Journal of Cellular Biochemistry 85:146–157 (2002)
DOI 10.1002/jcb.10107

Interaction of the ADP-Ribosylating Enzyme From the Hyperthermophilic Archaeon *S. solfataricus* With DNA and ss-Oligo Deoxy Ribonucleotides

Maria Rosaria Faraone-Mennella,[1]* Gennaro Piccialli,[2] Paola De Luca,[3] Sabrina Castellano,[1] Anna Giordano,[4] Daniela Rigano,[5] Lorenzo De Napoli,[6] and Benedetta Farina[1]

[1]Dipartimento di Chimica Biologica, Facolta' di Scienze, Universita' ''Federico II,'' via Mezzocannone, Napoli, Italy
[2]Facoltà di Scienze, Università del Molise, via Mazzini, Isernia, Italy
[3]Istituto di Genetica e di Biofisica (CNR), via Marconi, Napoli, Italy
[4]Istituto di Biochimica delle Proteine ed Enzimologia, CNR, via Toiano, Arco Felice, Napoli, Italy
[5]Dipartimento di Chimica delle Sostanze Naturali, Facoltà di Farmacia, Università ''Federico II,'' via Montesano, Napoli, Italy
[6]Dipartimento di Chimica Organica e Biochimica, Facoltà di Scienze, Università ''Federico II,'' Complesso Universitario di Monte S. Angelo, via Cynthia, Napoli, Italy

Abstract The DNA-binding ability of the poly-ADPribose polymerase-like enzyme from the extremely thermophilic archaeon *Sulfolobus solfataricus* was determined in the presence of genomic DNA or single stranded oligodeoxyribonucleotides. The thermozyme protected homologous DNA against thermal denaturation by lowering the amount of melted DNA and increasing melting temperature. The archaeal protein induced structural changes of the nucleic acid by modifying the dichroic spectra towards a shape typical of condensing DNA. However, enzyme activity was slightly increased by DNA. Competition assays demonstrated that the protein interacted also with heterologous DNA. In order to characterize further the DNA binding properties of the archaeal enzyme, various ss-oligodeoxyribonucleotides of different base composition, lengths (12-mer to 24-mer) and structure (linear and circular) were used for fluorescence titration measurements. Intrinsic fluorescence of the archaeal protein due to tryptophan (excitation at 295 nm) was measured in the presence of each oligomer at 60°C. Changes of tryptophan fluorescence were induced by all compounds in the same range of base number per enzyme molecule, but independently from the structural features of oligonucleotides, although the protein exhibited a slight preference for those adenine-rich and circular. The binding affinities were comparable for all oligomers, with intrinsic association constants of the same order of magnitude ($K = 10^6\ M^{-1}$) in 0.01 M Na-phosphate buffer, pH 8.0, and accounted for a ''non-specific'' binding protein. Circular dichroism analysis showed that at 60°C the native protein was better organized in a secondary structure than at 20°C. Upon addition of oligonucleotides, enzyme structure was further stabilized and changed towards a β-conformation. This effect was more marked with the circular oligomer. The analysed oligodeoxyribonucleotides slightly enhanced enzyme activity with the maximal increase of 50% as compared to the control. No activation was observed with the circular oligomer. J. Cell. Biochem. 85: 146–157, 2002.

Key words: Archaea; poly(ADPribose)polymerase; thermozyme; DNA-binding; ss-oligodeoxyribonucleotides

Abbreviations used: PARP, poly-adenosine diphosphate ribose polymerase; PARPss, PARP-like enzyme from *S. solfataricus*; PMSF, phenyl methyl sulphonyl fluoride.

Grant sponsor: Consiglio Nazionale delle Ricerche (CNR); Grant number: CTB98.01098CT14.

*Correspondence to: Maria Rosaria Faraone-Mennella, Dipartimento di Chimica Biologica, Facolta' di Scienze, Universita' "Federico II," via Mezzocannone, 16-80134 Napoli, Italy. E-mail: faraone@unina.it

Received 1 August 2001; Accepted 30 November 2001

S. solfataricus belongs to the domain of Archaea, which with Eukarya and Eubacteria kingdoms, groups all living organisms [Woese et al., 1990]. Archaea are able to tolerate very extreme conditions in their habitats, as hyperthermophily of acidic hot springs where *S. solfataricus* lives [De Rosa and Gambacorta, 1975]. Neverthless, the chemistry of archaeal macromolecules is not very different from that

of eukaryotic organisms [Barinaga, 1994; Klenk and Doolittle, 1994; Rowlands et al., 1994].

A question to address is which mechanism the cells of hyperthermophilic Archaea use both to preserve the genome against high temperatures and to relax the nucleoid allowing metabolic enzymes to get access to DNA [Peak et al., 1995]. DNA/protein interactions seem to be among the factors involved in the regulation of the nucleic acid metabolism [Marguet and Forterre, 1994]. Several histone-like and non-histone DNA-binding proteins were purified and characterized [Reddy and Suryanarayana, 1989; Ronimus and Musgrave, 1995]. From an evolutionary point of view, some of these proteins are related to eukaryotic histones [Grayling et al., 1994] or non-histones [Bauman et al., 1995] and play structural roles, such as DNA bending at specific sites [Sandman et al., 1994], or catalyse reactions involving DNA [Marguet and Forterre, 1994].

In the hyperthermophilic archaeon *S. solfataricus* a family of small, basic non histone-like proteins (7 kDa) are expressed [Thomm et al., 1982; Grote et al., 1986]. These proteins can form compacted nucleoprotein particles with both double- and single-stranded DNA [Bauman et al., 1995; Agback et al., 1998], and one of them, Sso7d, is able to renature in vitro homologous single strands of the nucleic acid at a temperature at which the spontaneous hybrid is not stable [Guagliardi et al., 1997].

In the last few years, from the hyperthermophilic archaeon *S. solfataricus*, we have purified and partially characterized a highly thermophilic (80°C) and thermostable poly (ADPribose) polymerase-like enzyme [Faraone-Mennella et al., 1996, 1998, 2000] that shares some properties with the thermolabile eukaryotic poly(ADP-ribose) polymerase (PARP; EC 2.4.2.30). This finding, reported for the first time by our group, seems unusual since PARP has been described so far only in Eukaryotes.

