In Vitro Induction of H1−H1 Histone Cross-Linking by Adenosine Diphosphate−Ribose Polymers†

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Received December 28, 1999; Revised Manuscript Received May 19, 2000

ABSTRACT: It is well-known that H1−H1 interactions are very important for the induction of 30 nm chromatin fiber and that, among all posttranslational modifications, poly(ADP-ribosyl)ation is one of those capable of modifying chromatin structure, mainly through H1 histone. As this protein can undergo both covalent and noncovalent modifications by poly(ADP-ribosyl)ation, our aim was to investigate whether and how ADP-ribose polymers, by themselves, are able to affect the formation of H1−H1 oligomers, which are normally present in a condensed chromatin structure. The results obtained in our in vitro experimental system indicate that ADP-ribose polymers are involved in chromatin decondensation. This conclusion was reached as the result of two different observations: (a) H1 histone molecules can be hosted in clusters on ADP-ribose polymers, as shown by their ability to be chemically cross-linked, and (b) H1 histone has a higher affinity for ADP-ribose polymers than for DNA; ADP-ribose polymers compete, in fact, with DNA for H1 histone binding.

Poly(ADP-ribosyl)ation is a posttranslational modification carried out by the enzyme poly(ADP-ribose) polymerase (*1*), which uses NAD^+ as substrate to introduce ADP-ribose polymers into chromatin proteins that, by assuming negative charges, change their affinity for DNA and for other proteins. It has been suggested through electron microscopy and sedimentation velocity experiments that poly(ADP-ribosyl)-ation is involved in chromatin decondensation (*2*−*4*). In fact, this posttranslational modification produces, in chromatin structure, a relaxed state similar to that of a chromatin fiber from which H1 histone has been removed with a consequent "beads on a string" conformation. Despite this, previous experiments had demonstrated, by measuring the amount of H1 histone present in poly(ADP-ribosyl)ation-dependent decondensed fiber, that H1 histone remains within the chromatin structure (*2*). The same conclusion was also supported by other experiments (*5*) showing that the methyl-accepting ability of linker DNA is not increased by H1 poly-(ADP-ribosyl)ation. At that time, H1 histone was thought to be modified by poly(ADP-ribosyl)ation in a covalent way (*6, 7*), and only recent reports have demonstrated that H1 histone can be modified also in a noncovalent way (*8*−*10*). This noncovalent modification foresees that long and branched polymers, covalently attached to poly(ADP-ribose) polymerase, engage in noncovalent interactions with H1. The resulting binding is strong enough to resist high salt concentrations, strong acids, detergents, and chaotropes (*8*). Moreover, the affinity between H1 and ADP-ribose polymers is high enough to compete for H1−DNA interactions (*11*, *12*). Other chromatin proteins have also been shown to interact noncovalently with ADP-ribose polymers and it is noteworthy that most of these proteins, such as, e.g., p53, which plays an important role in carcinogenesis, can also be covalently ADP-ribosylated (*13*−*15*). Among the acceptors of ADP-ribose polymers, H1 histone is the most studied: all H1 genetic variants undergo both covalent (*5*) and noncovalent (*10*) modifications. Since proteins such as those present in the nuclear matrix structure can form noncovalent bonds with long and branched ADP-ribose polymers (*16*), it seems likely that the modification of these proteins plays an important functional role.

The aim of this research was to investigate whether ADP-ribose polymers, through noncovalent association with H1 histone, interfere in the formation of the appropriate H1−H1 interactions critical for the attainment of the folded 30 nm chromatin structure. Our in vitro results indicate that ADP-ribose polymers are able to induce by themselves, like DNA, H1−H1 interactions, suggesting that more than one histone molecule can be hosted on a poly(ADP-ribose) chain. Thus, due to the high affinity for poly(ADP-ribose), and the transient nature of the latter, H1 could be temporarily removed

† This work was supported by the Italian Ministry of University and Scientific and Technological Research (Progetti di Interesse Nazionale and Progetti di Ateneo of the Universities of l'Aquila and of Rome "La Sapienza") and by National Research Center (CNR) Italy.

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10.1021/bi992977q CCC: $19.00
Published on Web 08/04/2000

from DNA to allow for local chromatin unfolding, in agreement with the histone shuttle model proposed by Althaus (*17, 18*).

MATERIALS AND METHODS

Materials. L929 mouse fibroblast cells were a kind gift of Roger L. P. Adams (University of Glasgow, U.K.). All materials for cell culture were obtained from Gibco−BRL Life Technologies; [^{32}P]NAD^+ (specific activity 800 Ci/mmol) was from Du Pont−New England Nuclear; NAD^+, proteinase K (EC 3.4.21.14), and trypsin (EC 3.4.21.4) were from Boehringer Mannheim; 3-aminobenzamide (3-ABA)[1] was from Sigma; *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and glutaraldehyde were from Fluka Chemie AG (Buchs, Switzerland); Sephadex G-50 was from Pharmacia LKB Division; dithiobis(succinimido propionate) (DSP) and Gelcode color silver stain were obtained from Pierce; and nitrocellulose membrane (0.2 μm pore size) was from Bio-Rad. All the other chemicals used were of the highest purity commercially available.

