Journal of Chromatography, 648 (1993) 275–278
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 381

Short Communication

Rapid method for the fractionation of nuclear proteins and their complexes by batch elution from hydroxyapatite

A. Zagariya

Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294 (USA)

S. Khrapunov

Department of General and Molecular Genetics, Kiev University, Vladimirskaya Street 64, Kiev 252601 (Ukraine)

W. Zacharias*

Department of Biochemistry and Molecular Genetics, BHSB 407, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294 (USA)

(First received February 16th, 1993; revised manuscript received June 15th, 1993)

ABSTRACT

A new procedure for the separation and purification of nuclear proteins and their complexes by batch elution from hydroxyapatite is presented. This method allows to isolate such proteins with different basic character faster and more efficiently than procedures using column chromatography, while showing high selectivity, sensitivity, simplicity, mild conditions of purification, reproducibility and protein stability.

INTRODUCTION

Fractionation and purification of nuclear proteins (histones and non-histones) are among the most versatile tools in modern molecular biology. Here we describe a simple and widely applicable new method for the purification of nuclear proteins and their complexes from mammalian chromatin. Our method is designed to fractionate nuclear proteins bound to hydroxyapatite by batch elution at different ionic strengths instead of the conventional fractionation by hydroxyapatite column chromatography [1]. This method may be used to study the heterogeneity and DNA-binding properties of nuclear proteins. It allows processing of many samples simultaneously and permits the isolation of proteins with different affinities to DNA (histones, their complexes, and non-histone proteins) very efficient-

* Corresponding author.

0021-9673/93/$06.00

ly, compared to previously published procedures using column chromatography [1,2]. These conditions help to avoid redistribution of proteins in the nucleohistone and are accompanied by minimum levels of proteolysis.

EXPERIMENTAL

Chemicals and solutions

The calf thymus nucleohistone material and Sephadex G-100 were obtained from Sigma (St. Louis, MO, USA). DNA 1 kilobase-pair size markers were from Bethesda Research Labs. (Gaithersburg, MD, USA), and total histones from Boehringer Mannheim Biochemicals (Indianapolis, IN, USA). Water was obtained from a Milli-Q system (Millipore, Milford, MA, USA). All other chemicals were analytical grade [Tris, ammonium sulphate, sodium dodecyl sulphate (SDS), acrylamide, bisacrylamide, Coomassie Blue, ethidium bromide, sodium chloride, acetic acid, methanol, boric acid, EDTA] and were obtained from Mallinckrodt (Paris, KY, USA) or Sigma. The 0.5 *M* phosphate buffer (pH 7.5) was prepared by combining 0.5 *M* NaH_2PO_4 and 0.5 *M* Na_2HPO_4 up to pH 7.5.

Hydroxyapatite preparation

Hydroxyapatite was prepared according to Tiselius *et al.* [3], with some modifications [4], by slow mixing (500 ml/h) of 0.5 *M* sodium phosphate (pH 7.5) and 0.5 *M* $CaCl_2$. We measured a protein content of 70% and DNA content of 30% in our calf thymus nucleohistone material. A 100-mg sample of this nucleohistone was mixed with 21 g of hydroxyapatite in 1 m*M* Tris–HCl (pH 7.9). Proteins with different DNA-binding affinities were eluted by washing and centrifugation in 10-ml fractions of different NaCl concentrations in 50 m*M* sodium phosphate buffer (pH 7.5).

Separation of histone complexes

Eluted histone complexes were loaded onto a Sephadex G-100 column (10 cm × 1 cm) equilibrated with 5 m*M* Tris–HCl (pH 8.0). Stepwise elutions of histone dimer by 1 *M* NaCl and tetramer by 2 *M* NaCl were precipitated overnight by $(NH_4)_2SO_4$ at 100 and 80% saturation, respectively. Histone octamer was eluted by 2 *M* NaCl, without previous elutions of histone dimer and tetramer, and was precipitated by $(NH_4)_2SO_4$ at 60% saturation.

Protein and DNA analysis

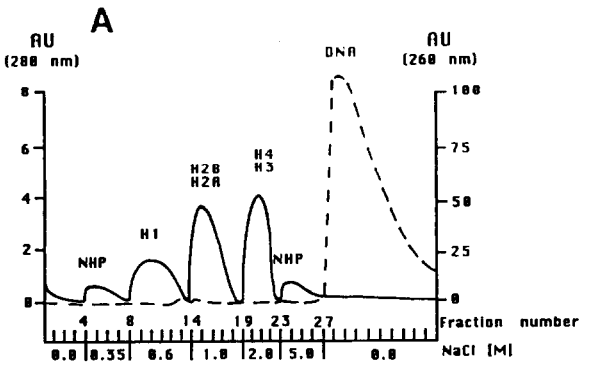
Protein concentrations were determined with the Bradford protein assay kit (Bio-Rad, Melville, NY, USA) by using a standard curve established with purified total calf thymus histone. DNA concentrations were measured by the UV absorbance at 260 nm (1 AU = 50 μg/ml).

