NON-PREFERENTIAL LOCALIZATION OF POLY(ADP-RIBOSE) POLYMERASE ACTIVITY IN TRANSCRIPTIONALLY ACTIVE CHROMATIN

Munehiko YUKIOKA, Yasuji OKAI, Tadayoshi HASUMA and Akira INOUE
Department of Biochemistry, Osaka City University Medical School, Abeno-ku, Osaka 545, Japan

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1. Introduction

In eukaryote nucleus, DNA exists as a highly ordered nucleoprotein complex, chromatin, whose organization regulates ultimately various functional states of the cell. To better understand this organization, it is of interest to know the subchromatin localization of proteins which influence functional activity of chromatin. Poly(ADP-ribose) polymerase, which is a ubiquitous nuclear enzyme, provides a suitable probe for this purpose, because it is firmly integrated in eukaryotic chromatin structure [1].

A chromatin fractionation technique, which yielded a fraction greatly enriched in nascent RNA and in DNA sequences transcriptionally active in vivo was developed [2,3]. The technique involves selective shearing of chromatin with DNAase II followed by differential precipitation. The nucleolytic cleavage of chromatin DNA is a mild treatment and does not lead to detectable level of chromosomal protein rearrangement relative to specific DNA sequences, which is observed during mechanical shearing of chromatin [4,5].

In this report, using this procedure, we studied the distribution of poly(ADP-ribose) polymerase activity in transcriptionally active and inactive chromatin regions. It was shown that the enzyme is not preferentially localized in transcriptionally active chromatin region.

2. Materials and methods

2.1. *Preparation and fractionation of chromatin*

Rat liver chromatin was prepared using a modification of the method in [6]. Livers were homogenized in a loose glass–Teflon homogenizer in 5 vol. 0.25 M sucrose/5 mM $MgCl_2$/10 mM Tris–HCl (pH 8.0). The homogenate was filtered through cheesecloth and centrifuged for 15 min at 1000 × *g*. The pellet was then homogenized in the same buffer containing 0.1% Triton X-100. The homogenate was layered over 6–7 vol. 2.2 M sucrose/5 mM $MgCl_2$/10 mM Tris–HCl (pH 8.0), and nuclei were pelleted by centrifugation for 60 min at 76 000 × *g* in Hitachi RP-30 rotor. The nuclear pellet was homogenized briefly in 40–50 vol. 1 mM Tris–HCl (pH 7.0)/0.1 mM EDTA/0.5 mM dithiothreitol/12.5% glycerol to lyse nuclei. Chromatin was pelleted at 15 000 × *g* for 15 min. The gel was resuspended by homogenization in the same buffer to yield a chromatin solution having an A_{260} 1 cm of 30 (measured in 1.0 N NaOH). The solution was brought to 37°C and DNAase II (Worthington, HDAC) was added to a final concentration of 400 units/ml. After incubation at 37°C, the reaction was terminated by the addition of 0.1 M Tris–HCl (pH 11) to pH 8.0 and cooling on ice. Unsheared chromatin (P_1) was removed by centrifugation at 30 000 × *g* for 60 min at 4°C. To the supernatant (S_1), 1/99th vol. 0.2 M $MgCl_2$ was added dropwise with rapid stirring. After 30 min of additional stirring, the turbid suspension was centrifuged at 30 000 × *g* for 30 min yielding a pellet (P_2) and supernatant (S_2) fraction.

2.2. *Assay for poly(ADP-ribose) polymerase*

The poly(ADP-ribose) polymerase assay contained in vol. 0.25 ml: 25 μmol Tris–HCl (pH 8.0), 0.5 μmol dithiothreitol, 0.5 μmol $MgCl_2$, 0.1 μCi [*adenine*-2,8-^{3}H]NAD (3.4 Ci/mmol, New England Nuclear) and an appropriate amount of nuclease digested chromatin, as indicated in each experiment. The reactions were

performed at 37°C for 10 min and terminated by addition of 3.0 ml ice-cold 20% trichloroacetic acid containing 1% sodium pyrophosphate. The insoluble material was collected on Whatman GF/C filter discs and the radioactivity determined in a Packard Model 3385 liquid scintillation spectrometer.

2.3. *Sucrose gradient fractionation*

The size distribution of nucleoprotein fragments produced by DNAase II digestion was analyzed by centrifugation on linear 7.5–22.5% sucrose gradient in 25 mM Tris–HCl (pH 8.0)/0.1 mM EDTA/0.5 mM dithiothreitol/12.5% glycerol. Centrifugation was performed at 25 000 rev/min in a Hitachi RPS-25 rotor for 40 h at 4°C. The approx. $s_{20,w}$ values were calculated assuming that the velocity and density corrections are linear with distance over short regions of the tube, using catalase (11 S) as an internal standard.

