tris–HCl pH 7.6, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM PMSF, 1 mM DTT and 50% glycerol. Protein concentration was determined using Bradford's dye-binding method.

The hairpin endonuclease activity of SbcCD against a single-stranded loop was measured on HP78 (5′-GTTTCTATTCAGCCCTTTGACGTAATCCAGCCCCGGGTTTTCCCGGGGCTG GATTACGTCAAAGGGCTGAATAGAAAC-3′) and HP56 (5′-GCCTTTGACGTAATCCAG CTTGCTGGAACGAGTTCCAAGCTGGATTACGTCAAAGGGC-3′) stem-loop substrates stabilized at ambient temperature (Fig. S3), labeled with [^{33}P] at the 5′-end and assayed under suboptimal conditions at 16 °C in presence of ATP-γS as discussed [14]. The 3′ → 5′ exonuclease activity was detected both on linear plasmid and on stem-loop substrates (HP56) labeled at the 5′-end as described [14]. The products were analysed on a 10% urea–PAGE gel.

2.6. DNA degradation and DNA repair studies

Genomic DNA of *Deinococcus* was labeled with [^{3}H] thymidine as described earlier [29]. In brief, early logarithmic phase cells were incubated with 20 μCi/ml [^{3}H] thymidine and allowed to grow for 18 h at 30 °C. Labeled cells were spun and resuspended in fresh TGY medium and divided in two sets. One set was irradiated with 6 kGy γ radiation and the other set was used as an unirradiated control. Both were allowed to grow at 32 °C at 200 rpm. 200 μl aliquots were collected in triplicate at a regular intervals and TCA precipitable [^{3}H] counts were measured as described earlier [22]. For this, the cells were spun and washed with butanol-saturated 0.5 M EDTA solution. The pellet was suspended in sterile PBS and spotted on Whatman GFC filter discs. Cells were lysed by incubating the filter discs in 10% ice-chilled TCA solution for 30 min, which

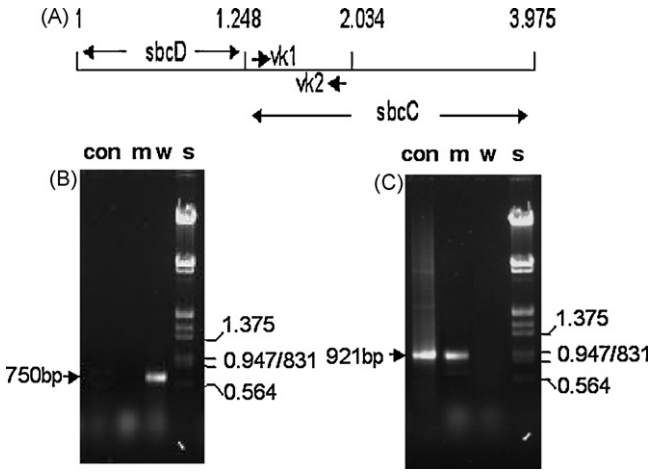

Fig. 1. Confirmation of *sbcCD* deletion in *Deinococcus radiodurans* genome. Genomic DNA was isolated from wild type (**w**) and from the putative *sbcCD* deletion mutant (**m**) generated using the pNOKDC (**con**) construct. The presence of *sbcCD* (B) and *nptII* (C) was confirmed by PCR amplification using gene-specific internal primers (*vk1* and *vk2*) for the *sbcCD* genes (A) and nptII specific primers.

also precipitates DNA on the discs. Discs were washed with ethanol, dried, and radioactivity was counted using scintillation counter. For pulsed-field gel electrophoresis, cells were irradiated with 6 kGy γ radiation (5.89 kGy/h) and aliquots were collected at different time intervals during post-irradiation recovery (PIR). Cell lysis and restriction digestion were carried out in agarose plugs and DNA fragments were separated by pulsed-field gel electrophoresis using a modified protocol of an earlier method [27]. In brief, the cells were washed with 70% ethanol in PBS (pH 7.5) for 5 min. Agarose plugs containing these cells were incubated with lysis buffer I (2 mg/ml lysozyme in 50 mM EDTA, pH 8.0) for 5 h at 37 °C, followed by overnight incubation at 55 °C in lysis buffer II (0.5 M EDTA, pH 8.0, 1% sodium sarcosine and 2 mg/ml proteinase K). Plugs were washed three times with TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA pH 8.0) followed by restriction enzyme buffer for 1 h each at 55 °C. For restriction digestion, the plugs were transferred to a fresh tube containing enzyme buffer and 30 units of NotI and incubated overnight at 37 °C. DNA fragments were analysed on 0.8% multipurpose agarose (Roche Biochemicals, Germany) for 25 h as described earlier [30].