Cell Culture. L929 mouse fibroblast cells were grown in Glasgow minimal essential medium (GMEM), supplemented with 10% newborn calf serum and 1 mM glutamine, in a humidified 5% CO_2 atmosphere at 37 °C. Confluent cells at a density of 1.2×10^5 cells/mL were scraped off the flasks, collected by centrifugation at 1000*g* for 5 min, and washed twice with cold phosphate-buffered saline (pH 7.2).

Isolation of H1 and H1−3ABA Histone. H1 histone was extracted from nuclei isolated from 7×10^7 L929 mouse fibroblast cells. To obtain the poly(ADP-ribose)-free isoform of H1 histone (named H1−3ABA), mouse fibroblasts were preincubated for 24 h with 2 mM 3-ABA. Cellular lysates were obtained by resuspension of the cellular precipitate into a buffer containing 10 mM Tris-HCl, pH 6.5, 50 mM sodium bisulfite, 1% Triton X-100, 10 mM $MgCl_2$, 8.6% sucrose, and 1 mM PMSF and repeated passage, through a 1 mL pipet tip, of the suspension kept on ice for 10 min. The suspension was then pelleted by centrifugation at about 10000*g* for 10 min in a microfuge. The nuclear pellet washed in PBS was then extracted overnight in 0.2 M H_2SO_4 at 4 °C. The supernatant was pelleted with 6 vol of acetone. The pellet, resuspended in water, was reextracted in 10% perchloric acid (w/v), according to Johns (*19*).

H1 histone concentration was determined spectrophotometrically with an extinction coefficient of 1.8 mg^{-1} cm^2 at 230 nm (*20*) and its purity was examined by SDS−polyacrylamide gel electrophoresis.

Isolation of DNA from Plasmid pVHCk and pMG. Plasmid pVHCk (Stefan Kass construct) and pMG (derivative of pBR322 with DNA methyltransferase insert; Bestor construct) were isolated from *Escherichia coli* JM 109 by means of a modified cleared-lysate method and purified by phenol−chloroform−isoamyl alcohol extraction. DNA from pVHCk was obtained by *Bam*HI linearization; having a general C + G content near to 50% and a CpG frequency of 229 pairs over 5025 bp, it can be considered as CG-rich and CpG island-like DNA. DNA from pMG is a *Bam*HI restriction fragment recovered from agarose gel by Qiaex II kit (Qiagen); this 4964 bp long fragment corresponds to the DNA methyltransferase gene with a typical genomic sequence rich in A + T (60%) and with the expected level of CpGs. The DNA concentration was estimated by measuring absorbance at 260 nm.

Purification of ADP-Ribose Polymers. Permeabilized cells were treated with 15 μM *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) to activate endogenous poly(ADP-ribose) polymerase (PARP) and incubated with 50 μM NAD^+ in 50 mM Tris-HCl, pH 7.4, containing 45 mM KCl, 0.1 mM PMSF, and 5 mM dithiothreitol at room temperature for 15 min. The reaction was blocked by addition of trichloroacetic acid to a final concentration of 20% (w/v).

ADP-ribose polymers were purified according to the method of Aboul-Ela et al. (*21*) with some modifications as indicated by Malanga and Althaus (*22*). After repeated extractions with chloroform/isoamyl alcohol (24:1), ADP-ribose polymers in the aqueous phase were loaded on a Sephadex G-50 column equilibrated in water and eluted with water (*23*), to remove low-molecular weight contaminants and to get a preliminary fractionation. Each fraction (0.4 mL) was monitored by absorbance at 260 nm and was subjected to electrophoresis according to Panzeter and Althaus (*24*). Following electrophoretic separation, polymers were stained with Gelcode color silver stain as described in the manual (*25*). Poly(ADP-ribose) chain lengths were expressed with respect to the mobility of the bromophenol blue and xylene cyanol dyes (*26*). Poly(ADP-ribose) concentrations were estimated by assuming that 40 μg of poly(ADP-ribose)/mL in water has an absorbance of 1.0 OD.

In Vitro Automodification of Poly(ADP-ribose) Polymerase. Poly(ADP-ribose) polymerase (PARP) (0.6 unit/3.4 μg) purified from calf thymus was incubated with 2 μg of CG-rich DNA in 100 μL of buffer containing 1 mM phosphate, 0.2 mM EDTA, 10 mM $MgCl_2$, and 1 mM dithiothreitol, pH 7.4, in the presence or in the absence (mock-automodified PARP) of 0.1 mM NAD^+ + 100 μCi of [^{32}P]NAD^+. The reaction was stopped after 20, 30, or 40 min of incubation by loading the mixtures on small Sephadex G-50 columns in order to remove low-molecular weight contaminants that could interfere with the subsequent cross-linking with DSP. Native or automodified PARP recovered from the column was lyophilized and dissolved in water.

