Molecular Interactions between DNA, Poly(ADP-ribose) Polymerase, and Histones*

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Molecular interactions between purified poly(ADP-ribose) polymerase, whole thymus histones, histone H1, rat fibroblast genomic DNA, and closed circular and linearized SV40 DNA were determined by the nitrocellulose filter binding technique. Binding of the polymerase protein or histones to DNA was augmented greatly when both the enzyme protein and histones were present simultaneously. The polymerase protein also associated with histones in the absence of DNA. The cooperative or promoted binding of histones and the enzyme to relaxed covalently closed circular SV40 DNA was greater than the binding to the linearized form. Binding of the polymerase to SV40 DNA fragments in the presence of increasing concentrations of NaCl indicated a preferential binding to two restriction fragments as compared to the others. Polymerase binding to covalently closed relaxed SV40 DNA resulted in the induction of superhelicity. The simultaneous influence of the polymerase and histones on DNA topology were more than additive. Topological constraints on DNA induced by poly(ADP-ribose) polymerase were abolished by auto ADP-ribosylation of the enzyme. Benzamide, by inhibiting poly(ADP-ribosylation), reestablished the effect of the polymerase protein on DNA topology. Polymerase binding to in vitro-assembled core particle-like nucleosomes was also demonstrated.

Chromatin structure plays an important role in the control of gene expression in eukaryotic cells (cf. 1–3). A variety of interactions between double-stranded DNA and various nuclear proteins may be involved in gene regulation (cf. 4–6). Protein-DNA associations can influence at least two types of related phenomena. First, the availability of certain DNA sequences for transcription may depend on proteins binding to DNA. Examples include the nucleosome octamer (7, 8) and histone H1-DNA interactions (9) which have been proposed to repress or curtail transcription. The second type of DNA-protein associations may result in conformational alterations of DNA itself, as documented in prokaryotes for the CAP-lac promoter (10, 11), the cro-operator (12, 13), and EcoRI endonuclease recognition sequence interactions (14), and in eukaryotes for nucleosome-DNA (15), RNA polymerase III transcription factor-rRNA* gene (16), and a heat shock transcription factor-promoter DNA binding (17).

Our interest in this subject arose as a consequence of studies concerned with the biological function of the poly(adenosine diphosphate ribose) polymerase enzyme molecule (EC 2.4.2.30), a DNA-binding nuclear protein (cf. 18–20) which has been implicated in the control of a variety of cellular functions such as differentiation, toxic damage to DNA (18, 19), aging (21), hormonal effects (22), and tumorigenic growth (23, 24). The catalytic activity of poly(ADP-ribose) polymerase enzyme has an absolute requirement for DNA (18, 19). Besides the polymerase protein itself, histones can also serve as ADP-ribose acceptors (18). The present paper deals with the molecular association of poly(ADP-ribose) polymerase with histones and DNA and with coinciding topological changes in DNA. An analysis of macromolecular binding processes is a prerequisite to an interpretation of enzymatic mechanisms of poly(ADP-ribose)ylation (25) in terms of cell physiology.

**MATERIALS AND METHODS**

**Proteins and DNA—**Poly(ADP-ribose) polymerase was isolated from calf thymus by a novel procedure (26) and also by a published method (27). The protein was at least 95% homogeneous, containing only traces of small peptide degradation products of the enzyme protein, as determined by SDS-polyacrylamide gel electrophoresis (28) and immunoblotting, and had a specific activity of 1000–1200 nmol of ADP-ribose mg⁻¹ min⁻¹ as tested in a standard assay (29). Calf thymus whole histones were purchased from Boehringer Mannheim. Histone H1, chicken core histones, and HMGs (1 + 2) were a gift from Dr. David Cole (University of California, Berkeley, CA). *Escherichia coli* recA protein was from USB Corp. (Cleveland, OH). [³⁵S]labeled proteins was performed with Iodobeads as recommended by the manufacturer (Pierce Chemical Co.). The molecular masses of labeled proteins (10⁶–10⁷ cps/µg) was verified by SDS-polyacrylamide gel electrophoresis (28) and autoradiography. Isolation of DNA of high molecular mass was carried out by a standard method (29) from confluent cultures of 14C cells (10⁶ cells), a cell line derived from rat-1 fibroblasts (24). SV40 DNA was purchased from Bethesda Research Laboratories. The high molecular weight 14C DNA was digested partially with *MboI* (New England Biolabs) at 0.1 unit/µg of DNA for 30 min at 37 °C to give DNA fragments ranging between 0.5 and 15 kilobase pairs as determined by agarose gel electrophoresis (29). SV40 DNA (Form II) was linearized with EcoRI (New England Biolabs) and labeled at the 5' ends with [³²P] ATP and T4 polynucleotide kinase (Pharmacia LKB Biotechnology Inc.) (30). Aliquots of this end-labeled DNA were ligated with T4 DNA ligase (New England Biolabs) at low DNA concentrations (50 µg/ml) (29) to minimize the formation of multimers and linear concatemers (Fig. 3B, inset).

