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High Stability Binding of Poly(ADPribose) Polymerase-Like Thermozyme From *S. solfataricus* With Circular DNA

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Abstract The poly(ADPribose) polymerase-like thermozyme from the hyperthermophilic archaeon *S. solfataricus* was found to bind DNA with high affinity and non-specifically. Binding was independent of base composition and length of the nucleic acid, and the protein showed a slight preference for the circular structure. By using pCMV-Neo-Bam plasmid as experimental model, the behaviour of the thermozyme upon binding with either circular or linear plasmid was analyzed. pCMV-Neo-Bam has a single HindIII site that allows to obtain the linear structure after digestion with the restriction enzyme. Intrinsic tryptophan-dependent fluorescence of poly(ADPribose) polymerase-like thermozyme noticeably changed upon addition of either circular or linear plasmid, showing the same binding affinity ($K = 2 \times 10^9\ M^{-1}$). However, experiments of protection against temperature and DNase I gave evidence that the thermozyme formed more stable complexes with the circular structure than with the linear pCMV-Neo-Bam. Increasing temperature at various DNA/protein ratios had a double effect to reduce the amount of circular DNA undergoing denaturation and to split the melting point towards higher temperatures. Nil or irrelevant effect was observed with the linear form. Similarly, DNase acted preferentially on the linear plasmid/protein complexes, producing an extensive digestion even at high protein/DNA ratios, whereas the circular plasmid was protected by the thermozyme in a dose-dependent manner. The complexes formed by archaeal poly(ADPribose) polymerase (PARPss) with the circular plasmid were visualized by bandshift experiments both with ethidium bromide staining and by labelling the circular plasmid with ^{32}P. The stability of complexes was tested as a function of enzyme concentration and in the presence of a cold competitor and of 0.1% SDS. From the performed experiments, a number of 3–10 base pairs bound per molecule of enzyme was calculated, indicating a high frequency of binding. The presence of circular DNA was also able to increase by 80% the poly(ADPribose)polymerase-like activity, as compared to 25% activation induced by the linear pCMV-Neo-Bam. J. Cell. Biochem. 85: 158–166, 2002.

Key words: poly(ADPribose)polymerase; thermozyme; DNA-binding; hyperthermophiles; Archaea; *S. solfataricus*

In the Archaea, the study of DNA–protein interactions is an important tool to understand the mechanisms involved in the structural changes of DNA organization, accompanying metabolic processes like DNA repair, transcription, and gene expression and ability to balance the environmental stress induced by the extreme geothermal conditions of their habitats [Marguet and Forterre, 1994; Grogan, 1998, 2000]. Genome integrity must be preserved at any living temperature and with either resting

Abbreviations used: ADPR, adenosine diphosphate ribose; DNase, deoxyribonuclease; EMSA, electrophoretic mobility shift assay; PARP, poly-ADPribose polymerase; PARPss, PARP from *S. solfataricus*; PMSF, phenyl methyl sulphonyl fluoride; SDS, sodium dodecyl sulphate; TBE, Tris-borate buffer/EDTA.

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or metabolically active DNA. The condensed state of the archaeal nucleoid is stabilized by several factors, including a number of structural proteins interacting with the DNA [Reddy and Suryanarayana, 1989; Ronimus and Musgrave, 1995; Pereira et al., 1997].

Archaeal DNA is packaged, at least in part, into a chromatin-like structure with the help of histone-like and non-histone proteins [Reddy and Suryanarayana, 1989; Sandman et al., 1990; Mc Afee et al., 1995; Ronimus and Musgrave, 1995; Pereira et al., 1997]. The DNA binding and nuclease protection properties of histone-like proteins from *Methanothermus* were shown to be consistent with the formation of nucleosome-like structures [Pereira et al., 1997]. In *Sulfolobales*, structural DNA-binding proteins play a crucial role in thermoprotecting the archaeal genome [Reddy and Suryanarayana, 1989; Mc Afee et al., 1995; Ronimus and Musgrave, 1995]. Among these, the most studied Sso7d and Sac7d from *S. solfataricus* and *S. acidocaldarius*, respectively, are chromosomal proteins able to bind with high affinity and to kink DNA independently of base sequence [Agback et al., 1998; Gao et al., 1998; Lopez-Garcia et al., 1998; Robinson et al., 1998].

