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Immunoaffinity fractionation of the poly(ADP-ribosyl)ated domains of chromatin

(DNA repair/single-strand DNA breaks)

NAJMA MALIK*, MASANAO MIWA†, TAKASHI SUGIMURA†, PETER THRAVES*, AND MARK SMULSON*

*Department of Biochemistry, Schools of Medicine and Dentistry, Georgetown University, Washington, D.C. 20007; and †National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo 104, Japan

Contributed by Takashi Sugimura, February 2, 1983

ABSTRACT Antibody to poly(ADP-ribose) has been covalently coupled to Sepharose and utilized to isolate selectively oligonucleosomes undergoing the poly(ADP-ribosyl)ation reaction from the bulk of chromatin. Approximately 12% of the unfractionated oligonucleosomes were bound to the immunoaffinity column and these represented essentially 100% of the original poly(ADP-ribosyl)ated nucleosomal species in the unfractionated chromatin. Poly(ADP-ribosyl)ated chromatin was not bound by preimmune IgG columns. KSCN eluted the modified nucleosomes in the form of nucleoprotein complexes. The eluted chromatin components were shown to contain poly(ADP-ribosyl)ated histones as well as automodified poly(ADP-ribose) polymerase. By using [^{3}H]lysine- and [^{3}H]arginine-labeled chromatin, it was shown that the poly(ADP-ribosyl)ated histones, attached to stretches of oligonucleosomes bound to the column, had a 6-fold enrichment of the modification compared to histones of the unfractionated chromatin. This indicated that non-poly(ADP-ribosyl)ated nucleosomes, connected and proximal to the modified regions, were copurified by this procedure. This allowed characterization of the oligonucleosomal DNA around poly(ADP-ribosyl)ated chromatin domains to be compared with the unbound bulk chromatin. The data indicated that immunofractionated poly(ADP-ribosyl)ated oligonucleosomal DNA contained significant amounts of internal single-strand breaks compared with bulk chromatin. The bound nucleoprotein complexes were found to be enzymatically active for poly(ADP-ribose) polymerase after elution from the antibody column. In contrast, the unbound nucleosomes, representing 90% of the unfractionated chromatin, were totally inactive in the poly(ADP-ribosyl)ation reaction.

Various indirect approaches have been used in the past to purify domains of chromatin undergoing specialized functional activities. The development of such enrichment procedures is important for an understanding of how the structure of chromatin and its associated nuclear proteins correlates with the functional activity (i.e., synthesis of DNA or RNA) of the genome. In the past, the poly(ADP-ribosyl)ation modification reaction of nuclear protein has been particularly difficult to study because of the high rate of turnover of the poly(ADP-ribose) covalently attached to nuclear proteins. The poly(ADP-ribosyl)ation modification may occur only on those regions of chromatin possessing single-strand DNA breaks accumulated during either DNA replication or repair (1–4). The modification may exist on only a small percentage of the chromatin (usually $<1\%$) undergoing these reactions at any one time.

Our development of highly active antisera directed specifically against the poly(ADP-ribose) polymer (5, 6) led us to test whether an immunoaffinity method could be used to bind selectively those domains of chromatin undergoing this reaction. The aims of the current studies were to develop this method and to characterize the nuclear proteins and DNA unique to poly(ADP-ribosyl)ated domains of chromatin.

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MATERIALS AND METHODS

Chemicals. CNBr-activated Sepharose-4B was purchased from Pharmacia; L-[^{3}H]lysine, L-[^{3}H]arginine, and β-[^{32}P]NAD$^+$ were from New England Nuclear. $(NH_4)_2SO_4$-fractionated anti-poly(ADP-ribose) IgG was obtained from rabbits as described (5, 6). Lysine- and arginine-deficient Eagle medium for suspension culture was obtained from the National Institutes of Health Media Unit.

Coupling of Anti-Poly(ADP-ribose) IgG to CNBr-Activated Sepharose-4B. CNBr-activated Sepharose-4B (3 g) was soaked for 2 hr in 1 mM HCl. This swollen, equilibrated Sepharose was washed with 1 mM HCl and was suspended in 15 ml of coupling buffer (0.1 M $NaHCO_3$, pH 8.5/1 M NaCl) containing 12 mg of anti-poly(ADP-ribose) IgG. The gel suspension was mixed end-over-end for 2.5 hr. The IgG-Sepharose was suspended in 1 M ethanolamine (pH 8.0) for 2 hr and subsequently washed alternately with low pH buffer (0.1 M acetate/1 M NaCl) and high pH buffer (1% ammonium bicarbonate). The washed gel was suspended and equilibrated in phosphate-buffered saline.

