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POLYAMINE INDUCED CHANGES IN THE ADP-RIBOSYLATION OF NUCLEAR PROTEINS FROM RAT LIVER

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SUMMARY: Purified rat liver nuclei were incubated in vitro with [^{3}H]NAD. Altered patterns of ADP-ribosylation of nuclear proteins occurred with 1 mM spermidine or spermine with the latter polyamine causing the greater change. Spermine treated nuclei showed a two-fold increase in ADP-ribose incorporation into H1 histones and a decrease in the other histones. Likewise, the incorporation into the more acidic non-histone nuclear proteins was greater with spermine than spermidine. These results suggest that polyamines may exert a regulatory function by altering the pattern of ADP-ribosylation of both histone and non-histone nuclear proteins.

Mammalian cell nuclei contain an enzyme that will incorporate the ADP-ribose moiety of NAD into nuclear proteins either as a monomer or a homopolymer of several units (1). Recently, it was observed that polyamines increased the rate of ADP-ribosylation in rat liver nuclei incubated with NAD (2). This observation may link the relationships with DNA synthesis which have been reported for both polyamine concentration (3) and ADP-ribosylation of nuclear proteins (1). The purpose of the present study was to explore the extent to which polyamines influence not only the rate of ADP-ribosylation of histone and non-histone nuclear proteins but also the pattern of ADP-ribosylation within these major protein classes. We wish to report that 1 mM spermine alters the distribution of ADP-ribosylation in both histone and acidic nuclear proteins, whereas, the effects of spermidine under identical conditions are less marked.

MATERIALS AND METHODS

Materials: White male rats of the Buffalo strain, weighing between 150-200 g, were used in all experiments. The [adenosine-2,8-^{3}H]NAD was purchased from New England Nuclear. NAD$^+$, spermine, spermidine, and putrescine were products of Sigma Chemical Company.

Isolation of rat liver nuclei: The animals were sacrificed by decapitation, followed by rapid excision of the livers. Nuclei were isolated by a modification of the procedure of Lynch et al. (4). All subsequent procedures were performed at 0-4°C. Approximately 2 g of minced tissue was homogenized in 7 volumes of 0.3 M sucrose-4 mM $CaCl_2$. The homogenate was filtered through 8 layers of cheesecloth and centrifuged at 1000 g for 7 min. The crude nuclear pellet was suspended in 15 ml 2.0 M sucrose-1 mM $CaCl_2$ and

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layered over 20 ml of 2.2 M sucrose. The nuclei were sedimented by centrifugation for 60 min in a Beckman SW27 rotor at 24,000 rpm. Finally, the nuclei were washed once with 1 M sucrose-0.5 mM $CaCl_2$ and suspended in 0.3 M sucrose.

Assay for protein and DNA: Protein and DNA were estimated by the methods of Lowry et al. (5) and Burton (6), respectively.

Assay of poly(ADP-ribose) polymerase: The standard reaction mixture contained 0.5 mM [^{3}H]NAD (1.0 μCi/ml reaction mixture), 50 mM Hepes buffer (pH 7.5), 25 mM KCl, 25 mM NaCl, 2 mM $MgCl_2$, 1 mM dithiothreitol, and 2-6 x 10^7 nuclei per ml reaction mixture. After a preincubation of 5 min at 20°C in the presence of polyamines, [^{3}H]NAD was added and the mixture incubated an additional 30 min at 35°C. The reaction was stopped by the addition of 15 ml ice-cold 0.3 M sucrose and nuclei were washed once with 0.3 M sucrose. Nuclear proteins were extracted from nuclei by the procedure of Lea and Koch (7) with the exception that the H1 histones were obtained by the method of Johns (8)

Electrophoresis and isoelectric focusing: The polyacrylamide gel electrophoresis and staining of histones with amido black were performed according to Panyim and Chalkley (9). In addition, sodium dodecyl sulfate-15% polyacrylamide gel electrophoresis was used for separation of histones (10). Isoelectric focusing was performed essentially in accordance with the procedure of Gronow and Griffiths (11).