*Nitrocellulose Filter Binding Assay—*The association of proteins with DNA in solution took place within 1 min; therefore, the filter binding technique as applied here determined end points. The macromolecular binding processes is a prerequisite to an interpretation of enzymatic mechanisms of poly(ADP-ribose)ylation (25) in terms of cell physiology.

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*The abbreviations used are: SDS, sodium dodecyl sulfate; bp, base pairs; HMG, high mobility group; RSA, bovine serum albumin; HEPES, 4-(2-hydroxyethyl)-1-piperazineneethanesulfonic acid.
romolecular complexes formed in solution were stable for at least 2 h and a 2-5-fold dilution did not dissociate them. It was determined in preliminary experiments that the largest quantities of proteins or DNA employed in these assays, either alone or in combination, did not saturate the membrane filter, a criterion strictly followed in all binding assays. Based on these considerations, the following technique was employed: varying quantities (see "Results") of [32P]DNA (in 1-5 μl) were added to 0.2 ml of the ice-cold binding buffer (31) (25 mM Tris-HCl, pH 8.0, 10 mM MgCl2, 0.5 mM dithiothreitol, 0.1 mM NaCl) and mildly agitated at 25 °C for 1 min. Binding of proteins to DNA was assessed by adding aliquots of the proteins (5-10 μl) to the DNA solution, followed by incubation for 10 min at 25 °C. Samples were transferred onto ice, followed by filtration through nitrocellulose filters (BA 85, Schleicher & Schuell, 0.47 μm pore size, 27 mm diameter), presoaked in the binding buffer for 30-60 min. Each filter was washed four times under suction with 2.5 ml of ice-cold binding buffer containing 5% dimethyl sulfoxide, which considerably reduced the background counts. The amount of radioactive material bound to the filters was determined by scintillation spectrometry following drying of filters. When the association of [32P]-labeled proteins with unlabeled DNA or proteins was determined, the filters were washed 10 times with 2.5 ml of the binding buffer to minimize nonspecific associations. All tests were carried out in duplicates. Typically, <5% of input [32P]DNA (counts/min) was bound to filters in the absence of protein and ≥25% of input protein (counts/min) was retained in the absence of DNA.

RESULTS

Promotion of Binding of Poly(ADP-ribose) Polymerase to DNA by Histones—Initially, the effects of whole thymus histones were determined because we could not predict if any of the histone subfractions might exhibit selectivity in terms of their interaction with DNA and the polymerase enzyme. The poly(ADP-ribose) polymerase protein is not known to display, in terms of DNA binding, any DNA sequence specificity, although enzymatic studies reveal that DNA molecules of differing sequence appear to stimulate enzymatic activity to various degrees (35). Therefore, rather than experimenting with specific DNA sequences as substrates for DNA binding, we first employed genomic DNA as binding ligand to provide broad-based DNA sequences.

