FRACTIONATION OF CALF THYMUS DNA BASED ON ITS INTERACTION WITH HOMOLOGEOUS f_1 HISTONE. MELTING CURVES OF THE OBTAINED FRACTIONS

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Summary

Calf thymus DNA fractions were obtained by precipitation with the homologeous f_1 histone and their melting curves were investigated. An increase of the melting temperatures of DNA remaining in the supernatants was observed. Within the range of 5-50 % of the DNA precipitated the melting temperatures and the melting intervals of DNA in the sediment remained constant. The obtained values /38 mole % GC, ΔT_m = 7.0°C/ suggest that DNA found in the precipitates corresponds to the main calf thymus DNA. Despite its heterogeneity this group of DNA molecules does not undergo fractionation using f_1 histone. We assume that the molecules of the main DNA all contain specific areas to which the f_1 histone attaches in our experimental conditions. The main DNA molecules, regardless of their base composition, seem to contain these specific areas in amounts causing equal precipitation probability. They seem to differ in this respect from some GC rich fractions, possibly those of satellite DNA.

Introduction

According to Šponar and Šormova /1/, supernatants obtained by precipitation of mixtures of DNA molecules differing in their AT/GC ratios by means of the f_1 histone, are more GC - rich as compared with the initial average base composition. This observation was made both for artificial mixtures of bacterial DNA's differing in their base composition and for calf thymus DNA. In the latter case, an accummulation of satellite DNA in the supernatant as judged on the basis of ultracentrifugation was observed. This work did not explain, however, how fractionation processes depend on the percentage of calf thymus DNA precipitated. The authors did not explain, either, whether the main calf thymus DNA component /38-39 mole % GC /2//, heterogeneous in respect to its base composition /3, 4/, undergoes any fractionation. The aim of our work was to answer these questions.

Materials and Methods

Calf thymus DNA was obtained by means of the detergent method of Kay et al. /5/ but DNA was dissolved and diluted in the media given by Welsh and Vyska /6/. An additional deproteinisation of DNA was performed before the detergent treatment by precipitating it from the solution of DNP in 2.5 M NaCl at $-5^{\circ}C$ by adding ethyl alcohol up to 33% concentration /7/. Protein content in DNA, estimated according to Lowry et al./8/, amounted to 0.6%. The hyperchromic effect amounted to 41%. The molecular weight was estimated on the basis of viscosity measurements /9/ and was found to be 16.10^6.

The lysine-rich f_1 histone fraction from calf thymus was isolated according to Johns /10/. Its homogeneity was tested by electrophoresis in the polyacrylamide gel according to Ilyin et al. /11/.

f_1-DNA complexes were obtained by mixing increasing volumes of the f_1 histone solution /ca. 3 mg/ccm/ and a constant volume of DNA solution /ca. 1 mg/ccm/, both in 1.5 M NaCl containing 0.013 M phosphate buffer, pH 6.8 /1/, and by filling up all samples to a constant volume by means of the above given solvent. The samples were subsequently dialyzed: 3 hours in 0.5 M NaCl, 3 hours in 0.3 M NaCl and 12 hours in 0.15 M NaCl, all solutions containing 0.013 M phosphate buffer, pH 6.8. The obtained precipitates were centrifuged for 30 min. at 25000 x g.

Deproteinisation of f_1-DNA complexes and the procedure for increasing the concentration of the DNA solutions. Both steps involved the use of hydroxyapatite prepared according to Miyazawa and Thomas /12/. The sediments were previously dissolved in 1.5 M NaCl. Salt concentration in the supernatants was increased to 1.5 M NaCl. The mixtures of f_1 with DNA were filtered through a thin layer of hydroxyapatite /2-3 mm/ covering Pyrex G3 /Ø ca. 2 cm./ glass filter. The efflux was forced out by a peristaltic pump working at a suction rate of 5 ccm/min. Hydroxyapatite with the adsorbed DNA was washed 4-5 times with 25 ccm volumes of 1.5 M NaCl. DNA was eluted with a 0.5 M phosphate buffer, pH 6.8, using the same suction system. If DNA concentration in the supernatants was too low for the estimation of melting profiles it could be concentrated several times on the hydroxyapatite by applying a large volume of the supernatant

and using only 5 ml of the phosphate buffer for elution. The hydroxyapatite/DNA layer was carefully mixed for 1-2 minutes with elution buffer before suction was applied.

Melting profiles of DNA preparations were estimated on the Unicam SP 500 spectrophotometer in 0.015 M NaCl + 0.0015 M sodium citrate /0.1 SSC/ /13/. The differences in the content of GC pairs were calculated from the formula $\Delta GC = 2.44\delta T_m$ /13/ where δT_m - difference between the melting temperatures of the given DNA fraction and its initial preparation.