RESULTS AND DISCUSSION

The polyamines spermine and spermidine when incubated with isolated nuclei, as described in Materials and Methods, exhibit a dose dependent effect on ADP-ribosylation (Fig. 1). Of the two polyamines, spermine is more effective in stimulating the incorporation of ADP-ribose into isolated nuclei. At a concentration of 1 mM spermine increased the incorporation of [^{3}H]NAD into isolated nuclei by approximately 50%. In contrast, putrescine does not cause a stimulation under the same conditions. We have found in our in vitro system that 2 mM spermine maximally stimulates ADP-ribosylation in the presence of 2 mM Mg^{2+}. At higher Mg^{2+} concentrations the effect of spermine is less marked. Furthermore, 2 mM spermine and 4 mM Mg^{2+} stimulates ADP-ribosylation to the same extent as 4 mM Mg^{2+} alone. Thus, high Mg^{2+} concentrations mask the effect of spermine on ADP-ribosylation. It appears that a dynamic equilibrium may exist in the nucleus between free and bound polyamines which is dependent upon the concentration of divalent cations. Therefore, we have chosen 2 mM as our optimal Mg^{2+} concentration .

The effect of polyamines on the incorporation of ADP-ribose into nucleoproteins was determined by incubating nuclei with [^{3}H]NAD in the presence of either spermine or spermidine. Following extraction of nucleoproteins, the distribution of labeled ADP-ribose was determined in the histone fraction, as well as, in the nucleoprotein fraction extracted in 0.35 M NaCl, as described in Materials and Methods. Using urea-acetic acid polyacrylamide gel electrophoresis, the distribution of labeled ADP-ribose was determined among the

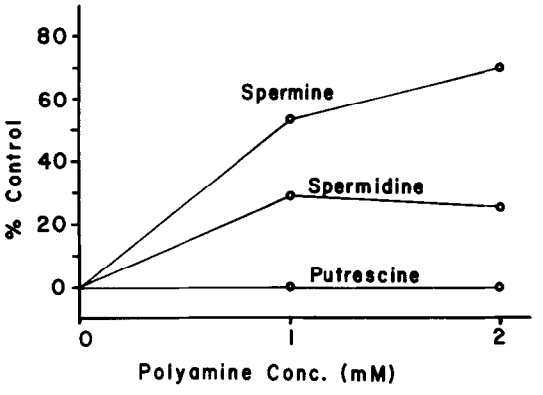

Fig. 1. Effect of polyamine concentration on ADP-ribosylation in isolated nuclei. The incorporation of [adenosine-^{3}H]NAD into acid-insoluble material after incubation for 15 min. at 35^{0}C is expressed as the per cent increase relative to control incubations without polyamines. Other assay conditions are as described in Materials and Methods.

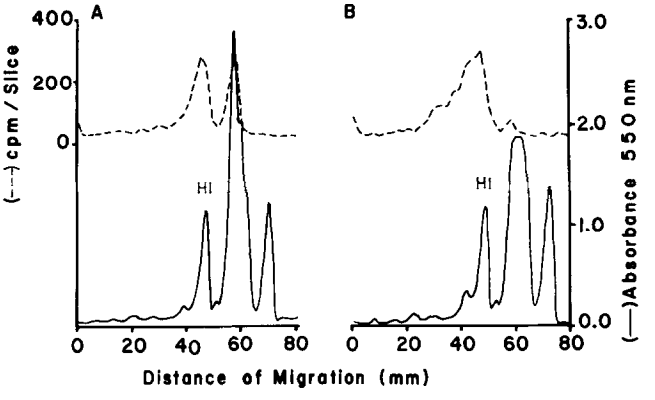

Fig. 2. Distribution of [^{3}H]poly(ADP-ribose) on acetic acid-urea polyacrylamide gels. Electrophoretic analysis was performed using histones extracted from isolated nuclei that were incubated with [^{3}H]NAD in the absence (A) or presence (B) of 1mM spermine as described in Materials and Methods. 100 µg of histone preparation was applied to each gel. Electrophoresis was carried out as described in Methods.

histones. As can be seen from Fig. 2, maximal incorporation was found among histones H1, H3, and H2b when nuclei were incubated in the absence of spermine. However, when spermine was added at a concentration of 1 mM, a shift in incorporation was observed. The greatest incorporation occurred in the H1 histone fraction with a concurrent decrease in incorporation in H3 and