However, the occurrence of these proteins to stabilize the condensed state of DNA does not address the question of how the genome might be thermoprotected during metabolic events requiring DNA relaxation (DNA replication, transcription, and gene recombination). Endogenous saving mechanisms must be hypothesized, like an efficient repair system, and possibly, post translational modification reactions, already described in this organism [Kennelly et al., 1993; Oxenrider and Kennelly, 1993; Faraone-Mennella et al., 1996].

In particular, the poly-ADPribosylation reaction might be involved in the modulation of structural changes of archaeal nucleoid, like it does in eukaryotes.

The poly-ADPribosylation reaction has a widespread occurrence in mesophyles [Althaus and Richter, 1987; Lowery and Ludden, 1990; De Murcia and Menissier-De Murcia, 1994; Ame' et al., 1999; Jacobson and Jacobson, 1999; Scovassi and Poirer, 1999; D'Amours et al., 1999; Berghammer et al., 1999; Burkle, 2000], and was demonstrated in the archaeon *S. solfataricus* [Faraone-Mennella et al., 1996, 1998, 2000]. The sulfolobal ADPribosylating thermozyme synthesizes, from the pyridinic substrate NAD^+, short oligomers of ADPribose and modifies specific acceptor proteins [Faraone-Mennella et al., 1998].

The interest in such an enzyme is that the eukaryotic counterpart, poly-ADPribose polymerase (PARP), is a DNA binding protein, strictly associated with chromatin and involved in the compaction/relaxation of DNA in the above mentioned metabolic processes, expecially DNA repair [De Murcia and Menissier-De Murcia, 1994; Berghammer et al., 1999; Scovassi and Poirer, 1999]. Targets of PARP are specific ADPR acceptors, including PARP itself, histones, and non histones, which all bind with DNA [De Murcia and Menissier-De Murcia, 1994; Berghammer et al., 1999; Scovassi and Poirer, 1999].

The finding that the ADPribosylating thermozyme shares with PARP the property to interact with homologous DNA, as demonstrated in the precedent article of this issue [Faraone Mennella et al., 2002], suggests that the archaeal protein might play a regulatory and a likely protecting role towards the bacterial nucleoid.

Supporting the possible protecting role, the PARP-like enzyme from *S. solfataricus* does not show DNA specificity, as demonstrated using ss-oligodeoxynucleotides of different length, base composition, and structure [Faraone Mennella et al., 2002].

In order to characterize further the DNA–protein interaction, the pCMV-Neo-Bam plasmid (6550 bp) was chosen as a more physiological and small-sized model [Van den Heuvel and Harlow, 1993]. The plasmid has a single HindIII restriction site and was used either circular or linearized after HindIII digestion. In this study, evidence is provided that PARPss binds both linear and circular extrachromosomal DNA with high affinity. However, the sulfolobal enzyme exhibits a preference for the circular structure of the DNA, producing more stable complexes than with linear plasmid.

MATERIALS AND METHODS

Strain and Growth of Microorganisms

S. solfataricus strain MT-4 (DSM N°5833) was grown at 87°C (pH 3.5) in a 90-L fermenter in standard medium and collected during stationary phase as described by De Rosa and Gambacorta [1975].

Enzyme Purification and Protein Assay

The pure enzyme was obtained from cell homogenate following the purification steps already described in the preceding article of this issue [Faraone Mennella et al., 2002]. Protein concentration was determined by Bradford's method [Bradford, 1976]. Bovine serum albumine was used as standard.

pCMV-Neo-Bam Plasmid

The plasmid used in the experiments of binding was a construct of pCMV-Neo-Bam containing a cdc2 insert at the Bam H1 restriction site [Van den Heuvel and Harlow, 1993].

pCMV-Neo-Bam was linearized by digestion with HindIII (Boehringher; 10 U/μg plasmid) according to the instructions provided by the firm. The reaction was nearly complete and about 90% plasmid was recovered after electrophoresis on 1% low melting agarose gel. For band shift experiments, after ^{32}P-labelling, the linear plasmid was circularized following standard protocols [Sambrook et al., 1989].