Results and Discussion

The melting temperatures of the particular supernatants gradually rise with the increasing percentage of the precipitated DNA /fig. 1,2/. The melting temperature of DNA in the precipitates remains constant within the range of 5-50 % of the precipitated DNA at the level of 1.5°C lower than that of the initial DNA preparation. It corresponds to 3.7 mole % drop in the content of GC pairs. The melting interval /ΔT_m/ of the precipitated DNA remains constant within this range of precipitation and amounts to 7.0°C. The content of GC pairs in the initial calf thymus DNA preparation amounts to ca. 42 mole % /14/. Thus, within the range of 5-50 % of the precipitated DNA, the DNA fractions in all sediments contain ca. 38 mole % of GC. The latter value as well as the value for the melting interval /ΔT_m = 7.0°C/ are in good agreement with those estimated for the main calf thymus DNA band obtained by ultracentrifugation /2/. They gradually increase up to those of the initial DNA if the degree of precipitation increases from 50 to 97 %. The observed stability of the base composition of DNA in sediments containing 5-50 % of the initial DNA strongly suggest that molecules of the main fraction undergo no fractionation by means of the f_1 histone despite their known heterogeneity in respect to the base composition /3,4/. If the affinity of f_1 versus AT content of the DNA molecules was the reason for their fractionation one should expect the difference between the melting temperatures of the initial DNA and of that from a given sediment to depend on the percentage of the precipitated DNA /see fig. 2, dotted line/.

The theoretical curves for sediments /fig. 2, dotted line/

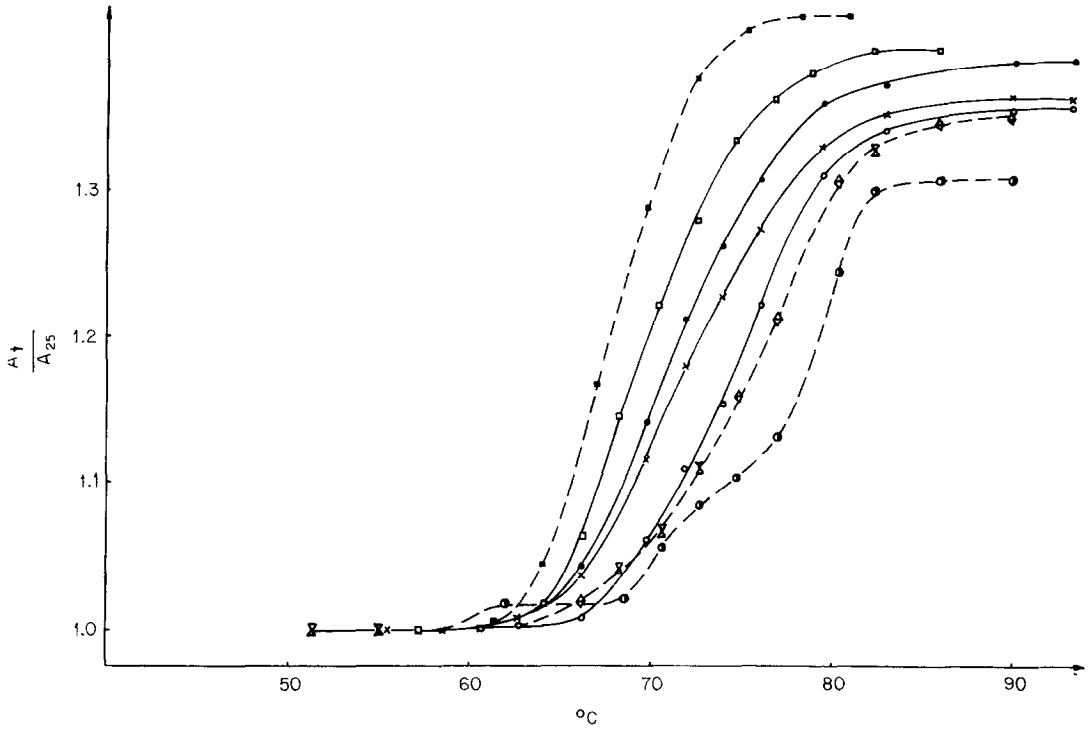

Fig. 1. Melting curves of DNA in 0.015 M NaCl + 0.0015 M sodium citrate /0.1 SSC/.

□——□ initial calf thymus DNA.
■— —■ DNA from the sediments obtained within the range of 5-50 % precipitated DNA.
DNA's from the supernatants obtained after precipitation of:
●——● 22.2 % DNA; ×——× 49.5 % DNA; ○——○ 69.0 % DNA;
△— —△ 83.5 % DNA; ▽— —▽ 89.0 % DNA; ◑— —◑ 95.0 % DNA.

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and for supernatants /fig. 2, dashed line/ were obtained on the basis of the following assumptions: 1/ the susceptibility of DNA molecules to precipitation by the f_1 histone increases parallelly to the increase of their AT content, 2/ the distribution of the molecules in total DNA depending on their base composition follows the Gaussian equation /16/.