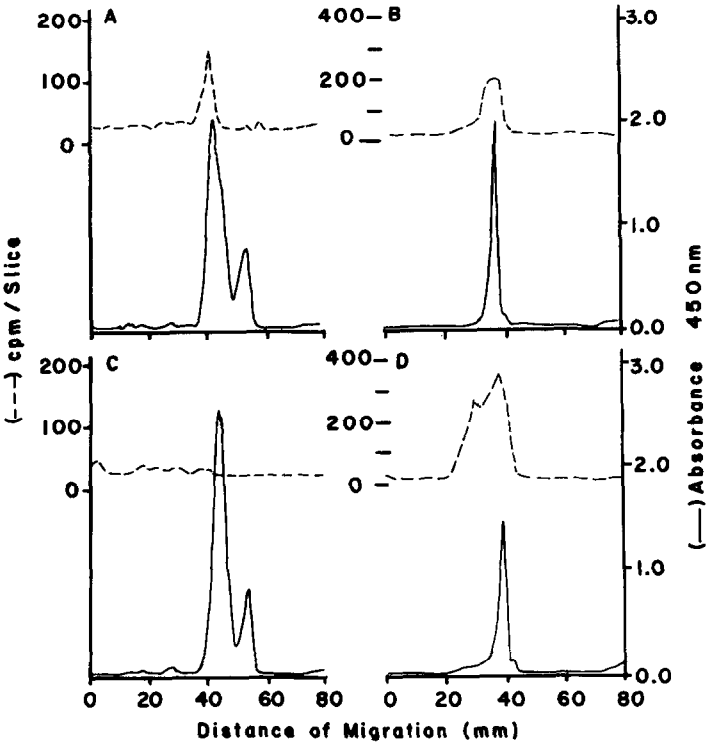

Fig. 3. Distribution of [^{3}H]poly(ADP-ribose) on acetic acid-urea polyacrylamide gels. Electrophoretic analysis was performed using either core nucleosome histones or H1 histones extracted from nuclei that were incubated with [^{3}H]NAD in the presence or absence of spermine. 100 µg of core histones and 25 µg of histone H1 were electrophoresed as described in Methods. (A) Control core histones, (B) Control histone H1, (C) Core histones extracted from nuclei incubated with 1mM spermine, (D) H1 histones extracted from nuclei incubated with 1mM spermine.

H2b histones. It appears, therefore, that spermine causes a change in the distribution of labeled ADP-ribose from the core histones (H3, H2b, and H2a) to the H1 fraction which is believed to have an internucleosomal location (12). Since the peak of radioactivity in the spermine treated nuclei is skewed to the left, there may exist a H1 histone-ADP-ribose complex larger than individual H1 histones and possibly existing in the form of a dimer (13, 14). This shift in labeled ADP-ribose incorporation was also observed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown).

To better evaluate the incorporation of ADP-ribose into H1 histones, we selectively extracted H1 histones from spermine treated and untreated nuclei using 5% $HClO_4$ (w/v) followed by the extraction of core histones with

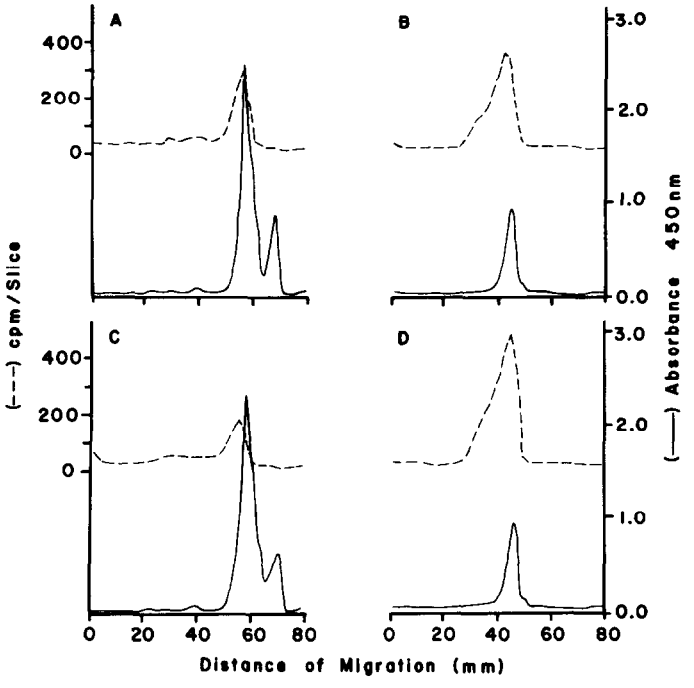

Fig.4. Distribution of [^{3}H]poly(ADP-ribose) on acetic acid-urea polyacrylamide gels. Electrophoretic analysis was performed as described in the legend of Fig. 3 with the substitution of spermidine for spermine. (A) Control core histones, (B) Control histone H1, (C) Core histones extracted from nuclei incubated with 1mM spermidine, (D) H1 histones extracted from nuclei incubated with 1mM spermidine.

