A C T A U N I V E R S I T A T I S L O D Z I E N S I S
FOLIA BIOCHIMICA ET BIOPHYSICA 8, 1991

Zofia Walter, Maria Gawrońska

CHROMATIN FRACTIONATION ON HYDROXYLAPATITE BY CENTRIFUGAL CHROMATOGRAPHY METHOD

Chromatin fractionation on hydroxylapatite (HAP) by centrifugal chromatography was presented. Eleven fractions different in respect to thermal stability, protein contents, DNA and nitrogen base composition were obtained. The centrifugal chromatography of chromatin on hydroxylapatite elaborated by our technique has all the advantages of thermal column chromatography but it is far less complicated and time-consuming.

INTRODUCTION

Structural and functional differentiation of chromatin implies search for the methods enabling separation of fractions with different composition and function. Some physicochemical properties characteristic for these fractions can be used in the research. Transcriptionally active chromatin is a subject to easier mechanical and enzymatic fragmentation. The separation of chromatin fractions following their fragmentation was applied by many researches. Thermal chromatography of chromatin on hydroxylapatite was elaborated by M c C o n a u g h y and M c C a r t h y [11] and affinity chromatography on Sepharose 6B by F e r r a r o et al. [6]. M c C o n a u g h y and M c C a r t h y [11] fractionated chromatin from avian erythrocytes by adsorption on hydroxylapatite and then elution in temperature gradient. The use of thermal precipitation of chromatin on hydroxylapatite column by M a r k o v et al. [10] led to three fractions dif-

ferent in the respect of structural and functional properties.

In this report we have tried to fractionate adsorbed chromatin on hydroxylapatite by centrifugal technique in temperature gradient.

MATERIAL AND METHODS

Pig peripheral blood and thymus taken immediately after slaughter. Pig peripheral blood leukocytes were isolated by sedimentation method according to W a l t e r [19]. Cell nuclei from leukocytes and thymus were isolated by A l l f r e y and M i r s k y's method [1]. Chromatin from cell nuclei was prepared by S p e l s b e r g and H n i l i c a's method [18]. Purity of chromatin preparations was examined spectrophotometrically. Chromatin spectrum was determined in 5 M urea, in the wave range 200-350 nm. Indicators elaborated by S p e l s b e r g and H n i l i c a [18] were used to determine purity i.e. A260/A290 in the range 3.12--3.91, A260/A230 in the range 0.91-1.14, A320/A260 below 0.1, A280/A260 in the range 0.6-0.7 and A260/A280 in the range 1.54--1.74.

Chromatin samples in 0.0012 M sodium phosphate buffer, pH 6.8 were sonicated in E.K. Klingenf sonicator (FRG). Sonication time used was 45 and 60 seconds with frequency 88 Khz.

Temperature melting curves were determined for chromatin solutions in 0.012 M sodium phosphate buffer pH 6.8 at DNA concentration 20-30 µg/ml. Absorption measurments (260 nm) were performed for each temperature change of 0.5°C during 0.5 min on Unicam SP 1700 spectrophotometer with automatic installation for temperature SP 876. Temperature melting curves were determined according to F e k e t e et al. [5].

Chromatin proteins were electrophoretically analyzed in 11.2% polyacrylamide gel containing SDS according to L a e m m l i [7]. Gel was stained Coomassie brillant blue according to F a i b r a n k s et al. [4].

