ON THE PRESENCE OF POLY(ADP-RIBOSE) POLYMERASE ACTIVITY IN METAPHASE CHROMOSOMES FROM HeLa S3 CELLS

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1. Introduction

In a number of different eucaryotic cells, interphase chromatin contains poly(ADP-ribose) polymerase [1–3]. It incorporates the ADP-ribose moiety of NAD into poly(ADP-ribose) of varying chainlength (1–40 residues). The polymer is covalently bound to histones and non-histone proteins. The polymerase has been found also in the cytoplasm of HeLa cells tightly bound to ribosomes [4].

This enzyme has also been detected in mitotic cells [5] but its location in isolated metaphase chromosomes not yet examined. Since the occurrence of this enzyme in chromatin from interphase cells does not necessarily mean that it is present in an enzymatically active form in metaphase chromosomes, it was considered important to establish this.

We show that metaphase chromosomes from HeLa S3 cells contain the enzyme in an active form. The enzyme seems to be an integral part of metaphase chromosomes and not merely a contamination of enzyme present in the cytoplasm [4], since metaphase chromosomes isolated by different methods yielded about the same result. Finally when isolated metaphase chromosomes were incubated in vitro with [^{14}C]NAD, the chromosomes became ADP-ribosylated and hence could serve as an acceptor for ADP-ribosylation. This might suggest that ADP-ribosylation of metaphase chromosomes plays a role during mitosis.

2. Methods and materials

2.1. *Harvesting of cells in metaphase*

The HeLa S3 cell line was maintained in suspension culture. Cells (10^7 from the suspension culture) were grown as monolayer at 37°C for 5 days in a roller bottle (Falcon 3027, 850 cm^2) in 250 ml Eagle minimum essential medium with Earle's salt) supplemented with 10% foetal calf serum, Hepes buffer (pH 7.3, final conc. 15 mM) 1 ml of Flow 100 × non-essential amino acids to 100 ml, benzylpenicillin 100 μg/ml and streptomycin 100 μg/ml. The bottle was then shaken to remove loosely attached cells and the medium replaced with 250 ml fresh medium containing 0.05 μg colcemid/ml. After further 6 h, change of medium was carried out as above; 16 h later the bottle was shaken gently to loosen metaphase cells. The yield was ~1.5×10^8 cells with 98% in metaphase.

2.2. *Isolation of chromosomes from cells in metaphase*

Two different methods were used. One (method A) based on that in [6,7] and the other (method B) on that in [8]. In method A, cells (7.5×10^7) were washed with 50 ml ice-cold Hanks solution and in method B cells (7.5×10^7) were suspended in 50 ml cold medium for 30 min. After centrifugation cell pellet was incubated at 25°C either for 20 min (method A) in 15 ml TMS buffer which contained 20 mM Tris–HCl (pH 7.3), 1 mM of, respectively, $CaCl_2$, $MgCl_2$, $ZnCl_2$ and 0.1% saponin or for 10 min (method B), in 15 ml 'Wray buffer' which consists of 1 M hexylenglycol, 0.5 mM Pipes (pH 6.8) and 0.5 mM $CaCl_2$. All further work was done at 0°C. Suspension of cells was homogenized with 4 bursts of 15 s duration and 15 s interval in the micro attachment of a Sorvall omnimixer. The homogenate was diluted 1:1 with, respectively, TMS or 'Wray buffer' and centrifuged for 5 min at 30–40 × *g* to remove nuclei. To spin down the chromosomes, the supernatant was centrifuged for 15 min at 1000 × *g*. In both

Abbreviations: PMSF, phenylmethylsulphonyl fluoride; DDT, dithiothreitol

methods the chromosomes were washed 3 times in 30 ml TM buffer (TMS buffer without saponin) and centrifuged as above. The chromosomes were finally suspended in 2 ml (method A) and 1 ml (method B) of TM buffer to give ~1 mg/ml of chromosomal DNA.

2.3. *Assay of poly(ADP-ribose) polymerase*

A solution (0.25 ml) containing 0.1 M Tris–HCl (pH 8.0), 12 mM $MgCl_2$, 0.1 mM [^{14}C]NAD (or ~8000 cpm/nmol) and 10 mM DTT was mixed with an aliquot of the suspension to be tested (usually 10 μl) and incubated for 15 min at 25°C. Trichloroacetic acid, 10% (2 ml) was added and the insoluble material collected on a Whatman GF/C filter, washed with 5% trichloroacetic acid and acetone. The radioactivity on the filter was measured in PPO in toluene (50 g/l). This method gave identical results to that in [3].