0.24 N HCl. Polyacrylamide gel electrophoresis of these fractions (Fig. 3) identified, more specifically, the shift of ADP-ribose to H1 histone complexes following spermine treatment. Furthermore, it is clearly seen that very little ADP-ribose remains with the core histones. Similar results were also observed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis of these fractions (data not shown).

Histones extracted from nuclei incubated with 1 mM spermidine showed trends similar to those of spermine, but to a lesser degree, as judged from Fig. 4. Rather than the almost complete loss of labeled ADP-ribose in core histones observed with spermine treated nuclei, spermidine caused only a 40% decrease in this fraction, however, a concomitant increase of 40% occurred in the H1 histone fraction. It appears from our observations that the incorporation of ADP-ribose into total histones is unchanged upon treatment with either spermine or spermidine, however, there exists a redistribution of ADP-ribose moieties. A recent preliminary communication

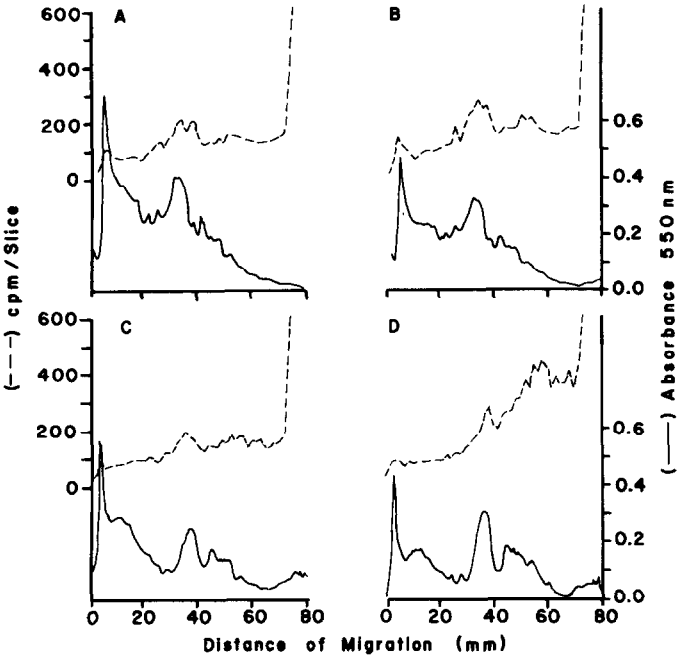

Fig. 5. Distribution of [^{3}H]poly(ADP-ribose) in nuclear proteins using isoelectric focusing in polyacrylamide gels. Nuclear proteins were extracted with 0.35 M NaCl from isolated nuclei that were incubated with [^{3}H]NAD in the absence (A and C) or presence of either spermidine (B) or spermine (D) at a concentration of 1mM, as described in Methods.

has indicated that polyamines may cause a change in the pattern of histone ADP-ribosylation in nuclei of HeLa cells which appears to be similar to that seen for rat liver nuclei in the present work (15).

Analysis of other nuclear fractions revealed that 1 mM spermine enhanced the incorporation of labeled ADP-ribose into non-histone acidic proteins extracted from nuclei in 0.35 M NaCl. Upon further analysis of this fraction using isoelectric focusing gel electrophoresis (Fig. 5) it was observed that the increase in incorporation occurred predominantly in the more acidic portion of the gel (pH 3.0-pH 6.0). In contrast, spermidine, under the same conditions, exhibited little effect on this fraction. The very acidic end of these gels contained large quantities of radioactive material that we believe to be free poly(ADP-ribose) (16) that was either non-specifically bound to or degraded from chromosomal proteins upon extraction from isolated nuclei.

In conclusion, the analysis of nuclear proteins from isolated nuclei revealed that polyamine specific modification of both histones and non-

histone proteins had occurred. Further analysis has shown that spermine enhanced the ADP-ribosylation of the more acidic nuclear proteins, as well as of H1 histones lying outside the core nucleosomal structure. Introduction of a negatively charged homopolymer like poly(ADP-ribose) to the highly basic regions of the H1 histone molecule might weaken the interaction of this histone with DNA, just as phosphorylation has been postulated as a mechanism involved in chromatin condensation (17). Although, the exact function of ADP-ribosylation remains unclear, it appears plausible to postulate that ADP-ribosylation may be a mechanism involved in extending or condensing chromatin.

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