PARP modifies specific nuclear proteins with polymers of ADP-ribose, synthesized from NAD^+ by hydrolysing the *N*-glycosidic bond [Althaus and Richter, 1987; De Murcia and Menissier-de Murcia, 1994; D'Amours et al., 1999; Jacobson and Jacobson, 1999]. PARP represents a family of enzymes, different in size and localization, most of which are involved in nuclear events like DNA repair [Jacobson and Jacobson, 1999]. Therefore, they are a class of DNA-binding proteins of various structural complexities as regard specifically the N-terminal domain interacting with the nucleic acid [Althaus and Richter, 1987; De Murcia and Menissier-de Murcia, 1994; D'Amours et al., 1999; Jacobson and Jacobson, 1999]. Human PARP1 (116 kDa), with two zinc fingers, is mainly responsible for enzyme activation by single-stranded DNA nicks. Human and mouse PARP2 (62 kDa) are able to bind DNA, although lacking the zinc-finger module. Their N-termini (aa 1–69) do not contain any obvious DNA-binding motif, but are enriched of basic residues, that might account for this function [Jacobson and Jacobson, 1999].

Previous results gave evidence that the PARP-like enzyme from *S. solfataricus* (PARPss; 46.5 kDa), is close to PARP2 in size and can be purified on DNA-Sepharose column, eluted at 0.5–1 M NaCl [Faraone-Mennella et al., 1998, 2000]. This result suggested a high salt resistance of DNA/protein interaction. Thus, aim of our research was to determine the features of DNA-binding ability of PARPss and whether DNA/protein interactions could be influenced in vitro by the kind of nucleic acid employed.

We chose both genomic DNA, a physiological model, and synthetic single stranded-oligodeoxyribonucleotides. In the present work, we report for the first time the results obtained by studying the effect of archaeal DNA and such oligomers, that differed by length, structure, and base composition, on DNA-binding properties of the ADPribosylating enzyme purified from *S. solfataricus*.

MATERIALS AND METHODS

Materials

PMSF, β-NAD, agarose, DNase I, and RNase A (type XIV) were purchased from SIGMA. [^{32}P-adenylate] NAD (1,000 Ci/mmol) and Sephacryl S-100 were products of Amersham-Pharmacia (U.K.). DNA-Sepharose was prepared according to Faraone-Mennella et al. (1998). SP6 promoter sequence (oligomer 3) was purchased from TIB-Molbiol (Centro Biotecnologie Avanzate, Genova, Italy).

Bacterial Strain

S. solfataricus strain MT-4 (DSM N5833) was obtained from Laboratorio di Fermentazioni of Istituto di Chimica M.I.B. (CNR, Arco Felice, Italy) directed by Dr. Agata Gambacorta. The

microrganism 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 Isolation and Purification

The purified enzyme was prepared from cell homogenate following the purification steps previously described [Faraone-Mennella et al., 1998, 2000]. The previous procedures were slightly modified in order to optimise the yield of pure PARPss and avoid the use of detergents and a time-consuming salt fractionation. Briefly, the crude homogenate (10 g cells; 60 mg protein/ml) was digested with DNase I in 30 mM Tris-HCl (pH 8.0), containing 0.15 M NaCl/0.1 mM PMSF/10 mM $MgCl_2$ at 37°C for 30 min. After addition of RNase A (20 μg/ml), incubation was carried on for further 30 min and the reaction was stopped adjusting the mixture to 20 mM EDTA. The following operations were carried out at room temperature. After centrifugation at 9,800*g* for 15 min, enzyme activity of the supernatant (DNase supernatant) was 32-times increased as compared to the homogenate. The supernatant was diluted to 10 mM Tris-HCl (pH 8.0)/0.05 M NaCl, adjusted to 0.1 mM PMSF and loaded onto a Sephacryl S-100 column (cm 4.5×80), which had been equilibrated in the same buffer. Flow rate of the column was 15 ml/h and 3 ml fractions were collected. Absorbance was read at 280 nm in a Cary 1 spectrophotometer (Varian) and enzyme activity was assayed as described below. The active fractions, eluted at a volume corresponding to a molecular mass of 50 kDa (fractions 40–57; 71% mU), were pooled and loaded onto a DNA-Sepharose column (5 mU/ml resin), which had been equilibrated with 10 mM Tris-HCl buffer (pH 8.0). The column was washed first with the equilibrium buffer and then with the same buffer containing 0.2 M NaCl, until $A_{280} = 0$. The elution was performed with 1 M NaCl in the same buffer. The active fractions (0.8 ml each) were pooled and stored at 4°C. The purity of enzyme was checked by SDS–polyacrylamide (12%) gel electrophoresis (Fig. 1). When needed, the fractions were dyalised against H_2O before use.

Enzymatic Activity Assay

The standard incubation procedure and the thermostability controls were described previously [Faraone-Mennella et al., 1998]. The reaction mixture contained PARPss (0.16 μU) in 100 mM Tris-HCl, pH 8.0/5 mM NaF and 640 μM [^{32}P] NAD (10,000 cpm/nmol), in a final volume of 62.5 μl. The activity was routinely assayed in sealed vials at 80°C and pH 8.0 for 10 min and determined as 20% trichloroacetic acid-insoluble radioactivity. Specific enzyme activity was calculated from the initial rates and expressed in mU/mg protein. One unit of enzyme was defined as the amount of PARPss required to convert 1 μmol of NAD/min under standard conditions (80°C and pH 8.0). At 80°C, NAD was 90% stable for 10 min incubation.

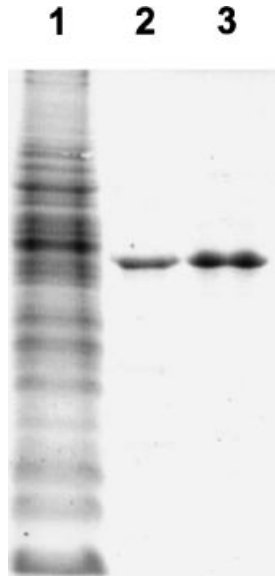


Fig. 1. SDS–PAGE of the ADPribosylating enzyme from *S. solfataricus*. **Lane 1**: DNase supernatant (50 μg); **lanes 2–3**, enzyme purified on DNA-Sepharose column, 2 and 5 μg, respectively. Coomassie blue (0.25%) staining.

Melting Curves

Thermal denaturation profiles of DNA in the absence and presence of protein were obtained by heating DNA in 0.5 ml 10 mM Na-phosphate buffer, pH 8.0/5 mM EDTA. Absorbance at 260 nm was read in a spectrophotometer Cary 1 (Varian) equipped with a thermoprogrammer. The rate of heating was 3 deg/min. The DNA/protein mixtures were left 1 min at starting temperature before heating. Separately blank and protein solutions were analysed under the same conditions, and negligible temperature-dependent variations were observed.