Fluorescence

Fluorescence titration measurements were performed on a Perkin-Elmer (mod.LS50B) spectrofluorimeter with 5 nm excitation and 10 nm emission slit widths. Binding titrations were performed with excitation at 295 nm and emission monitored in the range 310–400 nm. Reverse titrations were performed by adding aliquots of concentrate plasmid solutions to a known concentration of protein in a 1 ml fluorescence quartz cuvette. Mixtures (800 μl final volume) were left to stand 1 min before recording the spectra. Nucleic acid concentration was determined by densitometric analysis of the plasmid band obtained after 1% agarose gel electrophoresis [Sambrook et al., 1989], in comparison with that of a standard plasmid of known concentration. Binding parameters were obtained by using a simple, noncooperative model [Mc Ghee and von Hippel, 1974] and applying the following equation:

$$\nu/L = K(1 - n\nu)[1 - n\nu/1 - (n - 1)\nu]^{n-1}$$

where L is the concentration (mol/L) of free ligand; ν, ligand/protein (moles/moles); n, bp number at saturation.

DNA Stabilization

Thermal denaturation studies of plasmid and plasmid/PARPss complexes were performed on a Cary 1 (Varian) spectrophotometer equipped with a Peltier system in sealed 0.5 ml quartz cuvettes. Both linear and circular pCMV-neo-Bam (10 μg/ml) were analyzed in 10 mM sodium phosphate buffer, pH 8.0/5 mM EDTA, at increasing PARPss amounts, from 0 to 30 μg/ml, in a final volume of 300 μl. ΔA_{260} was monitored in a 20–100°C temperature range.

DNA Binding Studies by DNase Digestion

Interaction of PARPss with linear and circular plasmid was determined as effect of DNA protection by the protein after digestion with DNaseI.

Duplicate mixtures (50 μl) were prepared in 20 mM sodium phosphate buffer pH 8.0, containing plasmid (5 μg/ml) and PARPss from 0 to 10 μg. After incubation at 30°C for 10 min to allow the formation of DNA–protein complexes, one of the two aliquots was digested with DNaseI (Sigma, type II, 10 μg/ml) in the same buffer with addition of 17 mM $MgCl_2$ and 30 mM NaCl (final volume 60 μl) for 2 min at room temperature. Reaction was stopped adding 20 mM EDTA. Undigested and digested aliquots of each mixture were dryed and analysed by 1% agarose gel electrophoresis [Sambrook et al., 1989].

Electrophoretic Mobility Shift Assay (EMSA)

PARPss was mixed with circular plasmid (250 ng) in 10 mM sodium phosphate buffer, pH 8.0/5 mM EDTA (final volume 10 μl) and incubated 10 min at 40°C. The mixture was adjusted to 20 mM Tris-HCl buffer, pH 8.0/1 mM DTT/5 mM EDTA/100 μg BSA/ml in a final volume of 20 μl. After incubation for 10 min at 40°C, a loading solution, containing 0.25% bromophenol blue, 0.25% xylene cyanol, and 30% (v/v) glycerol was added to each mixture and the samples were electrophoresed on a 0.8% agarose gel at a constant voltage (4 V/cm) in 220 mM Tris-borate buffer, pH 7.5/6 mM EDTA (0.5 × TBE). DNA was evidenced with UV light after ethidium bromide (0.5 μg/ml) staining.

In one experiment, circular plasmid was ^{32}P-labelled according to standard techniques reported for circular DNA [Sambrook et al., 1989] and purified on Sephadex G-50 column. Plasmid probe ($6–8 \times 10^4$ cpm, 0.05 ng) was used for binding assay in a final volume of 5 μl,

10 mM Na-phosphate buffer, pH 8.00/10 mM EDTA. ^{32}P-plasmid was incubated at 30°C with increasing PARPss. After electrophoresis on 0.5% agarose gel in 0.5 × TBE, the gel was dried and analysed by molecular imager (FX, BioRad) or by exposure to RPV6 films (Amersham/Pharmacia).