Proteins (10 μg per lane) were analyzed in gels containing 15% acrylamide, 0.5% bisacrylamide, 0.38 *M* Tris–HCl (pH 8.8), 0.1% SDS; staining was done with 0.1% Coomassie Brilliant Blue R250 in acetic acid–methanol–water (10:25:65). Analysis of DNA (0.5 μg per lane) was done in 0.8% agarose gels in 89 m*M* Tris–borate, 2 m*M* EDTA (pH 8.3) at 50 V for 2 h, followed by staining in 10 μg/ml ethidium bromide for 1.5 min and destaining in water overnight.

RESULTS AND DISCUSSION

Fig. 1 shows the results obtained with the batch elution procedure. Hydroxyapatite-absorbed calf thymus nucleohistone complex was equilibrated with 50 m*M* sodium phosphate buffer (pH 7.5), and proteins were batch-eluted with different concentrations of NaCl in the same buffer. A small amount of basic non-histone proteins (NHP) was released in 0.35 *M* NaCl. Histone H1, which has more basic character than the other histones, was released in 0.6 *M* NaCl, histones H2A and H2B (with less basic character than H1) in 1.0 *M* NaCl, and histones H3 and H4 (with the least basic character) were eluted in 2 *M* NaCl (Fig. 1A). The purities of these fractions, as determined by gel electrophoresis (Fig. 1B), were 80–85% for H1, 90% for H2A and H2B, and 80–90% for H3 and H4. A small amount of weakly basic non-histone proteins was released in 5 *M* NaCl. The fractionation procedure could easily be performed in 1 day.

The recoveries of eluted histones were 12.5 mg



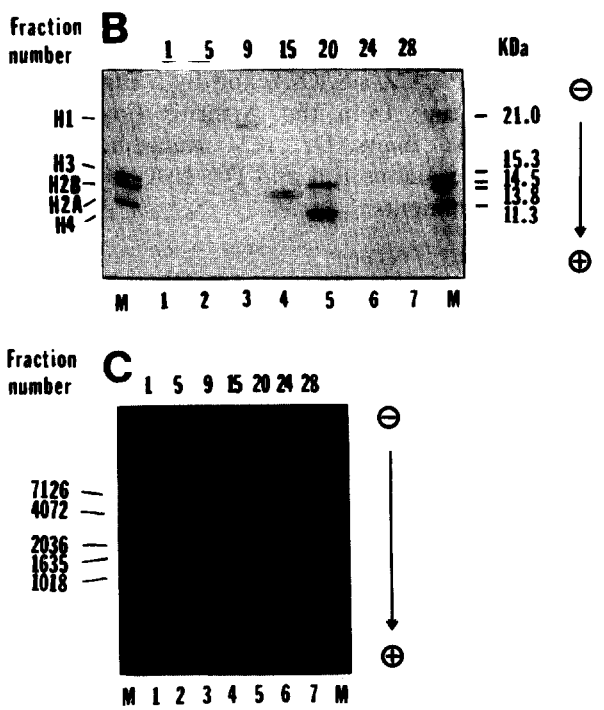


Fig. 1. Fractionation of histones from nucleohistone complex by batch elution from hydroxyapatite. (A) Elution profiles as determined by UV absorbance at 280 nm (protein; solid line) and 260 nm (DNA; dashed line). Fractions 1–27 were obtained at 0, 0.35, 0.6, 1.0, 2.0 and 5.0 *M* NaCl, respectively. Fractions 28–37 were obtained at 0.5 *M* sodium phosphate buffer (pH 7.5) without NaCl. (B) Analysis of eluted proteins by electrophoresis in denaturing polyacrylamide gels. Fraction numbers correspond to the numbers in the elution profile of Fig. 1A. M = Total histones (10 μg) from calf thymus nucleohistone. (C) Analysis of eluted DNA by agarose gel electrophoresis. Fraction numbers correspond to the numbers in the elution profile of Fig. 1A. M = DNA molecular mass marker.

for H1, 19.7 mg for H2A–H2B, and 22.5 mg for H3–H4. The yields of the non-histone proteins eluted by 0.35 *M* NaCl or by 0.5 *M* sodium phosphate (pH 7.5) without NaCl were 5.3 and 3.3 mg, respectively. Because of their low amounts, these non-histone proteins could not be detected in the Coomassie-stained gel (Fig. 1B). Altogether, the recovery of total proteins from 100 mg of nucleohistone complex was 63.3 mg, corresponding to an exellent yield of approximately 90%. For the column chromatography method, recoveries of 66% for H2A and