Synthesis of Oligodeoxyribonucleotides

The syntheses of oligos 1, 2, 4, 5 were performed on a Millipore Cyclone Plus automatic

DNA synthesizer following a standard phosphoramidite procedure and the oligomers 6–8 were prepared according to the previously reported solid phase procedure by De Napoli et al. [1995]. Analyses and purifications of the products were performed by HPLC on a Partisil 10 SAX column (Whatman, 250×4.6 mm, 10 μm), using linear gradients of KH_2PO_4 (20% CH_3CN, pH 7) from 1–350 mM. The collected peaks were desalted by gel filtration on a Biogel P2 column eluting with H_2O. A purity control on the isolated products was carried out by HPLC analysis on a Partisphere RP18 analytical column (Whatman, 125×4 mm, 5 μm) using a linear gradient (from 5 to 40%) of CH_3CN in 0.1 M aq. triethylammonium bicarbonate buffer (pH 7.0, flow rate 0.8 ml/min, detection at 260 nm).

Quenching of PARPss Intrinsic Fluorescence Upon Binding to DNA

Binding of DNA to the enzyme was measured by this assay in a reaction mixture (0.8 ml) containing the protein (10 μg/ml) in 10 mM Na-phosphate buffer, pH 8.0, with increasing amounts of single stranded oligodeoxyribonucleotides, added in 1–2 μl portions. The mixture was left to stand for 1 min, before monitoring the spectrum. The spectrum of each oligomer in the absence of PARPss was recorded as a control. The data represent mean values from three-five different experiments.

The excitation at 295 nm allowed us to specifically select the fluorescence emission due to tryptophan. The decrease in fluorescence intensity was measured in the range 300–400 nm at 60°C by a Perkin-Elmer spectrofluorimeter (mod. LS50-B). The data were corrected for dilution (max 5% of initial volume with the last addition). Artifacts due to inner filter were ruled out by titrating a control solution of *N*-acetyl-tryptophanamide.

The value at the wavelength (330 nm), corresponding to the maximal fluorescence emission of the protein in the absence of the ligand, was taken to draw the curves of ΔF versus oligonucleotide doses. The abscissa are given as 1–(F/Fo) at 330 nm, where Fo was the fluorescence of the protein and F that of the same solution of protein plus the oligonucleotide. The association constants were calculated from the curves applying the equation of Mc Ghee and von Hippel [1974] for non-cooperative binding. The number of bases per enzyme molecule at which saturation was observed by each oligomer was taken as the number of total binding sites.

Circular Dichroism

CD spectra were measured at different temperatures by using a spectropolarimeter (JASCO 750), equipped with a Philips computer, and 1 mm path-length quartz cuvette.

The conditions were a 16 s time constant, a slit width of 0.5 mm, and a scan rate of 10 nm/min (three accumulations/spectrum). CD spectra of homologous DNA, in the absence and presence of the protein, were performed in 10 mM Na-phospate pH 8, in the range 260–300 nm, setting the spectropolarimeter as above. The mixtures were left 10 min at the temperature of analysis before monitoring the spectra. A mean base pair weight of 660 was used to calculate molar ellipticity values of DNA. In order to ascertain that no change occurred in the formed DNA/protein complexes, monitoring of triplicate spectra was repeated three times, at 10 min intervals. The spectra were always overlapping, indicating that the analysed complexes were at equilibrium.

CD spectra of PARPss were recorded at both 20 and 60°C over 190–260 nm range. Protein solutions were prepared 0.08 mg/ml in 10 mM Na_2HPO_4, pH 8.0. Ellipticity values were converted into molar ellipticity by computational calculation (Θ; degrees $\cdot$ cm^2 $\cdot$ dmol^{-1}) on the basis of the protein molecular weight obtained from amino acid composition.

CD spectra in the presence of increasing amounts of oligonucleotides were performed as above. The mixtures were left 1 min at the temperature of analysis before monitoring the spectra.

Enzyme Kinetics With Oligonucleotides

The oligodeoxyribonucleotides were used in a range between 1 to 15 pmoles (up to 100 bases/protein molecule) with a fixed enzyme amount (0.16 μU). The addition of different compounds to the reaction mixture was as reported under Results. The data were mean values of 5–7 experiments in duplicate. Percent errors were calculated to be below 5% ($P = 1$).

Other Procedures

Protein determination and SDS–PAGE were performed as described previously [Faraone-Mennella et al., 1998]. DNA content was determined with the diphenylamine method

[Burton, 1968], by A260 and by 1% agarose gel electrophoresis [Sambrook et al., 1989]. ^{32}P-labelling of calf thymus DNA and DNA-binding filter assay were performed as described by Sambrook et al. [1989].

RESULTS

DNA-Protein Binding

The ability of the archaeal poly-ADPribose polymerase-like enzyme (PARPss) to bind DNA in vitro was analysed by using a physiological model like DNA from *S. solfataricus*.

Figure 2 depicts thermal denaturation profiles of DNA from *S. solfataricus* in the absence and presence of bound PARPss. Homologous circular DNA was thermally unstable above 60°C under the conditions of the experiment. Addition of scaled-up amounts of protein increased the thermal stability of DNA. Denaturation profiles showed melting points shifted toward high temperatures and Δ260 gradually decreased because lower amounts of DNA underwent denaturation as a consequence of protein thermoprotection. At 38 μg/ml PARPss irrelevant DNA denaturation was observed.

A further evidence that PARPss binds with DNA was obtained by circular dichroism analysis (Fig. 3). In the 240–310 nm range, naked DNA showed a high positive ellipticity, and a cross-over point shifted toward lower wavelengths (below 250 nm) due to a more relaxed conformational state of the nucleic acid. Upon addition of increasing amounts of PARPss, the positive ellipticity tended to be reduced and the cross-over point splitted towards higher wavelengths as a consequence of a more compact organization of the nucleic acid induced by the protein. The effect was dose-dependent and highly influenced by the temperature. At 90°C, positive ellipticity of naked DNA increased up to a maximum, around 280nm, that is more than three times higher than the corresponding value at 40°C (Fig. 3). At 1:0.2 (w/w) DNA:protein ratio ellipticity decreased from 10 to about 6 with a ΔΘ six times higher than the maximal

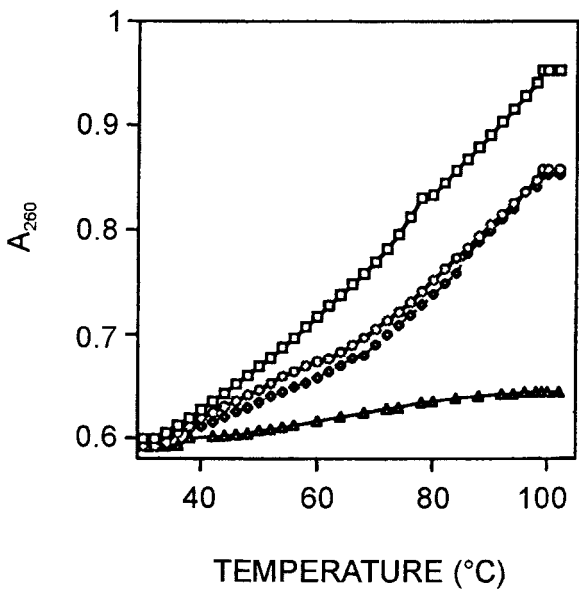


Fig. 2. Melting curves of archaeal DNA. *S. solfataricus* DNA (25 μg/ml) in the absence (□) and in the presence of 6(○), 12 (◇), and 38 (Δ) μg/ml PARPss. Reaction mixtures were in 10 mM Na-phosphate buffer pH 8.0/5 mM EDTA. A_{260} was measured in a Cary 1 spectrophotometer (Varian), equipped with a Peltier system. Temperature increase was 2 deg/min.

