Free Radical Biology & Medicine 48 (2010) 292–297

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Dps proteins prevent Fenton-mediated oxidative damage by trapping hydroxyl radicals within the protein shell

Giuliano Bellapadrona, Matteo Ardini, Pierpaolo Ceci, Simonetta Stefanini, Emilia Chiancone *

CNR Institute of Molecular Biology and Pathology, Department of Biochemical Sciences "A. Rossi-Fanelli," "Sapienza" University of Rome, 00185 Rome, Italy

ARTICLE INFO

Article history:
Received 8 July 2009
Revised 26 October 2009
Accepted 27 October 2009
Available online 3 November 2009

Keywords:
Dps proteins
Hydroxyl radicals
Fenton chemistry
Intraprotein radicals
DNA protection
Free radicals

ABSTRACT

Dps (DNA-binding proteins from starved cells) proteins belong to a widespread bacterial family of proteins expressed under nutritional and oxidative stress conditions. In particular, Dps proteins protect DNA against Fenton-mediated oxidative stress, as they catalyze iron oxidation by hydrogen peroxide at highly conserved ferroxidase centers and thus reduce significantly hydroxyl radical production. This work investigates the possible generation of intraprotein radicals during the ferroxidation reaction by *Escherichia coli* and *Listeria innocua* Dps, two representative members of the family. Stopped-flow analyses show that the conserved tryptophan and tyrosine residues located near the metal binding/oxidation center are in a radical form after iron oxidation by hydrogen peroxide. DNA protection assays indicate that the presence of both residues is necessary to limit release of hydroxyl radicals in solution and the consequent oxidative damage to DNA. In general terms, the demonstration that conserved protein residues act as a trap that dissipates free electrons generated during the oxidative process brings out a novel role for the Dps protein cage.

© 2009 Elsevier Inc. All rights reserved.

Formation of radicals within proteins is of crucial importance in biological systems, as radicals take part in a variety of processes that range from electron transfer to chemical modification of substrates [1–7]. However, free radicals are extremely dangerous because of their high chemical reactivity, which impairs the function of biological macromolecules [8,9]. The hydroxyl radical, which is generated via Fenton chemistry from the reaction of Fe(II) and hydrogen peroxide, is recognized in particular as the most harmful oxidative agent for DNA, lipids, and proteins [8,9]. To combat Fenton-mediated oxidative stress, all living cells express a variety of enzymes that either detoxify directly hydrogen peroxide or its products, such as catalase, superoxide dismutase, and peroxidase [9], or maintain free ferrous iron, such as ferritin, at low concentrations in the cytoplasm [10,11]. In recent years, the widespread bacterial Dps (DNA-binding proteins from starved cells) proteins were shown to remove both Fenton reagents concomitantly from solution [12]. Dps proteins belong to the ferritin superfamily, but unlike ferritins, which use molecular oxygen to catalyze Fe(II) oxidation, they use hydrogen peroxide as a physiological iron oxidant and thus reduce the production of hydroxyl radicals significantly [13,14].

Dps proteins are formed by 12 identical four-helix bundle monomers that assemble into spherical hollow structures endowed with 2:3 symmetry (Fig. 1). The internal cavity, where iron is stored after oxidation, is connected to the external medium by hydrophobic and hydrophilic channels formed at the junction between the threefold symmetry-related subunits. The hydrophilic channels are used by Fe(II) to enter the protein shell and reach the highly conserved ferroxidase center located at the twofold symmetry interface. Both symmetry-related subunits provide the iron ligands, namely two histidines, one aspartic acid, and one glutamic acid (Fig. 1). Because in all known proteins with ferroxidase activity the catalytic center is bimetallic, with a high-affinity A site and a low-affinity B site [15,16], the Dps ferroxidase center is believed to have a similar structure. However, in the available X-ray crystal structures the ferroxidase center contains one iron ion, coordinated by His, Asp, and Glu residues, in site A and a water molecule in site B, as in *Listeria innocua* Dps, in which the center was first identified [17], or two water molecules as in the prototypic *Escherichia coli* Dps [18].

