Intranucleosomal localization of the AP endodeoxyribonuclease of rat liver chromatin

Suzanne Bricteux-Grégoire and Walter G. Verly

Biochimie, Faculté des Sciences, Université de Liège, Sart Tilman B6, 4000 Liège I, Belgium

Received 15 March 1983; revision received 4 May 1983

The chromatin AP endodeoxyribonuclease of rat liver cells is located in the cores as well as in the linkers of the nucleosomes.

AP endodeoxyribonuclease *DNA repair* *Chromatin* *Nucleosome*

1. INTRODUCTION

AP (apurinic or apyrimidinic) sites are the most common lesions in DNA. The loss of bases may be spontaneous; it is enhanced by different chemical and physical agents; AP sites are also the result of the activity of DNA glycosylases which remove altered bases from DNA. The AP endodeoxyribonucleases nick the DNA strands near the AP sites and, in this way, they catalyze the first step of the repair of DNA containing AP sites.

We have shown that chromatin is the main location of the AP endodeoxyribonuclease activity in rat liver cells. Some activity is also found in nuclear sap and membranes; however, the latter species seem to be different from the chromatin enzyme [1].

This report shows that the chromatin AP endodeoxyribonuclease is associated with the cores as well as with the linkers in the nucleosomes.

2. MATERIALS AND METHODS

2.1. *Preparation of chromatin*

Preparation and purification of cell nuclei from rat liver and description of buffer A can be found in [1]. When the purified nuclei must be stored, 50% glycerol is added to buffer A before cooling at −80°C. To prepare chromatin, the nuclei are made to swell at 0°C in 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM Tris–HCl (pH 8.0), before proceeding as in [1].

2.2. *Chromatin digestion with micrococcal nuclease*

The chromatin gel from 24 g rat liver is suspended in 6 ml 0.5 mM PMSF, 1 mM $CaCl_2$, 10 mM Tris–HCl (pH 8.0); after addition of 90 units of micrococcal nuclease (Worthington), the mixture is incubated 6 min at 37°C. The reaction is stopped by addition of 200 μl 0.1 M EGTA and 200 μl 0.1 M EDTA. Aliquots are taken for identification of the histones and for analysis of the sizes of DNA fragments; the remaining solution (5.5 ml) is shared between 3 tubes for sedimentation analysis.

2.3. *Sedimentation analysis*

Samples (1.75 ml) of the digested chromatin are placed on top of 35 ml linear 5–30% sucrose gradients in 2 mM EGTA, 2 mM EDTA, 10 mM Tris–HCl (pH 8.0). The tubes are spun at 4°C for 20 h at 120000 × *g* in a Beckman centrifuge (rotor SW-27); they are emptied from the top and the transmittance at 260 nm is continuously recorded. The AP endodeoxyribonuclease activity is measured on each collected fraction.

2.4. *Gel electrophoresis of DNA*

The fraction to be analyzed (whole chromatin digest or fractions from the gradients) is dialyzed against 1 mM EDTA, 10 mM Tris–HCl (pH 8.0) and concentrated with polyethylene glycol.

Proteins are digested with proteinase K (Boehringer) and DNA is precipitated with ethanol [2] prior to electrophoresis.

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

2.4.1. Polyacrylamide gel electrophoresis

The electrophoresis is carried out in vertical 15 × 15 × 0.15 cm slab gels containing 6% acrylamide as in [3], at room temperature and 100 V for 3 h.

2.4.2. Agarose gel electrophoresis

The electrophoresis is performed in vertical 15 × 15 × 0.15 cm slab gels containing 1.7% agarose (Bio Rad) in Tris–acetate–EDTA buffer [4] at room temperature and 80 V until the bromophenol blue used as an indicator reaches the end of the gel.

In both cases, the gels are immersed in 0.1% ethidium bromide solution for 1 h, washed with water, and photographed under ultraviolet light. The gels are calibrated with a *Hin*cII digest of ϕX174 RF DNA (PL Biochemicals).

2.5. *Gel electrophoresis of histones*

Histones are extracted with 0.2 M H_2SO_4 from whole chromatin digest or fractions from the gradients [5]. Electrophoresis was done in vertical polyacrylamide slab gels as in [6].

2.6. *Assay for AP endodeoxyribonuclease activity*

The assay is described in [1]; one unit is the activity which hydrolyzes phosphoester bonds near 1 pmol AP sites/min at 37°C.

3. EXPERIMENTS AND RESULTS

Chromatin from rat liver was submitted to a limited digestion with micrococcal nuclease as in section 2. After a treatment with proteinase K, analysis of the DNA by agarose gel electrophoresis showed distinctly 10 bands corresponding to double-stranded DNA pieces of about 200 nucleotide pairs or multiples of this length (not shown).

When the whole chromatin digest was centrifuged at 110000 × *g* for 15 h so that nucleosomes were pelleted at the bottom of the tube, around 50% of the AP endodeoxyribonuclease activity was found in the supernatant instead of 10% when the chromatin was not digested. The effect of ionic strength on this distribution has been studied by adding NaCl to the standard buffer. The release of the enzyme activity in the supernatants was nearly complete when [NaCl] reached 0.2 M.

