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Automodification of PARP-1 mediates its tight binding to the nuclear matrix

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ABSTRACT

Poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme that catalyzes the NAD^+-dependent addition of ADP-ribose polymers on a variety of nuclear proteins, has been shown to be associated with the nuclear matrix. As yet, the properties and conditions of this association are unclear. Here, we show the existence of two PARP-1 pools associated with the nuclear matrix of rat liver and the ability of PARP-1 automodification to facilitate its binding to the nuclear matrix.

Introduction

Poly(ADP-ribosyl)ation is a posttranslational modification of proteins catalyzed by poly(ADP-ribose) polymerases (PARPs). Using nicotinamide adenine dinucleotide (NAD^+) as substrate PARPs catalyze the attachment of ADP-ribose units of NAD on various target proteins [1,2].

PARP-1 is the most abundant and the best described DNA-dependent enzyme of living cells which catalyzes poly(ADP-ribosyl)ation of numerous nuclear proteins including PARP-1 itself. PARP-1 is supposed to participate in the modulation of chromatin structure, transcription, repair of DNA etc. [3,4]. The presence of PARP-1 in isolated nuclear matrices of different tissues has been reported [5–8].

The nuclear matrix is a dynamic protein framework of the cell nucleus to which chromatin fibril folded in loops are attached. The nuclear matrix is likely to play an important role in basic genetic processes [9–11]. The content of PARP-1 in the matrix preparation depends on the tissue and, probably, on the matrix preparation conditions. For example, significant increase (5-fold) of PARP-1 content in the isolated nuclear matrix of the rat liver was observed when the nuclei were treated with the sulfhydryl cross-linking reagent sodium tetrathionate [12].

The aim of the present study was to determine PARP-1 content in nuclear matrices isolated by different methods, and define the role of PARP-1 automodification in the binding of the enzyme to the nuclear matrix.

Materials and methods

Materials. PARP-1 (A-20) goat polyclonal IgG, rabbit anti-goat IgG-HRP, Western Blotting Luminol Reagent were obtained from Santa Cruz Biotechnology (USA). Low melting point agarose, was obtained from Serva (Germany). β-NAD^+, PMSF, 1,5-isoquinolinediol, protease inhibitor cocktail, were purchased from Sigma–Aldrich Co., USA. [^{14}C] NAD^+ was obtained from Amersham (England). All other reagents used in this study were of the highest purity commercially available.

Preparation of nuclei and nuclear matrices. Male Wistar rats weighing approximately 150 g were used for this study. Isolation of rat liver nuclei was performed by the method of Chauveau et al. with some modifications [13], while preparation of the nuclear matrix by high-salt extraction was carried out as previously described [14], except the omission of β-mercaptoethanol from the buffer solutions. Electroelution method of nuclear matrix isolation was performed according to Postberg et al. [15]. About 2×10^8 nuclei were resuspended in 1 ml PBS containing 0.15 mM spermine and 0.5 mM spermidine, and then 1 ml 1.6% low melting point agarose was added at 30 °C. This mix was poured into disposable plug molds (Bio-Rad), each having a volume of 80 μl and containing 8×10^6 nuclei. For DNA digestion the agarose blocks were incubated in Tris–HCl buffer (pH 7.4) containing 0.15 M sucrose, 50 mM NaCl 5 mM $MgCl_2$ and 50 U/ml DNase I at 2 °C for 40 min and then at 30 °C for 10 min. Then the agarose blocks were washed once with PBS containing 0.2% Triton X-100 for 30 min and twice in PBS at 2 °C. DNA and protein not associated with the nuclear matrix were electroeluted from the agarose blocks in TAE buffer for 6 h at 50 V. After electroelution residual nuclear proteins were recovered by melting the agarose blocks at 60 °C in PBS containing 0.2% Triton X-100 and centrifugation 5 min at 2000*g*. All solutions

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used for the isolation of the nuclei and nuclear matrix contained 0.5 mM PMSF and protease inhibitor cocktail.

Western blotting analysis. Nuclear matrix (20 μg protein) or 1×10^6 nuclei and isolated from it nuclear matrices were separated by 7% SDS–PAGE according to Laemmli [16] and transferred onto a NC membrane using an electro-blotting apparatus. The membrane was incubated with anti-PARP-1 antibody diluted 1:200 and with HRP-conjugated secondary antibody diluted 1:2000. The bands labeled with the antibodies were visualized using a chemiluminescence luminol reagent by exposure to X-ray films. Quantitative analysis of the blots was performed using Scion Image Software.