Chromatin fractionation on hydroxylapatite (HAP) by centrifugal chromatography method. Sonicated chromatin was subject to fractionation. Chromatography was carried out in centrifugal tubes of 50 ml volume in which 10 g of suspended layer in 0.12 M sodium phosphate buffer, pH 6.8 were placed. Centrifugal tubes were then put in ultratermostate. 0.12 M sodium phosphate buffer pH 6.8 was the elution solution. Chromatin adsorption on the layer was carried out in 55°C mixing hydroxylapatite layer with chromatin disolved in 0.12 M sodium phosphate buffer, 1.5 mg chromatin to 1 ml layer for 20 min. It was then centrifuged at 5500 cpm/min. The collected supernatant contained a non-adsorbed fraction on layer at 55°C. The rest was treated with 10 ml elution buffer and extraction in the temperature range 60-100°C, every 5°C was done. After the elution in 0.12 M phosphate buffer pH 6.8 at 100°C the layer was extracted in the same temperature with 0.5 M sodium phosphate buffer, pH 6.8 and next with 8 M urea, 0.25 M sodium phosphate buffer, pH 6.8 0.01 M EDTA. A given fraction was obtained by using centrifugation following extraction at a given temperature. The fractions obtained were designated as 55°C, 60°C, 65°C. DNA content was determined by Burton's method [3] and protein by Lowry et al. method [8].

DNA was hydrolysed with 57% $HClO_4$ for 1h at 100°C. Bases were separated by thin-layer chromatography on cellulose MN 300 G. Isopropanol: HCl:H_2O was used as a solvent. Bases were identified directly in ultraviolet light using a suitable filter (254 nm). Next they were eluted with 0.1 N HCl and defined spectrophotometrically.

RESULTS AND DISCUSSION

In this report the chromatin isolated from two tissues of a pig thymus and leukocytes of peripheral blood, was fractionated by centrifugal chromatography method on

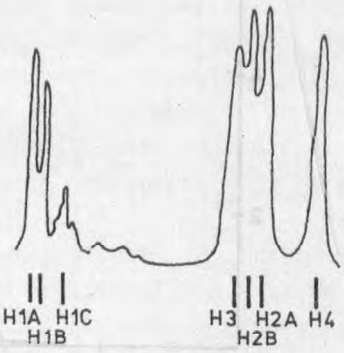

Fig. 1. The densitometric tracing of leukocyte histones distributed in 11.2% polyacrylamide gel (10 µg protein were applied on gel according to Laemmli[7] and stained Coomassie brillant blue)

