Fractionation of chromatin fragments on columns of Biogel A50-m at different salt concentrations

Hui-Chuan Huang and R. David Cole*

Department of Biochemistry, University of California, Berkeley, CA 94720, USA

Received 16 January 1985

Nuclease fragmented chromatin was chromatographed on Biogel at various NaCl concentrations. The yield of eluted chromatin, and its H1/core histone ratio was minimal at 0.18 M NaCl where the ratio of H1 subtypes H1c/H1ab was maximal. Therefore, the eluted material was aggregation-resistant chromatin while aggregatable chromatin remained on the columns. Previous results were interpreted as H1 depletion of chromatin by ion-exchange properties of Biogel, but the primary phenomenon is now seen as a separation of classes of chromatin that differ in sensitivity to salt-induced aggregation. At very low salt concentrations, Biogel chromatography can be used without concern for H1 depletion.

Chromatin *Gel permeation* *Chromatin fractionation*

1. INTRODUCTION

Gel permeation has been used in the preparation of chromatin in different size ranges. However, when Bates et al. [1] used columns of Biogel A150-m for the chromatography of chromatin that had been fragmented by mild treatment with micrococcal nuclease, the eluted chromatin was slightly depleted in H1 histone, and especially in one subtype. They suggested H1 depletion resulted from ion-exchange properties of the Biogel. Perhaps for this reason, there has been meager use of gel permeation in the fractionation of chromatin. Our recent studies on salt-induced aggregation of chromatin [2] suggested an alternative explanation for the apparent loss of H1 during chromatography, which if true, would indicate that at sufficiently low salt concentrations Biogel chromatography can be used, after all, to fractionate chromatin without loss of H1 histone.

H1 histone is non-uniformly distributed in chromatin in a stable pattern that probably correlates with the different degrees of chromatin condensation observed microscopically [2]. Chromatin, made 0.2 M in NaCl, was divided into an aggregatable fraction and an aggregation-resistant fraction. The latter fraction was apparently enriched in active genes and related to active chromatin fractions obtained by others. To isolate active chromatin Gottesfeld et al. [3] digested chromatin with DNase II and induced aggregation with Mg^{2+}, while Levy-Wilson and Dixon [4] digested with micrococcal nuclease and isolated very small oligonucleosomes after 0.1 M NaCl treatment. Bloom and Anderson [5] released non-aggregated small oligonucleosomes from nuclei by micrococcal nuclease treatment, while Rocha et al. [6] extracted active chromatin from nuclei with 0.2 M NaCl from nuclease treated nuclei. A correlation between DNase I sensitivity and salt-induced, H1-dependent folding of chromatin implicates chromatin condensation in gene regulation [7]. Thus salt-induced aggregation seems to separate chromatin into functionally distinct classes. Here we demonstrate that Biogel chromatography of chromatin at various salt concentrations accomplishes the same kind of fractionation into classes of chromatin that differ in H1 content. This

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

accounts for the low H1 content in the fractions of Bates et al. [1] without invoking ion exchange.

2. MATERIALS AND METHODS

2.1. *Buffers*

Buffer A: 0.3 M sucrose, 50 mM Tris-HCl, 25 mM KCl, 5 mM $MgCl_2$, and 1 mM phenylmethylsulfonyl fluoride (pH 6.5). Buffer B: 10 mM sodium phosphate and 1 mM EDTA (pH 6.5). Buffer C: 10 mM Tris-HCl, 10 mM NaCl, 3 mM $MgCl_2$, and 1 mM phenylmethylsulfonyl fluoride (pH 7.4).

2.2. *Preparation of nuclei*

Nuclei were isolated (0–4°C) from steer thymus glands as in [8] except that tris replaced triethanolamine.

2.3. *Preparation of soluble chromatin*

Micrococcal nuclease digestion was for 10 min at 37°C on calf thymus nuclei ($A_{260} = 50$) in buffer A. $CaCl_2$ was added to 1 mM before digestion, and 15 units/ml micrococcal nuclease (Worthington, 15 000 units/mg) was added). Digestion was quenched by NaEDTA (pH 6.5) to 5 mM on ice. Nuclei pelleted (5 min at $3000 \times g$) were lysed in 0.2 mM NaEDTA, pH 6.5, by incubation for 1–2 h at 0°C with intermittent agitation by a pasteur pipet. Lysed nuclei were centrifuged (6 min at $3500 \times g$) and the supernatant containing nucleosome oligomers was measured at A_{260} and stored at 0–4°C. This material, soluble at low salt concentrations, is usually referred to as soluble chromatin.