Preparation of HeLa Chromatin. HeLa nuclei were treated with micrococcal nuclease (30 units/1×10^8 nuclei) for 3 min at 37°C by the method of Butt and Smulson (7). This digestion renders 9–10% of the chromatin DNA soluble in acid.

Incubation with [^{32}P]NAD. Nucleosomes (approximately 1.5 A_{260}) were suspended in 10 mM Tris·HCl, pH 8.0/2 mM dithiothreitol/5 mM Mg^{2+}. The reaction was initiated by the addition of 5 μCi (1 Ci = 3.7×10^{10} Bq) of [^{32}P]NAD in 10 μM nonradioactive NAD; incubation was at room temperature for 5 min. The reaction was terminated by the addition of nicotinamide to a final concentration of 20 mM. The labeled nucleosomal sample was passed through a small column of Sephadex G-25 to remove unincorporated NAD.

Labeling of HeLa Cells. HeLa cells (2×10^5/ml) were adapted for growth in Eagle medium, 80% depleted in lysine and arginine, for 24 hr. Subsequently, the cells were grown for 24 hr in the same medium containing [^{3}H]lysine (5 μCi/ml, 60 Ci/mmol) and [^{3}H]arginine (30 Ci/mmol) as described by Annunziato *et al.* (8).

RESULTS

The anti-poly(ADP-ribose) IgG used in these studies is highly specific for poly(ADP-ribose) chains of length 3–200 units (5, 6). In a competitive titration assay, DNA, RNA, NAD, and other nucleotides did not show reactivity to the antibody (6). It was important to ascertain whether the purified antipoly(ADP-ribose) IgG fraction was capable of interaction with endogenous poly(ADP-ribosyl)ated nuclear proteins within the nucleosomal

structure. Accordingly, oligonucleosomes were incubated in the presence and absence of 10 μM NAD, subsequently separated by nondenaturating polyacrylamide gel electrophoresis, blotted (9), and allowed to react with anti-poly(ADP-ribose). Significant binding of the IgG to each chain size of nucleosomes was noted in the chromatin sample preincubated with NAD (data not shown).

Separation of Modified Nucleosomes by Immunoaffinity Chromatography. The antibody was covalently coupled to CNBr-activated Sepharose-4B and a column was prepared. As a control, a preimmune fraction of IgG was bound to Sepharose in a similar manner. To test the binding of poly(ADP-ribosyl)ated chromatin to the column, a preparation of oligonucleosomes (1.5 A_{260}) was incubated with [^{32}P]NAD (10 μM). Under these conditions, poly(ADP-ribose) from 2 units to >15 are enzymatically coupled to nucleosomal histone acceptors by the chromatin-associated polymerase (7, 10). However, only a limited domain of chromatin is accessible for poly(ADP-ribosyl)ation at any one time, accounting for approximately 0.5% of the total histone acceptors (11, 12).

After the *in vitro* incubation, the oligomer (4–8 nucleosomes; 4.95×10^6 cpm) in 1 ml was slowly applied to the column over a 45-min period (Fig. 1*A*). Washing was begun with phosphate-buffered saline; an A_{260} peak eluted between fractions 5 and 10. This represented >90% of the original polynucleosome sample. In addition to absorbance, acid-precipitable radioactivity representing poly(ADP-ribosyl)ated nucleosomal acceptors (see below) was monitored in all fractions. Negligible ^{32}P incorporation was noted in the unbound fractions, where the majority of nucleosomes eluted. When no further material with ^{32}P or absorbance at 260 nm eluted, the column was washed with 20 ml of phosphate-buffered saline. The bound material was released by the addition of 1.6 M KSCN (fraction 30 in Fig. 1). An almost immediate elution of acid-precipitable poly([^{32}P]ADP-ribosyl)ated material was noted. Approximately 95% of the total nucleosomal radioactive incorporation was represented in five 1.0-ml fractions (fractions 32–37 in Fig. 1). The presence of KSCN prevented accurate quantitation of absorbance; however, when bound fractions were dialyzed, a reliable measurement was possible, and nucleoprotein structures were observed (Fig. 1 *Inset*).

