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Research paper

A specific proteomic response of *Sulfolobus solfataricus* P2 to gamma radiations

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ABSTRACT

Sulfolobus solfataricus is an acidophilic hyperthermophilic crenarchaeon living at 80 °C in aerobic conditions. As other thermophilic organisms, *S. solfataricus* is resistant to gamma irradiation and we studied the response of this microorganism to this ionizing irradiation by monitoring cell growth, DNA integrity and proteome variations. In aerobic conditions, the *S. solfataricus* genome was fragmented due to the multiple DNA double strand breakages induced by γ-rays and was fully restored within a couple of hours. Comparison of irradiated and unirradiated cell proteomes indicated that only few proteins changed. The proteins identified by mass spectrometry are involved in different cellular pathways including DNA replication, recombination and repair. Interestingly, we observed that some proteins are irradiation dose-specific while others are common to the cell response regardless of the irradiation dose. Most of the proteins highlighted in these conditions seem to act together to allow an efficient cell response to γ-irradiation.

1. Introduction

In all living cells, chemical stability of DNA is limited and represents a major factor leading to spontaneous genetic instability. Obviously, particular environmental conditions such as high temperature and radiations increase the frequency of DNA damages, especially double strand breakages. Hyperthermophilic organisms living at temperatures above 70 °C are thus particularly exposed to these DNA damages [1–4]. However, in order to faithfully preserve their genetic information in these deleterious conditions, the hyperthermophiles presumably use different mechanisms to protect their chromosome(s), such as high salt concentration [5], polyamines [6] and DNA-binding proteins [7]. Different studies performed on the hyperthermophilic Euryarchaeota (such as *Pyrococcus* or *Thermococcus* genus) have shown that these organisms are particularly resistant to γ-radiations [8–10]. Moreover, no particular DNA protection was reported in hyperthermophiles since their chromosome fragmentation observed after irradiation was equivalent to that obtained in radiosensitive cells [9,11]. Therefore, hyperthermophiles must possess in counterpart a highly efficient repair system. Furthermore, proteome and transcriptome analyses suggest that proteins involved in DNA repair are constitutively expressed in these organisms [8,9,12]. This resistance property seems to be also shared by the Crenarchaeota, the second major kingdom of Archaea. Indeed, *Desulfurococcus amylolyticus* first and *Sulfolobus acidocaldarius* and *Sulfolobus solfataricus* more recently, have been described as resistant cells [13] but with significant differences in closely related Sulfolobales strains [14,15]. The studies focused on a limited set of proteins known to be involved in DNA repair, indicate that only some of them are affected by the γ-irradiation, essentially the RadA paralogues Ral1, Ral2 and Ral3 but in a lesser extent for the last one [15]. Moreover, even if the whole amount of both RadA and Mre11 is unchanged, these proteins are recruited onto DNA after γ-irradiations [14]. To determine whether γ-irradiations are able to significantly change or not the proteome of a hyperthermophilic crenarcheon, we monitored the proteome changes of the *S. solfataricus* P2 strain by using 2D-gel electrophoresis. We observed tiny proteome variations and we identified proteins either specifically implicated in the cell response to low and high γ-rays doses or common for both doses.

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2. Material and methods

2.1. Chemicals and enzymes used

Proteinase K and EDTA were purchased from Merck, *Rsr* II and PFGE II marker were from New England Biolabs. Modified frozen Trypsin was acquired at Promega. Urea Ultrapure was acquired at MP Biomedicals Europe. Certified megabase agarose, plugs molds, acrylamide/bisacrylamide, ReadyStrip™ IPG strips, Bio-Lyte ampholytes, Dodeca silver stain kit and Sypro Ruby protein stain were purchased from Bio-Rad. Agarose type V, CHAPS, DTT, iodoacetamide, gelrite and all other chemical products were purchased from Sigma.

2.2. Strains and media

S. solfataricus P2 (DSMZ 1617) was cultivated in liquid medium in Erlenmeyer flasks with long neck in rich media essentially as described previously [16]. *S. solfataricus* was plated at 80 °C on solid medium composed of a 30 ml layer of Brock's medium (Brock et al., 1972) with 0.64% gelrite (w/v) and of a 3 ml overlay of Brock's medium with 0.32% gelrite (w/v) containing diluted control or irradiated cells. Dilution of cells was carried out in minimum medium pH 5.5 containing 0.1% tryptone (w/v). All solutions were equilibrated at 80 °C before plating. After gelification, plates were placed in closed jars for 7 days at 80 °C.

2.3. Gamma irradiation

S. solfataricus cells grown at 80 °C in 100 ml of medium were harvested in exponential phase ($OD_{600nm} \approx 0.2$) by centrifugation at 3000 × g for 5 min at 4 °C. The pellets were resuspended in minimum medium (45.4 mM $(NH_4)_2SO_4$, 4.4 mM KH_2PO_4, 2.68 mM KCl, 0.28 μM $CuCl_2$, 0.04 μM $CoSO_4$, 9.1 μM $MnCl_2$, 11.74 μM $Na_2B_4O_7$, 0.12 μM Na_2MoO_4, 0.04 μM $NiSO_4$, 0.76 μM $ZnSO_4$, 1 mM $MgSO_4$, 0.3 mM $Ca(NO_3)_2$, 75 μM $FeSO_4$, 0.06 μM $VOSO_4$ adjusted at pH 5.5 by adding H_2SO_4) and centrifuged again as described above. The cells were resuspended in minimum medium (pH 5.5) to have a cell density of about 6.10^9 cells ml^{-1}. Cells were distributed in cooled glass tubes of 2 ml held in a glass cup filled with ice. Cooled cells, except cooled control cells (0 Gy), were irradiated with a ^{137}Cs γ-ray source (IBL 637 Cis bio International ^{137}Cs γ-irradiator) at a dose rate of 56.6 Gy min^{-1}. The time elapsed between cell preparation and cell reincubation at 80 °C was 2 h for both control and irradiated cells kept all the time at 4 °C. Cells were subsequently washed in cold minimum medium (pH5.5) before inoculation into 50 ml of fresh rich medium preheated at 80 °C so as to have about 24.10^7 cells ml^{-1} ($OD_{600nm} \approx 0.2$).