2.4. *Biogel A50-m chromatography*

Soluble chromatin, dialyzed against buffer B overnight, was concentrated in an Amicon concentrator with XM50 filter. 30 ml chromatin at $A_{260} = 50$ was fractionated on a column (4×100 cm) of Biogel A50-m, 100–200 mesh (Bio-Rad) in buffer B at a flow rate of 36 ml/h (7.5-ml fractions). Chromatin fractions from Biogel A50-m with average DNA size above 6 kbp were pooled and dialyzed overnight, and concentrated. Chromatin solutions adjusted to 20, 80, 150, 180, and 250 mM NaCl, respectively, with 5 M NaCl were equilibrated at each salt concentration at least 3 h before chromatography. Columns (0.45×18 cm) of Biogel A50-m, 100–200 mesh were equilibrated and eluted with buffer C at the same salt concentrations as the chromatin samples. Chromatin samples (0.65 ml at $A_{260} = 33$) applied were sometimes turbid, but the solutions were not clarified by centrifugation. Columns were eluted at 24 ml/h with fractions of 0.5 ml.

2.5. *Gel electrophoresis*

Chromatin samples were analyzed by SDS-polyacrylamide gel electrophoresis [9]. Slab gels with 4.5% acrylamide stacking gels and 12.5% acrylamide separating gels were run at 30 mA. Gels, stained with Coomassie blue, were scanned at 525 nm in a Kratos spectrodensitometer model SD3000.

Samples were deproteinized for DNA gel electrophoresis by dissolving in 1% SDS and 1 M NaCl, and then extracting twice with equal volumes of chloroform-isoamyl alcohol (24:1, v/v). The DNA, which precipitated overnight in 2.5 vols ethanol at −20°C, was centrifuged at $8000 \times g$ for 10 min and redissolved in electrophoresis buffer. Short double-stranded DNA fragments were fractionated at 15 mA in 2.5% polyacrylamide gels [10] as modified to contain 0.5% agarose [11]. Long DNA fragments were separated in 1% (w/v) agarose horizontal gels. The buffer for electrophoresis was 2 mM NaEDTA, 10 mM triethanolamine-HCl, pH 7.6 [12]. The gels stained with ethidium bromide (5 mg/l) were photographed under short-wavelength UV light through a red filter.

2.6. *Concentration measurement*

DNA concentration was determined by A_{260} assuming $E^{1\,cm,1\%} = 200$, aliquots of chromatin having been diluted 1:50 in 2% SDS before measurement.

3. RESULTS AND DISCUSSION

Salt-induced aggregation occurs maximally at 150–200 mM NaCl and the chromatin that resists aggregation has a low H1 histone content [2]. Since Bates et al. [1] observed low H1 contents in fractions obtained by gel permeation of chromatin fragments at 60 mM NaCl, we performed Biogel chromatography at several concentrations of NaCl

to learn whether their results might be explained by salt-induced aggregation. Chromatin solubilized by brief treatment with micrococcal nuclease, was applied to a column of Biogel A50-m equilibrated with buffer B (no NaCl) and eluted as a single broad peak. There was fractionation according to fragment size, as shown in fig.1. Chromatographic fractions represented by lanes a and b of fig.1 were pooled and aliquots were applied to columns of Biogel A50-m, equilibrated with 20, 80, 150, 180 and 250 mM NaCl in buffer C. The H1 contents of the eluted materials are shown in fig.2. The minor loss of H1 apparent in the chromatin eluted at 80 mM NaCl is reminiscent of the loss reported by Bates et al. However, there was no loss of H1 in the absence of salt, and the apparent loss of H1 was more severe as the NaCl concentration was increased to 180 mM, only to decrease at higher NaCl concentrations (250 mM).