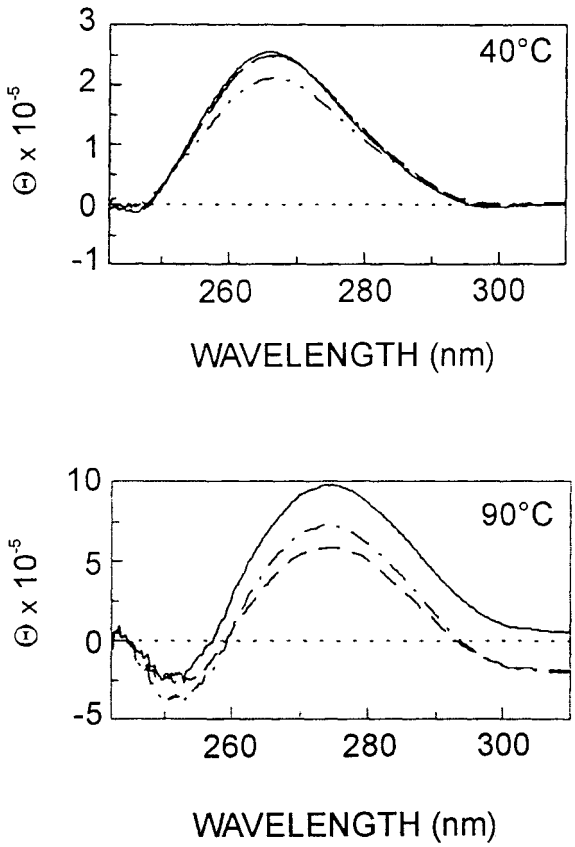


Fig. 3. Circular dichroism spectra of archaeal DNA at different temperatures. The spectra were recorded in the 242–310 nm range. Molar ellipticity is expressed as degrees × cm^2 ×$dmol^{-1}$. The experiments were performed in 10 mM Na-phosphate buffer, pH 8.0/5 mM EDTA (final volume 0.3 ml). DNA (___) was 70 μg/ml; PARPss was, at 40°C, 14 μg/ml (— —) and 140 μg/ml (—. . —. . —); at 90°C, 14 μg/ml (— . — . —), and 140 μg/ml (– – – –).

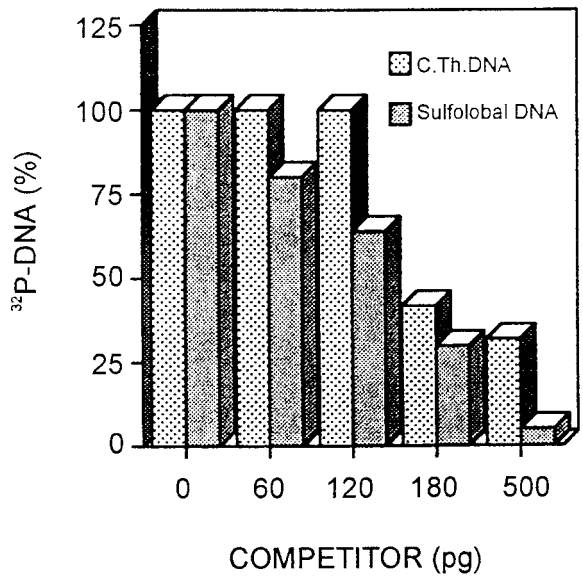


Fig. 4. Homologous and heterologous DNA binding filter assay. Standard reaction mixtures contained random priming ^{32}P-labelled calf thymus DNA (0.06 ng) digested with Eco-RI [Sambrook et al., 1989], and PARPss (50 ng) in 10 mM Na-phosphate buffer, pH 8.0/5 mM EDTA, in the presence of cold competitors (DNA from calf thymus and *S. solfataricus*) from 0 to 500 pg. After 5 min incubation at 30°C, the mixtures were filtered through nitrocellulose and the filters, washed three times with the incubation buffer, and suspended in a scintillation liquid. Radioactivity was measured in a liquid scintillation counter (Beckman mod LS 1701). The reported values represent mean of three different experiments. Percent error was below 5% for all samples.

reduction at 40°C reached at a 1:2 (w/w) DNA:protein ratio.

In order to obtain evidence that the archaeal enzyme was able to bind DNA from a eukaryotic source, we chose the nitrocellulose filter assay to perform competition experiments (Fig. 4). The ^{32}P-probe was calf thymus DNA digested with Eco-RI, and cold competitors were calf thymus and *S. solfataricus* DNA. Both nucleic acids displaced the probe from the enzyme, with an expected higher efficiency of the archaeal DNA, able to replace almost 100% ^{32}P-probe at the maximal DNA amount added.

DNA influenced also enzyme activity and doubled the value measured in the absence of nucleic acid (data not shown).

Fluorescence Titrations With ss-Oligonucleotides

The intrinsic fluorescence of PARPss was quenched upon binding of synthetic oligonucleotides to the protein, as a consequence of conformational changes modifying the environment of tryptophans upon excitation at 295 nm [Chen et al., 1969]. Hence, the formation of DNA/protein complexes was measured by this assay.

The single stranded oligodeoxyribonucleotides used for the binding assay and their features are listed in Table I. The compounds 1, 5, and 6 are very close in length, ranging from 12 to 16 nucleotides, but they differ in their base composition. Oligonucleotide 5 is enriched in adenine, whereas the oligomer 6 is completely devoid of this base and contains only cytosine and thymine in a 1:1 ratio. Oligonucleotide 1 has a more uniform composition of the four bases. Oligomers 2 (21-mer) and 5 (12-mer) are the truncated forms of oligonucleotide 4 (24-mer), and have similar adenine percentages. The three oligomers 6, 7, and 8 share identical base composition (50% C and 50% T), lacking adenine and guanine, and are different both in length (from 16- to 24-mer) and shape (linear and circular). Oligomer 3 corresponds to a promoter sequence.

