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The ADP-ribosylation of *Sulfolobus solfataricus* Sso7 modulates protein/DNA interactions in vitro

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This paper is dedicated to the dear Josianne Menissier-de Murcia.

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ABSTRACT

The 7 kDa Sso7 is a basic protein particularly abundant in *Sulfolobus solfataricus* and is involved in DNA assembly. This protein undergoes in vitro ADP-ribosylation by an endogenous poly(ADP-ribose) polymerase-like enzyme. The circular dichroism spectrum of purified ADP-ribosylated Sso7 shows that this modification stabilizes the prevalent protein β-conformation, as suggested by shifting of negative ellipticity minimum to 220 nm. Moreover, a short ADP-ribose chain (up to 6-mers) bound to Sso7 is able to reduce drastically the thermoprotective and DNA condensing ability of the protein, suggesting a possible regulatory role of ADP-ribosylation in sulfolobal DNA organization.

1. Introduction

Crenarchaeal sulfur-dependent *Sulfolobus solfataricus*, even though prokaryotic, shares many biochemical and genetic features with eukaryotes [1]. Still debated are questions about the chromosome-like structure of its DNA and the ability to maintain DNA structural integrity at very high temperatures [2,3]. The presence of a nucleosome-like structure has been suggested, although Sulfolobus does not contain histone-like proteins [3]. They are replaced by highly abundant small basic proteins, the so-called Sso7 protein family (named from a to f) [4,5]. These microheterogenous 7 kDa proteins have a characteristic N-terminal cluster of lysines in the order X-K-X-K-X-K resembling a similar sequence in the eukaryotic high mobility group proteins (HMGs) [6].

Sso7d from *Sulfolobus solfataricus* is a multifunctional protein that is bound to DNA, has a chaperone activity in renaturing protein aggregates, and exhibits ATPase activity [7,8]. It is worth noting that these functions depend on protein transition between ATPase-driven conformational states. Only the native conformation of Sso7d binds to protein aggregates, whereas complexing DNA, Sso7d lacks its ability to disaggregate proteins [7,8].

Purified Sso7 protein undergoes in vitro ADP-ribosylation by the sulfolobal poly(ADP-ribose) polymerase (PARP) – like enzyme (PARP*Sso*) [9]. In eukaryotes poly(ADP-ribosyl)ation reaction, catalysed by PARPs, a family of enzymes that use NAD^+ as substrate, is a versatile regulatory mechanism of several nuclear and cell processes, depending on which PARP is involved [10]. The most studied enzymes, PARP-1 and PARP-2, play a main role in both chromatin organization and DNA repair/metabolism [10]. Nuclear DNA-binding proteins (histones, HMGs, enzymes, PARP itself) are good substrates in this reaction and modification by ADP-ribose polymers modulates their biological functions (reviewed in [10]).

In this light the finding that Sso7 protein is modified by the sulfolobal PARP-like enzyme led us to hypothesize that ADP-ribosyl)ation might regulate the biological role(s) of the small basic protein. Therefore we have first studied whether the binding of ADP-ribose to Sso7 could modify one of the protein functions, i.e. the ability to interact and condense DNA. Here we provide evidence that in vitro ADP-ribosylation weakens the capacity of Sso7 to complex and to compact DNA.

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2. Materials and methods

2.1. Materials

[^{32}P]NAD$^+$, nicotinamide adenine dinucleotide di(triethylammonium)salt (adenylate-^{32}P), 1000 Ci/mmol, was supplied by GE Healthcare Europe GmbH, Bio-Sciences; DNAase I (EC 3.1.21.1), NAD$^+$, ADP-ribose, phenylmethyl sulfonyl fluoride (PMSF), protease inhibitors and electrophoretic markers (code P1677) were from SIGMA Chemical Company (Milano, Italy).

2.2. Cell culture and homogenate preparation

S. solfataricus strain MT-4 (DSM No. 5833) was grown at 87 °C (pH 3.5) in a 90-L fermenter in standard medium and collected during stationary phase. The crude homogenate was prepared from the collected cells as described in [11]. All protein solutions contained a cocktail of protease inhibitors (2 μg/mL).

2.3. Purification and electrophoresis of PARPSso and Sso7 proteins

PARP*Sso* was purified by a two-step chromatographic protocol [11]. Purification of Sso7 protein started from a 5% perchloric acid extract of sulfolobal homogenate and followed the procedure previously described [9]. Protein concentration was determined according to Di Maro et al. [12].

Homogeneity of the two proteins was checked by SDS–PAGE (12%) and silver staining of the gel [11].

2.4. PARPSso assay and ADP-ribosylation of Sso7 protein

Enzymatic activity of purified PARP*Sso* was assayed at 80 °C in the presence of 0.64 mM [^{32}P]NAD$^+$ (10000 cpm/ nmol) and pure Sso7 protein ranging from 0 to 1.6 (mol/mol), according to Refs. [9,11]. In a duplicate assay at 1:0.5 (mol/mol) PARP*Sso*/Sso7 ratio, after incubation and ice-blocking the reaction, Sso7 and PARP*Sso* were adjusted to 2 μg each in the reaction mixtures, precipitated with ice-cold 20% TCA, freed of NAD$^+$ with several cold ethanol washes, and analysed by SDS–PAGE [9]. Autoradiography of the gel was according to Faraone-Mennella and Farina [9].