Analysis of H1−DNA Complexes by Agarose Gel Electrophoresis. H1 histone was incubated with either CG-rich or AT-rich DNA at a histone:DNA ratio of 0.5 (w/w) in 36 mM TEA, 30 mM NaH_2PO_4, 1 mM EDTA, pH 7.55 (*27*), and 40 mM NaCl in a final volume of 7.2 μL. Where indicated, 0.05, 0.1, or 0.15 μg of ADP-ribose polymers were preincubated with 0.1 μg of H1 histone for 15 min before 0.2 μg of CG-rich or AT-rich DNA was added. Complexes were allowed to form for 1 h at room temperature in siliconized microfuge tubes. Since electrophoresis might destabilize macromolecular complexes held together by electrostatic interactions, we fixed samples overnight at 4 °C by adding glutaraldehyde to a final concentration of 0.1%.

Samples were mixed with 0.5 vol of glycerol and loaded on a slab of 1% agarose gel with 36 mM TEA, 30 mM NaH_2PO_4, and 1 mM EDTA, pH 7.55, as electrophoresis buffer (*27*). Electrophoresis was run at 10 V/cm.

[1] Abbreviations: 3-ABA, 3-aminobenzamide; DSP, dithiobis(succinimido propionate); MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; PBS, phosphate-buffered saline; PMSF, phenylmethanesulfonyl fluoride; TEA, triethanolamine; PARP, poly(ADP-ribose) polymerase.

Analysis of H1 Binding to DNA by Cross-Linking Assay. H1 histone was solubilized in 1 mM sodium phosphate and 0.2 mM EDTA, pH 7.4, since the presence of negative charges is important to induce a fairly stable ordered conformation (*28*). H1−DNA complexes were then allowed to form by adding a concentrated DNA solution into a dilute solution of H1 histone, to reduce aggregation (*29*).The final reaction mixture contained in 72 μL of incubation buffer (1 mM sodium phosphate, 0.2 mM EDTA, pH 7.4, and 40 mM NaCl) 1 μg of histone H1 (50 pmol) and 2 μg of either AT-rich or CG-rich DNA. Following incubation for 1 h at room temperature, the chemical cross-linking agent dithiobis-(succinimido propionate) (DSP) was added from a fresh stock solution (20 mg/mL) in dimethyl sulfoxide, to a final concentration of 0.2 mg/mL, and incubation was further continued for 13 min. The cross-linking reaction was stopped by addition of trichloroacetic acid (*30*). Samples were analyzed on 7% slab polyacrylamide gels in 1 mM sodium phosphate buffer, pH 6.6.

When the effect of poly(ADP-ribose) on H1 binding to DNA had to be tested, variable amounts of either protein-free or PARP-bound poly(ADP-ribose) were preincubated with H1 histone or H1−3ABA for 15 min at room temperature, before DNA addition. When the direct effect of ADP-ribose polymers on the formation of H1−H1 oligomers was investigated, DNA was omitted from the incubation mixture. Conditions for DSP treatment and analysis were as described above.

Synthesis and Purification of [^{32}P]Poly(ADP-ribose). Polymers of [^{32}P](ADP-ribose) (0.5 μCi/nmol of ADP-ribose) were synthesized in vitro essentially as described by Panzeter et al. (*8*), with partially purified poly(ADP-ribose) polymerase from bull testis.

Protein-free poly(ADP-ribose) was obtained by incubation at alkaline pH (10 mM Tris-NaOH/1 mM EDTA, pH 12.0) followed by extraction with $CHCl_3$/isoamyl alcohol (24:1). To get rid of any contaminating DNA, [^{32}P]poly(ADP-ribose) recovered in the aqueous phase was further purified by dihydroxylboronyl-Bio-Rex affinity chromatography (*22*) and stored as 100 pmol aliquots at −20 °C until use.

Poly(ADP-ribose) Blot Analysis. Purified H1 (1 μg) was dotted on nitrocellulose membrane (Bio-Rad, 0.2 μm pore size). The blots were air-dried, equilibrated in TBST (50 mM Tris-HCl, pH 7.4, 0.15 M NaCl, and 0.05% Tween-20) and then incubated in the same buffer containing [^{32}P](ADP-ribose) polymers (30−70 ng) in the absence (control) or presence of DNA, at poly(ADP-ribose):DNA ratios by weight indicated in the figure legends. The amount of radioactive poly(ADP-ribose) bound to H1 on nitrocellulose was determined by liquid scintillation counting.