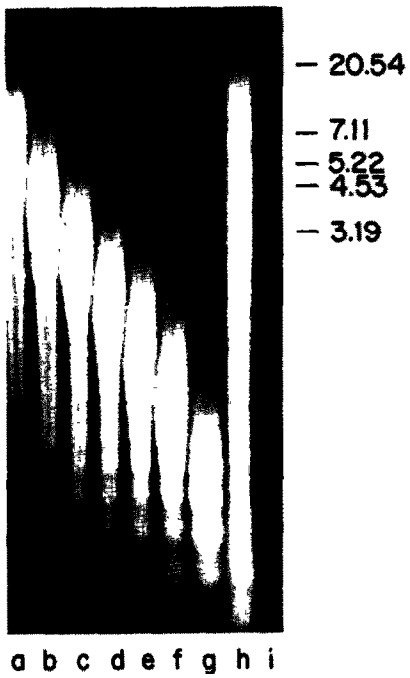

Fig.1. The size of DNA across the chromatographic profile. Soluble chromatin isolated from thymus nuclei after 10 min digestion with micrococcal nuclease was applied to a Biogel A50-m column (4 × 100 cm). DNA from fractions across the profile was analyzed in a 0.8% agarose gel and shown from lane a to g. Lane h shows the sample before chromatography, and lane i shows *Eco*R1 digest of λ DNA. Numbers refer to sizes of the restriction fragments in kbp.

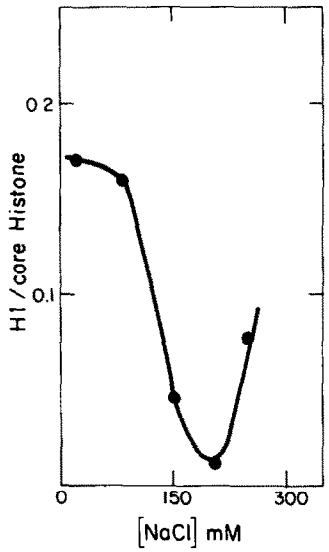

Fig.2. Content of H1 histone relative to core histones in the eluted fractions from Biogel A50-m columns. Equal amounts of chromatin were applied to columns equilibrated with the stated salt concentration. Histone ratios were measured by scanning a Coomassie blue-stained SDS 12.5% polyacrylamide slab gel.

The eluted chromatin was analyzed for the ratio of H1 subtypes (fig.3). Similar to the report of Bates et al., the ratio of H1 subtypes was different in chromatin eluted at 80 mM NaCl from that in

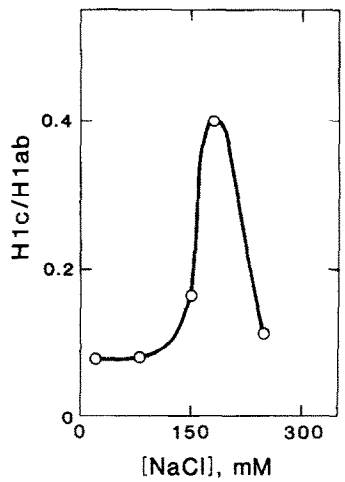

Fig.3. Ratios of H1 subtypes in the fractions from Biogel A50-m columns at various salt concentrations. The ratios were determined by scanning electrophoretic SDS-polyacrylamide gels.

the starting material. Once again, however, the difference in H1 subtype ratio became progressively more pronounced as salt concentration was increased, until it passed through a maximum at about 180 mM NaCl.

The salt dependence of the H1 content, and the H1 subtype ratio in the eluted fractions of chromatin resembled the behavior of the class of chromatin that resists salt-induced aggregation [2], so we tested the notion that aggregated chromatin had remained on the column. In fact, the yield of chromatin was strongly influenced by NaCl with a minimum at about 180 mM (fig.4). The correlations among H1 content, H1 subtype ratio, and yield are striking. Moreover, these correlations present the same pattern as that observed when aggregation was induced with NaCl in free solution [2]. We conclude, therefore, that the apparent depletion of H1 from chromatin that was passed through Biogel was not due primarily to ion-exchange properties of the matrix, but that it was simply an expression of the solubility of different classes of chromatin that contain different amounts H1 histone. It has been shown previously [2] that the different solubility classes of chromatin are discrete species that are not generated by exchange of their H1 histones. Therefore, it is not necessary to suppose a process of H1 depletion by the Biogel chromatography. Instead, it may be concluded that the chromatography results in a fractionation of pre-existing classes of chromatin. To avoid such fractionation, the salt concentration should be kept below 50 mM. Biogel chromatography at ~180 mM NaCl could provide a valuable tool for the preparation of unaggregated chromatin, but the fraction eluted would not represent total chromatin. It appears [2–6] that it would be enriched in active chromatin, and therefore that Biogel chromatography at 180 mM NaCl might be used to prepare chromatin that was enriched in active genes.