Fig. 1A (lower curves) shows that addition of increasing amounts of poly(ADP-ribose) polymerase (▲) or histones (●) to a constant concentration of [32P]-labeled 14C DNA causes increased retention of [32P]DNA on nitrocellulose filters. Similarly, if increasing amounts of [32P]-labeled DNA is added to a constant amount of protein, a proportional increase in retention of [32P] label on filters occurs (Fig. 1B). This indicates quantitative binding of both the polymerase and histones to restricted [32P]-labeled 14C DNA to a constant amount of enzyme or histones. ▲, polymerase; ●, histones. The concentration of the enzyme or histones (0.1 μg) was constant and DNA varied (abscissa). ▲, enzyme; ●, histones. The concentration of the enzyme or histones (0.1 μg) was constant and DNA varied (abscissa).
1A, upper curves). The experimental points in the upper curves of Fig. 1A have been obtained by subtracting the percent radioactive material contributed by the protein present in constant amounts from the total amount of radioactivity retained on the filter as a result of the binding of both proteins to DNA. This method of presentation permits a direct comparison, yielding the lower and upper curves in Fig. 1. Since the nitrocellulose binding technique does not permit the exact determination of the DNA-protein complexes, nor the identification of the nature of the interactions between individual macromolecular components of the reaction mixture (36-38), instead of cooperativity, it may be more correct to propose a mutual promotion of DNA binding between polymerase and histones. As observed by others (39), binding of histones to DNA at mass ratios of histone to DNA >1.0 seems to decrease the extent of retention of 32P label on the filters (Fig. 1A, C). Such an effect is not observed with either the polymerase alone (Fig. 1A, A) or when larger amounts of the polymerase are added with a small amount of histones (Fig. 1A, C).

The Specificity of Histones in Promoting the Binding of Poly(ADP-ribose) Polymerase to DNA—The specificity of histones in the promotion of the binding of the polymerase to DNA was tested by replacing histones with a nonspecific protein like BSA or by the DNA-binding high mobility group (HMG 1 + 2) proteins (40) or by the rec4 protein (41, 42) of E. coli. Fig. 1C shows that the polymerase-enhanced filter binding of [32P]DNA is not observed when BSA is employed in conjunction with the polymerase protein. BSA by itself does not bind to the DNA, but it binds directly to the polymerase protein as deduced from experiments employing 125I-labeled BSA (not shown). This direct binding to the polymerase apparently involves the blocking of DNA binding sites on the polymerase, hence the inhibition of the binding of the polymerase to DNA in the presence of BSA. Since BSA is not a physiologically occurring nuclear protein, we did not pursue this problem further. HMG (1 + 2) proteins, when present between 0.05-0.15 μg/test, resulted in the retention of 11-36% of input DNA. Varying concentrations of poly(ADP-ribose) polymerase (0.04-0.15 μg/test) added simultaneously with a small amount (0.04 μg) of HMGs affected DNA retention only slightly (10-15% retention). This is in contrast to a 75-85% increase in DNA retention observed upon addition of histones (Fig. 1A). Similar results were obtained with varying quantities of rec4 protein (data not shown). Polyarginine (M, 60,000) or polylysine (M, 50,000) at a mass ratio of 0.5 (polypeptide:14C DNA) did not simulate the effect of histones. At higher mass ratios (>0.5), these polypeptides inhibited the binding of the enzyme to DNA. Polyarginine and polylysine do not bind appreciably to DNA (43). These results are consistent with an apparent specificity of histones in promoting the binding of poly(ADP-ribose) polymerase to DNA.

Significance of the Order of Addition of Macromolecular Components—As illustrated in Fig. 1D, the histone-promoted binding of poly(ADP-ribose) polymerase to DNA depends on the order of addition of macromolecular components. Sequential addition of DNA + histones + enzyme, or DNA + enzyme + histones, resulted in promoted binding. On the other hand, the sequence: enzyme + histone + DNA, or histones + enzymes + DNA, failed to exhibit promotional binding kinetics.

Association of Histones and the Enzyme Protein—Since the apparent promotion of binding of the enzyme to DNA required histones as a third macromolecular component, it was of interest to explore a direct association between the enzyme and histones in the absence of DNA. Fig. 2A illustrates the association of varying concentrations of histones with a fixed concentration of poly(ADP-ribose) polymerase (C), and the binding of increasing concentrations of polymerase to a fixed concentration of histones (D). At histone:polymerase mass ratios of <1.0 the retention of increasing amounts of 32P-histones is observed, but at histone:polymerase mass ratios approaching 2.0, a lesser amount of the labeled polymerase is retained on the filters (Fig. 2A, C). On the other hand, when increasing amounts of 125I-polymerase are added to unlabeled histones, larger amounts of 125I-polymerase is retained on the filters, even at mass ratios >1.0. The addition of unlabeled 14C DNA to the reaction mixture results in enhanced retention of the 125I-polymerase on the filters (Fig. 2B).