When a similarly prepared column containing preimmune IgG coupled to Sepharose was utilized (Fig. 1*B*), the majority of the acid-insoluble poly([^{32}P]ADP-ribosyl)ated material remained unbound to the column and eluted with the bulk of the nucleosomes which passed directly through the column (fractions 3–7).

The procedure appeared to make possible the isolation of nucleosomal domains containing this specifically modified region of chromatin. Subsequent experiments were designed to characterize the poly(ADP-ribosyl)ated chromatin remaining bound to the column in comparison to unbound chromatin.

Analysis of Immuno-Complexed Radioactive Histones. Fractions of unbound and bound material from an experiment similar to that in Fig. 1*A* were acid extracted and subjected to electrophoresis and autoradiography to examine histone acceptors. In the experiment in Fig. 2 (lane 1), histones isolated from the labeled nucleosomes prior to immuno-fractionation showed the modification of core histones demonstrated previously (13). No poly(ADP-ribosyl)ated acceptors were detected in the histone fraction isolated from the unbound fraction (lane 2), although this fraction constituted the vast majority of the chromatin applied to the column. In contrast, well-defined poly(ADP-ribosyl)ated histones were noted in the region near the peak radioactive fraction of bound material (lane 4). The best-resolved modified histones were noted to elute slightly after the

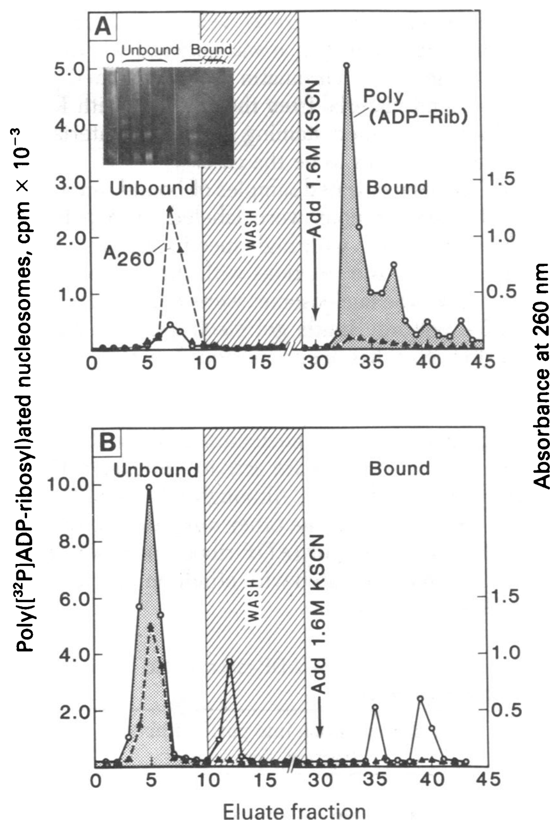

FIG. 1. Selective retention of modified nucleosome domains by anti-poly(ADP-ribose) IgG-Sepharose. (*A*) Oligonucleosomes (4–8 units) were poly(ADP-ribosyl)ated with [^{32}P]NAD. Nucleosomes (1.0 ml, 1.5 A_{260} units) were immunofractionated on anti-poly(ADP-ribose) IgG-Sepharose 4B. The column was washed in 1-ml fractions with phosphate-buffered saline. The bound material was eluted with 1.6 M KSCN at fraction 30. Absorbance at 260 nm (▲) was monitored on fractions prior to addition of KSCN. After addition of KSCN, absorbance was then measured after dialysis. Acid-precipitable radioactivity (○) was determined as described (7, 10). (*Inset*) Nondenaturating 3% polyacrylamide electrophoretic separation of dialyzed and concentrated samples of unbound and bound chromatin, stained with ethidium bromide. (*B*) Similarly prepared poly(ADP-ribosyl)ated oligonucleosomal sample analyzed on preimmune IgG-Sepharose column.

