Physico-Chemical Characteristics of DNA Chromatin Fractions from Calf Thymus

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Two DNA fractions with different physico-chemical properties were obtained from calf thymus. On the basis of our results as well as some literature data we suppose that these are eu- and heterochromatin DNAs.

Introduction

Structural and functional differentiation of chromatin implies the search for the methods of isolation and characterization of DNA bound with chromatin fractions. Preliminary fractionation of chromatin followed by the isolation of DNA from these fractions are the methods frequently used. The isolation procedure put forward by Strazhevskaya and Struchkov [1] makes it possible to isolate DNA fractions directly from the tissue homogenate. The preparations obtained in this way can be useful for the examination of functional and structural properties of chromatin DNAs. Strazhevskaya and Struchkov [1] isolated two DNA fractions from different mammalian tissues by their own method. They found that the fractions had different physico-chemical properties (specific viscosity, hyperchromic effect) and metabolic activity. The contents of these fractions in particular tissues differed and depended on the specificity of cell metabolism. On the basis of the results obtained they assumed that the isolated fractions correspond to eu- and heterochromatin DNAs. However, they characterized only some physico-chemical properties of these fractions. Literature data report the differences in the nucleotide composition of DNAs from eu- and heterochromatin. It was shown that satellite DNAs are mostly located in heterochromatin [2–6]. Nucleic acids of both fractions differ in the respect of methylation degree [7]. The differences of T_m values between eu- and heterochromatin DNAs were pointed out by Frenster *et al.* [8], McConaughy *et al.* [9]. The literature data presented above inspired, apart from further physico-chemical characteristics of eu- and heterochromatin DNAs, the investigation of the degree of pyrimidine nucleotide tracts in both fractions.

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Materials and Methods

Calf thymus obtained from Municipial Slaughter-House in Łódź directly after killing was used for experiments.

Isolation and purification of eu- and heterochromatin DNAs. DNA preparations of eu- and heterochromatin fractions were isolated according to Strazhevskaya and Struchkov's method [1] with slight modifications, *i.e.* a preliminary washing of cytoplasmic RNP with 0.14 SSC and DNA deproteinization both with a mixture of chloroform and isoamyl alcohol in the proportion of 24:1 and pronase.

The purity of DNA preparations was determined – spectrophotometrically by measuring spectra in the range of 200–300 nm, – chemically by calculating DNA content by Burton's [10], RNA by Schneider's method [11] and protein by Lowry *et al.* method [12] with our modification [13].

Determination of nucleotide DNA composition. DNA was hydrolysed with 57% $HCLO_4$ for 1 h at 100°. 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 which was passed through a suitable filter (254 nm). Next they were eluted with 0.1 N HCl and defined spectrophotometrically.

Chromatographic separation on hydroxyapatite (HAP) was carried out at the concentration gradient 0.05–0.3 M potassium phosphate buffer. 3 ml fractions were collected.

Determination of temperature melting curves (T_m). DNA was disolved in 0.01 M NaCl solution at the concentration 20–30 μg/ml. Absorption measurements were performed for each temperature change of 1° during 1 min on Unicam SP 1700 spectrophotometer with automatic installation for temperature SP 876.

DNA hydrolysis to pyrimidine tracts. Pyrimidine tracts of the formula Pir_nP_{n+1} were obtained after hydrolysis with 66% HCOOH and 2% diphenylamine for 17 h, at 30° according to Burton and Petersen's method [14].

Fractionation of pyrimidine tracts. Oligonucleotides obtained after acid hydrolysis were fractionated on DEAE-cellulose column [15, 16] at the concentration gradient LiCl 0.0–0.32 M acetate buffer, pH 5.1, at a flow rate of 32.0 ml per h (6.4 ml fractions were collected). Following separation oligonucleotides of similar length were rechromatographed on DEAE-cellulose column using concentration gradients for monopyrimidine: 0.0–0.1 M NaCl, dipyrimidine 0.0–0.2 M NaCl, tri- and tetrapyrimidine 0.0–0.3 M NaCl and 0.0–0.4 M NaCl for pentapyrimidine in 0.01 M acetate buffer, pH 3.0, at a flow rate of 36 ml per h (3.0 ml fractions were collected). Individual oligonucleotides were identified spectrophotometrically by determining the ratio A_{280}/A_{260} in pH 1.0 and their content calculated according to Beaven *et al.* [17].