An overall scheme of the ferroxidation reaction by hydrogen peroxide, which leads to the production of water, has been proposed in several papers [12,19–24]. Importantly, upon iron oxidation only slight amounts of hydroxyl radicals are released into solution as shown by EMPO spin trapping experiments on *E. coli* [12] and *L. innocua* Dps [20]. The possible generation of radicals within Dps proteins has never been investigated despite the strong similarity of the Dps redox center to that of ribonucleotide reductase, in which the formation of radicals involving tryptophan and tyrosine residues is known to occur [25–29]. This similarity suggested that a reason for the presence of a highly conserved tryptophan residue (in 99% of all known Dps sequences) at ~3 Å

* Corresponding author. Fax: +39 064440062.
E-mail address: emilia.chiancone@uniroma1.it (E. Chiancone).

0891-5849/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2009.10.053

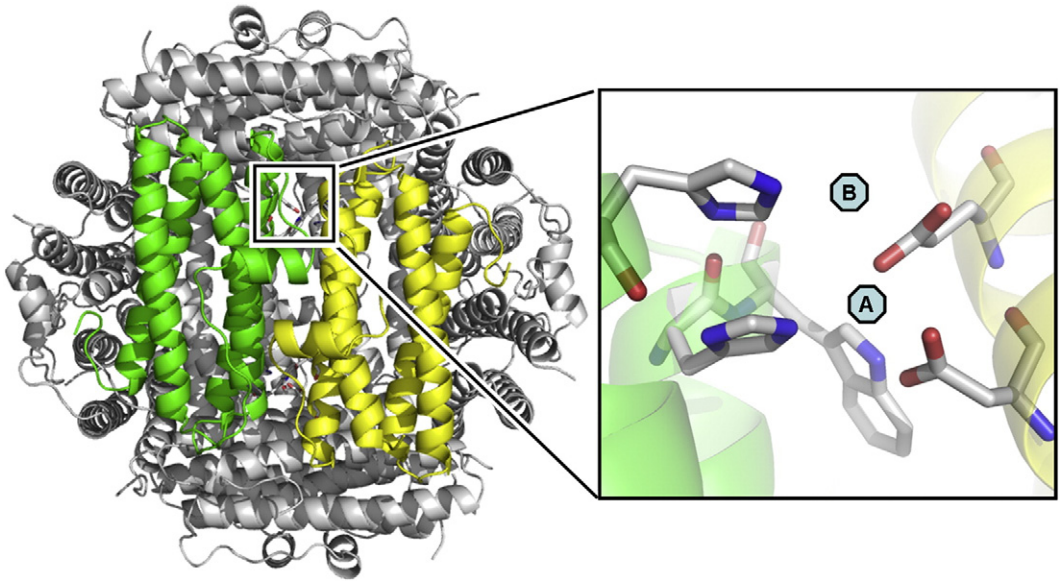

Fig. 1. Dodecameric assemblage of Dps proteins evidencing a twofold symmetry interface (left) and the intersubunit ferroxidase center located at this interface (right). The positions of the high-affinity A binding site and of the putative low-affinity B binding site are indicated along with those of the conserved iron ligands at site A, namely two histidine residues, one aspartic acid, and one glutamic acid. The tryptophan residue present in 99% of all known Dps sequences and located at ~3 Å from site A is also shown. This figure was generated by PyMOL (version 0.99; Delano Scientific LLC, Palo Alto, CA, USA).

from the A iron-binding site (Fig. 1) and of a nearby tyrosine residue (in ~50% of the sequences) could lie in their involvement in the formation of intraprotein radicals.

Materials and methods

Protein preparation

Site-specific mutants of *L. innocua* Dps (*Li*Dps), namely W32L, Y50R, and W32L-Y50R, were obtained with a Quick Change site-directed mutagenesis kit (Stratagene) using as template the pET-11a vector containing the *L. innocua fri* gene. *E. coli* Dps (*Ec*Dps), *Li*Dps, and the *Li*Dps mutants were overexpressed in the *E. coli* BL21(DE3) strain and purified as previously described [12,21]. Protein concentration was determined by the absorbance at 280 nm using the following molar extinction coefficients calculated using the Gill and Von Hippel equation [30]: *Ec*Dps, 0.83 M^{-1} cm^{-1}; *Li*Dps, 0.94 M^{-1} cm^{-1}; W32L, 0.64 M^{-1} cm^{-1}; Y50R, 0.86 M^{-1} cm^{-1}; W32L-Y50R, 0.56 M^{-1} cm^{-1}.