The fluorescence spectra were monitored at a temperature (60°C) that favoured the structuring of PARPss [Faraone-Mennella et al., 2000]. At this temperature no secondary structure of oligonucleotides occurred, i.e., no hairpin formation was favoured. The binding of PARPss to all oligonucleotides was associated to a significant quenching of the intrinsic fluorescence of

TABLE I. List and Properties of Single Stranded Oligodeoxyribonucleotides

Oligo	Sequence (5′→ 3′)	−mer	% A	% T	% G	%C	Shape	K (M^{-1}) × 10^6
1	GGCTTGGGCAAAACT	15	27	20	33	20	Linear	6.2
2	AAAAAGAAATCAAGAAAATCA	21	70	10	10	10	Linear	4.8
3	CATACGATTTAGGTGACACTATAG[a]	24	33	29	21	17	Linear	4.9
4	AAAAAGAAATCAAGAAAATCATCT	24	63	17	8	12	Linear	4.8
5	AAAAAGAAATCA	12	75	8.3	8.3	8.3	Linear	4.8
6	HO-T-$(CT)_7$-C-PO_4^{-2}	16	—	50	—	50	Linear	4.7
7	HO-T-$(CT)_{11}$-C-PO_4^{-2}	24	—	50	—	50	Linear	4.9
8	c(CT)12	24	—	50	—	50	Circular	4.7

[a]SP6 promoter sequence.

the tryptophans, with comparable results for oligonucleotides of similar length or base composition. As an example, the spectra of the enzyme binding to various amounts of oligonucleotides 1 and 2 are shown in Figure 5. The fluorescence of tryptophan, excited at 295 nm, was quenched, at 330 nm, upon binding of the oligonucleotide, up to a maximum value of 50 and 60%, respectively, and the maxima of emission spectra were slightly shifted to lower wavelengths.

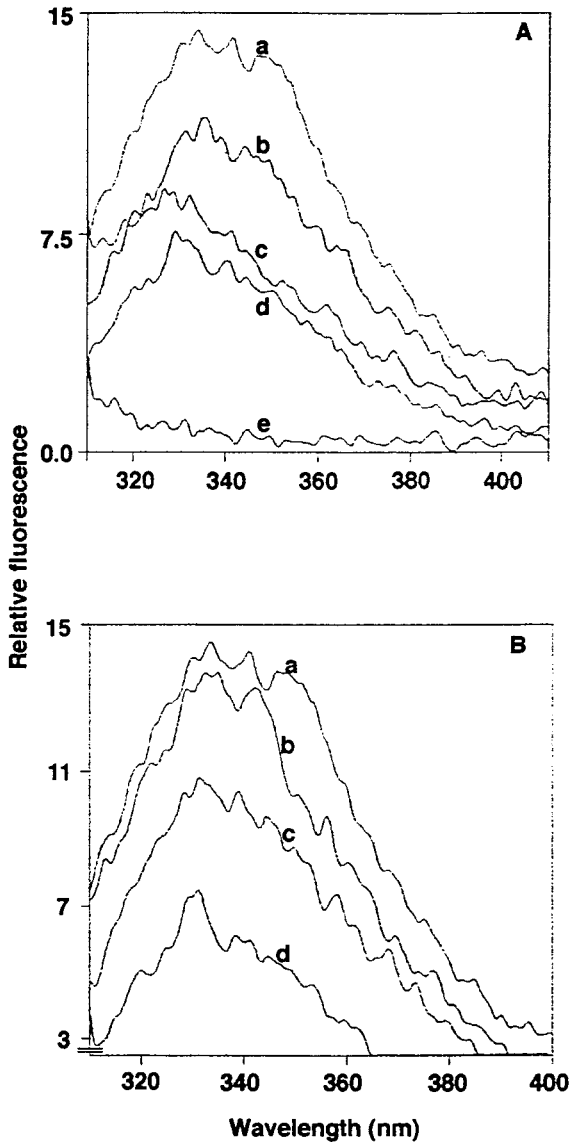


Fig. 5. Effect of oligomers 1 (**A**) and 2 (**B**) on the fluorescence spectrum of purified PARPss at 60°C. **a**: spectrum of the enzyme (10 μg/ml) in the absence of ligand. In A: oligomer 1 has been added to the protein at a concentration of 60 (**b**), 122 (**c**), 488 (**d**) pmoles in a final volume of 0.8 ml. (e) the spectrum of oligomer 1 at 488 pmoles/0.8 ml. In B: oligomer 2 added at a concentration of 9 (**b**), 18 (**c**), 30 (**d**) pmoles in a final volume of 0.8 ml. The indicated concentrations correspond to the base/enzyme molecule ratios reported in Figure 6.

In order to study the equilibrium binding of PARPss with each oligonucleotide, the titrations were performed keeping the protein concentration constant (2, 12 μM). The results were grouped taking into account the similarity of oligonucleotides regarding base composition, length, and structure (Fig. 6).

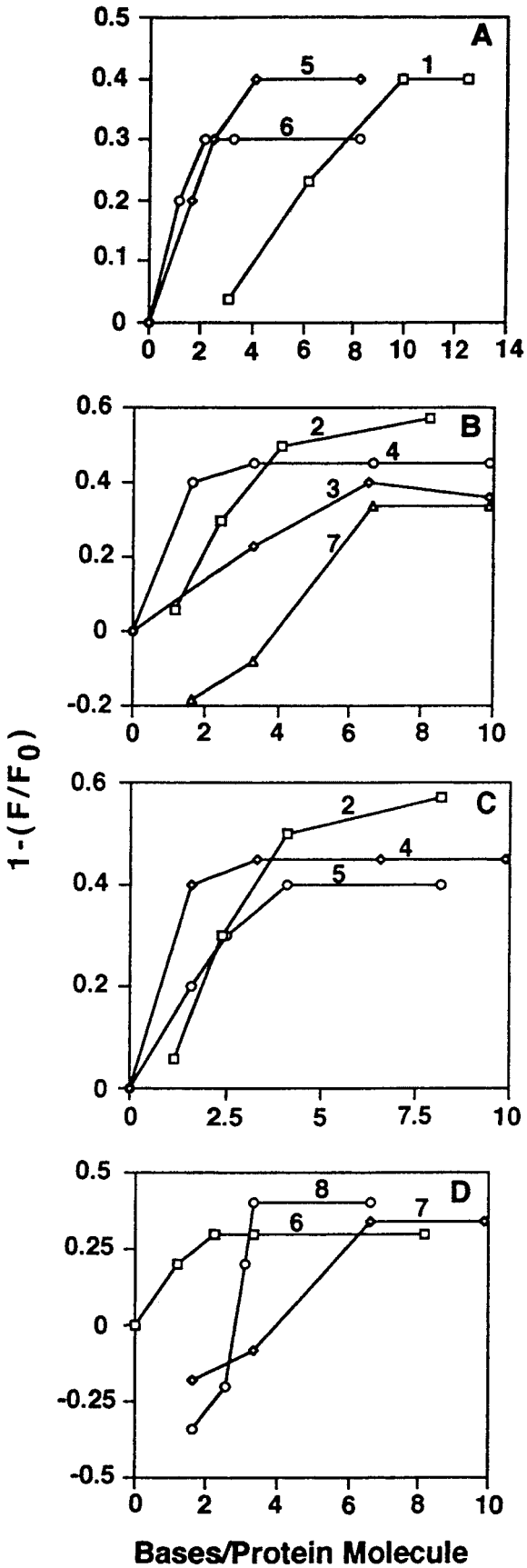
In Figure 6A, the curves of reverse fluorescence changes for short oligomers (oligonucleotides 1, 5, and 6) are shown. The ΔF was comparable for oligos 1 and 5, although with the latter the maximum value occurred at a 4–5 time lower DNA/protein ratio. The curve obtained with oligomer 6 showed a behaviour similar to that of oligomer 5, but with different decreases in fluorescence.