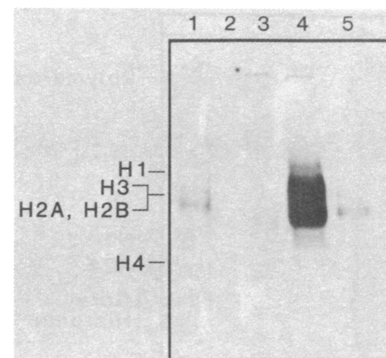

FIG. 2. Identification of poly(ADP-ribosyl)ated histones in immunoaffinity-bound chromatin. Separation of [^{32}P]NAD-labeled nucleosomes on the anti-poly(ADP-ribose) column was performed as in Fig. 1*A*. Heat-inactivated nucleosomes were added as a carrier to selected fractions of bound and unbound samples. The material soluble in 0.2 M H_2SO_4 was separated on acetic acid/urea/polyacrylamide gels and exposed for autoradiography as described (7, 12). Lanes: 1, nucleosomes applied to column; 2, unbound nucleosome; 3–5, bound nucleosomes, from another experiment, representing fractions similar to those of tubes 34–36 (Fig. 1), respectively.

peak; however, this varied to some extent among experiments. These results would imply a high level of enrichment of the limited domains of modified chromatin, and this was directly quantitated in subsequent experiments.

Association of Nonhistone Protein with Enriched Nucleosomes. Data from this laboratory and others have shown that the major nonhistone protein (112,000 daltons) acceptor for poly(ADP-ribose) polymerase is the enzyme itself, undergoing automodification (14–16). The automodified polymerase has been shown by various criteria to be reasonably tightly bound to the internucleosomal region of chromatin (7, 10, 13–15), and antibody to the polymerase has been shown to react with blots of electrophoretically separated nucleosomes (9).

Total poly(ADP-ribosyl)ated acceptors were examined in both bound and unbound chromatin by ethanol precipitation of the respective labeled fractions and NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 3). As before (Fig. 2), all poly(ADP-ribosyl)ated acceptors were detected only in the bound fractions (lanes 4–6). Labeled histones and possibly protein A-24 (17) were noted in these fractions, although the resolution of the core histones was more accurate in the data in Fig. 2.

The data also demonstrate that nucleosomes containing the poly(ADP-ribosyl)ated 112,000-dalton acceptor [as well as several lower molecular weight minor nonhistone protein acceptors (13, 14)] were effectively purified by the anti-poly(ADP-ribose) IgG-Sepharose (lanes 4–6). These results suggest that the immunoaffinity column partitions not only poly(ADP-ribosyl)ated domains of chromatin but, in addition, enzymatically active regions of chromatin containing the 112,000-dalton poly(ADP-ribose) polymerase. This possibility was explored in greater detail.

Degree of Enrichment of Poly(ADP-ribosyl)ated Nucleosomes. Not all nucleosomes within an oligonucleosome chain would be expected to contain poly(ADP-ribosyl)ated histones. To quantitate precisely the level of enrichment by the antibody of nucleosomal chains in which some or all histones contain poly(ADP-ribose), the amount of poly(ADP-ribose) relative to histone mass was determined.

Endogenous nucleosomal histones from HeLa cells were labeled with [^{3}H]lysine and [^{3}H]arginine as described by Annunziato *et al.* (8). ^{3}H-Labeled nucleosomes were prepared, incubated with 10 μM [^{32}P]NAD, and applied to an anti-poly(ADP-ribose) IgG-Sepharose column. After collection of the unbound material, the ^{32}P- and ^{3}H-labeled nucleosomes were eluted with KSCN. Of the ^{3}H radioactivity (i.e., amino acid-labeled nucleoproteins) applied to the column, 85.1% was recovered in the unbound fraction and 11.7% in the bound fraction (Table 1). In contrast, 95% of the poly([^{32}P]ADP-ribosyl)ated material remained bound. The relative specific activity (i.e., ^{32}P/^{3}H) of the bound material (before histone isolation) was 15; and that of the unbound was 0.0645, in contrast to 1.9 for the nucleosomes applied to the column.

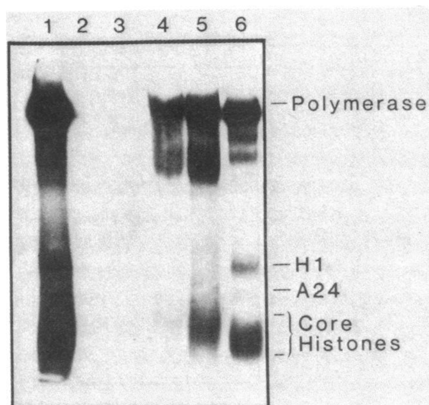

FIG. 3. Total nucleosomal poly(ADP-ribose) acceptors of chromatin purified by use of the anti-poly(ADP-ribose) column. The experiment was performed essentially as described in Fig. 2 except that carrier-containing samples were ethanol precipitated and subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography as described (14, 15). Lanes: 1, nucleosomes applied to column; 2 and 3, unbound nucleosomes; 4–6, bound nucleosomes, from another experiment, representing fractions essentially similar to those of tubes 34–36 (Fig. 1).