Stopped-flow experiments

An Applied Photophysic stopped-flow instrument equipped with a Spectra/kinetic monochromator was employed in the sequential mixing setup. Protein solutions at 66.8 μM (on a dodecamer basis) in 200 mM Mops–NaOH, pH 7.0, were mixed first with 400 μM $FeSO_4$ in 1 mM HCl and then, after 500 ms in the aging loop, with 100 μM H_2O_2. Spectra were acquired every 2.5 ms in the wavelength range of 350–700 nm at 20°C for a total time of 255 ms. Spectra were also recorded at the end of the reaction (65 s). The kinetic data were analyzed with Origin 8 PRO (OriginLab, Northampton, MA, USA) and/or MatLab 6.0 (Mathworks, Natick, MA, USA) using the following double-exponential equation: $Y = Y_0 + A_1[(1-\exp(-k_1 t)] \pm A_2[1-\exp(-k_2 t)]$, where Y_0 is the absorbance at $t=0$, A_1 and A_2 correspond to the amplitude of the first and second phase, respectively, and k_1 and k_2 are the first-order rate constants in s^{-1}. The yield of the various radicals formed was calculated on the basis of published molar extinction coefficients, namely 2300 ± 150 M^{-1} cm^{-1} for $Trp^{\bullet}$ at 510 nm [31] and 2600 M^{-1} cm^{-1} for $Tyr^{\bullet}$ at 410 nm [32].

DNA protection assay

DNA protection from hydroxyl radicals was assessed in vitro using pET-11a plasmid DNA (5600 bp, 10 nM) purified by a Quiaprep Spin plasmid miniprep kit (Qiagen). The total reaction volume was 16 μl in 50 mM Mops–NaOH, pH 7.0. Four microliters of 66.8 μM *Li*Dps or its site specific mutants in 200 mM Mops–NaOH, pH 7.0, was sequentially mixed with 4 μl of 400 μM $FeSO_4$ in 1 mM HCl, 4 μl of 40 nM pET-11a, and 4 μl of 200 μM H_2O_2. The reaction mixture was incubated for 15 min at room temperature. Plasmid DNA was resolved by electrophoresis on 1% agarose gel in Tris–base–EDTA. The gel was stained by ethidium bromide and imaged by ImageMaster VDS (Pharmacia Biotech).

MALDI-ToF MS analyses

Samples of *Li*Dps and *Ec*Dps were analyzed by MALDI-ToF MS before and after exposure to $FeSO_4$ and H_2O_2 (final concentrations: 16.7 μM protein dodecamer in 50 mM Mops–NaOH, pH 7.0, 100 μM $FeSO_4$, and 50 μM H_2O_2). The protein solutions were subjected to proteolysis at 37°C overnight with trypsin (about 50 ng) in a 25 mM ammonium bicarbonate digestion buffer. A 1-μl aliquot of each tryptic mixture was mixed with the α-cyano-4-hydroxy-*trans*-cinnamic acid matrix solution (10 mg/ml) in 70% acetonitrile containing 0.2% trifluoroacetic acid (v/v) and deposited onto a MALDI target. MALDI-ToF MS analyses were performed on a Voyager-DE STR instrument (Applied Biosystems, Framingham, MA, USA) equipped with a 337-nm nitrogen laser and operating in reflector mode. Mass data were obtained by accumulating several spectra from laser shots with an accelerating voltage of 20 kV. All mass spectra were calibrated externally using a standard peptide mixture containing des-Arg-bradykinin (m/z 904.4681), angiotensin I (m/z 1296.6853), and 1–17 (m/z 2093.0867), 18–39 (m/z 2465.1989), and 7–38 (m/z 3657.9294) adrenocorticotropic hormone fragments.

Results and discussion

The possible formation of intraprotein radicals in Dps proteins was investigated by a stopped-flow spectroscopic analysis of the iron oxidation reaction by hydrogen peroxide carried out on *Ec*Dps

Download English Version:

https://daneshyari.com/en/article/1909539

Download Persian Version:

https://daneshyari.com/article/1909539

Daneshyari.com