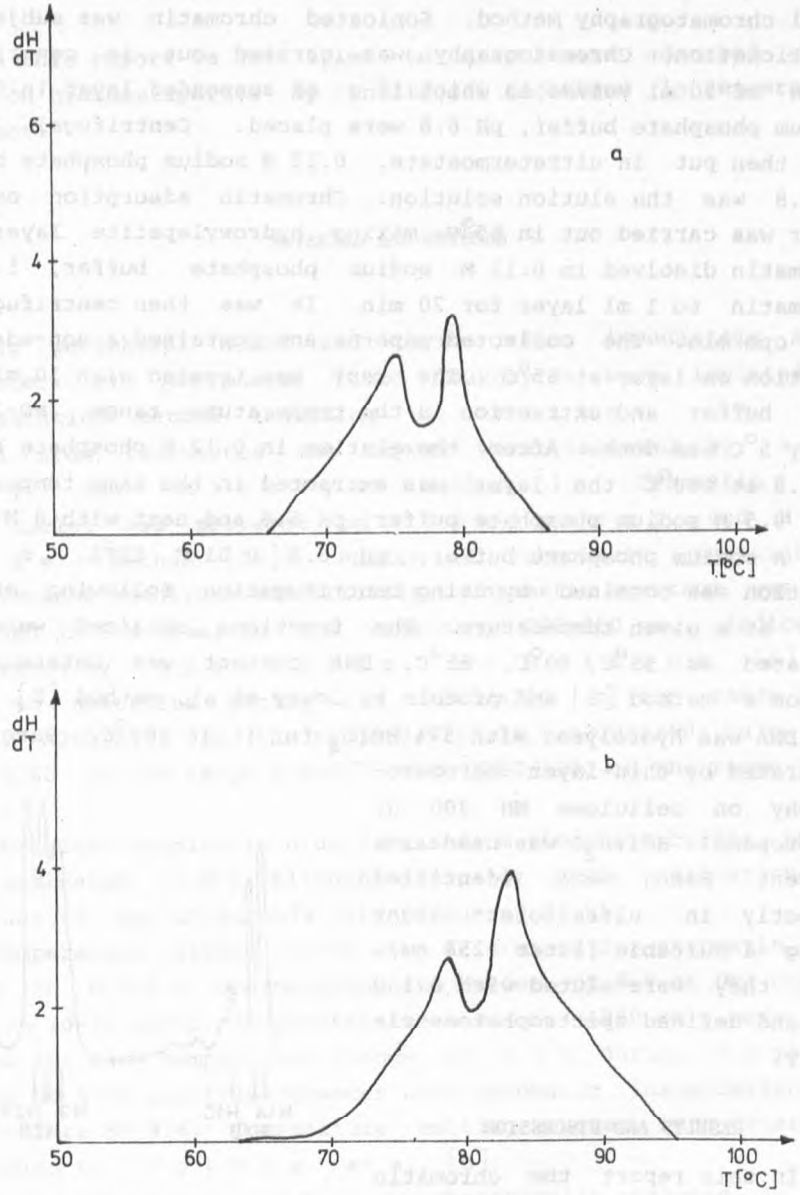

Fig. 2. Derivative melting profiles of sonicated (45 seconds), a) and native, b) pig leukocyte chromatin

hydroxylapatite gradient of temperature. Centrifugal technique on DEAE cellulose was used to determine the damage caused by gamma radiation in DNA (O l i ń s k i [14]) and the changes in DNA by organophosphate compounds (W o j t y s i a k, W a l t e r [23]). The fractionated chromatin was characterized spectrophotometrically. Indicators for solution of chromatin contained in borders settled from pure preparations worked out by S p e l s b e r g and H n i l i c a [18]. Absorption coefficients A260/A290 3,36 ±0.19, A320/A260 0.059 ±0.025, A280/A260 0.65 ±0.019, A260/A230 0.1 ±0.05, A260/A280 1.57 ±0.07 and A260/A290 3.46 ±0.26, A320/A260 0.043 ±0.015, A280/A260 0.60 ±0.05, A260/A290 1.18 ±0.08, A260/A280 1.65 ±0.07 were observed in the case of pig leukocyte and thymus chromatin, respectively. Electrophoretic analysis of proteins in 11.2% polyacrylamide gel showed presence of all histones as ilustrated on Fig. 1.

Temperature melting curves of isolated native and sonicated chromatin in 45 and 60 seconds were investigated. Melting profi-