To analyze the degree of enrichment of unbound and bound fractions for poly(ADP-ribosyl)ated histone acceptors, samples were acid extracted and histones were purified (Table 1). The ^{32}P/^{3}H ratio of histones extracted from the nucleosomes prior to fractionation on the antibody column was 2.4. After fractionation, the specific activity of the bound fraction was 14.1, representing a 5.9-fold enrichment. These results were expected because not all nucleosomes within a domain of chromatin might be anticipated to undergo poly(ADP-ribosyl)ation at any one time.

Analysis for DNA and Nucleoprotein. It had been established previously that poly(ADP-ribosyl)ated histones and poly(ADR-ribose) polymerase remain tightly associated with oligonucleosomes during their separation by nondenaturating polyacrylamide gel electrophoresis, velocity sedimentation, or gel filtration (7, 10). However, it was not clear whether the nucleoprotein structure of chromatin was maintained after dissociation of bound material from the antibody with KSCN, although ethidium bromide staining of bound material (Fig. 1 *Inset*) had indicated this to be the case.

Dialyzed and concentrated samples of bound, unbound, and unfractionated preparations were labeled, in a poly(ADP-ribose) polymerase assay, with high-specific activity [^{32}P]NAD. After termination of the reactions with the polymerase inhibitor nicotinamide, the nucleosomes were subjected to nondenaturating 3.5% polyacrylamide gel electrophoresis and autoradiography (Fig. 4). It has been reported (7, 9) that, under these labeling conditions, incorporated ^{32}P migrates with polymerase-active oligonucleosomal species. The unbound nucleosomes contained the majority of the nucleosomal chromatin applied to the column, yet no polymerase activity remained with these classes of chromatin (lane 2). Although the quantities of material were much less than the other samples, the bound fraction showed considerable poly(ADP-ribose) polymerase activity (lanes 1 and 3) which migrated with ethidium bromide-staining chromatin bands (not shown).

Table 1. Enrichment of poly(ADP-ribosyl)ated histones in bound oligonucleosomes

Nucleosome fraction	Incorporation into purified histone fraction, dpm × 10^{-3}: ^{3}H	^{32}P	^{32}P/^{3}H	Fold enrichment	Protein recovered, %
Unfractionated (i.e., applied)	29.2	70.0	2.4	1	—
Unbound	25.5	0.1	0.004	0.002	85.1
Bound	3.5	49.4	14.1	5.9	11.7

The oligonucleosomes fractionated on the anti-poly(ADP-ribose) IgG-Sepharose column were derived from HeLa cells which had been labeled during growth with [^{3}H]lysine and [^{3}H]arginine. The nucleosomes were incubated under conditions optimal for poly(ADP-ribosyl)ation with [^{32}P]NAD prior to immunofractionation. After fractionation, the original sample and bound and unbound samples were extracted for histones with 0.2 M H_2SO_4. Gel electrophoretic analysis indicated that >90% of the ^{3}H and ^{32}P incorporated in these samples resided in histones.

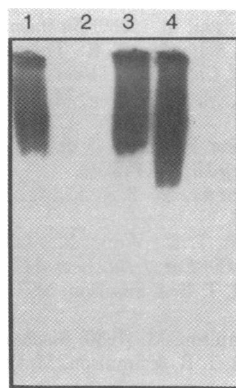

FIG. 4. Autoradiograms showing poly(ADP-ribosyl)ation of enzymatically active nucleoprotein complexes before and after immunofractionation. Lanes 1–4 represent samples of bound, unbound, bound (duplicate), and unfractionated (i.e., sample before column) chromatin fractions, respectively. After fractionation using nonradioactive NAD, samples (i.e., lanes 1–3) were dialyzed overnight against phosphate-buffered saline and concentrated. The chromatin preparations were poly([^{32}P]ADP-ribosyl)ated and electrophoresed on polyacrylamide gels.