Enzyme Activity Assay

PARPss activity (16 mU/ ml; 0.16 μU/assay) was tested incubating samples in 100 mM Tris-HCl buffer, pH 8.00/2 mM NaF, in the presence of 0.64 mM ^{32}P-NAD (Amersham/Pharmacia, 10,000 cpm/nmol) in a final volume of 62.5 μl. Reaction mixtures were incubated at 80°C for 10 min, and TCA-insoluble radioactivity was measured as described in Faraone-Mennella et al. [1998]. At 80°C for 10 min, the substrate was 90% stable.

RESULTS

Changes of PARPss Fluorescence Induced by pCMV-Neo-Bam

Spectrofluorimetric analysis of PARPss in the presence and in the absence of increasing amounts of plasmid is shown in Figure 1.

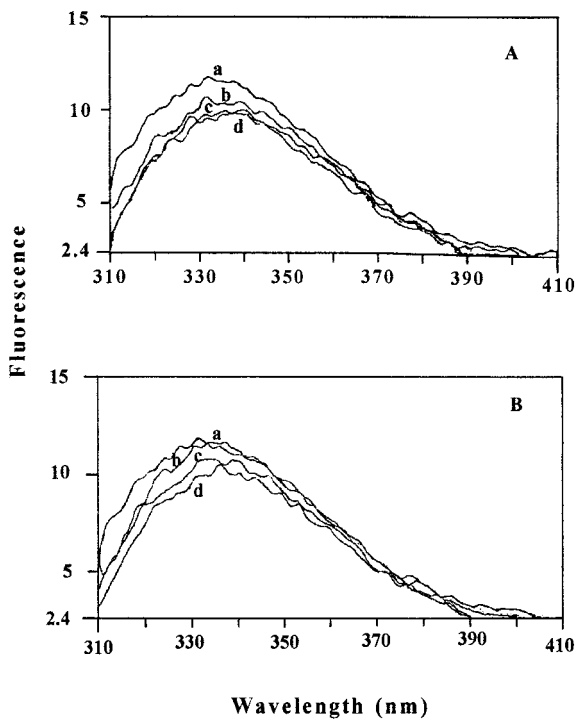

Fig. 1. Fluorescence spectra of PARPss in the presence of circular (**A**) and linear (**B**) plasmid. A: a, PARPss (5 μg); b–d, circular plasmid 160, 320, 480 ng, respectively. B: a, PARPss (5 μg); b–d, linear plasmid 141, 280, 440 ng, respectively.

Upon excitation at 295 nm, emission of the intrinsic fluorescence of PARPss was recorded at 60°C in the range 310–410 nm and showed a maximum around 330 nm, typical of tryptophan residue (Fig. 1A–B, a).

At a fixed protein concentration (5 μg), the first amount of added circular plasmid (160 ng) reduced the intrinsic fluorescence of the protein (Fig. 1A,b). This effect depended on the presence of plasmid that produced changes in the environment of Trp, probably masking fluorescence emission. Increase of plasmid was accompanied by further lowering of fluorescence. Between 320 and 480 ng plasmid, the curves nearly overlapped, suggesting that the last concentration was close to saturation (Fig. 1A, c–d).

Taking into account plasmid concentration giving the maximal effect (480 ng; about 8 bp/protein molecule) and the fluorescence value at 330 nm, the affinity constant was calculated to be $K(M^{-1}) = 2 \times 10^9\ M^{-1}$, in the order of nanomolar.

An analog experiment with the linear plasmid showed a fluorescence change of PARPss comparable to that obtained with the circular plasmid in the same range of plasmid concentration as indicated by the affinity constant, which gave the same identical value as that reported above (Fig. 1B).