Data presented were from a typical experiment. All the experiments were repeated at least three times and results were reproducible.

3. Results and discussion

3.1. The sbcDC mutant showed sensitivity to γ radiation

The *sbcDC* operon was deleted from the *D. radiodurans* genome and the homozygous replacement of *sbcDC* genes with the nptII was confirmed by PCR amplification using gene-specific primers (Fig. 1). The survival of these cells in response to DNA damage was monitored. Results showed nearly 100-fold decrease in mutant cell survival in response to γ radiation as compared to wild type (Fig. 2A). No effect of UVC radiation was observed until a dose of 800 Jm^{-2}, the mutant showed nearly 10-fold decrease in UVC resistance at a dose of approximately 1000 Jm^{-2} (Fig. 2B). There was no effect of hydrogen peroxide on mutant cell survival (data not shown), but the levels of MMC tolerance decreased by 4-fold as compared to wild type (Fig. 2C). Our results showed a similar pattern but a higher sensitivity of *sbcCD* mutant to γ radiation in comparison with an earlier report [21]. A 100-fold decrease in γ tolerance at a 20.6 kGy dose was reported, while our data showed

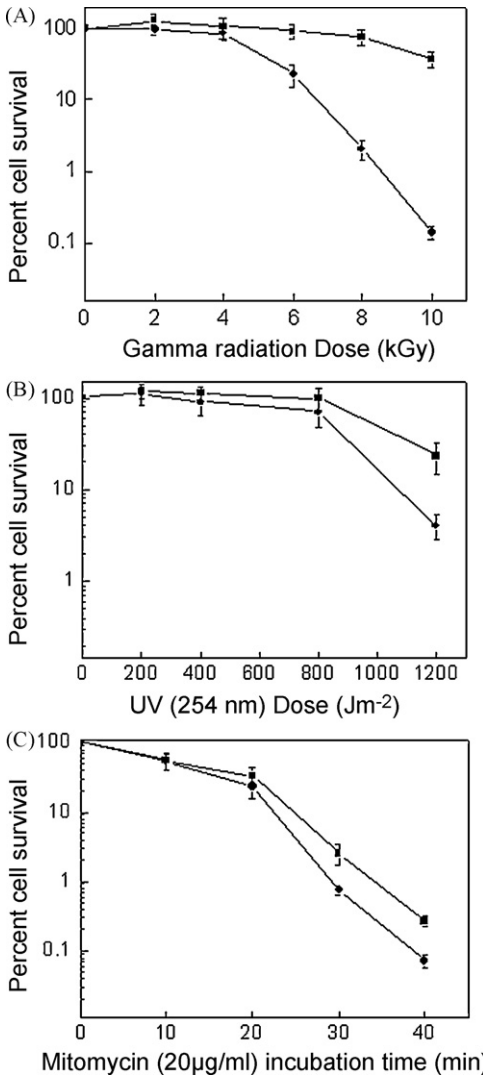

Fig. 2. Effect of *sbcCD* deletion on DNA damage response of *Deinococcus*. Wild type (-■-) and *sbcCD* deletion mutant (-●-) cells were treated with gamma radiation (A), UVC radiation (B) and mitomycin C (C) and cell survival was monitored. The one hundred percent cell survival in different samples was 1.23–3.5 × 10^7 cells/ml in wild type and 2.1–2.8 × 10^7 cells/ml in the mutant.

a similar lethality at only an 8 kGy dose. In addition, the earlier report found no effect of UV radiation on mutant survival, while we observed a nearly 10-fold decrease in UVC tolerance at high doses. This difference in radiation dose effects for similar bacterial strains could be the effect of different condition employed for gamma irradiation. For example, the dose rate of 60.6 Gy/min was used earlier while 98.17 Gy/min was used in the current study. Sensitivity of these mutants to γ, MMC and higher doses of UVC, but nearly no hydrogen peroxide sensitivity, suggests a role of SbcCD proteins primarily in DNA double-strand break repair. The roles of these proteins in bacterial tolerance to DNA strand breaks [31], as well as that of their orthologs like Rad50 (SbcC) and Mre11 (SbcD) in non-homologous end joining (NHEJ) in higher organisms [32,33], have been demonstrated.