Figure 6B depicts the titration curves in the presence of four oligonucleotides longer than those in Figure 6A (21–24-mer) and heterogenous in base compositions, i.e., oligomers 2, 3, 4, and 7. The binding profiles showed saturation at an approximate concentration of 1:6 protein:DNA bases for oligomers 3 and 7, and 1: 2.5–3.0 for compounds 2 and 4. This difference might account for a rather higher affinity of the protein towards the oligomers enriched in adenine (Table I).

The comparison of the curves obtained for oligonucleotides with similar base composition and different length (oligomers 2, 4, and 5) is shown in Figure 6C. All three compounds induced the maximal quenching of fluorescence at a DNA: protein ratio very close to each other, with a slightly lower ΔF for oligomer 5.

Figure 6D shows the extreme situation regarding the three oligomers 6, 7, and 8 with identical base composition (50% C and 50% T), which differ both in length and shape. The maximal decrease of fluorescence was comparable, although slightly higher with oligo 8, but saturation occurred at different DNA: protein ratios (1: 2–3 for compounds 6 and 8; 1:6 for oligomer 7).

The affinity of PARPss to the various oligonucleotides was quantified calculating the intrinsic association constants (Table I). These values were obtained from the maximum binding site density measured for each oligonucleotide that ranged between 2 and 10 bases/enzyme molecule. The order of magnitude was the same for all constants ($K = 10^6\ M^{-1}$).



Modulation of Enzyme Activity by Oligonucleotides

PARPss activity was assayed in the presence of oligonucleotides in the range 160–240 nM, at fixed enzyme concentration (3.3 nM, corresponding to 0.16 μU/assay). Figure 7 depicts the maximal activation induced by each oligo at the given base/protein molecule number.

In the presence of ligands, PARPss activity was not stimulated to a large extent. The maximal level (80% increase) was reached only with oligomer 1 (Fig. 7). The lowest effect (no activation) was observed with oligomers 6, 7, 8 (Fig. 7), which differ in length (oligo 6 and 7) and in structure (oligonucleotides 7 and 8 are linear and circular, respectively), but have in common a peculiar base composition, made only by cytosines and thymines (Table I).

Generally the increase of enzyme activity ranged between 100% (no activation) and 180%, occurred at higher oligomer concentrations than those needed for binding, and seemed to be affected by both base composition and length (Fig. 7).

Circular Dichroism Analysis

The effect of ss-oligonucleotides on the secondary structure of PARPss was investigated by circular dichroism. The spectra were monitored at 20 and 60°C (Fig. 8). At 60°C (Fig. 8A,b), the enzyme showed to be better organized in the secondary structure than at 20°C (Fig. 8A,a). The shape of the spectrum was more regular and the negative ellipticity reached lower values between 210–225 nm. The effect induced by oligonucleotides was quite common to all compounds analysed. In particular, the minimum of ellipticity became more negative and tended to be centered in correspondence to a specific wavelength, indicating a further stabilization of the secondary structure towards a β-conformation.

As a representation, Figure 8 shows the spectra of PARPss with oligomer 1 (Fig. 8A) and oligomers 6, 7, 8 (Fig. 8B,C). The behavior of

Fig. 6. Reverse titrations of PARP-like enzyme with different oligodeoxyribonucleotides at pH 8.0 (0.01 M Na-phosphate buffer) and 60°C. The site size ranges between 2 and 10 bases, maximal quenching is 80%, and association constants are 4–6 × 10^6 M^{-1}. The curves have been grouped to show the effect of short (**A**), long (**B**), base (**C**), and shape-differing (**D**) oligomers. The numbers refer to the oligonucleotides listed in Table I.

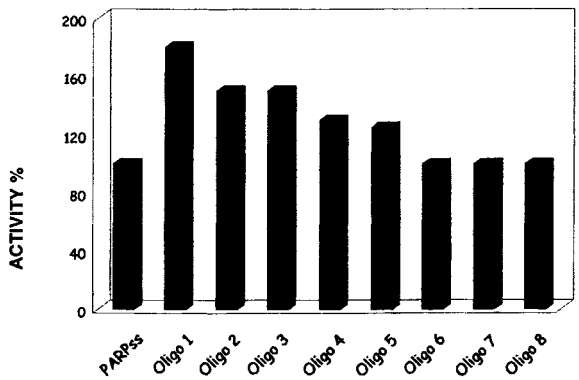


Fig. 7. Activity of PARPss in the presence of ligands. The numbers refer to the oligonucleotides listed in Table 1. The enzyme (0.16 μU) was assayed as reported in Materials and Methods. The addition of different compounds to the reaction mixture was as reported under Results. Data refer to the maximal activation measured and are mean values of 5–7 experiments in duplicate. Percent errors were calculated to be below 10% ($P=1$). Bases/protein molecule were: oligos 1, 2, 5, 50; oligos 3, 4, 6, 7, 8, 100.

PARPss in the presence of oligomer 1 was representative of the results obtained with most oligonucleotides.