To rule out the possibility that the increased retention of labeled DNA could be due to nonspecific clogging of the filters by the proteins (polymerase + histones; Fig. 1A), since both proteins can by themselves, be retained on the filters (Fig. 2, A and B), the following experiment was performed. Polymerase and histones, separately or together, were added to the binding buffer (as in Fig. 2A). This solution was first added
to the filters without washing. A solution of [32P]DNA was then added to these filters and the filters were washed (see "Materials and Methods"). It was observed that <10% of the input [32P]DNA was retained on the filters, a value which was approximately equal to the background level of [32P]DNA retained in the absence of the proteins. This shows (a) that the association of DNA with proteins occurs only in solution, and (b) that the proteins do not saturate the filters so as to prevent the DNA from passing through the filters.

Substitution of Whole Histones by H1—Fig. 2C shows the binding of the polymerase to H1 in the absence of DNA. In the presence of DNA, H1 and polymerase yielded results which were essentially indistinguishable from those obtained when whole histones were employed (compare Fig. 2D with 1A).

The Influence of DNA Topology—The binding of the polymerase protein and histones was determined with two forms of SV40 DNA. First, SV40 DNA (Form I) was linearized with EcoRI and labeled at the 5' ends. The second type of DNA ligand (Fig. 3, inset) consisted of religated linear SV40 DNA. Under comparable conditions, more polymerase enzyme protein was bound to circular (0) than to linear (●) DNA (Fig. 3). A fixed concentration of whole thymus histones (0.04 μg) promoted the association of the polymerase protein to SV40 DNA, in a manner similar to the enzyme binding to genomic DNA (compare Figs. 1A and 3). The apparent differences between the shape of the isotherms observed with genomic (Fig. 1A) and SV40 DNAs (Fig. 3) may reflect sequence and size differences in the DNAs which could influence protein binding. Since the DNA-background counts of the two forms of DNA were nearly identical, artifacts due to a preferential binding of circular over linear DNA to nitrocellulose membranes are unlikely. It has been reported that poly(ADP-ribose) polymerase also binds to supercoiled ColEl (44) and pBR322 DNAs.3

Binding of the Polymerase to SV40 DNA Restriction Fragments as a Function of the Ionic Strength—It is known generally that protein-DNA interactions are sensitive to the ionic strength of the binding medium. Therefore, we tested the effect of increasing concentrations of NaCl on the binding of the polymerase to various restriction fragments of SV40 DNA. SV40 DNA was digested with MboI and the fragments were 32P-labeled at their 5' ends. Filter binding assays were performed at a constant polymerase:DNA mass ratio but with increasing concentrations of NaCl in the binding and washing buffers. Filter-bound DNA was eluted and the DNA fragments separated on a polyacrylamide gel. Fig. 4 shows that all the restriction fragments were retained on the filters, indicating binding of the polymerase to these fragments even at a relatively high NaCl concentration (0.6 M, lane 12). Maximal binding to all the fragments occurred at ≈0.02 M NaCl (lane 3). The strength, or the amount, of polymerase bound to the fragments decreased as the salt concentration was raised (Fig. 4). Only a 50-60% reduction in the strength (or amount) of polymerase binding to the DNA fragments occurred even as the NaCl concentration was raised from 0.02 to 0.4 M. Variation in the amount of DNA retained on the filters was not an effect of the salt concentration on the filters since control experiments demonstrated that very little of the input radioactivity was retained in the absence of the protein (lanes 6 and 11, Fig. 4), irrespective of the concentration of NaCl. Densitometry revealed that the polymerase exhibited 3-4-fold preferential binding to the 610-bp fragment (position 4100-4710 bp on the SV40 sequence), followed by the 237-bp fragment (2534-2771 bp), at all NaCl concentrations tested. It is possible that the preferential binding of the polymerase to the 610- and 237-bp fragments is a reflection of the sequence or conformational specificity (or both). The sequence (32) of the 610-bp and the 237-bp fragments reveals several contiguous 6-16 bp A + T stretches and the fragments have an overall A + T richness of 50-66%. A + T-rich regions are known to bind proteins (45), occur in regulatory contexts (46), and have been implicated in unusual DNA conformations (47, 48). Competition binding and DNase I footprinting experiments with specific DNA fragments are underway to study the sequence specificity, if any, of the polymerase to DNA.