3. Results

3.1. *Time course of chromatin fractionation with DNAase II and poly(ADP-ribose) polymerase activity in S_2 fraction*

Figure 1 illustrates the time course of chromatin digestion with DNAase II. At 2.5–5.0 min enzymatic treatment, all the DNA liberated into fraction S_1 is soluble in 2 mM $MgCl_2$ and recovered in fraction S_2.

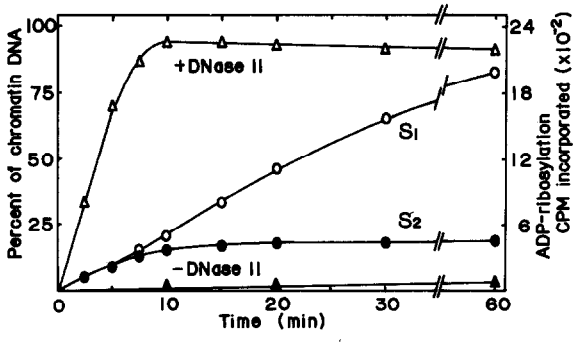

Fig.1. Time course of chromatin fractionation with DNAase II and poly(ADP-ribose) polymerase activity in S_2 fraction. Chromatin was fractionated as described in section 2, and the amount of nucleic acid in each fraction determined by A_{260} of an aliquot diluted in 1.0 N NaOH. Poly(ADP-ribose) polymerase activity analyses were performed as described in section 2 on individual S_2 fractions (0.1 ml), (○——○, ●——●) per cent of chromatin DNA; (△——△, ▲——▲) radioactivity.

On longer digestion, Mg^{2+}-insoluble material (P_2) begins to come out in fraction S_1. At 10 min, as much as 15–18% chromatin DNA is found in fraction S_2 (Mg^{2+}-soluble) and 2–3% in fraction P_2. Further digestion (60 min) liberates about 80% of the chromatin into S_1 with small increase in S_2 chromatin. These features of DNAase II treatment of rat liver chromatin resemble that reported [7].

The S_2 chromatin is reported to be enriched in the DNA sequences that code for cellular RNA, i.e., transcriptionally active chromatin; whereas the P_2 fraction contains predominantly the transcriptionally inactive chromatin [2].

As shown in fig.1, the poly(ADP-ribose) polymerase activity in S_2 fraction reached a maximum in 10 min DNAase II treatment where Mg^{2+}-insoluble material (P_2) accumulated only slightly in S_1 fraction. Further enzymatic treatment did not result in increase in poly(ADP-ribose) polymerase activity of S_2 fraction. These results indicated that 10 min digestion was sufficient to release the enzyme activity into S_2 fraction by DNAase II. No significant liberation of poly(ADP-ribose) polymerase activity was observed from chromatin that was incubated in the absence of DNAase II.

The chromatin used in the present studies was isolated by a very brief, gentle procedure ensuring preservation of the integrity of chromatin [6]. We examined the RNA polymerase activity in the chromatin fractionated by DNAase II. A considerable amount of RNA polymerase was liberated from chromatin in the absence of DNAase II, which it made difficult to draw an unequivocal conclusion as to the preferential localization of RNA polymerase in the active chromatin fraction.

3.2. *Distribution of poly(ADP-ribose) polymerase activity in template active and inactive chromatin regions*

To examine the distribution of poly(ADP-ribose) polymerase in the template active and inactive chromatin regions, a 10 min DNAase-treated chromatin was fractionated, and assayed for poly(ADP-ribose) polymerase activity in each fractionated chromatin. As indicated in table 1, the major proportion of poly(ADP-ribose) polymerase activity was found in fraction P_1 or transcriptionally inactive chromatin.

Some 15–18% of the activity was released into the supernatant (S_1) by the nuclease treatment.

Table 1
Distribution of poly(ADP-ribose) polymerase activity in fractionated chromatin

Chromatin fraction	%DNA	%Poly(ADP-ribose) polymerase activity	Poly(ADP-ribose) polymerase activity unit of DNA
Experiment 1			
S_1	17.5	17.1	0.98
P_1	82.5	82.9	1.00
Experiment 2			
S_1	14.8	16.2	1.09
P_1	85.2	83.8	0.98

Chromatin was digested with DNAase II for 10 min at 37°C, and was fractionated as described in section 2. The poly(ADP-ribose) polymerase activity in each chromatin fraction was determined as given in section 2. The amount of DNA and the poly(ADP-ribose) polymerase activity in each fractionated chromatin were expressed as % of original undigested chromatin.