The comparison of the oligomers 6, 7, and 8 (identical base composition) in Figure 8B shows their behaviour dependent on length and shape. With oligonucleotide 1, the negative ellipticity increased by comparable values either at 20°C (Fig. 8A,d) or at 60°C (Fig. 8A,c), although the shape of the spectra was slightly different. In the presence of oligomer 8, the temperature seemed to exert a higher influence. At 20°C (Fig. 8B,d), the minimum was centered at 220 nm and the ellipticity reached more negative values. At 60°C (Fig. 8B,c), the effect of oligomer 8 was lower as compared with the spectrum of native PARPss. The change of negative ellipticity was more pronounced at 20°C than at 60°C probably because at 60°C, the native protein was already well organized and oligomer 8 exerted a lower effect. A comparison

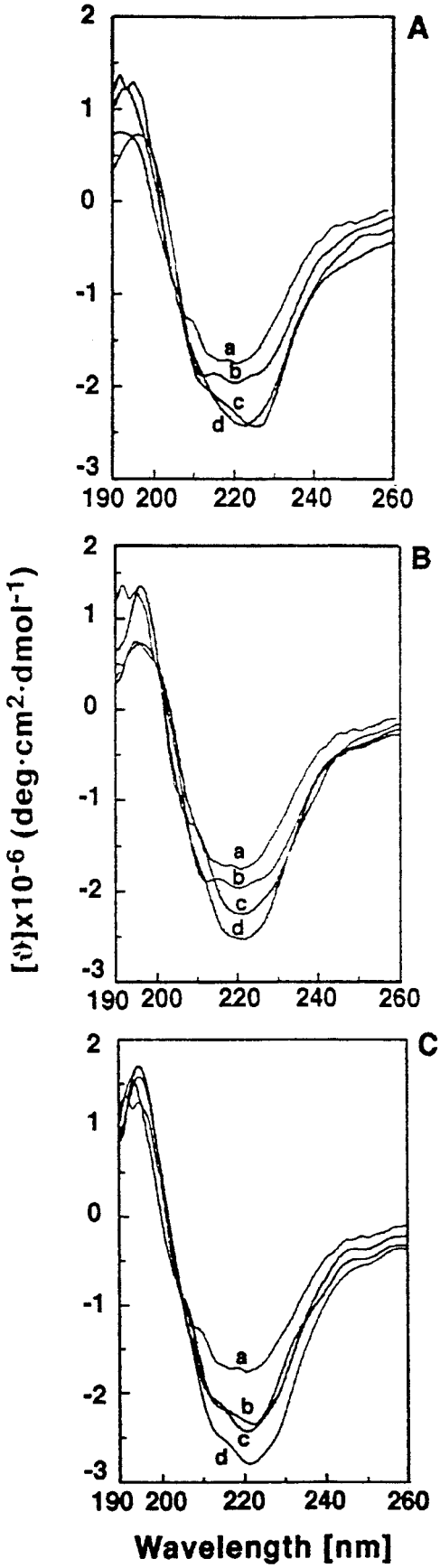


Fig. 8. Circular dichroism spectra of PARPss with different oligomers at 20 and 60°C. **A**: The enzyme was analysed in the absence (**a,b**) and presence (**c,d**) of oligomer 1 (200 nM), at 20°C (a,d) and 60°C (b,c). **B**: CD spectra of the enzyme (**a,b**) modified upon addition of the circular oligomer (**c**, 20°C; **d**, 60°C), at a concentration of 200 nM. **C**: At 20°C, the maximal effect of structure stabilization seems to be due to the circular shape of oligomer 8 (d). At the same base/enzyme molecule ratio, oligomers 6 (b) and 7 (c) induce a similar change of negative ellipticity. CD spectrum of the purified protein were measured over 190–260 nm range as described under Materials and Methods.

with oligonucleotides 6 and 7 revealed that oligomer 8 was the most effective (Fig. 8C). In fact, although the length of oligos 6 and 7 was different, both induced the same conformational changes and to the same extent as demonstrated by the identical spectra (Fig. 8C). The superimposition of oligomer 8 revealed a deeper negative minimum, when added at the same concentration of oligonucleotide 7 (the same length and base composition as oligomer 8, but linear).

The general effect by all analysed oligos was to stabilize the structure of PARPss and to induce a β-like conformation.

DISCUSSION

In this paper, for the first time, we present evidence that the PARP-like enzyme from *S. solfataricus* exhibits a non-specific binding with DNA.

The sulfolobal poly-ADPribose-like thermozyme, purified and characterized in our laboratory, shares some features with the eukaryotic PARP, including the ability to interact with DNA [Faraone-Mennella et al., 1998]. Eukaryotic PARP plays a main role in many nuclear functions involving the regulation of the conformational state of chromatin, and is able to recognize breaks in DNA, promoting the DNA repair [Jacobson and Jacobson, 1999]. Like the mesophilic counterpart, PARPss might be involved in the mechanism of condensation/decondensation of the archaeal nucleoid. This observation raises from previous results that the DNA-condensing 7kDa protein from *S. solfataricus* is in vitro ADPribosylated by PARPss [Faraone Mennella and Farina, 1995].

The previous evidence that the archaeal enzyme had affinity for DNA as it binds to the DNA-Sepharose column was confirmed by the present results. The experiments reported in this study showed that DNA/protein complexes were formed both with circular (homologous) and linear (heterologous) DNA, and that the binding of PARPss with homologous DNA was temperature-dependent. This finding is not surprising, since the protein was better structured at a high temperature and it is likely that a correct conformation favoured its binding with DNA. Such a hypothesis is supported by the result that at 90°C, lower amounts of protein were needed to reach a large extent of DNA compaction.

Moreover, in this study, we reported the results that the enzyme from *S. solfataricus* was able to bind also to single stranded oligodeoxyribonucleotides at a temperature (60°C), where the compounds do not have any secondary structure and independently from their base composition, length, and shape. They revealed a "non-specific" way of DNA/protein interaction. The intrinsic binding constants had the same order of magnitude (10^6 M^{-1}) for all oligomers and were comparable to the values reported for other DNA-binding proteins from *S. solfataricus* [Mc Afee et al., 1995]. The number of bases per enzyme molecule ranged between 2 and 10, demonstrating a high frequency of binding, although the protein showed a slight preference for some features of oligomers, like the circular shape.

The structure of PARPss was deeply influenced by the presence of oligonucleotides. These compounds induced a strong quenching of the fluorescence, but the positions of the fluorescence maxima at increasing concentrations of oligodeoxyribonucleotides indicated a hydrophobic environment of tryptophans. The blue shift of the fluorescence maxima pointed to a more pronounced exclusion of the solvent or to a more effective stacking of aromatic residues.

Although the CD spectrum of PARPss in the peptide region 190–260 nm was typical for $\alpha + \beta$ proteins, upon addition of oligodeoxyribonucleotides the dichroic spectra reflected pronounced conformational changes of the archaeal enzyme (Fig. 8). The shape of the curves indicated a reduction of the α-helix content and showed to change toward a typical β-conformation. These results clearly suggested that a conformational adaptation of PARPss occurred upon interaction with DNA. However, the structurally modified protein showed 100% activity, or, with some oligomers, was slightly activated. Thus, in the presence of oligonucleotides, the structural change of the enzyme did not influence the active site, but likely modified the overall conformation of PARPss favouring the binding with DNA.