Total cellular levels of poly(ADP-ribosyl)ation have been shown to be greatly increased by various treatments that lead to the production of DNA strand breaks (1–4). The status of internal single-strand DNA breaks was compared in chromatin totally lacking poly(ADP-ribosyl)ated species (i.e., unbound chromatin) and in bound chromatin. DNA from the respective fractions was phenol extracted, treated with alkaline phosphatase (to cleave external and internal phosphomonoesters), and ^{32}P-labeled with polynucleotide kinase. The small quantity of DNA in the bound fraction necessitated the use of the labeling procedure. The DNA was analyzed on a nondenaturating 1.6% agarose gel in the first dimension and on an alkaline agarose gel (18), to detect strand breaks, in the second dimension. Ethidium bromide staining of the first dimension of the unbound DNA (Fig. 5 *Top*) showed distinct stained nucleosomal DNA species at approximately 270 and 400 base pairs. The autoradiograph (Fig. 5 *Middle*) of the first dimension indicated a range of base pairs from 200 to 800; however, because of the variety of required experimental treatments of the sample, distinct labeled nucleosomal repeats were not observed. In the second dimension (denaturing), the unbound sample migrated as a diagonal. Faster bands appearing off the diagonal and the occurrence of diffuse labeled DNA in this area indicated that the unbound chromatin contained some oligomers with internal strand breaks. The bound nucleosomal DNA was more diffuse, and a minor diagonal band (denoted by arrows) was noted. However, the bulk of the bound DNA migrated considerably faster than the diagonal and appeared to be composed almost entirely of nucleosomal DNA with considerable internal strand breaks.

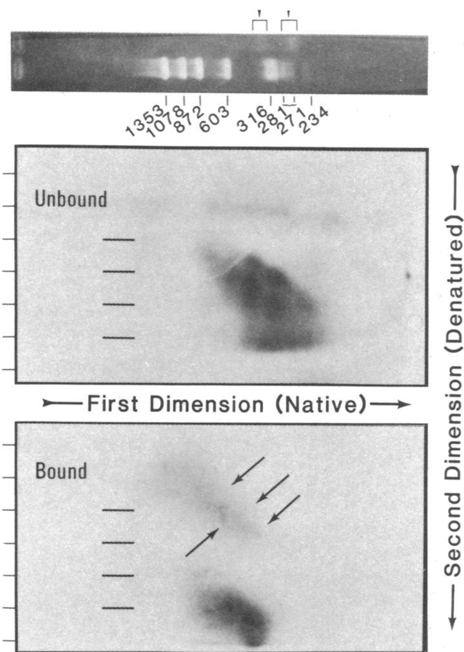

FIG. 5. Poly(ADP-ribosyl)ated nucleosomes contain significant internal DNA strand breaks. DNA from unbound (4.4 A_{260} units) and bound nucleosomes (0.47 A_{260} unit) was extracted and end labeled by using [^{32}P]ATP and polynucleotide kinase. The ethanol-precipitated labeled DNA samples were subjected to nondenaturating 1.6% agarose gel electrophoresis as described by Modak and Beard (18). A duplicate lane of unbound DNA and a lane of *Hae* I-restricted ϕX174 DNA were stained with ethidium bromide (*Top*). The arrows indicate staining at mono- and dinucleosome regions of the gel. The first-dimension lanes were electrophoresed under denaturating conditions in the second dimension (18), and the gels were dried and autoradiographed. The arrows (*Bottom*) outline a low level of nucleosomal DNA "diagonal," which is in the same position as the major diagonal in the unbound DNA sample.

DISCUSSION

Experimentation aimed at relating biological function to structural features of nucleosomal chromatin has suffered from the lack of methods to isolate large quantities of highly enriched nucleoprotein domains undergoing such reactions. In the present study, the use of anti-poly(ADP-ribose) antibody covalently attached to Sepharose permitted the selective enrichment of chains of oligonucleosomes proximal to poly(ADP-ribosyl)ation sites in chromatin. We have not tested any other forms of material except nuclease-prepared chromatin.

The steady-state concentration of poly(ADP-ribose) within chromatin is exceedingly small, representing approximately 1–5% of potential histone acceptors at any one time (11, 12). This is due in part to the apparent cellular functional requirements for this modification (i.e., sensing DNA strand breaks) via modulation of the synthetic and degradative enzymes involved in poly(ADP-ribose) metabolism. The immunoaffinity method described here allowed us to isolate and to analyze these dynamic domains of chromatin. Additionally, the "unbound species" of chromatin, readily obtainable in native form by the present procedure, possesses negligible poly(ADP-ribosyl)ated nucleosomes or binding domains containing measurable enzymatic potential for poly(ADP-ribosyl)ation.