2.4. *Incubation of ADP ribosylated chromosomes with phosphodiesterase*

Chromosomes were incubated with [^{14}C]NAD as described above, and to the mixture was added 20% trichloroacetic acid. The precipitate was recovered by centrifugation, washed 3 times with 10% trichloroacetic acid and once with 90% aqueous acetone. The dry solid was dissolved in 0.1 N NaOH incubated at 37°C for 30 min, neutralized with HCl and incubated with 50 μg phosphodiesterase in 20 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 25 mM NaCl and 1 mM AMP in 0.1 ml total vol. After 1 h at 37°C labelled AMP and phosphoribosyl AMP were separated by thin-layer chromatography on PEI-cellulose plates (Macherey and Nagel) and detected by radioautography [9].

2.5. *Protein and DNA analysis*

The protein was estimated by Lowry's method after precipitation of the protein with trichloroacetic acid in the presence of deoxycholate [10]. The DNA was determined by the modified method of Burton [11]. Bovine serum albumin and calf thymus DNA were used as standards.

2.6. *Labelled material*

Nicotinamide [U-^{14}C]adenine dinucleotide was purchased from the Radiochemical Centre, Amersham.

2.7. *Other materials*

Phosphodiesterase was from Worthington Biochem. Corp.

3. Results

When isolating metaphase chromosomes, it is often customary to incubate cells in the isolation buffer at 37°C [6,8]. However, it was found that incubation at this temperature reduced the activity of the poly-(ADP-ribose) polymerase, particularly in the 'Wray buffer' where the final chromosome preparation exhibited very low activity. Addition of 1 mM PMSF did not reduce the loss of activity at 37°C. Reducing the temperature to 25°C was sufficient to stabilize the activity during the incubation.

To liberate individual chromosomes it was necessary to homogenize the cells in the micro attachment of the omnimixer. The TMS buffer (see section 2) gave chromosomes with good morphology whereas the 'Wray buffer' (see section 2) gave lower yield of chromosomes and a preparation which consisted of fibers and stretched chromosomes [12].

The poly(ADP-ribose) polymerase activity was assayed by measuring the amount of acid insoluble radioactivity incorporated into the chromosomes (per μg DNA) when incubated with NAD labelled in the adenine moiety (tables 1,2) The incorporation was found to be proportional to the amount of

Table 1
Incorporation of [^{14}C]NAD into acid-insoluble material in a suspension of HeLa S3 metaphase chromosomes isolated using the TMS buffer (see section 2)

Prep. no.	nmol [^{14}C]NAD incorp. μg DNA^{-1} . 15 min^{-1}	Protein / DNA
1	0.06	6.4
2	0.060	4.3
3	0.060	6.2
4	0.050	6.4
5	0.04	7.9
6	0.02	5.2
7	0.090	7.1
Av.	0.05	6.2

Chromosome suspension 10 μl (5–10 μg DNA) was added to 0.25 ml incubation mixture. The protein/DNA ratio of the various preparations is given

Table 2
Incorporation of [^{14}C]NAD into acid-insoluble material in a suspension of HeLa S3 metaphase chromosomes isolated using the 'Wray buffer' (see section 2)

Prep. no.	nmol [^{14}C]NAD incorp. μg DNA^{-1} . 15 min^{-1}	Protein / DNA
1	0.08	4.3
2	0.10	4.0
3	0.07	5.9
4	0.02	3.0
5	0.03	5.7
Av.	0.06	4.6

Chromosome suspension 10 μl (8–10 μg DNA) was added to 0.25 ml incubation mixture (see section 2). The protein/DNA ratio of the various preparations is given

chromosomes added over a 4-fold range. Table 1 shows that in 5 preparations, the incorporation varied between 0.04–0.06 nmol/μg DNA. The remaining two preparations (6,7) differed. The results obtained with chromosomes isolated using the 'Wray buffer' are given in table 2. It appears that the incorporations varied between 0.07–0.1 nmol in 3 preparations and that the incorporations in the last 2 preparations were lower. The average value for preparations in table 1 and table 2, respectively, was 0.05 and 0.06 nmol and hence nearly the same.

Digestion of chromosomes with phosphodiesterase after incubation with [^{14}C]NAD, liberated labelled material which chromatographed on polyethylenimine impregnated cellulose as AMP and phosphoribosyl AMP.

4. Discussion

The results show that HeLa S3 cells arrested with colcemid in metaphase contain the enzyme poly-(ADP-ribose) polymerase in an active form. Furthermore, metaphase chromosomes isolated by two very different procedures yielding preparations with some differences in the protein/DNA ratio (see tables 1,2), gave for a number of preparations an average value of poly(ADP-ribose)polymerase activity, which was about the same. Hence, the enzyme activity seemed to be an integral part of the metaphase chromosomes and not merely a contamination by enzyme which could have been present in the cytoplasm.

The metaphase chromosomes became ADP-ribosylated when incubated with [^{14}C]NAD. Thus, metaphase chromosomes contain sites which can serve as acceptors for ADP-ribosylation. This finding suggested that ADP-ribosylation of metaphase chromosomes could play some role during mitosis.

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