A hypothesis to explain this high affinity and non-specific binding with the deoxyribonucleic acid that did not inhibit enzymatic activity is that PARPss might play a double function. At least 100% PARPss activity upon binding with DNA accounts for its full catalytic function that allows to modify acceptor proteins like the DNA-binding 7 kDa-protein [Faraone Mennella and

Farina, 1995]. The modification reaction might loosen protein interaction with the nucleic acid. Meanwhile, PARPss might exert a protective effect by binding with DNA that becomes transiently exposed prior to undergo metabolic events. The "non specific" binding with DNA would enable PARPss to recognize any sequence along the whole nucleic acid molecule. In this way, PARPss would allow DNA relaxation during metabolic events preventing the hydrolysis of the nucleic acid.

How can the slight preference of PARPss for the circular structure of nucleic acid be explained?

The results reported in the following article of this issue [Faraone-Mennella et al., 2002] are an approach aimed to answer this question.

ACKNOWLEDGMENTS

We thank the "Servizio Fermentazioni" of Istituto di Chimica M.I.B. (CNR), Arco Felice, Napoli, directed by Dr. Agata Gambacorta for providing homogenate from *S. solfataricus* cells.

REFERENCES

Agback P, Baumann H, Knapp S, Ladenstein R, Hard T. 1998. Architecture of non specific protein-DANN interactions in the Sso7d-DANN complex. Nature Struct Biol 5(7):579–584.

Althaus FR, Richter C. 1987. ADPribosylation of proteins. Enzymology and biological significance. Berlin: Springer-Verlag. pp 12–37.

Barinaga M. 1994. Archaea and Eukaryotes grow closer. Science 264:1251.

Bauman H, Knapp S, Karshikoff A, Ladenstein R, Hard T. 1995. DNA-binding surface of the Sso7d protein from *S. solfataricus*. J Mol Biol 248:2836–2843.

Burton K. 1968. A study of the conditions and mechanism of diphenilamine reaction for the colorimetric estimation of DNA. Cell 23:585–593.

Chen RF, Edelhoc H, Steiner RF. 1969. Physical principles and techniques of protein chemistry part A. In: Leach SJ, editor. New York: Academic Press. pp 171–244.

D'Amours D, Desnoyers S, D'Silva I, Poirer GG. 1999. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J 342:249–268.

De Murcia G, Menissier-de Murcia J. 1994. Poly(ADPribose) polymerase: A molecular nick sensor. Trends Biochem Sci 19:172–176.

De Napoli L, Galeone A, Mayol L, Messere A, Montesarchio D, Piccialli G. 1995. Automated solid phase synthesis of cyclic oligonucleotides. A further improvement. Bioorg Med Chem 3:1325–1329.

De Rosa M, Gambacorta A. 1975. Extremely thermophilic bacteria convergent with *S. acidocaldarius*. J Gen Microbiol 86:156–164.

Faraone Mennella MR, Farina B. 1995. In vitro ADPribosylation of a DNA-binding protein from *S. solfataricus*. Biochim Biophys Res Commun 208:55–62.

Faraone-Mennella MR, De Luca P, Giordano A, Gambacorta A, Nicolaus B, Farina B. 2002. High stability binding of poly(ADPribose)polymerase-like thermozyme from *S. solfataricus* with circular DNA. J Cell Biochem 85:158–166.

Faraone-Mennella MR, Gambacorta A, Nicolaus B, Farina B. 1996. Immunochemical detection of ADPribosylating enzymes in the archaeon *S. solfataricus*. FEBS Lett 378: 199–201.

Faraone-Mennella MR, Gambacorta A, Nicolaus B, Farina B. 1998. Purification and biochemical characterization of a poly(ADPribose) polymerase-like enzyme from the thermophilic archaeon *S. solfataricus*. Biochem J 335: 441–447.

Faraone-Mennella MR, Discenza A, Gambacorta A, Nicolaus B, Farina B. 2000. Purification of the ADPribosylating enzyme from *S. solfataricus* by SDS–PAGE and electroelution. Prep Biochem Biotechn 30(1): 61–67.

Grayling RA, Sandman K, Reeve JN. 1994. Archaeal DNA binding proteins and chromosome structure. System Appl Microbiol 16:582–590.

Grote M, Dijk J, Reinhardt R. 1986. Ribosomal and DNA binding proteins of the thermoacidophilic archaebacterium *S. acidocaldarius*. Biochim Biophys Acta 873:405–413.

Guagliardi AM, Napoli A, Rossi M, Ciaramella M. 1997. Annealing of complementary DNA strands above the melting point of the duplex promoted by an archaeal protein. J Mol Biol 267:841–848.

Jacobson MK, Jacobson EL. 1999. Discovering new ADP-ribose cycles: protecting the genome and more. Trends Biochem Sci 24:415–417.

Klenk HP, Doolittle WF. 1994. Archaea and eukaryotes versus bacteria? Curr Biol 4(10):920–923.

Marguet E, Forterre P. 1994. DNA stability at temperatures typical for hyperthermophiles. Nucl Acids Res 22: 1681–1686.

Mc Afee JG, Edmondson SP, Datta PK, Shriver JW, Gupta R. 1995. Gene cloning, expression, and characterization of the Sac 7 protein from the hyperthermophile *S. acidocaldarius*. Biochemistry 34:10063–10077.

Mc Ghee JD, von Hippel PH. 1974. Theoretical aspects of DNA-protein interactions: Co-operative and non-co-operative binding of large ligands to a one-dimensional homogenous lattice. J Mol Biol 86:469–489.

Peak MJ, Robb FT, Peak JG. 1995. Extreme resistance to thermally induced DNA backbone breaks in the hyperthermophilic archaeon pyrococcus furiosus. J Bacteriol 177:6316–6318.

Reddy TR, Suryanarayana T. 1989. Archaebacterial histone-like proteins J Biol Chem 264:17298–17308.

Ronimus RS, Musgrave DR. 1995. A comparison of the DNA binding properties of histone-like proteins derived from representatives of two kingdoms. FEMS Microbiol Lett 134:79–84.

Rowlands T, Baumann P, Jackson SP. 1994. The TATA-binding protein: A general transcription factor in eukaryotes and Archaea. Science 264:1326–1329.

Sambrook J, Fritsch EF, Maniatis T. 1989. In Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press.

Sandman K, Grayling RA, Dobrinski B, Lurz R, Reeve JN. 1994. Growth phase-dependent synthesis of histones in the archaeon methanothermus fervidus. Proc Natl Acad Sci U S A 91:12624–12628.

Thomm M, Stetter KO, Zillig W. 1982. Histone-like proteins in eu- and archaebacteria. Zentral Bakteriol Mikrobiol Hyg I Abt Orig C3:128–139.

Woese CR, Kandler O, Wheelis ML. 1990. Towards a natural system of organisms: Proposal for the domains of Archaea, bacteria, and Eukarya. Proc Natl Acad Sci U S A 87:4576–4579.