RESULTS

Formation of H1 Histone−DNA Complexes. Purified total histone H1 and H1−3ABA were incubated with either CG-rich or AT-rich DNA, at a H1:DNA ratio of 0.5 (w/w), and then analyzed by agarose gel electrophoresis, as described under Materials and Methods. Under our experimental conditions, the formation of H1−DNA complexes is revealed by the shift of the band corresponding to free DNA to the origin of the gel: an increase of H1−DNA complexes is paralleled by a decrease of unreacted DNA. As shown in Figure 1, total H1 seems to bind more efficiently AT-rich DNA. On the other hand, all CG-rich DNA molecules are engaged in binding H1−3ABA while a part of AT-rich DNA is unreacted. H1−3ABA shows therefore a strong preference for CG-rich DNA with respect to AT-rich DNA, whereas total H1 shows a weak preference for AT-rich DNA.

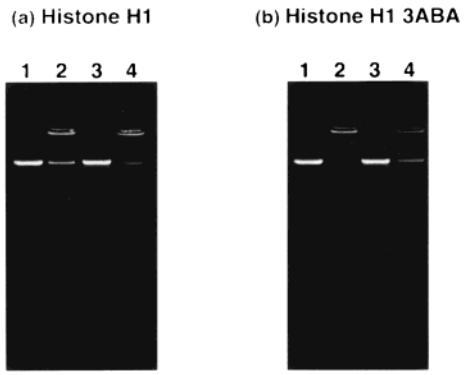

FIGURE 1: Electrophoretic analysis of H1−DNA complexes. Total H1 histone and H1−3ABA were incubated with either CG-rich (lanes 2a and 2b) or AT-rich DNA (lanes 4a and 4b) at protein:DNA ratios of 0.5 (w/w) as described under Materials and Methods. Following fixation with glutaraldehyde, histone−DNA complexes were electrophoresed on 1% agarose gels and stained with ethidium bromide. CG-rich naked DNA controls are in lanes 1a and 1b; AT-rich naked DNA controls are in lanes 3a and 3b.

Effect of ADP-Ribose Polymers on H1-DNA Interaction. Chemical cross-linking is a sensitive assay for H1 binding to DNA since cross-linked H1 oligomers are formed only when H1 molecules, upon binding to DNA, place themselves at the right distance for the cross-linking to occur. Bound and unbound H1 are easily distinguishable, since after treatment with the cross-linking agent dithiobis(succinimido propionate) (DSP) H1 oligomers migrate as discrete bands in SDS−polyacrylamide gels, while unbound H1 migrates faster and the band tends to smear (*29*). To investigate whether poly(ADP-ribose) may affect the formation of H1−H1 oligomers on DNA (i.e., H1 interaction with DNA) both protein-free and PARP-bound ADP-ribose polymers were used in our in vitro system.

Protein-free polymers of ADP-ribose isolated by gel-filtration chromatography and separated on 20% polyacrylamide gels showed a typical ladder of variously sized poly(ADP-ribose) molecules (data not shown). For our experiments we collected the fractions corresponding to branched and long linear ADP-ribose polymers with an average chain length of 38 residues. These polymers engage in noncovalent interactions with H1 histone (*8*).

Figure 2 shows that both CG-rich and AT-rich DNA promote the formation of H1 oligomers up to $(H1)_5$. The addition of 0.5 μg of protein-free poly(ADP-ribose) to the reaction mixture caused a reduction of histone cross-linking leading to the disappearance of the largest H1 oligomers, $(H1)_5$. However, increasing the amount of poly(ADP-ribose) up to 1.5 μg had no further effect on H1 oligomer formation.

In the experiments shown in Figure 3, PARP, either native (lanes 3−5) or poly(ADP-ribosyl)ated (lanes 6−8), was preincubated with H1−3ABA before addition of CG-rich DNA. We observed that although native (mock-automodified) PARP alone somewhat reduced the extensive H1 cross-linking of the control (lane 2), such an effect was more pronounced when automodified PARP was added to the

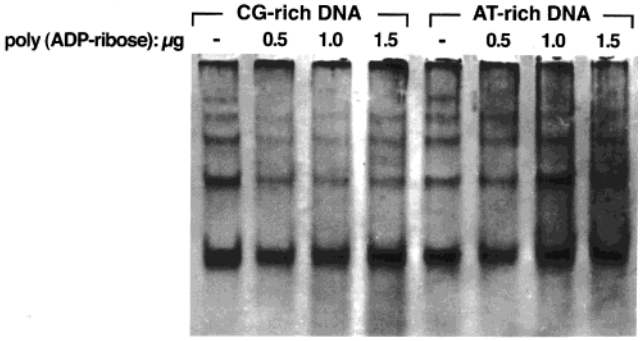

FIGURE 2: Effect of protein-free poly(ADP-ribose) on H1 binding to DNA. H1 binding to DNA in the absence or presence of increasing amounts of ADP-ribose polymers (as indicated in the figure) was investigated by a chemical cross-linking assay. Total H1 histone (1 μg) was incubated with 2 μg of either CG-rich (on the left) or AT-rich DNA (on the right) and subsequently treated with DSP, as described under Materials and Methods. When present, poly(ADP-ribose) was preincubated with H1 before DNA addition. Proteins were separated by SDS−PAGE and visualized by silver staining.