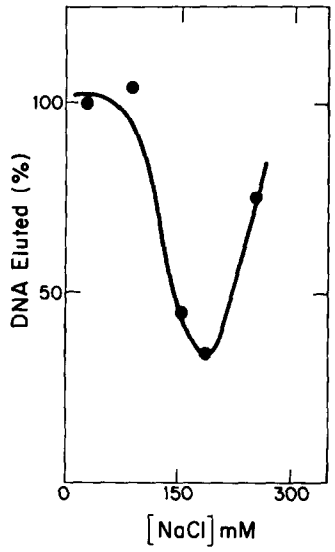

Fig.4. The yield of chromatin eluted from Biogel A50-m columns. The yield was measured by DNA content (A_{260}) of the fractions from Biogel A50-m columns at various salt concentrations.

The extent of apparent H1 depletion seems more severe in chromatographic fraction eluted by 150–180 mM NaCl than reported previously for the supernatants of chromatin preparations sedimented at these ionic strengths. The difference is due not to the chromatographic process per se. The difference is explained by the fact that the samples applied to the Biogel columns were at a concentration of $A_{260} = 30$, while the experiments in free solution were done at $A_{260} = 2$–6. The effect of chromatin concentration on apparent H1 depletion is shown in fig.5. Solutions of chromatin at several concentrations were adjusted to 160 mM NaCl and centrifuged (1000 × g, 15 min) after 2 h. The chromatin remaining in the supernatants

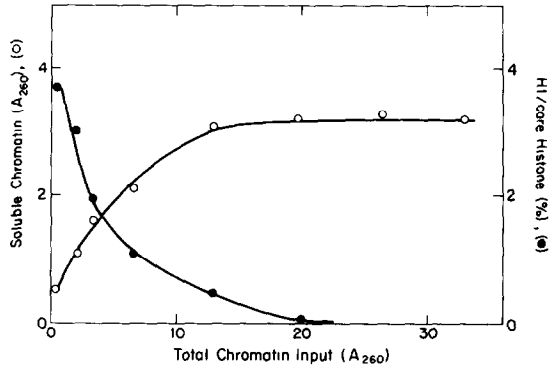

Fig.5. The effect of chromatin concentration on chromatin solubility and the content of H1 relative to core histones in dissolved chromatin. Chromatin at several concentrations was adjusted to 160 mM NaCl in buffer B, and centrifuged after 2 h. The supernatant fractions were measured by A_{260} (○) and the proteins of the supernatants were analyzed by SDS gel electrophoresis (●).

(A_{260}) showed saturation when the input chromatin exceeded $A_{260} = 15$. Not surprisingly, the heterogeneous population of chromatin fragments behaves as a non-ideal solute and as the overall chromatin concentration increases, the composition of the supernatant changes will respect to the H1 content of its chromatin fragments. Apparently, H1-poor fragments are more effective than H1-rich ones in the competition that develops for the solvent. Not only was the H1/core histone ratio decreased in the unaggregated chromatin as the total chromatin concentration was increased, but the H1c/H1ab ratio increased as well (not shown).

ACKNOWLEDGEMENTS

This work was supported by US Public Health Service grant GM20338 and by the Agricultural Research Station. We appreciate the kindness of Dr Charles Dekker for the use of his densitometer.

REFERENCES

[1] Bates, D.L., Butler, P.J.G., Pearson, E.C. and Thomas, J.O. (1981) Eur. J. Biochem. 119, 469.
[2] Huang, H.-C. and Cole, R.D. (1984) J. Biol. Chem. 259, 14237–14242.
[3] Gottesfeld, J.M., Garrard, W.T., Bagi, G., Wilson, R.F. and Bonner, J. (1974) Proc. Natl. Acad. Sci. USA 71, 2193–2197.
[4] Levy-Wilson, B. and Dixon, G.H. (1978) Nucleic Acids Res. 5, 4155–4164.
[5] Bloom, K.S. and Anderson, J.N. (1978) Biochemistry 17, 2086–2095.
[6] Rocha, E., Davie, J.R., van Holde, K.E. and Weintraub, H. (1984) J. Biol. Chem. 259, 8558–8563.
[7] Smith, R.D., Yu, J., Annunziato, A. and Seale, R.L. (1984) Biochemistry 23, 2970–2976.
[8] Todd, R.D. and Garrard, W.T. (1977) J. Biol. Chem. 252, 4729–4738.
[9] Laemmli, U.K. (1970) Nature 277, 680–685.
[10] Loening, U.E. (1967) Biochem. J. 102, 251–257.
[11] Peacock, A.C. and Dingman, C.W. (1968) Biochemistry 1, 668–674.
[12] Varshavsky, A.J., Bakayev, V.V., Chumacker, P.M. and Georgiev, G.P. (1976) Nucleic Acids Res. 3, 2101–2113.