The chromatin digest was also submitted to centrifugation on a sucrose gradient containing EGTA and EDTA as in section 2. The transmittance at 260 nm showed several peaks (fig.1). After digestion with proteinase K, the content of each peak was submitted to DNA analysis using polyacrylamide gel electrophoresis (fig.2). The first peak gave a smear of small DNA fragments; this peak likely contained pieces of linker DNA and proteins liberated by the digestion of this DNA. The size of the DNA from the second peak was 160–200 nucleotide pairs; it is the peak of monosomes; histone H1 together with the core histones was identified when an acid extract of these monosomes was submitted to gel electrophoresis. The size of the DNA from the third peak was about 400 nucleotide pairs; it is the peak of disomes. The analysis was not carried out further; the fourth, fifth and sixth peaks of the

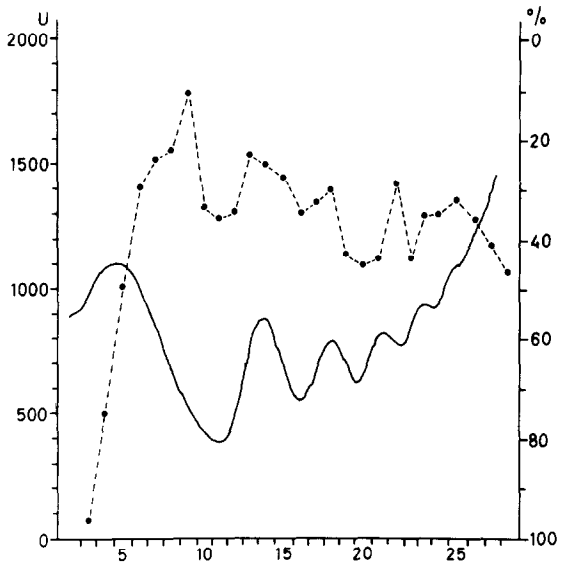

Fig.1. Sedimentation of the chromatin digest through a linear sucrose gradient. Rat liver chromatin was digested for a short time with micrococcal nuclease. An aliquot (1.75 ml) of the digest was placed on top of a 35 ml linear 5–30% sucrose gradient in Tris–HCl (pH 8.0) containing EGTA and EDTA. After 20 h at 120000 × *g*, the tube was emptied from the top and the transmittance at 260 nm recorded (continuous line [%]); the AP endodeoxyribonuclease activity was measured on each of the collected 1.2 ml fractions (dots and discontinuous line; U, enzyme units/fraction). Monosomes are in fractions 12–15, disomes in fractions 17–19, and trisomes in fractions 20–22.

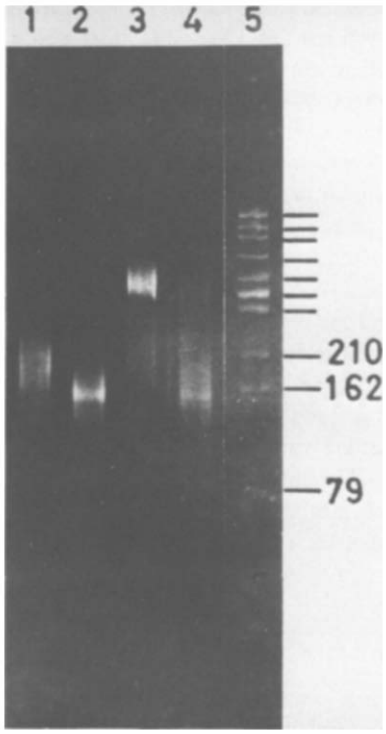

Fig.2. Acrylamide gel electrophoreses of DNA. The chromatin has been treated with micrococcal nuclease and the digest submitted to sucrose gradient centrifugation (fig.1). The DNA from the second peak in the gradient (lane 1) and the third peak (lane 3) were analyzed by gel electrophoresis. Monosomes from the second peak were redigested with micrococcal nuclease and the new digest submitted to a second centrifugation (fig.3); the DNA from the monosome peak was analyzed by gel electrophoresis (lane 2). Disomes from the third peak of the first centrifugation (fig.1) were also redigested; the DNA from this digest was analyzed by gel electrophoresis (lane 4). Calibration was made with the *Hin*cII digest of ϕX174 RF DNA (lane 5); the scale at right indicates the positions of DNA pieces of 210, 162 and 79 nucleotide pairs.

transmittance profile probably correspond to trisomes, tetrasomes and pentasomes.

Determination of the AP endodeoxyribonuclease activity in the fractions from the gradient indicated that some enzyme was associated with the proteins liberated by digestion of the linker DNA; this is followed by several peaks coincident with the nucleosome peaks.

The fractions from the gradient containing the monosomes were pooled and submitted to a second digestion with micrococcal nuclease. When this second digest was submitted to another analysis on sucrose gradient, some AP endodeoxyribonuclease activity was found in the top fractions, but most of it remained associated with the monosome peak (fig.3). Acrylamide gel electrophoresis after treatment with proteinase K showed that the DNA had been reduced to about 145 nucleotide pairs (fig.2); no histone H1 could be found in the monosome peak.