The polymerase catalyzes the successive transfer of the ADP-ribose moiety of NAD to histone acceptors to generate poly(ADP-ribose) on these proteins. A significant feature of the catalytic reaction of the purified enzyme, which perhaps accounts for the minute domain of chromatin undergoing the reaction at any instance, is the strict requirement for DNA for enzymatic activity (19). Additionally, Benjamin and Gill have demonstrated that the ability of DNA to support poly(ADP-ribose) synthesis is completely dependent upon the number of breaks of phosphodiester bonds in the DNA sample (4). A substantial body of recent literature has suggested that one key biological role of this system lies in the modification of regions of chromatin containing strand breaks and undergoing DNA repair/replication (1–4).

The fractionation procedure allowed the nucleosomal DNA of ADP-ribosylated chromatin regions to be analyzed and compared with unbound nucleosomal DNA (Fig. 5). The data suggest that considerable regions of internal single-strand DNA breaks existed in antibody-bound chromatin fractions, an ob-

servation consistent with a role for poly(ADP-ribosyl)ation in some step of DNA repair. An important prediction in such a biological function, partially answered above, is that chromatin domains proximal to DNA strand breaks should be extensively poly(ADP-ribosyl)ated compared to sites distal to the breaks.

1. Smulson, M. E., Schein, P., Mullins, D. W., Jr., & Sudhakar, S. (1977) *Cancer Res.* **37**, 3006–3012.
2. Sudhakar, S., Tew, K. D. & Smulson, M. E. (1979) *Cancer Res.* **39**, 1405–1410.
3. Durkacz, B. W., Nduka, N., Omidiji, O., Shall, S. & Zia'ee, A. A. (1980) in *Novel ADP-Ribosylations of Regulatory Enzymes and Proteins*, Developments in Cell Biology, eds. Smulson, M. E. & Sugimura, T. (Elsevier North Holland, New York), Vol. 6, pp. 207–216.
4. Benjamin, R. C. & Gill, D. M. (1980) *J. Biol. Chem.* **255**, 10502–10508.
5. Kanai, Y. & Sugimura, T. (1980) in *Novel ADP-Ribosylations of Regulatory Enzymes and Proteins*, Developments in Cell Biology, eds. Smulson, M. E. & Sugimura, T. (Elsevier North Holland, New York), pp. 153–162.
6. Kanai, Y., Miwa, M., Matsushima, T. & Sugimura, T. (1974) *Biochem. Biophys. Res. Commun.* **59**, 300–306.
7. Butt, T. R. & Smulson, M. (1980) *Biochemistry* **19**, 5235–5242.
8. Annunziato, A. T., Schindler, R. K., Thomas, C. A., Jr., & Seale, R. L. (1981) *J. Biol. Chem.* **256**, 11880–11886.
9. Malik, N., Bustin, M. & Smulson, M. (1982) *Nucleic Acids Res.* **10**, 2939–2950.
10. Butt, T. R., DeCoste, B., Jump, D. B., Nolan, N. & Smulson, M. (1980) *Biochemistry* **19**, 5243–5249.
11. Stone, P. R., Lorimer, W. S. & Kidwell, W. R. (1977) *Eur. J. Biochem.* **81**, 9–18.
12. Nolan, N. L., Butt, T. R., Wong, M., Lambrianidou, A. & Sumulson, M. E. (1980) *Eur. J. Biochem.* **113**, 15–25.
13. Jump, D. B., Butt, T. R. & Smulson, M. (1979) *Biochemistry* **18**, 983–990.
14. Jump, D. B. & Smulson, M. (1980) *Biochemistry* **19**, 1024–1030.
15. Jump, D. B., Butt, T. R. & Smulson, M. (1980) *Biochemistry* **19**, 1031–1037.
16. Ogata, N., Ueda, K., Kawaichi, M. & Hayaishi, O. (1981) *J. Biol. Chem.* **256**, 4135–4137.
17. Goldknopf, I. L., Taylor, C. W., Baum, R. M., Yeoman, L. C., Olson, M. O. J., Prestayko, A. W. & Busch, H. (1975) *J. Biol. Chem.* **250**, 7182–7187.
18. Modak, S. P. & Beard, P. (1980) *Nucleic Acids Res.* **8**, 2665–2678.
19. Hayaishi, O. & Ueda, K. (1977) *Annu. Rev. Biochem.* **46**, 95–116.