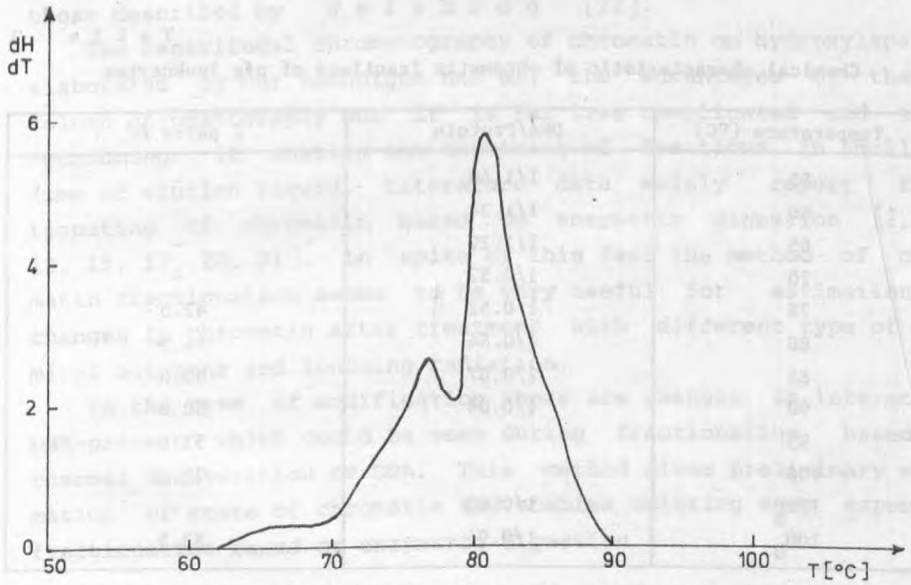

Fig. 3. Derivative melting profile pig thymus chromatin

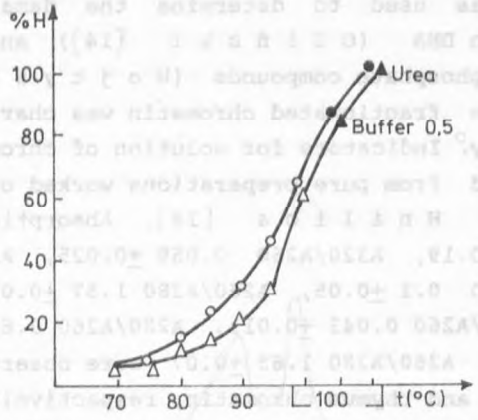

Fig. 4. Chromatography of pig leukocyte and thymus chromatin on HAP in temperature gradient. Chromatin sample in 0.12 M sodium phosphate buffer (pH 6.8) subjected to sonication 45 seconds, ●●●●●● thymus chromatin, -Δ-Δ-Δ leukocyte chromatin were eluted with 0.12 M sodium phosphate buffer (pH 6.8), buffer 0.5 M then with 0.5 M sodium phosphate buffer (pH 6.8) and later with 8 M urea, 0.25 M sodium phosphate buffer (pH 6.8), 0.01 M EDTA

Table 1

Chemical characteristic of chromatin fractions of pig leukocytes

Temperature (°C)	DNA/Protein	% pairs GC
55	1/1.40	-
60	1/1.24	-
65	1/1.29	-
70	1/0.52	-
75	1/0.52	42.5
80	1/0.54	44.5
85	1/0.07	49.0
90	1/0.04	50.0
95	-	51.0
100	-	73.2
100_B	1/0.05	-
100_U	1/0.94	83.5

Abbreviations: 100_B - Eluted with 0.5 M sodium phosphate buffer pH 6.8, 100_U - Eluted with 8 M urea, 0.25 M sodium phosphate buffer pH 6.8, 0.01 M EDTA.

les of the chromatin have two phases, their derivatives (dH/dT) are presented on Fig. 2 and 3. The melting profile of chromatin from pig leukocytes of peripheral blood has shown two transitions at 77.5°C and 82.5°C while the chromatin of pig thymus at 76.8°C and 83.8°C. During sonication temperature melting (Tm) shifted of 2-3°C for the chromatin preparations, what corresponds with experiments described by M a c i e w i c z and L i [9]. Eleven fractions different in respect to thermal stability, protein contents, DNA and nitrogen base composition during centrifugal chromatography on hydroxylapatite in temperature gradient 55-100°C were obtained (Table 1 and Fig. 4). Chromatography on HAP by centrifugal technique showed difference between chromatin from pig blood peripheral leukocytes and thymus.

It´s well known, that fraction of chromatin eluted in range of 70-80°C was designated as transcriptionally active [11, 16]. Chromatin fraction eluted in temperature range of 70-80°C consists of about 11 and 17% of nuclear DNA in pig blood peripheral leukocytes and thymus, respectively. The amounts of transcriptionally active chromatin obtained in this report are similar to those described by W e i s b r o d [22].

The centrifugal chromatography of chromatin on hydroxylapatite elaborated by our technique has all the advantages of thermal column chromatography but it is far less complicated and time-consuming. It enables the obtaining of fractions in small volume of elution liquid. Literature data mainly report fractionating of chromatin based on enzymatic digestion [2, 12, 13, 15, 17, 20, 21]. In spite of this fact the method of chromatin fractionation seems to be very useful for estimation of changes in chromatin after treatment with different type of chemical mutagens and ionizing radiation.

In the case of modification there are changes in interaction DNA-protein which could be seen during fractionation based on thermal denaturation of DNA. This method gives preliminary estimation of state of chromatin and enables omitting very expensive fractionation based on enzymatic digestion.