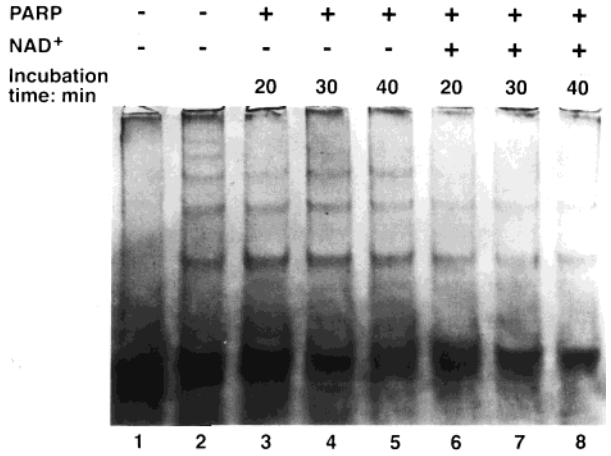

FIGURE 3: Effect of PARP-bound poly(ADP-ribose) on H1 binding to DNA. Conditions were as described in the caption for Figure 2 and under Materials and Methods. PARP was incubated in either the absence or presence of ^{32}P−NAD$^+$ for the indicated times and separated from unreacted NAD$^+$ by Sephadex G-50 chromatography, before being added to the reaction mixture. H1−3ABA was then preincubated with either mock-modified PARP (lanes 3−5) or ADP-ribosylated PARP (lanes 6−8) before CG-rich DNA addition. Migration of free histone and histone oligomers formed in the absence of PARP is shown in lanes 1 and 2, respectively.

reaction mixture. As already observed with protein-free poly(ADP-ribose), an increase of the extent of PARP automodification [i.e., an increase of the amount of poly(ADP-ribose) bound to the enzyme] by increasing time of incubation with [^{32}P]NAD$^+$ did not cause a parallel further decrease in the extension of H1−H1 cross-linking. A possible explanation for this observation is given by the results illustrated in Figure 4. In these experiments, H1 was incubated with ADP-ribose polymers in the absence of DNA for 1 h and then treated with DSP. Surprisingly, oligomers of H1 were found to be formed. Moreover, the increase of the amount of poly(ADP-ribose) allowed a higher recovery of cross-linked protein, without affecting the length of H1 oligomers. Scanning densitometry of the oligomer pattern showed that the amount of trimers and dimers formed vs monomers (taken as 100%) decreased with increasing amounts of poly(ADP-ribose). Relative to the monomer, dimers are 25%, 23%, and 12.6% and trimers are 17%, 11.2%, and 4.6% in the presence of 0.5, 1.0, and 1.5 μg of poly(ADP-ribose), respectively. These data suggest that, in the presence of a high amount of ADP-ribose polymers, H1 molecules tend to bind to different polymer molecules, preferring a distributive binding. This makes it more difficult for H1 molecules to be at the correct distance for cross-linking.

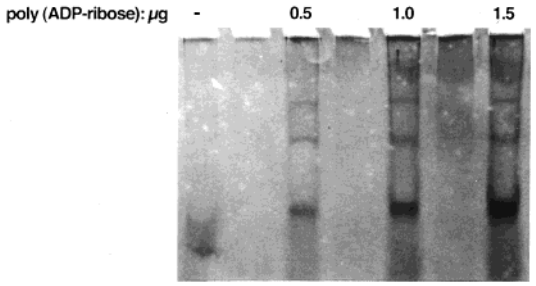

FIGURE 4: Cross-linking of H1 histone in the presence of ADP-ribose polymers. H1 histone was incubated in the absence or presence of the indicated amounts of ADP-ribose polymers and subsequently treated with DSP. Analysis was accomplished as indicated in the caption for Figure 2.