Torsional Constraints Induced by the Polymerase on Relaxed DNA—It is known that the binding of histones, especially H3 and H4, to relaxed covalently closed circular DNA molecules induces superhelicity (39, 49, 50), and it has been proposed that DNA wraps around the polymerase protein (51). Therefore, we determined topological changes in DNA following the binding of the enzyme protein and histones alone and in combination to relaxed covalently closed circular SV40 DNA. SV40 DNA (Form I, supercoiled duplex) was converted to relaxed covalently closed circular DNA (Form Ir) by treatment with topoisomerase I. The DNA was then deproteinized.

3 Dr. Gilbert de Murcia, University of Strasbourg, France, personal communication.
Proteins to be tested (polymerase and histones) were mixed with Form I DNA, and the reaction mixture was incubated at 25°C for 1.5 h. Topoisomerase I was added to relax any DNA twists that were not due to protein binding, and the DNA was deproteinized and run on an agarose gel (78). The addition of increasing amounts of enzyme protein to a constant concentration of DNA resulted in increased superhelical twisting of relaxed SV40 DNA (lanes 3–6, Fig. 5A). The number of superhelical turns induced by the polymerase appeared to plateau as the enzyme:DNA mass ratio approached 1:0, and raising the mass ratio further produced no significant increase (compare lanes 5 and 6, Fig. 5A). Histones, when present at a histone:DNA mass ratio of 0.5, induced the same number of superhelical turns as poly(ADP-ribose) polymerase at an enzyme:DNA mass ratio of 1.2 (compare lanes 6 and 7, Fig. 5A), suggesting a greater effectiveness of histones as compared to the polymerase protein. The sequential addition of first histones, followed by the enzyme protein, to DNA exceeded the additive effect of histones or the enzyme protein alone (compare lanes 6 and 7 with lanes 8 and 9, Fig. 5A). This more-than-additive effect is reminiscent of the mutually promoting influence of histones and the polymerase on their binding to DNA (Figs. 1–3). For example, at an enzyme:DNA mass ratio of 0.8, the number of superhelical turns induced was about 10, whereas at an enzyme + histone to DNA mass ratio of 0.6, the number of superhelical turns increased to 15 (compare lanes 5 and 9, Fig. 5A). From these data it was calculated (52, 53) that, in the presence of topoisomerase I (nicking and closing enzyme) and 1 mol of polymerase protein (molecular mass 120,000/mol SV40 DNA (molecular mass 3.2 x 10^6, 2543 bp), a change in the linking
number of 0.5 occurred. Since the linking number is always an integer, it is estimated that a change of 1 unit in linking number corresponds to the binding of 2 mol of enzyme in the presence of the nicking and closing activity of topoisomerase I. Resolution of the topoisomeres on ethidium bromide-containing agarose gels indicated that the superhelical turns had a negative sign (52).