Poly(ADP-ribosyl)ation reaction in nuclei. Nuclei (4 mg protein/ml) were incubated in a buffer solution containing 50 mM Tris–HCl, pH 8.0, 10 mM $MgCl_2$, 0.25 M sucrose, 0.5 mM PMSF, protease inhibitor cocktail and 0.2 mM and 1 mM NAD^+ at 20 °C for 10 min. The reaction was stopped by the immersion of samples into an ice bath and simultaneous addition of five volumes of the ice cold buffer solution without NAD^+. The samples were centrifuged at 1000*g* for 10 min.

Detach of poly(ADP-ribose) from proteins. Nuclear matrix (4 mg protein/ml) was suspended in 2 mM Tris–NaOH, pH 12 and incubated at 60 °C for 2 h. The pH was neutralized by adding of 1 volume 50 mM Tris–HCl, pH 6.8.

Nuclear matrix PARP activity assay. Matrix PARP activity was determined by incorporation of [^{14}C] NAD^+ in acid insoluble material [14].

Protein and DNA assay. Protein concentration was determined using the Bradford protein assay with bovine serum albumin as a standard [17], while DNA was measured by the diphenylamine reaction using calf thymus DNA as a standard [18].

Results and discussion

For determination of quantitative distribution of PARP-1 between matrix and nonmatrix regions of rat liver nuclei, the high-salt extraction method of matrix preparation, as well as less invasive method of matrix isolation under physiological conditions was used.

It has been established that the nuclear matrix prepared by high-salt extraction method contains ~10% of nuclear PARP-1, while nuclear PARP-1 content in the matrix isolated from agarose encapsulated nuclei by electroelution markedly increases up to ~50% (Fig. 1). These results indicate the probable existence of various pools of PARP-1 associated with different strength (high-salt resistant and nonresistant) to the nuclear matrix.

It has been shown that poly(ADP-ribose) binds noncovalently, though tightly and specifically to the nuclear matrix proteins [19,20]. Therefore we decided to elucidate the influence of automodification of PARP-1 in the attachment of the enzyme to the nuclear matrix. For this purpose purified liver nuclei were incubated in the absence and presence of 1 mM NAD^+ for catalyzing the reaction of ADP-ribosylation. After these, the nuclear matrices have been isolated using high-salt extraction method with the content of PARP-1 being afterwards detected by the analysis of Western blots. Fig. 2 shows that 113 kDa band corresponding to PARP-1 of the matrix isolated from preincubated with NAD^+ nuclei is of significantly lower intensity than in the matrix prepared from control, nonincubated with NAD^+ nuclei. On the other hand decrease of 113 kDa band intensity in the matrix isolated from preincubated with NAD^+ nuclei is accompanied with the formation of smear from immunoreactive band towards the top of the gel. PARP inhibitor 1,5-isoquinolinediol (0.2 mM) partially removes this effects.

Because automodification of PARP-1 with poly(ADP-ribose) molecules causes a slower migration of PARP-1 during electrophoresis and reduces its immunoreactivity, to release long PAR chains

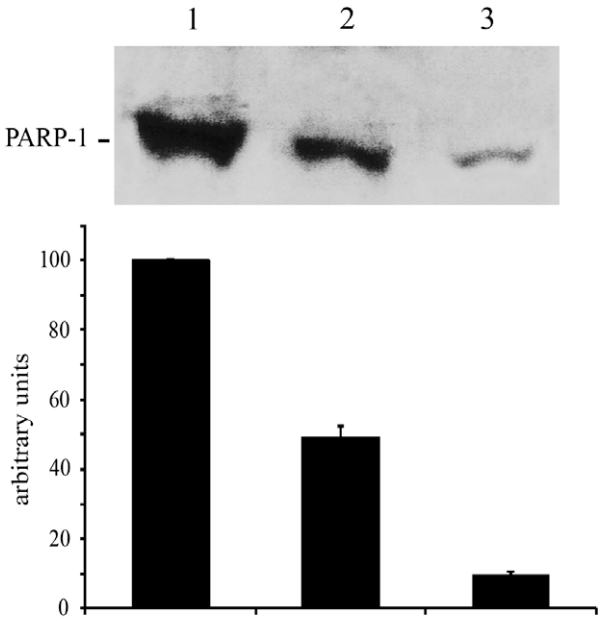

Fig. 1. Detection of PARP-1 content in the nuclear matrices isolated from rat liver. About 1×10^6 purified rat liver nuclei (lane 1) and the nuclear matrices corresponded to the same amount of nuclei by electoelution (lane 2) and high-salt extraction (lane 3) methods were separated in 7% SDS–PAGE and PARP-1 content was determined by Western blot analysis. The abundance of PARP-1 is expressed in arbitrary units as a percentage of nuclear PARP-1 content (100%). The data are presented as mean ± SE.

