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Nuclear matrix associated poly(ADP-ribose) metabolism in regenerating rat liver

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We have examined a possible role for protein poly(ADP-ribosylation) during in vivo DNA replication by studying the metabolism of poly(ADP-ribose) in the nuclear matrix fraction from normal and regenerating rat liver. This fraction contains the newly replicated DNA and thus allows for the examination of the events closely associated with the replication process. It was found that 55% of the total nuclear protein-bound poly(ADP-ribose) and 15–35% of the total nuclear poly(ADP-ribose)-polymerase activity were tightly associated with this subnuclear compartment in normal liver. Surgical removal of two-thirds of the liver initiated a time-dependent decrease in nuclear matrix associated polymers of ADP-ribose and poly(ADP-ribose) polymerase activity which reached a minimum of 40% of control livers after 24 h, before returning to normal levels at 41 h post-partial hepatectomy. In contrast, the total levels of poly(ADP-ribose) in intact liver and the total polymerase activity of isolated nuclei exhibited a 2-fold increase over basal levels. These results are consistent with the conclusion that the nuclear matrix is a major poly(ADP-ribosylation) site within the nucleus and that this metabolic reaction may be closely connected with the events modulating DNA replication in this fraction.

Nuclear matrix; DNA replication; Poly(ADP-ribosylation)

1. INTRODUCTION

The chemical modification of proteins is a common mechanism for regulation of macromolecular function in cells. Poly(ADP-ribosylation) of proteins in eukaryotic organisms is recognized as an important reversible modification reaction that modulates the structure and function of nuclear proteins [1,2]. This reaction is catalyzed by poly(ADP-ribose) polymerase (EC 2.4.2.30), a chromatin-bound enzyme that uses NAD^+ as a substrate to assemble highly branched polymers of ADP-ribose [3] covalently bound to proteins in the presence of DNA. This reversible reaction has been suggested to be involved in the regulation of various physiological functions of the nucleus by modulating changes in chromatin architecture [4,5] that occur either during DNA replication [6], gene expression [7,8] or DNA-excision repair [9,10].

It has recently been reported that a significant portion of endogenous poly(ADP-ribose) is tightly associated with the nuclear matrix [11]. This subnuclear fraction, which contains less than 2% of the total cellular DNA, is typically obtained by sequential nuclease digestion and high-salt extraction of isolated nuclei [12]. An active fraction of DNA-replication enzyme complexes is also associated with the nuclear matrix [13]. In addition, radiolabeled precursors of DNA synthesis are incorporated first into nuclear matrix DNA [14,15]. Some of the major polypeptide components of the nuclear matrix, i.e. the lamins, have also been shown to be acceptors for poly(ADP-ribose) in vitro following incubation of isolated nuclei with radiolabeled NAD^+ prior to nuclear matrix isolation [16,17].

Here, we have determined the levels of protein-bound poly(ADP-ribose) and poly(ADP-ribose)

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polymerase activity associated with the nuclear matrix fraction in normal and regenerating rat liver, following partial hepatectomy, as an initial step to examine a possible role for this metabolic reaction in nuclear matrix associated DNA replication in vivo.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats (180–200 g) were subjected to surgical removal of two-thirds of the liver to induce liver regeneration. At different times following partial hepatectomy, animals were killed and the livers were excised and processed for nuclear matrix isolation.

2.1. *Isolation of nuclear matrices*

The nuclear matrix fraction of adult rat liver was isolated essentially as in [12]. Briefly, nuclei were first isolated by centrifugation through 2.2 M sucrose. Nuclei were subsequently incubated for 45 min at 37°C [18] to digest endogenously DNA and the pellet obtained following centrifugation was sequentially extracted with a low-salt-containing buffer, a high-salt-containing buffer and 0.1% Triton X-100. These nuclear matrices typically contained less than 2% of the total DNA and approx. 12–15% of the total nuclear protein.

2.2. *Poly(ADP-ribose) polymerase assay*

The activity of poly(ADP-ribose) polymerase was determined by incubating 50 μg total nuclear matrix protein with 100 μM NAD^+ at 30°C for 5 min in the presence of 10 mM $MgCl_2$, 10 mM dithiothreitol, 100 mM Tris-HCl buffer (pH 8.0), as well as 100 μg/ml of calf thymus DNA (Sigma, St. Louis, MO) and 1 μCi ^{3}H-labeled NAD^+ (New England Nuclear, Boston, MA). The reaction was terminated by adding trichloroacetic acid to 20% (w/v) final concentration at 0°C for 15 min. The acid-insoluble material was collected by filtration on GF/C filter papers and the radioactivity retained on the filters was determined by liquid scintillation counting.

2.3. *Preparation of acid-insoluble liver powder*

Livers were excised and immediately dropped in liquid nitrogen. Frozen tissue was then ground with a mortar and pestle in liquid nitrogen and placed in 20 vols of 20% trichloroacetic acid (w/v). The acid-insoluble material was then resuspended in a Polytron homogenizer and pellets were collected by centrifugation and washed three times with 20% trichloroacetic acid. The acid-insoluble material was also washed twice with ethanol, twice with ether, and finally dried at room temperature under vacuum for 48 h.

