JOURNAL OF PROTEOMICS 75 (2012) 2588–2600

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

A comparative proteomic approach to better define *Deinococcus* nucleoid specificities

Magali Toueille[a,1], *Boris Mirabella*[b,c,d,1], *Philippe Guérin*[e,1], *Claire Bouthier de la Tour*[a,1], *Stéphanie Boisnard*[a], *Hong Ha Nguyen*[a], *Laurence Blanchard*[b,c,d], *Pascale Servant*[a], *Arjan de Groot*[b,c,d,2], *Suzanne Sommer*[a,2], *Jean Armengaud*[e,*,2]

[a]*Univ. Paris-Sud 11, CNRS UMR 8621, CEA LRC 42V, Institut de Génétique et Microbiologie, Bâtiment 409, Université Paris Sud, F-91405 Orsay Cedex, France*

[b]*CEA, DSV, IBEB, Lab Ecol Microb Rhizosphere & Environ Extrem, Saint-Paul-lez-Durance, F-13108, France*

[c]*CNRS, UMR 6191 Biol Veget & Microbiol Environ, Saint-Paul-lez-Durance, F-13108, France*

[d]*Aix-Marseille Université, Saint-Paul-lez-Durance, F-13108, France*

[e]*CEA, DSV, IBEB, Lab Biochim System Perturb, Bagnols-sur-Cèze, F-30207, France*

ARTICLE INFO

Article history:
Received 19 January 2012
Accepted 1 March 2012
Available online 15 March 2012

Keywords:
Comparative proteomics
Deinococcus
DNA compaction
Nucleoid
Nucleoid associated proteins

ABSTRACT

Compared to radiation-sensitive bacteria, the nucleoids of radiation-resistant *Deinococcus* species show a higher degree of compaction. Such a condensed nucleoid may contribute to the extreme radiation resistance of *Deinococcus* by limiting dispersion of radiation-induced DNA fragments. Architectural proteins may play a role in this high degree of nucleoid compaction, but comparative genomics revealed only a limited number of *Deinococcus* homologs of known nucleoid-associated proteins (NAPs) from other species such as *Escherichia coli*. A comparative proteomic approach was used to identify potentially novel proteins from isolated nucleoids of *Deinococcus radiodurans* and *Deinococcus deserti*. Proteins in nucleoid enriched fractions were identified and semi-quantified by shotgun proteomics. Based on normalized spectral counts, the histone-like DNA-binding protein HU appeared to be the most abundant among candidate NAPs from both micro-organisms. By immunofluorescence microscopy, *D. radiodurans* HU and both DNA gyrase subunits were shown to be distributed throughout the nucleoid structure and absent from the cytoplasm. Taken together, our results suggest that *D. radiodurans* and *D. deserti* bacteria contain a very low diversity of NAPs, with HU and DNA gyrase being the main proteins involved in the organization of the *Deinococcus* nucleoids.

Abbreviations: 1D, one-dimensional; DAPI, 4,6-diamidino-2-phenylindole dihydrochloride; FM 4-64, N-(3-triethylammonium-propyl)-4-(6-(4-(diethylamino)phenyl) hexatrienyl)pyridinium dibromide; HA, hemagglutinin peptide; LTQ, linear trap quadrupole; NAP, nucleoid-associated protein; NSAF, normalized spectral abundance factor; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; PVDF, polyvinylidene difluoride; SDS, sodium dodecyl sulphate; SPA, sequential peptide affinity.

* *Corresponding author at:* CEA, DSV, IBEB, Laboratoire de Biochimie des Systèmes Perturbés, Bagnols-sur-Cèze, F-30207, France. Tel.: +33 4 66 79 68 02; fax: +33 4 66 79 19 05.
E-mail address: jean.armengaud@cea.fr (J. Armengaud).

[1] These authors contributed equally to this work and should be considered co-first authors.

[2] These authors contributed equally to this work as senior authors and should be considered co-last authors.

1874-3919/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jprot.2012.03.002

1. Introduction

Deinococcus radiodurans [1] and *Deinococcus deserti* (the latter recently isolated from desert sand) [2] belong to a family of bacteria characterized by an exceptional capacity to cope with the lethal effects of DNA-damaging agents, including ionizing radiation, UV light and desiccation. The radioresistance of these bacteria is linked to their extraordinary ability to reconstruct a functional genome from hundreds of radiation-induced chromosomal fragments, whereas the genome of most organisms is irreversibly shattered under the same conditions [3,4]. Different factors (efficient DNA repair processes, accumulation of manganese in place of iron to prevent protein oxidation, nucleoid organization) are intimately combined to enable survival from stresses (for review see [5–8]). Different DNA repair pathways were proposed for the reconstruction of an entire genome from DNA fragments in *D. radiodurans*: extended synthesis-dependent strand annealing (ESDSA) [9–11], homologous recombination (HR) [12,13], single-strand annealing (SSA) [14,15] and non-homologous end joining (NHEJ) [16,17].