REFERENCES

[1] Allfrey U. G., Mirsky D. T. (1967), "Methods in Enzymology" (S. P. Colowick, N. O. Kaplan, eds), New York 12, 418-420.

[2] Baibi C., Abelmoschi M. L., Gogioso L., Parodi S., Barboro P., Cavazza B., Patrone E. (1989), "Biochemistry", 28, 3220-3227.

[3] Burton K. (1956), Biochem. J., 62, 315-322.

[4] Faibranks G., Steck T. L., Wallach D. F. (1971), "Biochemistry", 10, 2606-2617.

[5] Fekete A., Ronto G., Feigin L. A., Tikhonychev V. V., Modos K. (1982), Biophys. Struct. Mech., 9, 1-9.

[6] Ferraro J. H., D'Erme M., Cinquepalmi M., Noce A., Padula F., Turano G. (1982), Biochem. Biophys. Res. Commun., 106, 961-966.

[7] Laemmli U. K. (1970), "Nature" (London), 227, 680-685.

[8] Lowry O. H., Rosenbrough N. H., Farr A. L., Randall R. J. (1951), J. Biol. Chem., 193, 265-275.

[9] Maciewicz R. A., Li J. H. (1978), "Biochemistry", 17, 962-967.

[10] Markov C. G., Ivanov J. G., Pashev I. G. (1975), J. Mol. Biol., 93, 405-413.

[11] McConaughy B. L., McCarthy B. J. (1972), "Biochemistry", 11, 998-1003.

[12] Nickel B. F., Allis C. D., Davie J. B. (1989) "Biochemistry", 28, 958-963.

[13] Notbohm H. (1986), Int. J. Biol. Macromol., 8, 114-119.

[14] Oliński R. (1977), Acta Univ. Lodz., ser. II, 16, 75-90.

[15] Reeves R. (1984), Biochim. Biophys. Acta, 782, 343-393.

[16] Saffitz J. E., Caplan A. J. (1978), "Biochemistry", 17, 3487-3495.

[17] Seligy V. L., Poon N. H. (1978), Nucl. Acids Res., 5, 2233-2252.

[18] Spelsberg T. C., Hnilica L. S. (1971), Biochim. Biophys. Acta, 228, 202-211.

[19] Walter Z. (1970), *Chemiczne i fizykochemiczne badania kwasu dezoksyrybonukleinowego leukocytów*, Łódź.

[20] W a l t e r Z., G a w r o ń s k a M. (1987), Kiel. Stud. Biol., 4, 143-154.
[21] W a l t e r Z., G a w r o ń s k a M. (1988), Kiel. Stud. Biol., 5, 127-137.
[22] W e i s b r o d S. (1982), "Nature", 297, 289-295.
[23] W o j t y s i a k M., W a l t e r Z. (1989), Bull. Soc. Sci. Lett., 39, 19, 1-10.

Department of Molecular Genetics
Institute of Biochemistry
University of Łódź

Zofia Walter, Maria Gawrońska

FRAKCJONOWANIE CHROMATYNY METODĄ CHROMATOGRAFII WIRÓWKOWEJ NA HYDROKSYAPATYCIE

Chromatynę izolowaną z grasicy i leukocytów krwi obwodowej świni rozfrakcjonowano metodą chromatografii wirówkowej na HAP. W wyniku frakcjonowania otrzymano 11 frakcji różniących się termiczną stabilnością, zawartością białka, DNA oraz składem zasad azotowych. Wykazano różnicę międzytkankową profili chromatograficznych preparatów chromatyny izolowanych z badanych tkanek. Przedstawiona przez nas w niniejszej pracy metoda chromatografii wirówkowej na hydroksyapatycie zachowuje wszelkie zalety termicznej chromatografii kolumnowej, a jest znacznie prostsza i skraca czas rozdziału. Pozwala na uzyskanie frakcji o dużym stężeniu w niewielkiej ilości płynu elucyjnego.