Results and Discussion

Structure and physico-chemical properties of DNA from eu- and hererochromatin are still contradictory mostly because of different methods of isolation applied by particular authors. Many experimental data speak for structural and functional heterogeneity of DNA in animal cells [18–21]. The question arises therefore if this heterogeneity is not an artifact since all generally accepted methods of DNA isolation are connected with degradation of these molecules. To obtain well defined DNA fractions many authors isolated them directly from cell nuclei. Strazhevskaya and Struchkov's procedure [1] diminishes the possibility of enzymatic and mechanical degradation of DNA due to DNAs isolation directly from the tissue homogenate. Our experiments showed that DNAs isolated by this method from eu- and heterochromatin constituted 15% and 85%, respectively. Hyperchromic effect [ΔH] determined on the basis of melting curves for euchromatin DNA was about 4% lower than the one for hetero-

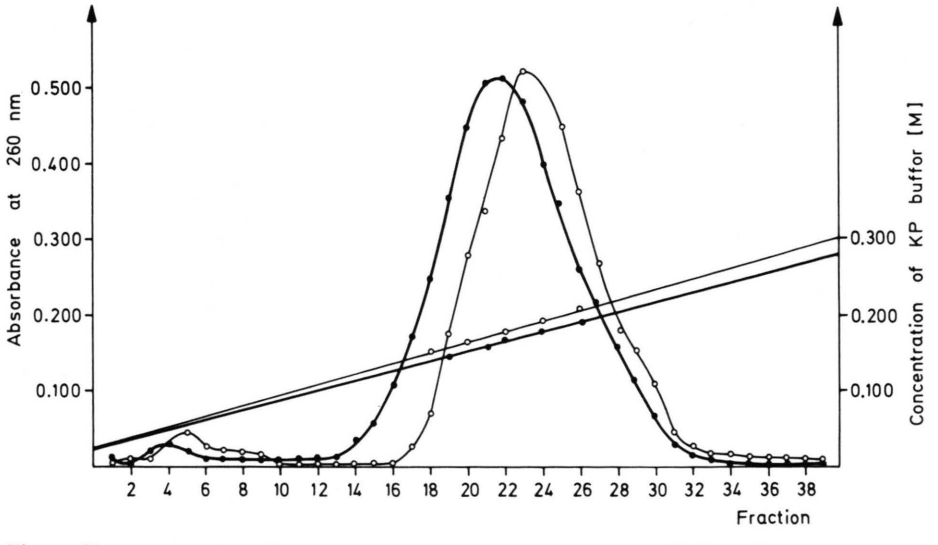

Fig. 1. Chromatography of eu- and heterochromatin DNAs on HAP in linear concentration gradient of potassium phosphate buffer. DNA in 0.14 M NaCl at the concentration 50 μg/ml subject to sonication was applied on the column (1 × 3 cm). DNA was eluted in the linear concentration gradient of 0.05–0.3 M potassium phosphate buffer, pH 6.9 at the speed rate 2.5 ml/5 min. –●–● euchromatin DNA, –○–○– heterochromatin DNA.

Table I. Physico-chemical characteristics of eu- and heterochromatin DNAs.

Estimated values	DNA eu-chromatin	DNA hetero-chromatin
T_m [°C]	72.0 ± 0.5	74.0 ± 0.5
ΔH	38.0 ± 3.0	30.0 ± 2.0
% of renaturation[a]	19.0 ± 1.0	30.0 ± 1.0

[a] % of renaturated DNAs obtained after separation on HAP.

chromatin DNA (Table I). Chromatography on hydroxyapatite demonstrated that native fraction of euchromatin DNA is eluted in the range of 0.14–0.2 M potassium phosphate buffer while of heterochromatin DNA in the range of 0.16–0.23 M potassium phosphate buffer (Fig. 1). Melting temperatures determined for the preparations purified on HAP were higher of about 1.5–3.0° than for nonfractionated preparations. The difference between T_m values of eu- and heterochromatin DNAs remains unchanged after purifications on HAP. The percent of renaturated DNAs from eu- and heterochromatin obtained after separation on hydroxyapatite was found to be 30% and 20%, respectively. Elution of renaturated fractions from eu- and heterochromatin DNAs was carried out at the concentration 0.1–0.16 M potassium phosphate buffer. The differences between deoxyribonucleic acids of eu- and heterochromatin manifested among others by changes of thermal denaturation curves and chromatography on HAP would indicate the differences both in secondary structure and sequence of nucleotides. Hence DNA of both fractions was characterized in respect of bases composition as well as distribution of pyrimidine tracts. Yunis and Yasmineh [22] pointed out that differences in nucleotide composition of eu- and heterochromatin DNAs can mostly be ascribed to the presence of satellite DNAs in heterochromatin fraction. Satellite DNA of calf thymus (type II) is a nucleic acid of GC type. According to Solage and Cedar [23] high repetitive sequences of satellite DNAs contain 85% of total 5-methylcytosine. Bases composition of the preparations was determined by thin-layer chromatography. Euchromatin DNA was characterized by the following composition of nitrogen bases: A 27.7 ± 0.4%, T 27.8 ± 0.5%, G 21.5 ± 0.7%, C 22.4 ± 0.4%, 5-methylcytosine 0.6 ± 0.1%, AT/GC+MC = 1.25 ± 0.01%, GC pairs content 44.5 ± 0.3%, Heterochromatin DNA contained: A 26.9 ± 0.4%, T 26.7 ± 0.3%, G

