Targeting of Histone Tails by Poly(ADP-ribose)*

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After Zn²⁺ finger-mediated binding to a DNA break, poly(ADP-ribose) polymerase becomes autodegraded with long polymers of ADP-ribose. These nucleic acid-like polymers may facilitate DNA repair by noncovalently interacting with neighboring proteins. Using a novel screening technique, we have identified histones as the predominant poly(ADP-ribose)-binding species in human keratinocytes, rat hepatocytes, frog eggs, and yeast. Polymer binding is confined specifically to the histone domains responsible for DNA condensation, i.e. histone tails. Our results indicate that polymers of ADP-ribose are targeted to sites of DNA strand breaks by poly(ADP-ribose) polymerase and subsequently function to alter chromatin conformation through noncovalent interactions with histone tails.

Poly(ADP-ribose) formation by poly(ADP-ribose) polymerase is required for repair of damaged plasmids in human cell-free extracts (1). After Zn²⁺ finger-mediated binding to a DNA single- or double-strand break (2), the enzyme is activated to catalyze: 1) cleavage of NAD⁺ to nicotinamide and ADP-ribose, 2) attachment of an initial ADP-ribose residue to a modification site on the polymerase molecule (automodification), and 3) elongation of the initial residue to form polymers of >200 units (3–5). These nucleic acid-like polymers may facilitate DNA repair by noncovalently interacting with neighboring proteins to influence chromatin function. We have therefore developed a simple assay to screen complex protein mixtures for poly(ADP-ribose)-binding proteins. In all nuclear lysates tested and in a frog egg lysate, histones proved to be the predominant polymer-binding proteins. Furthermore, histone binding to poly(ADP-ribose) was mediated solely by the domains responsible for DNA condensation, thus supporting a role for poly(ADP-ribose)ylation in DNA repair-associated chromatin relaxation (6, 7).

MATERIALS AND METHODS

Lysates—Lysates were prepared from 500 µl of sedimented human keratinocyte nuclei (8), rat liver nuclei (9), and Saccharomyces cerevisiae nuclei (10) after digestion with 200 units of DNase I (Sigma) for 2 h at 25 °C. Nuclei were lysed upon addition of 0.6 ml of 0.2 mm EDTA (pH 7.9) and repeated passage through a 200-µl pipette tip. Insoluble debris was pelleted by centrifugation at 7,400 × g for 10 min at 4 °C. The cleared nuclear lysates were divided into 100-µl aliquots, lyophilized, and stored at −20 °C. Xenopus laevis egg lysate was prepared according to Almouzni and Machali (11) and stored at −80 °C.

Histone Fragments—Histone fragments were prepared by chemical cleavage of electrophoretically pure histones (Boehringer Mannheim) as follows (see Fig. 3). H1 was cleaved with N-bromosuccinimide (12); H2B, H3, and H4, respectively, were cleaved by the cyanogen bromide reaction (13). All reactions proceeded to >90% completion as determined by polyacrylamide gel analysis. Each lyophilized peptide mixture was dissolved in water to a final concentration of 1 ng/ml protein and stored at −20 °C.

ADP-ribose Polymer Blot Analyses—ADP-ribose polymer blot analyses were performed after separation of duplicate protein samples on SDS-polyacrylamide gels (14) as indicated in the figure legends. One channel of each gel was stained with Coomassie Blue. Proteins in the other half were transferred to nitrocellulose (0.45-μm pore size for lysate proteins, 0.2-μm pore size for histone fragments; Schleicher & Schuell) using the SemiPhor transfer system (Bio-Rad) with SDS-polyacrylamide gel electrophoresis running buffer (14). The nitrocellulose membrane was rinsed with three changes of TBS-Tween 20 (10 ms Tris, 0.15 mM NaCl, 0.05% v/v Tween 20, pH 7.4) in 16 min. Blocking was performed for 1 h at room temperature or overnight at 4 °C in TBS-Tween 20 containing 3% bovine serum albumin after which the nitrocellulose was rinsed with three changes of TBS-Tween 20 in 30 min. Note that the blocking step is not necessary for polymer blot analysis but was included in anticipation of subsequent protein detection using immunoblotting techniques.

Polymers of [32P]ADP-ribose (0.5 μCi/μmol ADP-ribose; 0.5–1 nmol of total ADP-ribose; mean polymer size 49 residues; Ref. 15) were diluted to 10 ml with TBS-Tween 20 and added to the nitrocellulose. After incubation for 1 h at room temperature with gentle agitation, the membrane was washed with TBS-Tween 20 until no radioactivity could be detected in the supernatant. The nitrocellulose was dried and subjected to autoradiography.