The fractions from the first sucrose gradient containing the disomes were pooled and also submitted to a second digestion with micrococcal nuclease. When this second digest was submitted to analysis on sucrose gradient, the greater part of the AP endodeoxyribonuclease activity was found in the top fractions, but some remained associated with a peak of monosomes. Analysis of DNA from the redigested disomes showed that most of the molecules had about 145 nucleotide pairs although some pieces of about 350 nucleotide pairs were still present (fig.2).

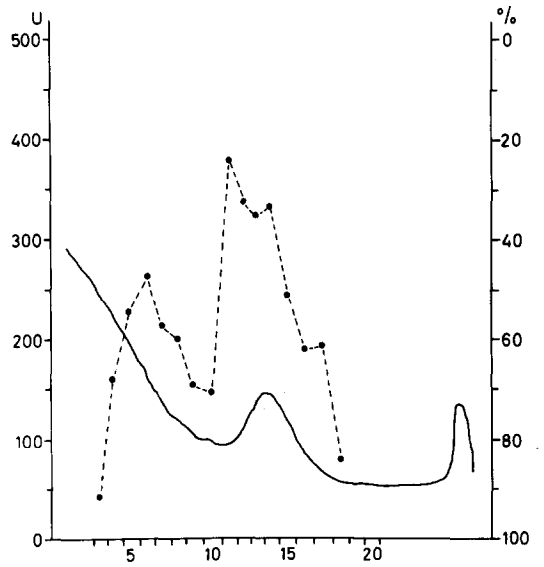

Fig.3. Sedimentation of the redigested monosomes through a linear sucrose gradient. Fractions 12–15 from the first centrifugation (fig.1) were pooled, dialyzed, concentrated with polyethylene glycol and submitted to a second treatment with micrococcal nuclease. Centrifugation was carried out as described in fig.1; the same symbols are used in the two figures.

4. DISCUSSION

The chromatin has a beaded structure; the constitutive units, called nucleosomes, contain a DNA piece of about 200 nucleotide pairs. The nucleosome is constituted of a core (145 nucleotide pairs surrounding a histone octamer) and a linker joining the core to the next nucleosome. Micrococcal nuclease preferentially digests linker DNA and separates fragments containing one or several nucleosomes. After a light digestion, the DNA pieces are multiples of about 200 nucleotide pairs. With increasing time of digestion, the monosomes become dominant and the DNA piece associated with them is first reduced to about 165 nucleotide pairs; there is still some linker DNA protected by histone H1. Later, histone H1 is lost and the DNA shortened to about 145 nucleotide pairs; the monosome has been transformed into a core [7].

Here, we have shown that a limited digestion of rat liver chromatin with micrococcal nuclease liberates a part of the AP endodeoxyribonuclease activity so that it can no longer be sedimented with the nucleosomes. This result suggests that some AP endodeoxyribonuclease is located in the linker region. Analysis of the digest by sedimentation on a sucrose gradient leads to the same conclusion: part of the enzyme activity was found in the top fractions with other proteins liberated by the nuclease digestion, but most of it remained associated with the monosomes, disomes, trisomes, etc.

Analysis of DNA from the monosomes showed that it was 160–200 nucleotide pairs long and histone analysis indicated that it was still associated with histone H1. These monosomes thus had still linker pieces; the AP endodeoxyribonuclease bound to these monosomes could be associated with the remaining linker as well as with the core. These monosomes were thus further digested with micrococcal nuclease until the DNA piece was reduced to about 145 nucleotide pairs. A sedimentation on sucrose gradient of this second digest showed that, if some enzyme appeared in the top fractions (which had likely been associated with the remaining linker pieces), most of it remained bound to the core particles.

The disomes from the first sucrose gradient were also submitted to a second digestion which reduced some of the DNA pieces to 145 nucleotide pairs. A second sucrose gradient analysis showed that part of the AP endodeoxyribonuclease activity remained with the core particles. The enzyme activity in the top fractions was relatively higher than with the redigested monosomes; this agrees with the presence of an intact linker in the disomes.

These results indicate that the AP endodeoxyribonuclease of rat liver chromatin is present in the linkers and in the cores of the nucleosomes.

ACKNOWLEDGEMENTS

This work was supported by grants from the Fonds Cancérologique de la CGER and the Fonds de la Recherche Scientifique Médicale.

REFERENCES

[1] Thibodeau, L. and Verly, W.G. (1980) Eur. J. Biochem. 107, 555–563.
[2] Todd, R.D. and Garrard, W.T. (1977) J. Biol. Chem. 252, 4729–4738.
[3] Loening, U.E. (1967) Biochem. J. 102, 251–257.
[4] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning; A Laboratory Manual, p.156, Cold Spring Harbor Laboratory, New York.
[5] Panyim, S., Bilek, D. and Chalkley, R. (1971) J. Biol. Chem. 246, 4206–4215.
[6] Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337–346.
[7] Kornberg, R.D. (1974) Science 184, 868–871.