3.2. The sbcDC deletion affects in vivo DNA degradation

The bacterial genome was labeled with [^{3}H] and TCA precipitable [^{3}H] counts were monitored during post-irradiation recovery (PIR). Mutant showed a very different pattern of *in vivo* DNA degradation as compared to wild type. While wild-type cells showed a

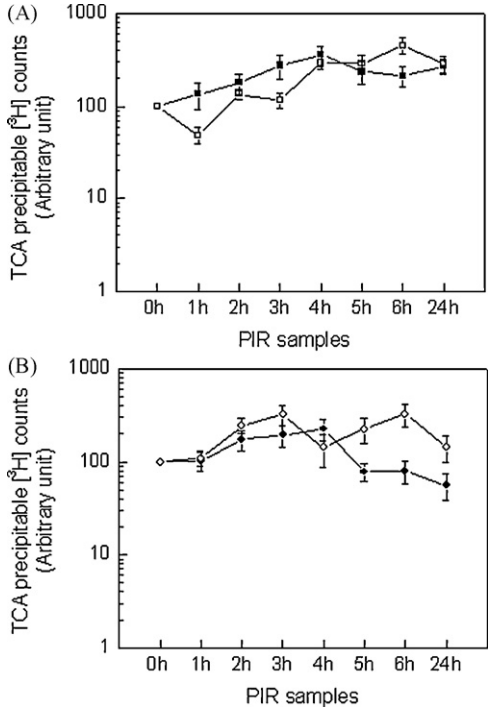

Fig. 3. Effect of *sbcCD* deletion on degradation of genomic DNA during post-irradiation recovery of *Deinococcus radiodurans*. Wild type (A) and *sbcCD* mutants (B) cells labeled with [^{3}H] were grown to exponential phase and irradiated with 6.5 kGy gamma radiation (-□- and -●-). The TCA precipitable [^{3}H] counts of irradiated samples were compared to unirradiated (-■- and -○-) controls. Experiments were repeated three times and results were reproducible.

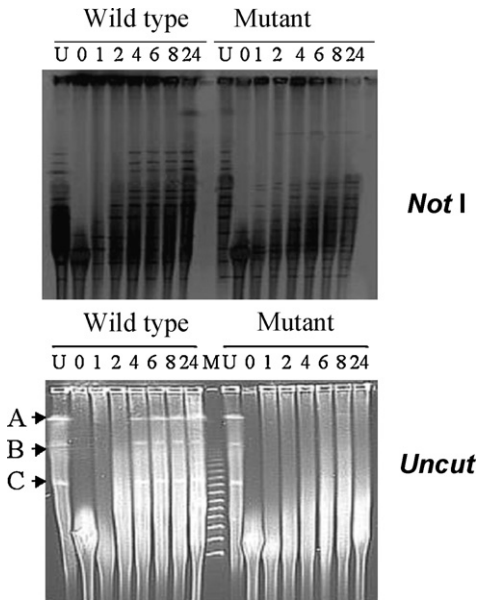

Fig. 4. DSB repair kinetics of wild type and *sbcCD* mutant. Wild-type and mutant cells grown exponentially were irradiated with 6.5 kGy gamma radiation and allowed to recover from radiation effects. Aliquots were taken at 0–2, 4, 6, 8 and 24 h post-irradiation recovery intervals and the pattern of DNA reassembly was studied by digesting the genome with NotI (**NotI**) or without digestion (**Uncut**). DNA from unirradiated (U) cells included as a control. Undigested control DNA in each case showing three DNA bands indicated with arrows A, B and C. Experiments repeated three times and were reproducible. Data given here are from a representative experiment.