RESULTS AND DISCUSSION

To identify proteins present in vivo which bind noncovalently to polymers of ADP-ribose, we have developed an ADP-ribose polymer blot assay (Fig. 1). The assay is similar to a conventional immunoblot but employs poly(ADP-ribose) as the probe in place of antibodies to rapidly screen cellular extracts for polymer-binding proteins. When nuclear lysates from human keratinocytes, rat liver, and S. cerevisiae and an egg lysate from X. laevis were screened for poly(ADP-ribose)-binding proteins, histones proved to be the predominant polymer-binding species (Fig. 2). Scanning densitometry of the gels and polymer blots revealed that, while representing only 10% of total protein in each lysate, histones accounted for almost 100% of poly(ADP-ribose) binding. In the keratinocyte and rat liver samples, H1 was the major polymer-binding protein followed by the core histones. Also visible were minor poly(ADP-ribose)-binding proteins with molecular mass values of 25–30 kDa, which may represent histone-like HMG proteins (16), and polymer-binding proteins larger than 45 kDa, which have yet to be identified.

Although the presence of a poly(ADP-ribose)ylation system in yeast is still a matter of controversy (17, 18), yeast nuclei contain poly(ADP-ribose)-binding proteins, all of which migrate as histones (Fig. 2). Despite evolutionary divergence (19), yeast H2B, H3, and H4 have retained affinities for poly(ADP-ribose). There was no indication of ADP-ribose polymer binding to any proteins larger than H3, thus corroborating the absence of a

The abbreviation used is: TBS, Tris-buffered saline.
were necessarily isolated using different protocols with differing effects on histone recovery (see "Materials and Methods"). However, the presence of histone quantities below the detection limit of Coomassie Blue were easily revealed by the polymer blot assay (Fig. 2). Not only does this result emphasize the sensitivity of the assay, but also the very high affinity of poly-(ADP-ribose) for histones (22). Third, evolutionary differences in a putative polymer-binding motif within each histone cannot be ruled out. Although ionic interactions may play a role in ADP-ribose polymer binding to immobilized proteins, they do not seem to be the sole determinants for binding, since the 14.4-kDa molecular size marker in our blots is lysozyme, a basic protein with a pI of 11, which does not bind the highly acidic polymers. Our results using the ADP-ribose polymer blot assay are in complete agreement with results obtained in solution which have shown noncovalent binding of pure histones to poly(ADP-ribose) (22, 23), indicating that immobilization of proteins on nitrocellulose has little effect on their binding to poly(ADP-ribose).

From the above results, it was concluded that histones are the primary protein species to which polymers of ADP-ribose bind noncovalently. In light of these findings, we have used the polymer blot assay to establish which histone domains are responsible for polymer binding to H1, H2B, H3, and H4, respectively (Fig. 3 and 4). As seen in the lanes containing H1 and its domains (Fig. 4), only the C-terminal fragment of H1 exhibited affinity for poly(ADP-ribose). Therefore, it seems likely that this domain is solely responsible for binding of ADP-ribose polymers (22). Likewise, the N-terminal regions of H3 and H4 exhibited signals of polymer binding comparable to the respective intact proteins, whereas binding to the C-terminal regions was not detected. The affinity of H2B for poly(ADP-ribose) was apparently abolished upon cleavage. Ponceau S staining after transfer revealed efficient transfer and binding of all histone fragments to the nitrocellulose except for the C-terminal fragment of H2B. Transfer and binding of this H2B fragment to diazotized cellulose was, however, successful, but no binding to poly(ADP-ribose) was detected (not shown).

The histone tails involved in polymer binding are also those responsible for DNA condensation and those most easily accessible for macromolecular interactions in a chromatosomal configuration (24, 25). Specifically, the C-terminal region of H1 and the N-terminal regions of H3 and H4 bend linker DNA to bring nucleosomes together, and their absence results in internucleosomal unwinding (26, 27). Furthermore, our results concerning H2B revealed that scission of the molecule within the globular region abolished affinity for poly(ADP-ribose). This correlates with a reported loss in the ability of H2B to participate in the formation of condensed chromatin (28).

Fig. 3. Chemical cleavage products of histones. Pure histones H1, H2B, H3, and H4 were chemically cleaved (see Methods) to yield the indicated fragments. The N-terminal/globular and the globular/C-terminal transition regions shown in this diagram are approximate (±5 amino acids). aa, amino acids.
Recent studies from our laboratory have shown that poly(ADP-ribose) formation allows DNA processing enzymes access to previously inaccessible DNA regions by disrupting histone-DNA interactions (29). These are the key steps of the histone shuttle mechanism catalyzed by the poly(ADP-ribose)ylation system of chromatin (29, 30). The present study reveals an amazing target specificity of this mechanism, i.e., to histone essential for the supranucleosomal organization of chromatin.

REFERENCES