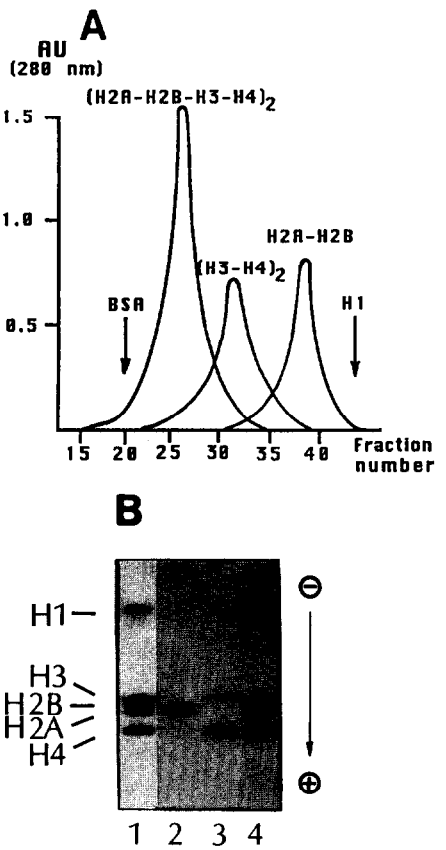


Fig. 2. Separation and identification of histone complexes after batch elution and Sephadex G-100 gel permeation. (A) Elution of histone dimer H2A–H2B by 1 *M* NaCl and tetramer $(H3–H4)_2$ by 2 *M* NaCl. Histone octamer was also eluted by 2 *M* NaCl, but without previous elution of histone dimer and tetramer. BSA = Bovine serum albumin. (B) Analysis of eluted fractions by electrophoresis in denaturing polyacrylamide gels. Lanes: 1 = total histones from calf thymus as marker; 2 = histone dimer; 3 = tetramer; 4 = octamer.

H2B, and 95% for H3 and H4 were reported [1].

Yields of DNA during the elutions up to 5 *M* NaCl (fractions 1–27) were negligibly small (Fig. 1A and C). Approximately 31 mg of free DNA was eluted only in 0.5 *M* sodium phosphate (pH 7.5) without NaCl up to fraction 37. This corresponds to a yield of DNA of approximately 100% from this nucleohistone complex.

The oligomeric complexes of histones were obtained in the following way (Fig. 2). First, a small amount of highly basic non-histone proteins was eluted in 0.35 *M* NaCl, and histone H1 was eluted by 0.6 *M* NaCl. The subsequent fractions were analyzed by Sephadex G-100 gel permeation for the oligomeric nature of the histone complexes (Fig. 2A). Histone dimers (H2A–H2B) were eluted in 1 *M* NaCl, and histone tetramers $(H3–H4)_2$ in 2 *M* NaCl. Histone octamers $(H2A–H2B–H3–H4)_2$ were eluted in a separate batch in 2 *M* NaCl without previous elution of histone dimers and tetramers. The purities of the histone complexes obtained were determined by electrophoresis in denaturing polyacrylamide gels (Fig. 2B). It was shown previously that at reduced ionic strength, histone octamers dissociate into two dimers and one tetramer [4].

CONCLUSIONS

Our data demonstrate some important advantages of protein separation by batch elution: good multiple peak resolution, high purity, excellent yield, and time-saving isolation. Although we have not tried samples of more than 100 mg of nucleohistone complex as starting material, this procedure can certainly be scaled up to larger amounts of nucleohistone. Thus, batch elution from hydroxyapatite can be a valuable and rapid alternative for the purification of nuclear proteins and their complexes, to be used for nucleosome reconstitution, gel shift assays of DNA-protein interactions, for obtaining total histones, and for many other molecular biological applications.

ACKNOWLEDGEMENTS

This work was supported by the General and Molecular Genetics Department, Kiev University, Ukraine (A.Z., S.Kh.), and by a Biomedical Science Grant from the Arthritis Foundation (W.Z.). We thank Dr. N.V. Kalashnikov for reading the manuscript and Dr. L.A. Sitailo for helpful comments.

REFERENCES

1 R.H. Simpson and G. Felsenfeld, *Nucleic Acids Res.*, 6 (1979) 689–696.
2 C. von Holt, W.F. Brandt, H.J. Greyling, G.G. Lindsey, J.D. Retief, J. de A. Rodrigues, S. Schwager and B.T. Sewell, *Methods Enzymol.*, 170 (1989) 431–503.
3 A. Tiselius, S. Hjertén and O. Levin, *Arch. Biochem. Biophys.*, 65 (1957) 132–155.
4 I.B. Dubrovsky and S.N. Khrapunov, *Ukr. Biokhim. J.*, 60 (1988) 8–13.