rapid decrease in TCA precipitable [^{3}H] counts in the first 30 min PIR, TCA precipitable [^{3}H] counts increased by 3-fold before DNA degradation was observed in mutant cells (Fig. 3). The wild type showed an increase in TCA precipitable [^{3}H] counts by nearly 4-fold at 6 h PIR. Since treated cells were allowed to recover from radiation stress in presence of [^{3}H] thymidine, both DNA synthesis and DNA degradation must be occurring simultaneously but at different rates during PIR. The change in [^{3}H] levels therefore, reflects the relative efficiency of DNA synthesis and DNA degradation in the cells collected at different PIR times. The increased incorporation of [^{3}H] thymidine during PIR could be accounted for by a faster rate of DNA synthesis as compared to degradation and *vice versa*. The rapid burst of DNA degradation in first 30 min of PIR in wild-type cells irradiated with 6.0 kGy followed by a rapid rate of DNA synthesis, is the typical characteristic of wild-type cells [19,22,34]. The molecular mechanism underlying the different DNA metabolism in the mutant is not clear and is intriguing. The *sbcCD* mutant lacks the 30 min PIR nucleolytic degradation, but showed a faster rate of degradation during later PIR, suggesting a role of these proteins in the regulated DNA processing during recovery from γ radiation. The *sbcD* and *sbcC* genes are organized as a dicistronic operon in *D. radiodurans* and are expressed under the *sbcD* promoter [35,36]. Although, *sbcD* is expressed constitutively, transcriptome analysis showed an approximately 4-fold increase in mRNA synthesis of these genes in response to γ irradiation ([37], H.S. Misra and colleagues unpublished data). Based on these findings, it is hypothesized that the SbcCD nuclease is involved in DNA processing, leading to DNA degradation followed by efficient DNA synthesis in wild-type cells. In mutant cells, DNA synthesis was initially higher but, possibly due to defect in DSB repair, could not be continued. The synthesized DNA was then degraded by yet an unexplored mechanism in the mutant.

3.3. The sbcDC mutant shows a different pattern of PIR repair of DSBs

The *sbcDC* deletion mutant, which showed a dynamic change in genome stability during PIR, was checked for reassembly of its shattered genome. Gamma irradiated cells were allowed to recover and the change in DSB repair kinetics was monitored by pulsed-field gel electrophoresis (PFGE). Interestingly, both the wild type and mutant showed similar NotI pattern in unirradiated cells and the same extent of DNA damage with 6-kGy γ radiation (Fig. 4). While some of the NotI fragments were common in both the cases, there were clear differences in the NotI profiles of mutant and wild-type cells at different PIR times. Certain NotI fragments of wild type were missing in mutant (Fig. 4A). Wild-type cells could reassemble a full-length genome by 4 h PIR; the mutant cells failed to do so till 24 h PIR. PFGE analysis of undigested genomes showed the maturation of circular genomes in wild type, while mutant cells did not show the corresponding DNA bands (Fig. 4B). This indicated that *sbcCD* mutant cells were as efficient as wild type in reassembling the genome during the early phase of PIR, but were inefficient in RecA-dependent phase II DSB repair and genome maturation. Earlier studies have shown a direct correlation between DSB repair and γ radiation tolerance [19,21,30] in this bacterium. The pattern of genome recovery in *sbcDC* mutant obtained here is slightly different from earlier study [19], where the full recovery of the mutant genome was demonstrated. This could be explained by accounting the experimental differences both in dose rate and buffer conditions used during radiation treatment. It has also been shown that *D. radiodurans* derivatives exhibiting low γ resistance are defective in DSB repair during later PIR [23] and have a higher rate of *in vivo* DNA degradation [22]. The higher sensitivity of the *sbcCD* mutant to γ radiation, the kinetic change in net nucleolytic activity during PIR and the difference in NotI pattern relative to wild-type suggest the involvement of the SbcCD proteins in DNA end processing that may be required for efficient phase II of DSB repair. This