2.4. *Determination of protein-bound poly(ADP-ribose)*

The levels of ADP-ribose in polymeric form were determined in both acid-insoluble liver powder (vide supra) and purified nuclear matrix according to Jacobson et al. [19]. Briefly, acid-insoluble material was dissolved in alkali to detach ADP-ribose from proteins and the polymers were purified by affinity chromatography. Pure polymers were subsequently digested to the unique nucleoside ribosyladenosine (rAdo) which was then chemically converted to the fluorescent etheno derivative. Etheno-rAdo was finally quantified by fluorescence detection following reversed-phase high-performance liquid chromatography.

2.5. *Miscellaneous*

Protein and DNA were determined by the methods of Lowry et al. [20] and Burton [21], respectively.

3. RESULTS AND DISCUSSION

3.1. *Changes in poly(ADP-ribose) polymerase activity and levels of protein-bound poly(ADP-ribose) in rat liver following partial hepatectomy*

We first determined whether hepatocellular proliferation and DNA synthesis in vivo were accompanied by changes in the total levels of poly(ADP-ribose) polymerase activity associated with isolated rat liver nuclei. Fig.1A shows that the activity of the polymerase more than doubled after 18 h of liver regeneration and these levels remained elevated even after 40 h. Due to the high sensitivity of poly(ADP-ribose) polymerase to stimulation by DNA strand breaks, it was possible that the increase in polymerase activity shown in fig.1A was due to the effects of DNA fragmentation that occurs during isolation of rat liver nuclei. To demonstrate that the increase in enzyme activity actually reflected the in vivo stimulation of the polymerase, livers were frozen immediately upon removal from the animals, and the levels of constitutive polymeric ADP-ribose residues were determined as indicated in section 2. As shown in fig.1B, the levels of protein-bound poly(ADP-ribose) increased to 2-fold the basal levels after partial hepatectomy. In contrast, neither enzyme activity in liver nuclei nor poly(ADP-ribose) levels in frozen tissue increased in laparotomized animals (not shown). These data suggested that the stimulation of poly(ADP-ribosylation) in isolated nuclei from regenerating liver actually corresponded to poly(ADP-ribose) polymerase and not to mono(ADP-ribosyl) transferase activity [22].

3.2. *Changes in the levels of nuclear matrix associated poly(ADP-ribose) polymerase activity and polymeric ADP-ribose residues following partial hepatectomy*

To assess further the possible involvement of ADP-ribosylation of proteins in replication events, we determined the level of poly(ADP-ribose)

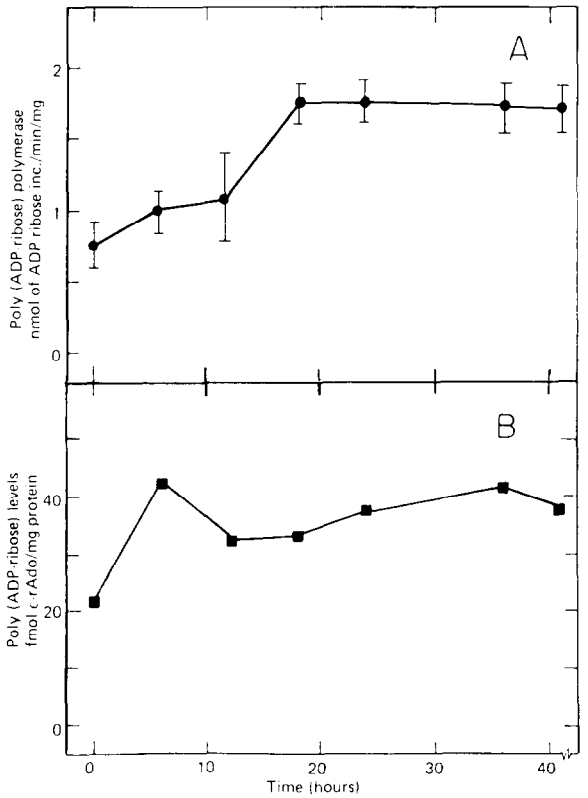

Fig.1. Time-dependent changes in poly(ADP-ribose) polymerase activity in isolated liver nuclei (A) and poly(ADP-ribose) levels in intact liver (B) following partial hepatectomy. Data points shown are the averages of duplicate determinations of three independent experiments. Error bars in (A) indicate the standard deviations from the mean. In (B), the standard deviations were smaller than the graphical space occupied by the data points.