Therefore, only a small fraction of the poly(ADP-ribose) polymerase activity was located in the transcriptionally active chromatin. However, the specific activity of poly(ADP-ribose) polymerase activity in fractions S_1 and P_1, which was normalized per unit of chromatin DNA present in each fraction, appeared to be identical (table 1, column 4). This result suggests that the poly(ADP-ribose) polymerase is not preferentially localized in the transcriptionally active chromatin region, but equally distributed between transcriptionally active and inactive regions of chromatin.

To assess whether or not the poly(ADP-ribose) polymerase activity presented in the active chromatin fraction is actually bound to the nuclease-resistant nucleoproteins [4], generated by DNAase II treatment, S_1 fraction was fractionated on sucrose density gradient centrifugation. After the fractionation, an aliquot of each fraction was assayed for poly(ADP-ribose) polymerase activity. As shown in fig.2, the peaks of poly(ADP-ribose) polymerase activity coincided with the absorbance profile of the nuclease-resistant nucleoprotein complexes, which had sedimentation coefficients approx. 16–22 S, but not with the small DNA fragments which appeared close to the top of the gradient. This result indicated that hte poly(ADP-ribose) polymerase activity in the transcriptionally active chromatin is truly associated with the nuclease-resistant nucleoprotein complexes.

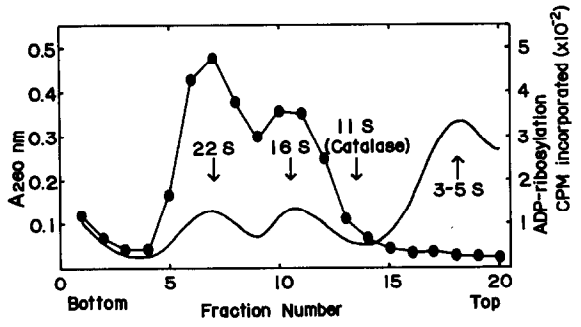

Fig.2. Sucrose gradient fractionation of the transcriptionally active chromatin and associated poly(ADP-ribose) polymerase activity. A sample of 10 min DNAase II-treated S_1 chromatin fraction (1.0 ml) was fractionated on a 30 ml 7.5–22.5% sucrose gradient, as described in section 2. Poly(ADP-ribose) polymerase activity analyses were performed as described in section 2 on individual fractions (0.2 ml). (——) A_{260}; (•——•) radioactivity.

4. Discussion

The present study clearly demonstrates that a chromatin-bound protein-modifying enzyme, poly(ADP-ribose) polymerase, is not preferentially localized in the transcriptionally active chromatin region, but equally distributed between transcriptionally active and inactive fractions. These results are contrary to the observation in [8]. They used a sonicated chromatin preparation from HeLa S_3 cells and showed that poly(ADP-ribose) polymerase activity was associated primarily with the transcriptionally active regions

of chromatin. The reason for the discrepancy is not clear, but it might be due to the fact that the chromatin used in [8] is prepared by conventional methods involving a mechanical shearing (sonication), which have been shown to cause a drastic change in chromatin structure [4,5].

A similar observation was reported as to the distribution of nuclear thyroid hormone receptor within chromatin regions; the thyroid hormone receptor had been reported to be enriched in the transcriptionally active chromatin fraction prepared by a mechanical shearing procedure [9], whereas the receptor protein was not found to be preferentially enriched in the transcriptionally active chromatin fraction prepared by shearing with DNAase II [10]. In fact, it has been shown that the chromatin fractionation technique employing mechanical shearing is not adequate for separation of chromatin into 'active' and 'inactive' material [11–13].

The physiological function of the poly(ADP-ribose) polymerase has only been elucidated partially. Much of the data on the ADP-ribosylation of chromatin proteins seem to indicate that poly(ADP-ribose) polymerase is involved in either DNA replication, DNA repair, or chromatin structure modification [1]. Even though we are not certain, at present, of the physiological meaning of the even distribution of poly(ADP-ribose) polymerase in the chromatin regions, it is possible that the enzyme might exert its function in some step in DNA replication or chromatin condensation, which occurs throughout the genome, rather than in RNA synthesis which, on the contrary, occurs at limited loci of chromatin. Elucidation on the enzyme affinity for the different regions of chromatin will give better understanding on the even distribution of the enzyme.

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