Sso7 was ADP-ribosylated endogenously by incubating the sulfolobal homogenate (60 mg/mL; 340 total mg, 40 mU), in 100 mM Tris–HCl, pH 8.0/5 mM NaF in the presence of 0.64 mM [^{32}P]NAD$^+$ (80000 cpm/nmol) in a final volume of 6.5 mL, at 80 °C for 10 min. The reaction was blocked by transferring the mixture on ice and adding 10% perchloric acid (PCA; v/v; 5% final concentration). This treatment solubilized quite exclusively Sso7, with few other small basic proteins (8–10 kDa). After 1 h stirring the sample was centrifuged at 10000 rpm for 15 min and the supernatant collected. Pellet was resuspended in 3 mL of 5% PCA and centrifuged as above. The two supernatants were pooled (5% PCA extract) and proteins precipitated by 20% (v/v) trichloroacetic acid (TCA, final concentration). After centrifugation as above, the acid-insoluble pellet was washed twice with cold ethanol and dried.

2.5. Purification of ADP-ribosylated Sso7

Purification of Sso7 was according to Alvarez-Gonzalez et al. [13] with few modifications. Briefly, the ethanol-washed pellet was suspended in a minimum volume of H_2O and equilibrated in 50 mM 3-(N-morpholine)-propane sulfonate (MOPS), pH 8.2/ 75 mM NaCl by a 2 h-dialysis. The sample was added in batch to phenyl-boronate resin (PROSEP-PB, Millipore) in the same buffer, and gently shaked for 1 h at 4 °C. Phenyl-boronate was transferred to a column (cm 1 × 10) and washed with the same buffer containing 1.0 M guanidine–hydrochloride to remove unmodified proteins. ADP-ribosylated proteins were eluted with the same buffer, pH 5.5, dialysed against H_2O and dried. In order to separate [^{32}P]ADP-ribose Sso7 from other modified proteins, the ethanol-washed eluate was extracted with 5% PCA and centrifuged (Section 2.4). The supernatant was precipitated with cold 20% TCA and the pellet ethanol-washed as above. A small amount of this pellet (3000 cpm) was used to analyse protein-free reaction products by PAGE [14] (data not shown).

2.6. DNA purification and melting curves

DNA was prepared from the homogenate of *S. solfataricus* according to Faraone-Mennella et al. [15]. DNA concentration was determined spectrophotometrically at 260 nm (1 O.D. = 50 μg DNA). DNA recovery was about 2.0 mg/g bacteria.

Melting experiments were performed according to Faraone-Mennella et al. [16] in a Cary 1 spectrophotometer, equipped with a Peltier system (Varian), in sealed 0.5 mL quartz cuvettes. Thermal denaturation of sulfolobal DNA (10 μg) was measured at 260 nm in the presence and absence of both native (0.15–1.5 μg) and ADP-ribosylated (0.1– 0.5 μg) Sso7 in 10 mM sodium phosphate buffer, pH 8.0/5 mM EDTA (final volume 0.5 mL). Temperature increased from 20 °C to 110 °C (3°/min). DNA/protein mixtures were equilibrated 1 min at 20 °C. Temperature was increased 3°/min. Hyperchromicity% was calculated as $A_{(X\,^\circ C)} - A_{(20\,^\circ C)}/A_{(100\,^\circ C)} - A_{(T20\,^\circ C)}$ ratio, ($A_{(X°C)}$, absorbance at 260 nm and temperature X °C).

Buffer and protein solutions were analysed under the same conditions as controls, and negligible temperature-dependent variations were observed.

2.7. Circular dichroism

The circular dichroism spectra of native and ADP-ribosylated Sso7 were determined in a spectropolarimeter (JASCO, mod J-750), equipped with a Peltier system, in the range 190–300 nm with an equal amount (15 μg; 80 μg/mL) of the two proteins in 10 mM sodium phosphate buffer, pH 8.0/5 mM EDTA. Molar ellipticity (Θ) was reported as degree $(cm^2)^{-1}$ decimol^{-1} [15].

Circular dichroism spectra of sulfolobal DNA (15 μg; 30 μg/mL) were determined in the presence and absence of native (5 μg) and ADP-ribosylated (5 μg) Sso7 in 10 mM sodium phosphate buffer, pH 8.0/5 mM EDTA (final volume 0.5 mL), in the range 240–310 nm.

Spectra were determined at a 16 s time constant, a scan rate of 10 nm/min and a slit width of 0.5 nm; three accumulations/ spectrum were performed [16]. In order to ascertain that no change occurred in DNA/protein complexes, each spectrum was monitored three times, at 10 min intervals. The spectra were always overlapping, indicating that the analysed complexes were at equilibrium.

3. Results

3.1. Protein electrophoresis and PARPSso assay

Homogeneity of native PARP*Sso* and Sso7 proteins was checked by SDS–PAGE (12%) and silver staining of the gel (Fig. 1A). Fig. 1B and C shows the autoradiographic pattern of both purified proteins electrophoresed after incubation with 0.64 mM [^{32}P]NAD$^+$ (10000 cpm/nmol) under PARP*Sso* assay conditions (in a duplicate experiment). Most labelling corresponded to Sso7 and a weak radioactivity was associated with PARP*Sso* (automodification).

PARP*Sso* assays, in the presence and absence of native Sso7, gave the results in Fig. 2. PARP*Sso* activity increased 4–5 times in the presence of Sso7 (Fig. 2, columns 1–3), with a maximum at 1:0.5 (mol/mol) PARP*Sso*/Sso7 ratio (Fig. 2, column 3).