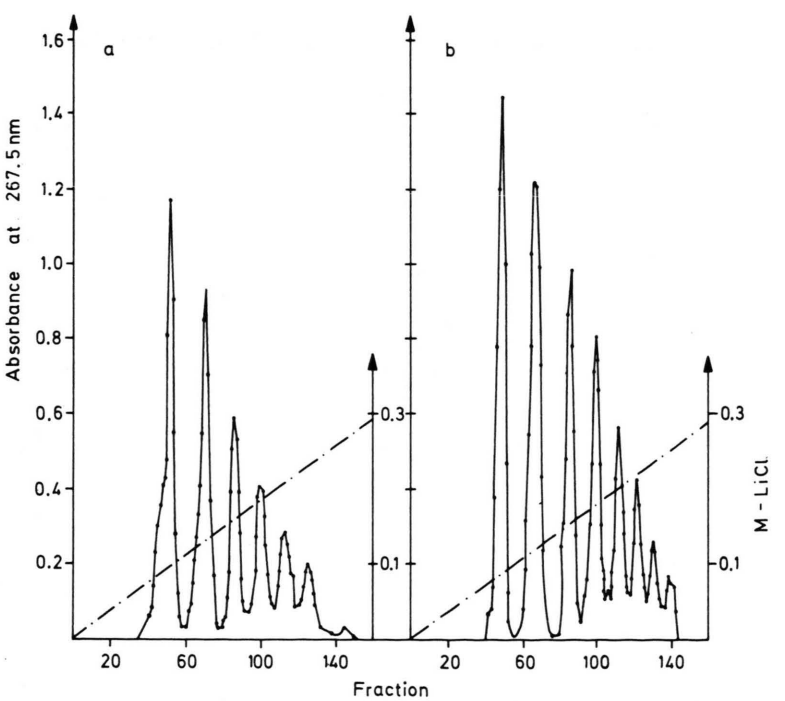

Fig. 2. Separation of products obtained from formic acid-diphenylamine treatment of calf thymus DNA on DEAE-cellulose column (1×15 cm). Oligonucleotides were eluted with the linear gradient of lithium chloride 0.0–0.32 M in 0.01 M lithium acetate buffer, pH 5.1. Flow rate was 32.0 ml per h, a) euchromatin DNA, b) heterochromatin DNA.

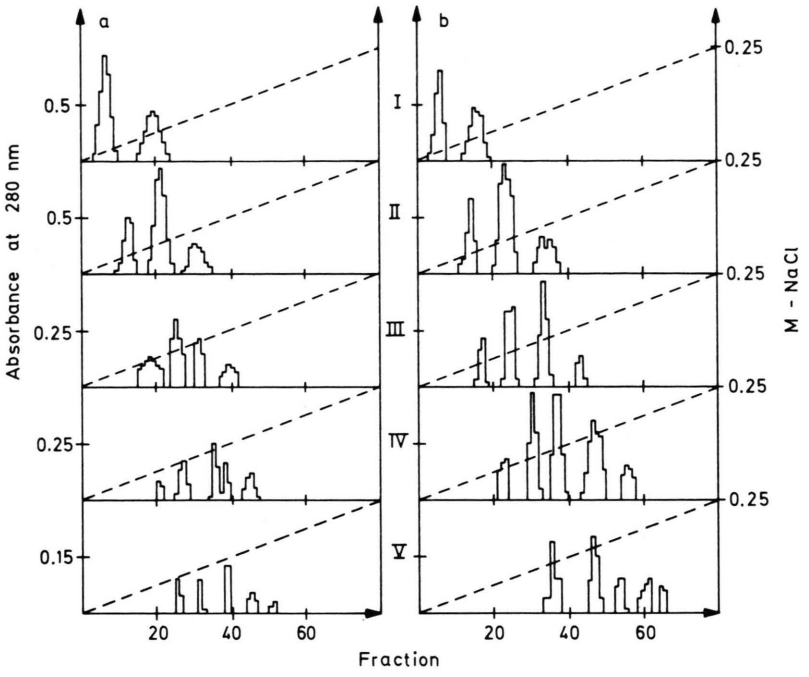

Fig. 3. Separation of pyrimidine tracts (I–V) into base compositional isomers on DEAE-cellulose column (1 × 14 cm) with the linear gradient of sodium chloride 0.0–0.4 M in 0.01 M sodium acetate buffer, pH 3.0. Flow rate was 36.0 ml per h, a) eucromatin DNA, b) heterochromatin DNA.