The specific condensed ring-like structure of *D. radiodurans* nucleoid was observed unaltered after 15 kGy γ-irradiation and it was proposed that this structure may contribute to the extreme radioresistance of this organism [18]. Such a condensed genome may provide suitable scaffolds for DNA repair by limiting dispersion of radiation-induced DNA fragments. More recently, the nucleoid morphologies of seven species of *Deinococcus*, of the radioresistant *Rubrobacter radiotolerans* and of the radiosensitive *Thermus aquaticus* and *Escherichia coli* bacteria were evaluated [19]. This study revealed a high degree of genome condensation in the radioresistant *Deinococcus* and *Rubrobacter*, whereas the genomic DNA is uniformly distributed in cells of radiosensitive *E. coli* and *T. aquaticus*. The nucleoids of the radioresistant *Deinococcus geothermalis*, *R. radiotolerans* and *D. deserti* do not adopt a fixed shape [4,19]. This suggests that the strong nucleoid condensation, rather than the shape of the nucleoid, may be the common trait among radioresistant organisms.

It is believed that architectural proteins associated with the nucleoid may play a role in organization and compaction of bacterial nucleoids [20]. Twelve small nucleoid-associated proteins (NAPs) from *E. coli* have been extensively studied for their recognition sequence specificity and DNA-binding affinities, *i.e.* HU, DnaA, Fis, H-NS, IHF, Lrp, CbpA, CbpB, Dps, Hfq, IciA and StpA [21]. For several of these mostly basic and small proteins, DNA bending or compacting activity has been demonstrated [20]. In exponentially growing *E. coli* cells, the proteins Fis and HU are the most abundant, whereas Dps is the major nucleoid component in the stationary phase [22]. Moreover, proteins belonging to the SMC (Structural Maintenance of Chromosome protein) family are present in all organisms and were shown to be involved in chromosome condensation, pairing, segregation and DNA repair (for review see [23]). In addition, topoisomerases such as DNA gyrase and DNA topoisomerase I play an important role in maintenance of steady-state levels of DNA supercoiling [24], and in *E. coli* it was shown that DNA gyrase plays a critical role in supramolecular loop organization [25]. As in other bacteria, the two subunits of DNA gyrase and DNA topoisomerase I are conserved in *Deinococcaceae* [4,26,27].

Based on amino acid sequence similarity analysis, homologs of only 4 of the 12 NAPs characterized in *E. coli*, HU (DRA0065), DnaA (DR0002), Dps (DR2263 called Dps1, the closest *D. radiodurans* homolog of the *E. coli* Dps protein, and DRB0092 called Dps2), and Lrp (DR1894, DR0200), have been identified in *D. radiodurans*. Also in *D. deserti*, only homologs of HU (Deide_2p01940 called HU1, Deide_3p00060 called HU2, Deide_00200 called HU3, and, Deide_3p00832 called HU4 in order of their degree of homology with the HU protein of *D. radiodurans*), DnaA (Deide_00010), Dps (Deide_21200) and Lrp (Deide_09020, Deide_18030, Deide_03140, Deide_3p00770) have been identified [4], but remarkably with four homologs of HU and Lrp. The HU protein was shown to be essential for *D. radiodurans* cell viability and it is localized all over the nucleoid [28]. When HU is expressed from a thermosensitive plasmid, its progressive depletion at the non-permissive temperature generates decondensation of DNA before fractionation of the nucleoid in several entities and finally lysis of the cells suggesting a major role of HU in nucleoid organization and DNA compaction in *D. radiodurans* [28]. In contrast to the drastic effect of HU depletion, no change in nucleoid morphology and cell viability was observed in cells devoid of the two Dps homologs [28] or in cells devoid of the SMC protein [29] compared to the wild type. The limited number of *Deinococcus* homologs of *E. coli* NAPs and the phenotype of the *smc* null and *dps* null mutant bacteria lead us to speculate that in conjunction with HU, other, not yet identified, proteins drive the compact organization of the *D. radiodurans* nucleoid.

Over the recent years, high throughput shotgun proteomics made possible with the most recent generation of high resolution mass spectrometers revealed new facets in microbial physiology and ecology [30–32]. For example, a higher diversity of protein virulence factors could be uncovered in pathogenic bacteria with shotgun approaches compared to traditional two-dimensional gel strategy [33]. New metrics in proteomic label free relative quantitation, such as the normalized spectral abundance factor (NSAF), allows comparing purified fractions of a proteome and follow-up protein enrichment [34–37]. Here, a comparative proteomic approach was used to identify proteins potentially involved in nucleoid compaction. For this purpose, we have isolated the nucleoids from *D. radiodurans* and *D. deserti* by adapting a nucleoid-isolation procedure described for *E. coli* [38,39]. The isolation of nucleoids was probed by microscopy after DNA staining with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI). NAPs were identified by nanoLC-MS/MS shotgun approach and semi-quantified by spectral count. Already known NAPs in *D. radiodurans* and in *D. deserti* (homologs of HU and Dps) were used as markers for our procedure. Their enrichment in the soluble fraction after extraction in presence of 2 M NaCl was monitored by differential quantitative proteomics. Candidate NAPs were then immuno-localized in *D. radiodurans* cells. The results of our comparative proteomic approach suggest that *D. radiodurans* and *D. deserti* bacteria contain a very low diversity of NAPs, with HU and DNA gyrase being the main proteins involved in the organization of the nucleoids in *Deinococcus*.