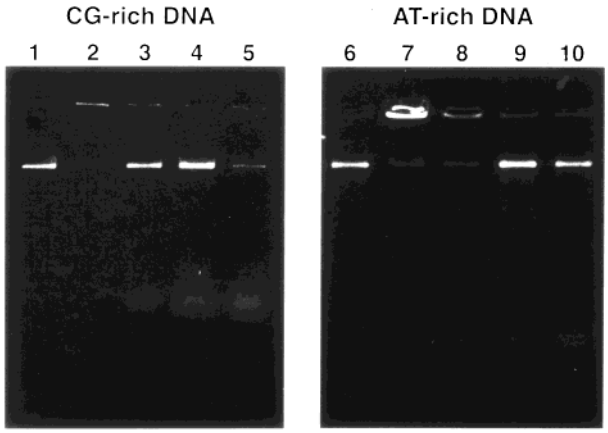

FIGURE 5: Effect of poly(ADP-ribose) on H1−DNA complex formation. H1 histone was incubated with either CG-rich or AT-rich DNA following preincubation in absence (lanes 2 and 7) or presence of increasing amounts of poly(ADP-ribose) at poly(ADP-ribose) to DNA ratios (w/w) of 0.25 (lanes 3 and 8), 0.5 (lanes 4 and 9) and 0.75 (lanes 5 and 10). Histone−DNA complexes were fixed by glutaraldehyde and analyzed by 1% agarose gel electrophoresis stained with ethidium bromide. Naked DNA controls are in lanes 1 and 6.

Since in the experiments illustrated in Figures 2 and 3 it was not possible to distinguish H1 oligomers due to H1 interaction with DNA from those due to H1 interaction with ADP-ribose polymers, it seemed important to examine the relative affinities of H1 for the two polyanions. To this end, two different approaches were followed. First, H1 histone was preincubated with ADP-ribose polymers before adding either AT-rich or CG-rich DNA, at poly(ADP-ribose):DNA ratios by weight ranging from 0.25 to 0.75. The effect of ADP-ribose polymers on the formation of H1−DNA complexes was monitored by DNA agarose gel electrophoresis. Alternatively, a poly(ADP-ribose) blot assay (*9*) was used.

The results in Figure 5 show that increasing amounts of ADP-ribose polymers interfere in a dose-dependent manner with the formation of H1−DNA complexes, up to a poly(ADP-ribose) to DNA ratio of 0.5 (w/w), at which value complete inhibition of complex formation was observed,

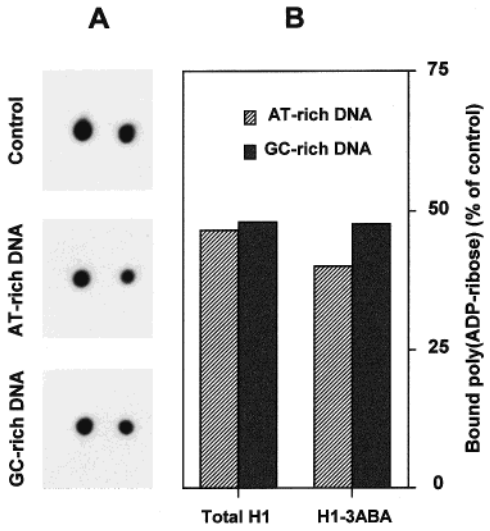

FIGURE 6: Poly(ADP-ribose) binding to histone H1 in the absence and presence of competitor DNA. H1 histone (1 μg) was dotted on nitrocellulose membrane and incubated in 5 mL of TBST containing [^{32}P]poly(ADP-ribose (33 ng) alone (control) or together with 495 ng of either AT-rich DNA or CG-rich DNA. (A) [^{32}P]poly(ADP-ribose)−H1 complexes (on the left) and [^{32}P]poly(ADP-ribose)−H1−3ABA complexes (on the right) were visualized by autoradiography. (B) [^{32}P]Poly(ADP-ribose) bound to either total histone H1 or H1−3ABA was quantified by liquid scintillation counting of the nitrocellulose sections corresponding to the autoradiographic dots. Results are expressed as percentages of control (no DNA added) and are averages of two separate experiments (maximum deviation from average 8%).

independently of DNA composition (Figure 5, lanes 4 and 9). At the lowest poly(ADP-ribose) concentration used [(poly(ADP-ribose):DNA ratio = 0.25], we observed a more pronounced inhibitory effect on the formation of DNA−H1 complexes involving CG-rich DNA (Figure 5, lane 3) as compared to AT-rich DNA (Figure 5, lane 8). With the highest amount of poly(ADP-ribose), we observed the unexpected formation of H1−DNA complexes (Figure 5, lanes 5 and 10). While highly reproducible, such a phenomenon is difficult to explain. The high concentration of poly(ADP-ribose) could either hinder its correct binding to H1 histone or induce a local rearrangement of H1 conformation so that the histone, although bound to poly(ADP-ribose), can still interact with DNA. Using antibodies specific for isolated individual histones, Thibeault et al. (*31*) found that the increased accessibility of H1 observed with a rather low quantity of ADP-ribose polymers was reversed when high concentrations of ADP-ribose polymers (250−1000 nM) were used. The results reported above suggest that H1 forms stable complexes with poly(ADP-ribose) and it is thus subtracted from the reaction mixture for its interaction with DNA.