Table II. Amounts of individual base compositional isomers obtained by separation of pyrimidine tracts I through V.

Pyrimidine Tract	Component	$\frac{A_{280}}{A_{260}}$	Oligonucleotide amount mol%	
			Euchromatin DNA	Heterochromatin DNA
monopyrimidine	d[Cp_2]	1.88	3.49 ± 0.25	3.96 ± 0.62
	d[Tp_2]	0.69	8.88 ± 0.62	8.75 ± 0.20
dipyrimidine	d[C_2p_3]	1.85	1.29 ± 0.36	1.85 ± 0.29
	d[CTp_3]	1.17	5.75 ± 0.38	5.65 ± 0.44
	d[T_2p_3]	0.63	3.38 ± 0.40	3.87 ± 0.35
tripyrimidine	d[C_3p_4]	1.72	0.72 ± 0.11	0.95 ± 0.13
	d[C_2Tp_4]	1.32	3.04 ± 0.20	2.85 ± 0.42
	d[CT_2p_4]	1.03	2.34 ± 0.31	2.65 ± 0.04
	d[T_3p_4]	0.72	1.94 ± 0.09	1.41 ± 0.21
tetrapyrimidine	d[C_4p_5]	1.88	0.45 ± 0.06	0.46 ± 0.04
	d[C_3Tp_5]	1.41	1.70 ± 0.31	1.50 ± 0.26
	d[$C_2T_2p_5$]	1.13	1.63 ± 0.24	2.10 ± 0.17
	d[CT_3p_5]	0.86	1.33 ± 0.06	2.24 ± 0.18
	d[T_4p_5]	0.63	0.64 ± 0.15	0.69 ± 0.18
pentapyrimidine	d[C_5p_6]	–	–	–
	d[C_4Tp_6]	1.37	0.83 ± 0.05	0.78 ± 0.10
	d[$C_3T_2p_6$]	1.21	1.25 ± 0.07	1.17 ± 0.22
	d[$C_2T_3p_6$]	1.07	0.92 ± 0.05	1.41 ± 0.20
	d[CT_4p_6]	0.92	0.49 ± 0.08	1.21 ± 0.03
	d[T_5p_6]	0.80	0.42 ± 0.05	0.45 ± 0.05

21.8 ± 0.4%, C 23.0 ± 0.7%, 5-methylcytosine 1.6 ± 0.3%, GC pairs content 46.4 ± 0.5%, AT/GC + MC = 1.16. The number of GC pairs determined by thin-layer chromatography confirmed their contents calculated from T_m values. DNA preparations obtained by us from heterochromatin had the nucleotide composition richer in G + C pairs than DNA from euchromatin. Eu- and heterochromatin DNAs were hydrolysed with 2% diphenylamine and 66% formic acid to oligonucleotide tracts containing thymine and cytosine. DNA hydrolysates separated on DEAE-cellulose by column chromatography gave 6–7 fractions (Fig. 2). Pyrimidine mono- and dinucleotides of eu- and heterochromatin DNAs constitute the largest part (22.8 ± 2.0 mol% and 24.1 ± 1.9 mol%, Table II). The distribution of shortest oligonucleotides is slightly different in DNAs from eu- and heterochromatin. There were 36.9 ± 3.53 mol% and 38.9 ± 3.53 mol% of mono-, di-, tri- and tetrapyrimidine nucleotides all together in DNAs from eu- and heterochromatin, respectively. Oligonucleotides from mono- to pentapyrimidine were rechromatographed on DEAE-cellulose, at pH 3.0 (Fig. 3). Heterochromatin DNA contained more CT_3p_5, $C_2T_3p_6$ and CT_4p_6 in comparison with euchromatin DNA (Table II). Moreover, the oligonucleotides, composed either of thymine or cytosine appear in heterochromatin DNA at greater amounts. Summarizing, it may be stated that the results of chromatography on HAP, temperature melting measurements, the analysis of base composition as well as the distribution of pyrimidine tracts indicate the differences in physico-chemical properties of two DNA fractions isolated by us. DNA from heterochromatin demonstrates higher thermostability/higher melting temperature, a shift of elution profile on HAP, a higher content of GC pairs, 5-methylcytosine and oligonucleotides of the composition CT_3p_5, $C_2T_3p_6$, CT_4p_6 in comparison with euchromatin DNA. The differences in the thermostability of GC pairs content between eu- and heterochromatin have been confirmed by earlier literature data [8, 9].

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