2.4. Pulse-field gel eletrophoresis (PFGE)

Irradiated and unirradiated cells back to 80 °C were harvested at different time points (including time point 0 corresponding to the cell resuspension with the 80 °C preheated rich medium) and centrifuged as described above. Then, they were washed twice in minimum medium (pH 5.5) then in buffer A (50 mM Tris–HCl pH 8, 50 mM EDTA) and finally in buffer B (50 mM Tris–HCl pH 8, 125 mM EDTA). Afterward, cells were resuspended in buffer B preheated at 50 °C to have a cell density of 10^9 cells ml^{-1}. An equal volume of 1.6% melted agarose (w/v) was added to cells kept at 50 °C. These cell preparations were molded in plug molds. Cells embedded in plugs were lyzed by incubation in buffer A supplemented with 1% N-Laurylsarcosine (w/v) and 1 mg Proteinase K ml^{-1} for 48 h at 42 °C. Plugs were washed twice for 30 min at room temperature in TE buffer (10 mM Tris pH 8, 1 mM EDTA) supplemented with 1 mM PMSF then washed four times for 30 min at room temperature in TE buffer only. Half plugs were equilibrated three times for 1 h in *Rsr* II enzyme buffer as described by the manufacturer. Embedded chromosomal DNA was hydrolyzed by 1 unit of *Rsr* II per half plug for 24 h at 37 °C then half plugs were rinsed twice for 30 min at room temperature in TE buffer. The half plugs were disposed onto a 1% agarose (w/v) gel in 0.5 x Tris-borate EDTA [17]. The electrophoresis was carried out at 10 °C at 5.5 V/cm for 28 h with a switch angle of 120° and a pulse time of 40 s in a Biorad CHEF Mapper® XA system. Gel was stained for 20 min in 2.5 μg ethidium bromide ml^{-1} of and rinsed in water before numerization using BioRad Molecular Imager® Gel Doc™ system.

2.5. 2D electrophoresis and analysis of proteome

3.10^9 cells (about 0.5 mg of protein) irradiated or not were collected at different time points up to 4 h and centrifuged for 5 min at 3000 × g at 4 °C. The pellets were resuspended in sterilized water, transferred in 1.5 ml microtubes and centrifuged as described above. Each pellet was resuspended in 200 μl of a solution containing 7 M urea, 2 M thiourea, 100 mM DTT, 4% CHAPS (w/v), 1% Triton X-100 (IEF solution). The microtube was placed in the center of a cup horn then treated in four cycles of 30 s of sonication (Vibracells sonicator at 440 W) spaced by 1 min ice chilling. Cell extracts were adjusted to 500 μl with IEF solution before adding pH 3–10 and pH 8–10 ampholytes to a final concentration of 0.3% (v/v) each. Isoelectric focusing was carried out essentially on pH 3–10 ReadyStrip IPG strips (17 cm of length) and using Bio-Rad PROTEAN® IEF Cell as follows: active rehydration of strips for 15 h at 50 V; 30 min at 250 V (rapid ramp); 2 h at 1000 V (rapid ramp); 6 h of pre-focusing (rapid ramping with a maximum of 10,000 V); 60,000 V h^{-1} focusing with a maximum of 10,000 V (rapid ramp). The strips were removed from the tray and equilibrated in 0.375 M Tris–HCl pH 8.8, 6 M urea, 130 mM DTT, 4% SDS (w/v), 20% glycerol (v/v) for 35 min at 37 °C under gentle agitation (200 rpm). A second equilibration was performed in 0.375 M Tris–HCl pH 8.8, 6 M urea, 135 mM iodoacetamide, 4% SDS (w/v), 20% glycerol (v/v) for another 35 min at 37 °C, under gentle agitation (200 rpm). The second dimension was carried out on 12.5% (37.5:1 acrylamide:bisacrylamide) SDS-PAGE gels (20 cm × 20 cm) at 80 V for 16 h in Bio-Rad PROTEAN® II xi 2-D cell. Proteins on gels were stained with Coomassie blue, Dodeca silver stain kit or Sypro Ruby protein stain. Coomassie blue and silver stained gels were equilibrated in 7.5% acetic acid (v/v) and 10% glycerol (v/v), dried between cellophane sheets and numerized for analysis using ImageMaster™ 2D Platinum v6.0 (GE Healthcare). We loaded twice the amount of proteins for gels stained with Coomassie blue. Sypro Ruby stained gels were visualized using a Typhoon scanner (GE Healthcare).

For each condition, three biological replicates were produced for proteome kinetics. The 2D gel triplicates were analyzed with ImageMaster™ Platinum software (GE Healthcare). Comparative analyses of protein spots were performed by matching corresponding spots from different gels. A protein variation was considered when the irradiated cells/control cells protein spot intensity ratio was above 2. Each of the matched protein spots was double-checked manually. The proteins of interest (proteins exhibiting variations) as well as their corresponding location on control gels were excised and subjected to subsequent identification by mass spectrometry (see supplementary method). Only spots present in all the experiments with an intensity ratio higher than 2, were selected. In addition, spots for which the analysis leads to the identification of several proteins in different conditions or when several identifications have failed, were rejected.