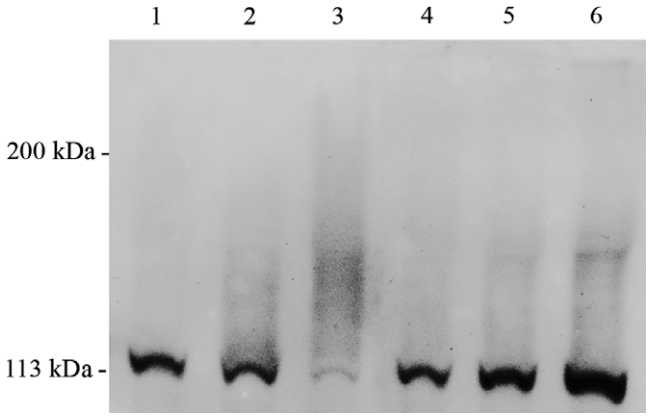

Fig. 2. Influence of PARP-1 automodification on its binding to the nuclear matrix. Nuclear matrices obtained by high-salt extraction method from the nuclei preincubated, in the absence of NAD^+ (lane 1), with 1 mM NAD^+ and 0.2 mM 1,5-isoquinolinediol (lane 2), 1 mM NAD^+ (lane 3) and the same preparations incubated under alkaline conditions, (lanes 4–6, respectively), were separated in 7% SDS–PAGE and PARP-1 content was determined by Western blot analysis.

from the nuclear matrix proteins, the matrices were incubated under alkaline conditions, at pH 12. After this treatment the PARP-1 content in the nuclear matrix isolated from preincubated with NAD^+ nuclei is much higher than in control matrix preparations isolated from nonincubated with NAD^+ nuclei. However, the smears do not disappear in the matrices isolated from the nuclei incubated with NAD^+ and are more intensive than in control preparation, which indicates that alkaline treatment does not completely release poly(ADP-ribose) molecules from PARP-1.

Interestingly, the tight binding of PARP-1 to the nuclear matrix after automodification is accompanied by the increase in the matrix PARP activity. As shown in Fig. 3, the PARP activity of the matrices isolated from incubated with 0.2 and 1 mM NAD^+ increases 1.7- and 2-fold correspondingly. This suggests that

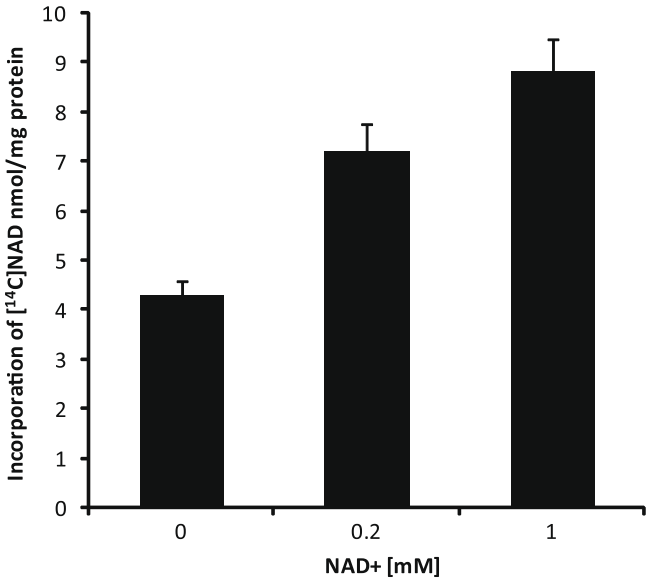

Fig. 3. Influence of automodified PARP-1 binding to the nuclear matrix on matrix PARP activity. PARP activity of rat liver nuclear matrices isolated after preincubation of the nuclei in the absence and presence of 0.2 and 1 mM NAD^+. Data are presented as mean ± SE.

automodified PARP-1 molecules associated with the nuclear matrix retain enzymatic activity and, probably, DNA binding ability.

These results clearly demonstrate that automodification of PARP-1 induces the translocation of the enzyme from nonmatrix regions to matrix regions of rat liver nuclei and provides tight binding of PARP-1 to the nuclear matrix structure or at least intensifies loosely associated PARP-1 binding to the matrix.

Based on the above mentioned, it can be proposed that automodified PARP-1, through binding to matrix proteins and, on the other hand, to DNA provides tight attachment of DNA to the nuclear matrix.

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