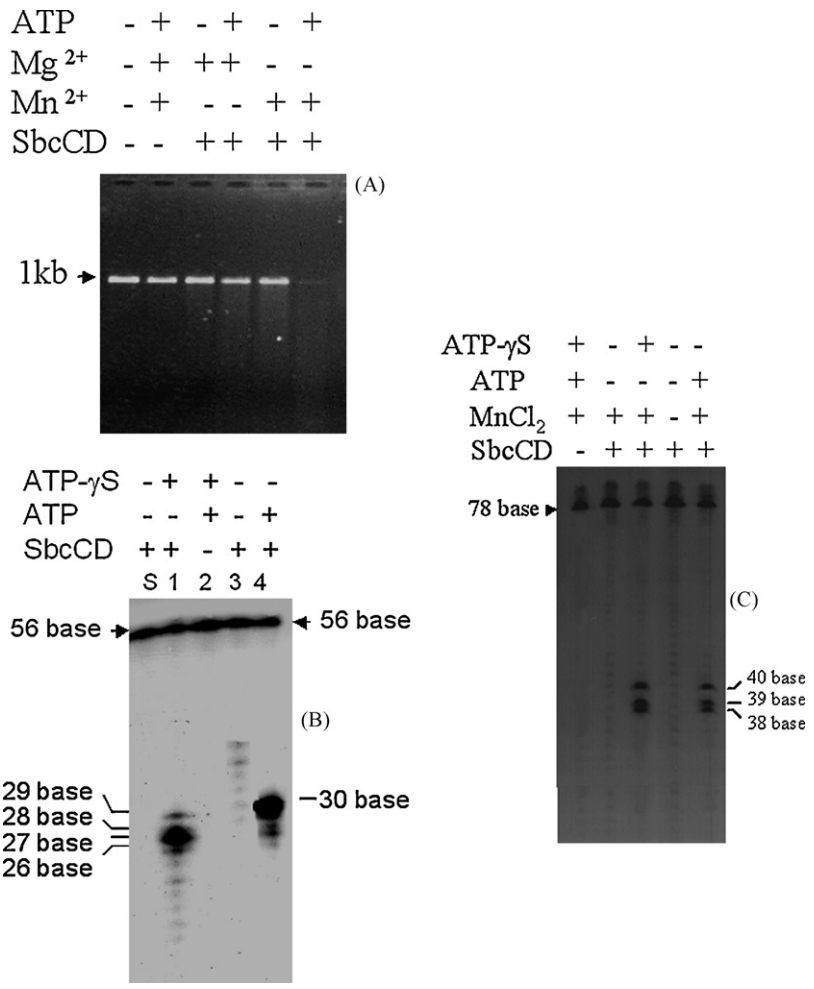

Fig. 5. Activity of recombinant SbcC and SbcD proteins. Purified SbcC and SbcD proteins were mixed and the complex was incubated with 1 kb PCR-amplified linear DNA substrate (**S**) at 37 °C, in presence of Mg^{2+} and Mn^{2+} with and without ATP. Products were analysed on a 1% agarose gel (A). The HP56 stem-loop DNA substrate (**S**) was labeled at 5′-end and incubated with the SbcCD in a buffer containing 5 mM $MnCl_2$ overnight at 16 °C (1 and 2) and 1 h at 37 °C (3 and 4) (B). Similarly HP78 substrate labeled at 5′-end was incubated with the SbcCD overnight at 16 °C (**C**). Products were analysed on 12% urea–PAGE as described in Section 2.

hypothesis is based on the assumption that the deinococcal–SbcCD complex is functionally similar to the *E. coli* SbcCD complex. Therefore, the functional characterization of the SbcCD complex from *D. radiodurans* was carried out *in solution*.

3.4. The deinococcal–SbcCD proteins showed typical Mre11–Rad50 activity in solution

The *sbcC* and *sbcD* genes of *D. radiodurans* were cloned (Fig. S2) and expressed in *E. coli*. Both the proteins were purified to near homogeneity (Fig. S3). The recombinant proteins were mixed in (SbcC):(SbcD)2 subunit ratio in order to constitute an active complex of these proteins as shown in *E. coli* [38]. It has been shown that SbcC and SbcD proteins of *E. coli* form a macromolecular complex in a 1:2 molar ratio in presence of Mn^{2+} and the DNA processing activity of *E. coli* SbcCD has an absolute requirement of Mn^{2+} and ATP [38]. Deinococcal–SbcCD complex was assayed for nuclease activity in presence of Mn^{2+} and Mg^{2+} ions in presence and absence of ATP. The complex showed low nuclease activity in presence of Mg^{2+} as compared to Mn^{2+}. However, the ATP stimulation of nuclease activity was observed with Mn^{2+} ions only (Fig. 5A). The single-stranded endonuclease and 3′ → 5′ dsDNA exonuclease activities were examined on 5′ labeled 78 (HP78) and 56 (HP56) nucleotides long substrates that form different sizes of stems and a 4 nucleotides loop structure at ambient temperature (Fig. S3). The incubation of these substrates with the SbcCD complex at 16 °C in presence of ATP-γS yielded single strand endonuclease activity. The results showed the generation of 26–29 nt products in presence of ATP-γS with HP56 (Fig. 5B), and 38–40 nt products with HP78 (Fig. 5C), at 16 °C. This demonstrates that SbcCD complex makes endonucleolytic cleavage randomly at any of three positions in the single-stranded loop region irrespective of its nucleotide sequence.