Thermostability of PARPss/Plasmid Complexes

The circular plasmid was analyzed at 260 nm and in a 30–100°C range. The thermal denaturation profile of circular plasmid showed a gradual reduction upon addition of increasing amounts of PARPss (Fig. 2A). A lower ΔA_{260} in the presence of PARPss indicated that the protein increased the stability of DNA in a dose-dependent manner. ΔA_{260} was determined at 1:1 and 1:2 DNA/protein ratio and reached a maximal reduction of 30%. Thermoprotection was already exerted at the lowest protein concentration, at about 3 bp/protein molecule (1:1, w/w). Another stabilizing effect of the protein regarded the fraction of plasmid undergoing denaturation (70%). In the presence of PARPss, it gradually melted toward higher temperatures. At 1:2 (w/w) DNA/protein ratio, T_m was splitted to 77°C, with an increase of the melting point of 7–8°C. An analog experiment with the linear plasmid, at the same DNA/protein ratio, showed no relevant reduction of A_{260} and a T_m increase of less than 2°C (Fig. 2B). It is likely that increasing temperature

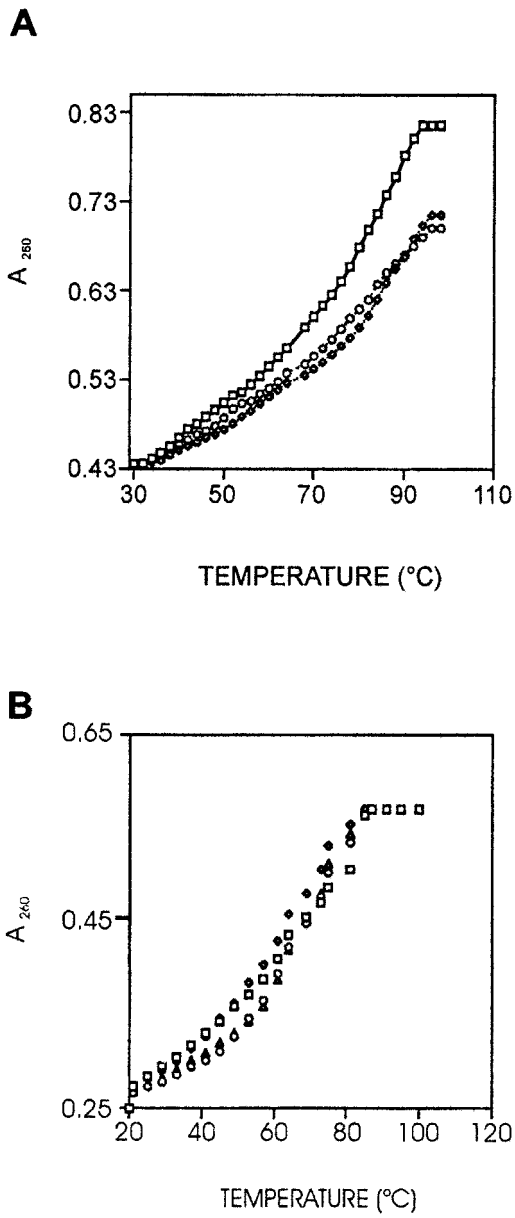

Fig. 2. Thermal denaturation of circular (**A**) and linear (**B**) pCMV-Neo-Bam plasmid (10 μg/ml). In A: PARPss 0 (□), 10 (○), 20 μg/ml (◇). In B: PARPss 0 (□), 3 (◇), 10 (○), 30 μg/ml (Δ). Mixtures were 300 μl final volume.