Let's assume that the precipitated part of DNA, /P/, equals

$$P = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{u_p} e^{-\frac{u^2}{2}} du \qquad \ldots /1/$$

where: $u = \frac{x - 42}{6}$, x = mole % content of GC in DNA in

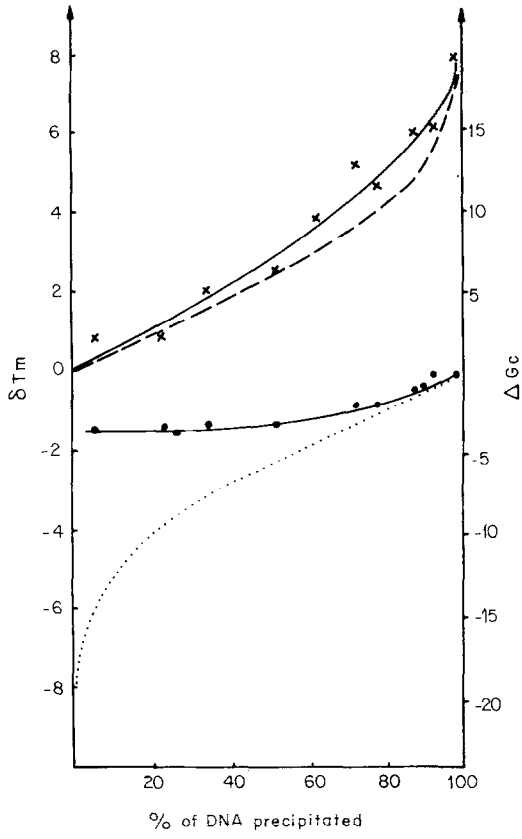

Fig. 2. The dependence of the differences of the melting temperatures, / T_m/, between DNA s in the sediments or supernatants and the initial preparation on the percentage of precipitated DNA. Protein from the sediments and the supernatants was removed on hydroxyapatite.

——— supernatants, theoretical curve;
×———× supernatants, experimental curve;
..... sediments, theoretical curve;
•———• sediments, experimental curve.

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the sediment, 42 - the average content of GC in calf thymus DNA, $6 = 1.22/\Delta T_m - 3/$ /16/, ΔT_m - the melting interval for calf thymus DNA.

Using the tables of the normal distribution, the u_p values for the given P /P = 0.01; 0.05; 0.1; 0.2 ... 0.9; 0.95; 0.99/ can be found. In order to calculate the average content of GC pairs in the sediment, one should calculate the mean value of u within the interval /$-\infty$, u_p/:

$$\bar{u}_p = \frac{\frac{1}{\sqrt{2\pi}} \int_{-\infty}^{u_p} u \cdot e^{-\frac{u^2}{2}} du}{P} = -\frac{e^{-\frac{u_p^2}{2}}}{P\sqrt{2\pi}} \quad \ldots /2/$$

and the same for the supernatants:

$$\bar{u}_s = \frac{\frac{1}{\sqrt{2\pi}} \int_{u_p}^{\infty} u \cdot e^{-\frac{u^2}{2}} du}{/1 - P/} = \frac{e^{-\frac{u_p^2}{2}}}{/1 - P/\sqrt{2\pi}} \quad \ldots /3/$$

Finally, the average contents of GC pairs in the sediments and in the supernatants can be calculated from the following equations:

$$\bar{x}_p = 42 + \bar{u}_p \cdot 6 \quad / \text{for sediments} / \quad \ldots /4/$$

$$\bar{x}_s = 42 + \bar{u}_s \cdot 6 \quad / \text{for supernatants} / \quad \ldots /5/$$

The value of 6 used in our calculations, $6 = 7.3$ mole %, was obtained on the basis of the value of the melting interval ΔT_m which, in our experiments, amounted to $9.0^{\circ}C$ for the initial calf thymus DNA.

The shape of the experimental curve for DNA from the sediments, if compared with the theoretical one, shows that no fractionation of the main DNA band molecules according to their base pair composition does occur. One of the possible explanations of this phenomenon could be based on the assumption that all molecules belonging to the main band, independent from their AT/GC ratio, are equally equipped in specific areas, possibly base sequences, responsible for binding of the f_1 histone. The existance of such areas, specifically binding the arginine-rich f_3 histone, was shown by Clark and Felsenfeld /17/. Another possible explanation is based on the assumption that the interaction between the "main band" DNA and the f_1 histone may influence the conformation of the f_1 molecules in a different way than the interaction between GC rich satellite subfractions and f_1. These conformational changes may stimulate the protein - protein interaction of the kind suggested by Bradbury et al. /18/. Thus, the similarity between the theoretical and the experimental curves obtained for the supernatants /fig. 2/

is purely accidental. The shape of the experimental curve does not reflect a fractionation of the main DNA according to its AT/GC content. The reason of the accumulation of GC rich DNA fractions in the supernatants, in agreement with the results of Šponar and Šormova /1/, seems to be the weaker ability of the more GC rich satellite DNA molecules to precipitate with the f_1 histone. At the beginning of DNA precipitation, the GC pair content in the subsequent supernatants and their melting temperatures show a linear increase /figs. 1 and 2/. At high degrees of DNA precipitation /83-90%/, the subsequent melting curves do not differ from each other . Further increase of the melting temperature of the supernatant can be observed only when 95% of DNA gets precipitated. Evidently, the nature of the interactions between the f_1 histone and various kinds of molecules of the calf thymus DNA is very complicated and it changes parallel to the change in the f_1/DNA ratio in the complexes.

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