The above results were obtained with an enzyme protein that was not exposed to NAD\textsuperscript{+}. Incubation of the enzyme with NAD\textsuperscript{+} in the presence of DNA results in the automation of the enzyme by covalently bound ADP-ribose oligomers (18, 19) and a concomitant decrease in the binding of the enzyme to DNA (31).\textsuperscript{3} Incubation of the polymerase protein with histones and relaxed SV40 DNA in the presence of NAD\textsuperscript{+}, which result in poly(ADP-ribosylation) of both proteins, prevented DNA supercoiling in the test system (Fig. 5B, lane 4). Benzamide, a specific inhibitor of poly(ADP-ribose) polymerase (56) abolished the inhibitory effect of poly(ADP-ribosylation) on DNA supercoiling in the above system and reestablished the topological change in DNA (lane 5, Fig. 5B). In the reaction containing no histones, only the enzyme protein, SV40 DNA, and NAD\textsuperscript{+} (lane 9, Fig. 5B), benzamide also prevented the inhibitory effect on DNA supercoiling (lane 10, Fig. 5B). On the other hand, histone-induced supercoiling of SV40 DNA (lane 7, Fig. 5B) was not affected by either NAD\textsuperscript{+} (lane 14, Fig. 5B) or benzamide (lane 15, Fig. 5B) or both (not shown). The activity of topoisomerase I was not influenced by NAD\textsuperscript{+} and benzamide (lane 11, Fig. 5B). These results show that the enzymatic activity of the polymerase protein regulates DNA topology in a system containing histones, poly(ADP-ribose) polymerase, NAD\textsuperscript{+}, and topoisomerase I. The effect of NAD\textsuperscript{+} and benzamide were also tested employing the filter binding assay, and the results indicated that there was a substantial decrease in the retention of 32P-labeled SV40 DNA under the conditions of auto ADP-ribosylation of the enzyme. Benzamide restored the retention of DNA onto the filters when included in the binding mixture (data not shown).

Binding of the Polymerase to in Vitro-assembled Core Particle-like Nucleosomes—In the preceding experiments, the binding of the polymerase to DNA in the presence of whole histones or H1 was determined. Under these conditions histones are presumed to form dimers or tetramers (55, 56), but in core particles and chromatin histones exist as octamers (3). Chromatin isolated from nuclei may contain tightly bound poly(ADP-ribose) polymerase (18). Therefore, it was considered important to determine if the polymerase protein would bind to nucleosome-like particles in vitro. Recent experiments have demonstrated convincingly that nucleosomes can be assembled in vitro onto restriction fragments of SV40 DNA (33, 57–59).

A core particle-like structure was assembled in vitro onto a 209-bp EcoRI-PstI fragment of SV40 DNA and these particles were employed as ligands for polymerase binding. Two recent studies have demonstrated that this fragment lies within a region of SV40 DNA where a unique array of stable nucleosomes can be reconstituted (33, 59); the EcoRI-PstI fragment is just sufficient in length to accommodate only one octamer of histones. The formation of an octamer of histones onto this fragment was verified by two methods. 1) Gel electrophoresis of histone-DNA complexes at histone-DNA mass ratios approaching unity revealed a distinctly retarded (as compared to the naked DNA) band (Fig. 6A). This band migrates at approximately 390 bp, as observed for core particles (60). 2) The sedimentation coefficient (\(\approx 11\) S) of these particles, which sedimented near the middle (Fig. 6C) of the sucrose gradients, closely agrees with the reported value for native core particles (10.5–11 S; 61, 62). These criteria show that the reconstituted core particle-like nucleosome resembled native core particles.

In the absence of histones, the binding of increasing amounts of poly(ADP-ribose) polymerase to the 209-bp-labeled SV40 DNA fragment results in a concomitant reduction in the electrophoretic mobility of the 32P-labeled DNA fragment (Fig. 6B). At low polymerase:DNA mass ratios (<1.4) three different retarded (as compared to free DNA) bands appear (Fig. 6B). At mass ratios (polymerase:DNA) of 1.4 and 1.8, only one retarded band and the complete absence of any free DNA is observed, indicating the binding of saturating amounts of the polymerase to the DNA. It is estimated that 2 or 3 polymerase molecules could be bound/DNA molecule at saturation, assuming full binding activity for all the macromolecules and the following molecular masses: polymerase = 120,000; 209-bp DNA = 138,000. The binding of 125I-labeled polymerase to the 209-bp 32P-labeled fragment was also assayed by sucrose density gradient centrifugation. When the mass ratio of 125I polymerase to 209-bp 32P-labeled DNA fragment was 1.0 to 1.8, and conditions comparable to the electrophoretic assay were employed for binding, the positions of the peaks containing the 125I label and the 32P label in the density gradient corresponded to the number of bands in the gel assay (data not shown). At a polymerase:209-bp fragment mass ratio of 1.8 only one peak containing the 125I label (representing the polymerase coinciding with a peak of 32P label (representing the DNA fragment) was observed. This 125I peak had an approximate sedimentation coefficient of 12.5 S (not shown).