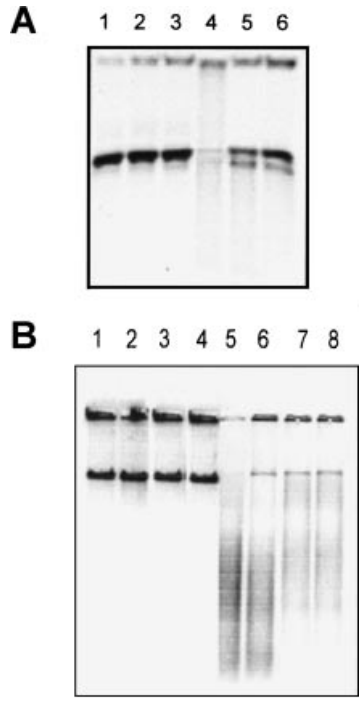

Fig. 3. Agarose (1%) gel electrophoresis of DNase digested and undigested circular (**A**) and linear (**B**) plasmid, in the presence of increasing amounts of PARPss. A: **Lane 1**, circular pCMV (250 ng); **lanes 2–3**, 2 and 4 μg PARPss, respectively; **lane 4**, DNase digested plasmid; **lanes 5–6**, complexes of lanes 2–3 after digestion with DNase. B: **Lane 1**, linear plasmid (250 ng); **lanes 2–4**, 2, 4, and 8 μg PARPss, respectively; **lane 5**, linear pCMV digested with DNase; **lanes 6–8**, complexes of lanes 2–4, after nuclease digestion.

destabilized the binding of PARPss with the linear plasmid.

Nuclease Protection of the Plasmid by PARPss

The DNA–protein complexes were evidenced by measuring the protecting effect of PARPss on plasmid upon digestion with DNaseI (Fig. 3). After incubation of circular pCMV-Neo-Bam with increasing amounts of PARPss under the conditions favouring the formation of complexes, reaction mixtures were digested with DNase, and thereafter, analysed by agarose (1%) gel electrophoresis (Fig. 3A).

The electrophoretic pattern of circular plasmid consisted of two bands migrating very close to each other. The retarded band was the major component (Fig. 3A, lane 1). In the absence of PARPss, plasmid was extensively digested by nuclease (Fig. 3A, lane 4). The intensity of the two bands was highly reduced.

The digestion patterns of the two plasmid/protein complexes (Fig. 3A, lanes 5, 6) showed a lower extent of DNA degradation, enhanced by the highest protein concentration (Fig. 3A, lane 6). Fluorescence of the major band reached the maximal intensity at the highest protein concentration, close to that of undigested samples (Fig. 3A, lanes 2, 3).

The binding of PARPss with the linearized plasmid was analysed under the same experimental conditions. In the absence of PARPss, the single band of the plasmid, after

DNase digestion, showed an extensive fragmentation (Fig. 3B, lane 5). In the presence of protein, fragmentation was limited and a subtle band appeared in correspondence to that in the control (Fig. 3B, lanes 1–4). However, increasing protein concentration did not lead to restore an intensity comparable to that of the same band in the control (Fig. 3B, lanes 1–4).

EMSA

A bandshift experiment was performed with pCMV-Neo-Bam and PARPss electrophoresed on a 0.8% agarose gel and complexes were evidenced by ethidium bromide staining (Fig. 4). In the presence of protein, the probe tended to broad, indicating the formation of heterogenous populations of DNA–protein complexes. At 1:8 (w/w) DNA/protein ratio an intermediate complex appeared (Fig. 4, lane 2), which was definitely formed at 1:16 (w/w) DNA/protein ratio (Fig. 4, lane 3).

Figure 5 shows the results of another experiment of EMSA where the probe was the circular plasmid ^{32}P-labelled and analysed at DNA/protein ratios from 0 to 65 bp/enzyme molecule (lanes 1–4). This experiment was performed incubating PARPss and the plasmid at 25°C for 30 min. PARPss was able to bind with the plasmid, giving rise to a main broad complex, likely including different populations of DNA/protein molecules. This band was highly retarded on 0.5% agarose gel at increasing protein concentration. At 65 bp/enzyme molecule ratio, the intermediate labelling disappeared and complexes were retained on the top of the gel (Fig. 5, lane 4).

Moreover, the DNA–protein complexes were highly thermostable. Lane 5 shows the complexes of lane 3 after exposure to 70°C for 10 min. The intensity of the radioactive bands did not change noticeably by increasing temperature.