The very high affinity of H1 for poly(ADP-ribose), as compared to DNA, was also confirmed by poly(ADP-ribose) blot assay (*9*). In these experiments, H1 immobilized on nitrocellulose membrane was incubated with [^{32}P]poly(ADP-ribose) in the presence of either AT-rich or GC-rich DNA. Figure 6 shows that despite the larger amount of DNA over poly(ADP-ribose) (15-fold, by weight), the latter still bound to H1: the amount of ADP-ribose polymers binding to H1 was 40−48% that of controls (no DNA added). Similar results were obtained for total H1 and H1−3ABA.

DISCUSSION

A relevant role of H1 histone in chromatin is to induce and maintain the 30 nm fiber through H1−H1 oligomer formation, which stabilizes the condensed structure (*32, 33*). To evaluate the involvement of poly(ADP-ribosyl)ation in modulating H1−DNA interactions, the behavior of H1 histone and of H1−3ABA—the poly(ADP-ribose)-free isoform of H1—in the formation of complexes with DNA was investigated. We demonstrated that the absence of poly(ADP-ribosyl)ation favors H1 histone binding to CG-rich DNA. Such a high affinity could have a role in the mechanism by which H1 histone participates in maintaining the methylation pattern on DNA (*23, 34−36*). According to these data, the investigation of how poly(ADP-ribosyl)ation modulates the structural role of H1 histone seemed to be of interest. Two contradictory theories have been proposed in this respect: a "condensing" one, based on the immunological evidence that H1−H1 dimers linked by ADP-ribose polymers of about 15 units in size occur in vivo (*37*), and a "decondensing" one based on results obtained by electron microscopy, analytical ultracentrifugation, and band-shift assays (*2−4, 17, 18*).

To address this question, we used a further experimental approach, i.e., cross-linking with DSP (*38*), a compound that is often employed to study H1−H1 interactions and their role in chromatin compaction (*29, 30, 39*).

Our previous experiments (*5*) had shown that the ability to induce H1−H1 interactions was lower when the H1e variant, which has the highest condensing effect on chromatin structure, was enriched in its covalently poly(ADP-ribosyl)ated isoform. The aim of this paper was to investigate whether long and branched polymers of ADP-ribose were able to affect the formation of H1−H1 oligomers through their noncovalent interaction with H1 histone. To this aim, we used two different experimental strategies which are the addition of either protein-free or PARP-bound ADP-ribose polymers to H1−DNA complexes. In both cases a slight decrease in the number of H1−H1 oligomers was observed. The degree of H1 histone cross-linking did not change as a function of DNA composition (i.e., either CG- or AT-rich). Under our experimental conditions we were unable to evaluate whether this decrease might have been greater, since the control carried out by incubating ADP-ribose polymers with H1 (without DNA) showed, to our surprise, that the ADP-ribose polymers are by themselves, like DNA, able to induce the formation of H1−H1 oligomers. This observation demonstrates that what was thought to be a prerogative of DNA can indeed be extended to ADP-ribose polymers.

H1−H1 interactions can be evidenced since ADP-ribose polymers are able to host more than one H1 histone molecule at a distance that allows their cross-linking by DSP. Assuming that the number of H1−H1 oligomers is dependent on ADP-ribose polymer size, this would explain the results of Wong et al. (*37*), who found only H1−H1 dimers associated with polymers of 15 units.

The fact that the ADP-ribose polymers are able to induce H1−H1 interactions as DNA does might suggest an involvement of poly(ADP-ribosyl)ation in chromatin condensation. However, this hypothesis was disproved both by previous data (*11−12*) and by our present results showing that the affinity of H1 histone is far higher for ADP-ribose polymers than for DNA. These data suggest that ADP-ribose polymers

might induce a local chromatin decondensation by attracting H1 histone molecules onto themselves, thus helping DNA in its functions.

Our findings reinforce the histone shuttle mechanism proposed by Althaus (*17*, *18*) to explain the role of poly(ADP-ribosyl)ation in DNA repair.

ACKNOWLEDGMENT

We thank Professor Guy Poirier for the kind gift of calf thymus poly(ADP-ribose) polymerase.