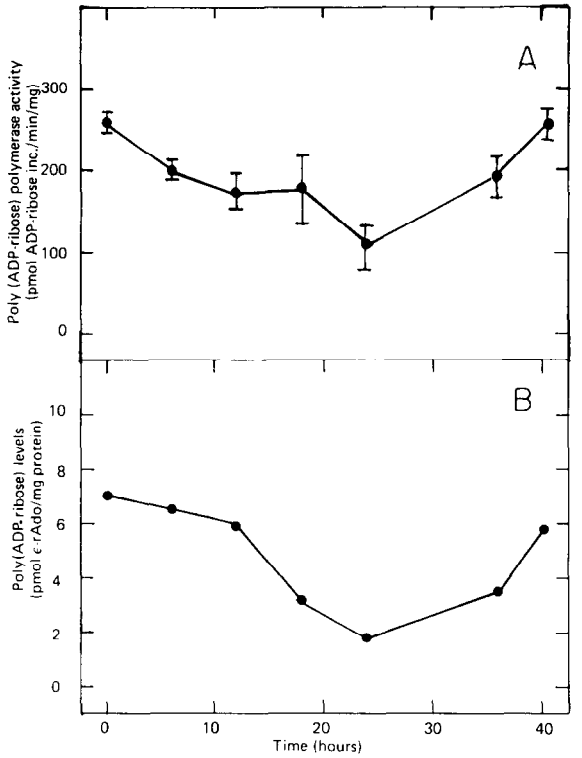

Fig.2. Time-dependent changes in the levels of poly(ADP-ribose) polymerase activity (A) and polymeric ADP-ribose residues (B) associated with the nuclear matrix fraction from adult rat liver following partial hepatectomy. Data points represent the average of triplicate samples of two independent experiments. Error bars in (A) indicate the standard deviations from the mean. In (B), the standard deviations were smaller than the graphical space occupied by the data points.

polymerase co-isolating with the nuclear matrix fraction of normal liver. Routinely, 15–35% of the polymerase activity associated with whole liver nuclei was found to co-isolate with the nuclear matrix. Other investigators have previously reported that only 2% of the enzyme is present in this nuclear compartment [17]. In our experiments to assay poly(ADP-ribose) polymerase activity in the nuclear matrix preparations, exogenous active DNA had to be added to the incubation mixture to detect the levels of enzyme reported here. As possible explanation for this discrepancy, it should be noted that a substantially different experimental protocol for the isolation of the nuclear matrix fraction was used in the previous report. Additional evidence that the level of incorporation of radiolabeled ADP-ribose into acid-insoluble material observed with our nuclear matrix preparation actually corresponded to poly(ADP-ribose) polymerase was obtained in experiments using competitive inhibitors of the polymerase. When nicotinamide, benzamide or thymidine were added to the enzyme assay, polymer biosynthesis was completely abolished (table 1).

Following partial hepatectomy, the levels of enzyme activity associated with this fraction, in contrast to the stimulation observed in enzyme activity in whole liver (fig.1A), decreased in the time-dependent fashion shown in fig.2A. Interestingly, the levels of the polymerase in this fraction

Table 1

Inhibition of nuclear matrix associated poly(ADP-ribose) polymerase activity by competitive inhibitors of the polymerase

[Inhibitor] (5 mM)	[^{3}H]ADP-ribose incorporated (cpm)	Inhibition (%)
Control	2819.9 ± 569 (n = 3)	–
Benzamide	25.5 ± 8 (n = 6)	99.1
Nicotinamide	250.0 ± 17 (n = 6)	91.1
Thymidine	120.8 ± 39 (n = 6)	95.7

Table 2

Fraction of protein-bound polymers of ADP-ribose co-isolating with the nuclear matrix following incubation of isolated rat liver nuclei with 100 μM NAD$^+$ at 30°C for 30 min

Fraction	Radioactivity associated with the fraction (cpm)	%
Nuclei	2818.9 ± 697.6	100
Nuclear matrix	1564.9 ± 418.5	55

recovered to basal levels in the post-replicative period of liver regeneration [23] 41 h after operation.

Due to the known intrinsic association of endogenous polymers of ADP-ribose with the nuclear matrix isolated from cells in tissue culture [11], we next determined whether poly(ADP-ribose) was associated with the nuclear matrix fraction. Normal liver nuclei were incubated with 100 μM [^{3}H]NAD$^+$ for 30 min at 30°C prior to nuclear matrix isolation, and the radioactivity incorporated following incubation, as well as that associated with the isolated nuclear matrix, was determined. About 55% of the in vitro synthesized polymers remained tightly bound to this subnuclear fraction (table 2) in agreement with previous observations [11].

The total levels of protein-bound poly(ADP-ribose) residues associated with the nuclear matrices from regenerating livers also decreased initially (fig.2B), and later recovered to basal levels with a time-dependent profile reminiscent of the fluctuations in poly(ADP-ribose) polymerase activity (fig.2A). The lowest levels of both polymerase activity and enzyme product associated with the nuclear matrix were observed at 24 h post-partial hepatectomy, the time at which the maximum rate of DNA synthesis occurs in regenerating liver [23]. These data suggest that significant changes in the nuclear compartmentalization of poly(ADP-ribose) polymerase activity occur during liver regeneration. In conclusion, our results are consistent with the proposal that modulation of nuclear matrix structure by poly(ADP-ribosylation) may be involved in the regulation of nuclear matrix associated DNA replication in vivo. Further studies are needed to identify the in vivo nuclear matrix acceptor proteins of polymeric ADP-ribose residues in order to understand better the molecular role of ADP-ribosylation in DNA replication in vivo.

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