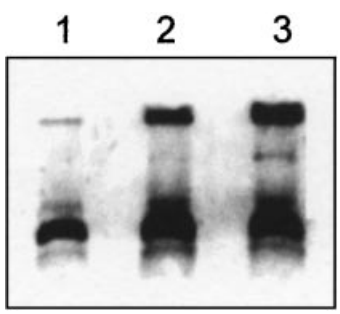

Fig. 4. Band shift of circular pCMV /PARPss complexes. **Lane 1**, circular plasmid (250 ng); **lanes 2–3**, 1:8 and 1:16 (w/w) plasmid/PARPss, respectively. Electrophoresis was performed on 0.8% agarose gel [Sambrook et al., 1989].

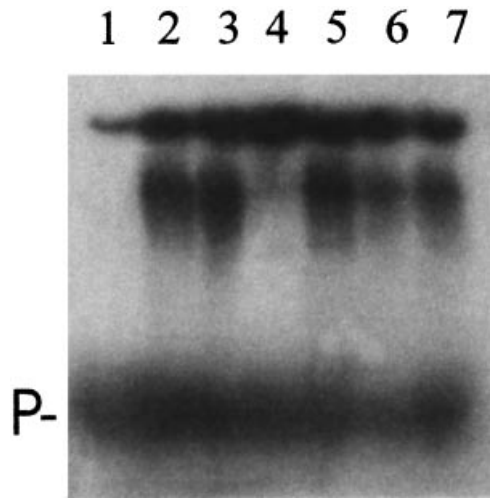

Fig. 5. EMSA of circular ^{32}P-pCMV/PARPss complexes. **Lane 1**, naked ^{32}P-probe (0.05 ng; 6×10^4 cpm); **lanes 2–4**, 0.05, 0.1, and 0.5 μg PARPss. **Lane 5**, ^{32}P-pCMV/PARPss as in lane 3, incubated 10 min at 70°C. **Lane 6**, ^{32}P-pCMV/PARPss as in lane 3, in the presence of cold pCMV-Neo-Bam (0.1 ng). **Lane 7**, as lanes 3, in the presence of 0.1% SDS. P, ^{32}P-probe.

A reduction of complexes was observed when incubation was performed in the presence of a cold competitor (circular plasmid, 0.1 ng) and 0.1% SDS (Fig. 5, lanes 6–7, respectively).

Enzyme Activity

Table I shows enzyme activity in the absence and presence of homologous DNA, circular, and linear plasmid at the nucleic acid concentrations giving maximal activation.

Both DNA and circular plasmid produced a high and comparable increase of enzyme activity, about 100 and 80% by DNA and plasmid, respectively.

A lower effect was observed in the presence of linear plasmid, giving only 25% increase of PARPss activity.

TABLE I. Activation of $PARP_{ss}$ by DNA

Nucleic acid	ng	mU/mg	% activity
—	—	16.0 ± 2	100
DNA from *S. solfataricus*	10	32.8 ± 3	205
Circular pCMV-Bam	50	28.8 ± 1	180
Linear pCMV-Neo-Bam	50	20.0 ± 3	125

DISCUSSION

The non-specific bond of PARPss to DNA, previously described [Faraone Mennella et al., 2002], was further characterized by the present study. The novelty of the results reported in this study consists in the evidence that beside the ability to recognize any DNA, PARPss forms very stable complexes with the circular structure of DNA. In fact, not standing the comparable affinity that PARPss exhibited for both linear and circular plasmids (Fig. 1), thermostability of plasmid/protein complexes strictly depended on the structure of the nucleic acid, and was favoured exclusively by the circular plasmid. This finding is in line with previous results from our laboratory showing that the circular structure of ss-oligodeoxyribonucleotides exhibited a preferential and temperature-dependent role in driving PARPss towards a β-conformation [Faraone Mennella et al., 2002]. Furthermore, the binding of archaeal genomic DNA with PARPss was favoured at high temperatures, where PARPss structure was highly stabilized [Faraone Mennella et al., 2002]. The replacement of ss-oligodeoxyribonucleotides and genomic DNA with pCMV-Neo-Bam plasmid gave the double advantage to work with a physiological and small-sized model, and with a single molecule available in both circular and linear structure, the latter by digestion of the plasmid at the single restriction site for HindIII enzyme.