The incubation of 5′-labeled HP56 with the SbcCD complex at 37 °C in presence of Mn^{2+}-ATP yielded a prominent 30 nt product (Fig. 5B), which could have arisen either by nicking at the single strand–double strand DNA junction of hairpin substrate or by 3′ → 5′ dsDNA exonuclease activity on the double-stranded stem region until the single-strand region in loop region is reached. In order to distinguish these potential activities of the complex, the HP56 oligo was labeled at the 3′-end and incubated with enzyme complex under identical conditions. Results showed the absence of both (i) the 26 nt product predicted by nicking of the DNA at ssDNA–dsDNA junction, and (ii) any other processive degradation products (data not shown). The SbcCD complex of *E. coli* has been characterized for Mre11–Rad50 activities and its preference to Mn^{2+} ions [14]. These results suggested that the SbcCD complex of *D. radiodurans* is similar to the SbcCD complex of *E. coli* and exhibits both single-stranded endonuclease and 3′ → 5′ exonuclease activity on dsDNA. Both the activities observed with deinococcal–SbcCD complex are similar to those of the Mre11–Rad50 (MR) complex of

eukaryotes [40,41], which are known to contribute to DNA strand break repair by end processing and removal of hairpin blocks at DNA ends. These results suggest that the SbcCD complex of *D. radiodurans* acts as a MR complex and is involved in DNA strand break repair via its DNA end processing activity, as known for MR proteins in eukaryotes. The roles of Mn^{2+} in the extreme radioresistance of *Deinococcus* through oxidative stress tolerance mechanisms have been demonstrated recently [39]. The higher concentration of Mn^{2+} present in this bacterium might make these proteins work more efficiently in processing its complex genome structures. These results are in good agreement with earlier findings on the roles of the SbcCD complex in radiation resistance and DSB repair of this bacterium. In addition, our results demonstrate the involvement of the SbcCD complex in the repair of DNA damage caused by higher doses of UVC, the possible mechanism of differential *in vivo* DNA degradation during PIR and involvement of these proteins in the DNA processing required for DSB repair.

It has been shown that DSB repair in *D. radiodurans* R1 is biphasic [42]. Phase I that involves the reassembling of shattered genome fragments to a larger size with extended synthesis dependent strand annealing (ESDSA) [34] as a major pathway and NHEJ as a backup pathway. Phase II is characterized as the RecA-dependent slow process of homologous recombination and is critical for the maturation of circular genomes [17,19,34]. As *D. radiodurans* lacks the RecBC recombination pathway, a role of the RecF recombination in γ radiation resistance and DSB repair of this bacterium has been suggested [19,28,43]. The extensive DNA end processing of the radiation-shattered genome would be required for efficient DSB repair by any of these pathways. DNA end-processing and end-tethering roles have been shown for the SbcCD proteins both in bacteria [44] and its functional homologues in higher organisms [45,46]. Furthermore, the *Deinococcus* cells expressing *sbcB* and the SbcCD mutant have same viability as wild-type cells, but DNA damage tolerance of both the cell types was severely affected [9,23]. The genetic interaction of transgenic *sbcB* and/or *recBC* with/*D* genes and its effect on the DNA damage response of the *sbcCD* deletion mutant will be interesting to investigate.

4. Conclusion

The RecBCD helicase/nuclease activity is required for the processing of double-strand breaks in *E. coli* [1]. This study presented evidence to suggest that deinococcal–SbcCD proteins exhibit the single-strand endonuclease and $3' \rightarrow 5'$ dsDNA exonuclease activities similar to the Mre11–Rad50 complex of higher organisms. These activities might help the cells to process DNA ends blocked either by a hairpin structure, by the end blocking proteins [15] or by DNA interstrand crosslinks [47] and thereby make the cells highly efficient in DSB repair. The kinetic change *in vivo* DNA degradation and a strong impairment in the later phase of DSB repair in *sbcCD* mutant could be explained by a major defects in its DNA end processing functions. Taken together, these results support the role of the SbcCD complex in DNA double-strand break repair through its contributions to DNA-end processing, very similar to that observed for Mre11–Rad50 complex in higher organisms. These observations also lead us to speculate that *recBC* suppressors function differently in *D. radiodurans* than in *E. coli*, and this will require independent investigations.

Conflict of interest

Nothing to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.dnarep.2010.01.012.

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