The addition of 125I-labeled polymerase to the 32P-labeled core particle-like nucleosomes results in a pronounced change in the sedimentation behavior of most of the 32P label from 11 S (core particle-like nucleosome), to a faster sedimenting species at 14.3 S (Fig. 6D). This peak of faster sedimenting 32P label also coincides with a peak of 125I-labeled, indicating binding of the polymerase to the core particle-like nucleosomes. Some of the 32P label appears as polymerase-free nucleosome-like particles. Approximately 50% of the input 125I polymerase is bound to the 32P core particle-like nucleosomes, as indicated by the distribution of the 125I label (Fig. 6D). The 125I-polynucleosome sediments at 4.8 S in agreement with a previous estimate (31). Assuming the molecular mass of a histone octamer to be 109,000, the approximate stoichiometry of 32P-nucleosomes to 125I-polymerase is 1:1, indicating considerable affinity of the polymerase to the in vitro-assembled nucleosomes.

**DISCUSSION**

The majority of experimental work concerned with the cellular function of poly(ADP-ribosylation) of nuclear proteins has been focused on enzymatic rates that are correlated with biological responses (18, 19). A notable exception is the cellular function of poly(ADP-ribosylation) of nuclear proteins (9, 12). The recA protein of E. coli, or polyamino acids, no promotion of the binding of the enzyme to DNA occurred. When histones were substituted by HMGs (1 + 2), the recA protein of E. coli, or polyamino acids, no promotion of the binding of the enzyme to DNA occurred. When histones were substituted by BSA, there was an inhibition of polymerase binding to DNA as discussed under "Results" (Fig. 1C).

It is possible that the torsional constraints imposed on DNA by the binding of histones can create a specific DNA
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conformation which is required for maximal polymerase binding and/or enzymatic activation (or both). Torsional constraints can be induced on both circular and linear molecules.

The formation of poly(DNA-ribose) polymerase-experimental evidence (Fig. 3) indicates that this constraint may be larger with circular DNA, as compared to linear DNA hence larger promotion binding to circular DNA. It has been suggested (64) that torsional constraints can be induced on linear DNA molecules, presumably between two protein binding sites. Thus, the promoted binding of histones + enzyme protein to genomic DNA (Figs. 1 and 2) could also be explained on this basis. As shown in Fig. 1D, the order of addition of macromolecules is critical to obtain promotional association with DNA. The results show that polymerase-DNA or histone-DNA complexes must be formed for subsequent promotional binding with the second protein component (either histones or polymerase). This behavior is compatible with the following scheme. Promotional binding to DNA is present in schemes 1 and 2, but not in scheme 3. As described under “Results,” binding of one protein (polymerase or histones) to DNA induces a topological change in the DNA which apparently precedes the promotional binding of the second protein according to this scheme.

E + DNA ➔ (E·DNA) + H ➔ E·DNA*·H

H + DNA ➔ (H·DNA) + E ➔ H·DNA*·E

E + H ➔ (E·H) + DNA ➔ (E·H)·DNA

*promotional (more than additive) binding properties; (E·H) = enzyme-histone complex (no promotional binding); H = whole thymus histones: E = enzyme, poly(ADP-ribose) polymerase.

Although we have not demonstrated directly in vivo if any of the phenomena described in this paper are important for cell physiology, the promotion of polymerase binding to DNA by histones and the accompanying changes in DNA topology could be of biological significance. Since histones are much more abundant than the enzyme in the cell, any changes in DNA topology due to the binding of the enzyme might be confined to relatively few DNA regions. The requirement for superhelicity in transcription in eukaryotes has been demonstrated in some instances (65–69), even though this subject remains controversial (70–72). Polymerase binding to DNA or nucleosomes could conceivably have a localized effect on the topology of DNA and thus may indirectly influence gene expression.

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