REFERENCES

1. D'Amours, D., Desnoyers, S., D'Silva, I., and Poirier, G. G. (1999) *Biochem. J. 342*, 249−268.
2. Poirier, G. G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C., and Mandel, P. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3423−3427.
3. de Murcia, G., Huletsky, A., Lamarre, D., Gaudreau, A., Pouyet, J., Daune, M., and Poirier G. G. (1985) in *ADP−Ribosylations of Proteins* (Althaus, F., Hilz., H., and Shall, S., Eds.) pp 180−189, Springer−Verlag, New York.
4. de Murcia, G., Huletsky, A., Lamarre, D., Gaudreau, A., Pouyet, J., Daune, M., and Poirier, G. G. (1986) *J. Biol. Chem. 261*, 7011−7017.
5. D'Erme, M., Zardo, G., Reale, A., and Caiafa, P. (1996) *Biochem. J. 316*, 475−480.
6. Poirier, G. G., and Savard, P. (1980) *Can. J. Biochem. 58*, 509−515.
7. de Murcia, G., Huletsky, A., and Poirier, G. G. (1988) *Biochem. Cell Biol. 66*, 626−635.
8. Panzeter, P. L., Realini, C. A., and Althaus, F. R. (1992) *Biochemistry 31*, 1379−1385.
9. Panzeter, P. L., Zweifel, B., Malanga, M., Waser, S. H., Richard, M. C., and Althaus, F. R. (1993) *J. Biol. Chem. 268*, 17662−17664.
10. Malanga, M., Atorino, L., Tramontano, F., Farina, B., and Quesada, P. (1998) *Biochim. Biophys. Acta 1399*, 154−160.
11. Mathis, G., and Althaus, F. R. (1987) *Biochem. Biophys. Res. Commun. 143*, 1049−1054.
12. Althaus, F. R., Collinge, M., Loetscher, P., Mathis, G., Naegeli, H., Panzeter, P., and Realini C. (1989) in *Chromosomal Aberrations−Basic and Applied Aspects* (Obe, G., and Natarajan, A. T., Eds.) pp 22−30, Springer-Verlag, Berlin.
13. Wesierska-Gadek, J., Schmid, G., and Cerni, C. (1996) *J. Cell. Biochem. 224*, 96−102.
14. Malanga, M., Pleschke, J. M., Kleczkowska, H. E., and Althaus, F. R. (1998) *J. Biol. Chem. 273*, 11839−11843.
15. Kumari, S. R., Mendoza-Alvarez, H., and Alvarez Gonzalez, R. (1998) *Cancer Res. 58*, 5075−5078.
16. Malanga, M., Kleczkowska, H. E., and Althaus, F. R. (1998) *J. Cell. Biochem. 70*, 596−603.
17. Realini, C. A., and Althaus, F. R. (1992) *J. Biol. Chem. 267*, 18858−18865.
18. Althaus, F. R. (1992) *J. Cell Sci. 102*, 663−670.
19. Johns, E. W. (1977) *Methods Cell Biol. 16*, 183−203.
20. Renz, M., and Day, L. A. (1976) *Biochemistry 15*, 3220−3228.
21. Aboul-Ela, N., Jacobson, E. L., and Jacobson, M. K. (1988) *Anal. Biochem. 174*, 239−250.
22. Malanga, M., and Althaus, F. R. (1994) *J. Biol. Chem. 269*, 17691−17696.
23. Zardo, G., D'Erme, M., Reale, A., Strom, R., Perilli, M., and Caiafa, P. (1997) *Biochemistry 36*, 7937−7943.
24. Panzeter, P. L., and Althaus, F. R. (1990) *Nucleic Acids Res. 18*, 2194.
25. Malanga, M., Bachmann, S., Panzeter, P. L., and Althaus, F. R. (1995) *Anal. Biochem. 228*, 245−251.
26. Alvarez-Gonzales, R., and Jacobson, M. K. (1987) *Biochemistry 26*, 3218−3224.
27. De Bernardin, W., Losa, R., and Koller, T. (1986) *J. Mol. Biol. 189*, 503−517.
28. Caiafa, P., Reale, A., Allegra, P., Rispoli, M., D'Erme, M., and Strom, R. (1991) *Biochim. Biophys. Acta 1090*, 38−42.
29. Clark, D. J., and Thomas, J. O. (1988) *Eur. J. Biochem. 178*, 225−233.
30. Clark, D. J., and Thomas, J. O. (1986) *J. Mol. Biol. 187*, 569−580.
31. Thibeault, L., Hengartner, M., Lagruex, J., Poirier, G. G., and Muller, S. (1992) *Biochim. Biophys. Acta 1121*, 317−324.
32. Zlatanova, J., and van Holde, K. (1996) *Prog. Nucleic Acid Res. Mol. Biol. 52*, 217−259.
33. Ramakrishnan, V. (1997) *Crit. Rev. Eukaryotic Gene Expression 7*, 215−230.
34. Zardo, G., and Caiafa, P. (1998) *J. Biol. Chem. 273*, 16517−16520.
35. de Capoa, A., Febbo, F. R., Giovannelli, F., Nivelau, A., Zardo G., Marenzi, S., and Caiafa, P. (1999) *FASEB J. 13*, 89−93.
36. Zardo, G., Marenzi, S., Perilli, M., and Caiafa, P. (1999) *FASEB J. 13*, 1518−1522.
37. Wong, M., Kanai, Y., Miwa, M., Bustin, M., and Smulson, M. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 205−209.
38. Lomant, A. J., and Fairbanks, G. (1976) *J. Mol. Biol. 104*, 243−261.
39. McArthur, M., and Thomas, J. O. (1996) *EMBO J. 15*, 1705−1714.

BI992977Q