The present results confirmed that the archaeal thermozyme was able to bind any DNA with no preference for either linear or circular structure. The binding constants determined by protein fluorescence assay gave an identical value, indicating a comparable affinity and a binding site density of PARPss, on both structures, of 3–10 bp/protein molecule (Fig. 1). Beside this high similarity of binding, the question remained whether circular plasmid and PARPss made more stable complexes than the linear pCMV-Neo-Bam was able to do. Experiments of thermodegradation demonstrated unequivocally that protein interaction with the linear structure of plasmid did not modify significantly the melting process, whereas the circular DNA bound with PARPss was more heat-resistant, suggesting that more stable complexes were formed. Furthermore by EMSA, it was demonstrated that complex size changed depending on PARPss concentration. Treatment of PARPss/plasmid at high temperatures (70°C) or with detergents (0.1% SDS) did not alter the stability of complexes. These complexes were also highly stable toward nuclease digestion and stabilization resulted to be dependent on structure, as the linear plasmid underwent a more extensive digestion even at high PARPss concentrations.

The behaviour of archaeal enzyme does not allow a direct comparison with the eukaryotic poly(ADPribose)polymerase. The nuclear protein is well known as a DNA nick sensor as it recognizes and links specifically DNA strand breaks [Althaus and Richter, 1987; Lowery and Ludden, 1990; De Murcia and Menissier-De Murcia, 1994; Ame' et al., 1999; Berghammer et al., 1999; D'Amours et al., 1999; Jacobson and Jacobson, 1999; Scovassi and Poirer, 1999; Burkle, 2000].

In contrast, we have demonstrated that the archaeal PARPss can bind any DNA, independently from sequence, structure and free ends, although it links the circular structure stronger than the linear one.

Beside the evolutionary divergence, it must be considered that the high diversity of habitats for Sulfolobales and Eukaryotes implies different structural and functional needs of the cell.

Thermoprotecting the genome in both the resting or metabolically active state is a main problem for the archaeal cell. Stabilization by structural DNA-binding proteins that maintain DNA condensed is a frequent mechanism in Archaea [Reddy and Suryanarayana, 1989; Ronimus and Musgrave, 1995; Pereira et al., 1997].

However, during metabolic events, DNA is transiently freed from protective structural proteins and is exposed to hydrolytic breakage [Mc Afee et al., 1995; Agback et al., 1998]. Thus, it is likely that hyperthermophiles possess specific mechanisms to regulate the conformational changes of the archaeal nucleoid and to avoid the damage of the nucleic acid, including an efficient repair system [Mc Afee et al., 1995; Agback et al., 1998]. In general, many biochemical processes, such as condensation, maintenance, and control of DNA structure are related to non-sequence specific DNA–protein interactions. Non-specific DNA binding proteins have been evolved to tolerate a wide range of nucleotidic sequences, that they bind with high affinity, and include structural and functional components. The 7 kDa protein family from

Sulfolobales belongs to the former, able to induce negative supercoil stabilizing and condensing DNA [Agback et al., 1998].

What happens during DNA decondensation that accompanies metabolic events is still unclear, and it is unlikely that efficient repair systems can be sufficient to preserve genome integrity at high temperatures. Enzymes intervening in metabolic events occurring along the whole DNA molecule, upon binding DNA, might exert a sort of protection of the nucleic acid [Marguet and Forterre, 1994]. Thus, it cannot be excluded that PARPss could be numbered among these enzymes. In the accompanying article of this issue, we gave evidence that PARPss binds all DNAs with the same affinity. It is a matter of fact that it forms more stable complexes with circular DNA, as demonstrated in the present study. These observations allow to hypothesize that while the thermozyme catalyzes the specific reaction to ADPribosylate acceptor proteins and likely destabilizes their binding with the nucleic acid, it might also cover the decondensing (still circular) region of DNA until metabolic enzymes have access on it. As far as these enzymes start to nick DNA, the interaction of PARPss might be loosened as suggested by the result that the thermozyme forms weaker complexes with linear DNA.

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