The fractionation of calf thymus DNA on histone KAP - Sepharose 4B columns

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ABSTRACT

It was found that fractionation of calf thymus DNA on homologous histone KAP covalently bound to CNBr activated Sepharose 4B depends on the molecular weight of DNA. The maximum of elution of high molecular DNA (m.wt. above 5×10^6) was observed at 0.56 M NaCl and that of degraded DNA (m.wt. 0.8×10^6) at 0.52 M NaCl. Significant differences in melting temperatures and melting intervals were observed among fractions obtained from low molecular DNA as a result of enrichment of some fractions in satellite DNAs. These differences were very small in DNA of m.wt. above 5×10^6 . The results are discussed in terms of specific areas which may exist on calf thymus DNA molecules playing the role of loci, where lysine-rich histone KAP is preferentially bound.

INTRODUCTION

The ability of the lysine-rich histone KAP to form specific complexes with DNA was shown by Šponar et al ¹. They observed that calf thymus DNA, which remained in the supernatant after centrifugation of the precipitated histone-DNA complexes, contained more GC pairs than the initial total DNA. This phenomenon was caused by accumulation in the supernatant of satellite DNAs which are GC-rich in this material. These observations were confirmed in our laboratory ². Furthermore, we found that the interaction of calf thymus main DNA with histone KAP does not lead to its further fractionation. The above findings led us to the assumption that affinity chromatography of DNA on histone KAP covalently bound to CNBr activated Sepharose 4B would give new information concerning the interaction between DNA and histone KAP. We also expected this technique to be useful for preparative purposes. Recently, Ayad and Parker ³ used some histone fractions for fractionation of DNA, reporting high species specificity of such interactions.

An explanation of this interesting observation seems difficult in view of small differences found among amino-acid sequences of histones from different sources ^{4, 5}.

In our work the species specificity was not considered and we concentrated our attempts on seeking for optimal conditions leading to the separation of GC-rich satellite DNAs from the rest of calf thymus DNA on KAP-HS ⁶ columns.

MATERIALS AND METHODS

KAP-HS was prepared by coupling calf thymus histone KAP, obtained according to Johns ⁷, to CNBr activated Sepharose 4B (Pharmacia Fine Chemicals). The homogeneity of the histone was tested by electrophoresis in polyacrylamide gel according to Ilyin et al. ⁸ The reaction of covalent binding of KAP histone to CNBr-Sepharose 4B was carried out in 0.1 M borate buffer pH 9.0 + 1 M NaCl for 4.5 hours at room temperature. The reaction mixture consisted of 1 g KAP histone and 50 ml of sedimented commercial CNBr-Sepharose 4B in a total volume of 100 ml. In these conditions 54% of KAP histone was attached to Sepharose. Before being used for column formation, KAP-HS was washed several times with 0.4 M NaCl in 0.02 M phosphate buffer, pH 6.8.

Calf thymus DNA was obtained and characterized in our laboratory as previously described ². Its average molecular weight, estimated on the basis of viscosity measurements ⁹, was found to be 29×10^6 . DNAs of lower average molecular weights were obtained by sonication of the above preparation in MSE ultrasonicator.

Fractionation was carried out on columns containing 1.5 x 27 cm KAP-HS beds. Up to 5 mg DNA was applied on each bed. Samples of DNA were applied on the columns in 0.4 M NaCl, 0.02 M phosphate buffer, pH 6.8. Step gradient of NaCl concentration (0.4 - 0.8 M NaCl + 0.02 M phosphate buffer, pH 6.8, in 0.04 M NaCl steps), formed by means of LKB "Ultrade" gradient former was used for elution at the rate of 10-12 ml/h. The absorbance of the effluent was controlled by means of a LKB Uvicord II absorptiometer.

Melting curves of DNA preparations were estimated on the Unicam SP 500 spectrophotometer in 0.015 M NaCl + 0.0015 M sodium citrate (0.1 SSC) ¹⁰. The differences in the GC pair content between individual fractions and total DNA were calculated from the formula $\Delta GC = 2.44 \Delta T_m$ ¹⁰, where ΔT_m = the difference between the melting temperatures of the given DNA fraction and the initial, total DNA. DNA in

effluents too much diluted for the estimation of melting curves was concentrated on hydroxyapatite, as described earlier². This method was also used for concentrating DNA fractions before their re-chromatography.

Regeneration of KAP-HS columns was easily performed by washing them with 2.0 M NaCl and equilibrating with 0.4 M NaCl + phosphate buffer pH 6.8.

No changes of the properties of the bed were observed after more than twenty regeneration procedures.

RESULTS

The elution of DNA took place in the range of 0.48 - 0.8 M NaCl. The shape of the elution profiles depended on the molecular weight of DNA. Preparations of average molecular weights 15×10^6 , 11×10^6 and 5×10^6 were eluted exactly in the same way as DNA of m.wt. 29×10^6 (fig. 1). In all cases the majority of DNA was removed in 0.56 M NaCl.

However, in the case of the highly degraded sample (m.wt. 0.8×10^6) most of the material was eluted in 0.52 M NaCl (fig. 2, 3). In comparison to about 20% of high molecular DNAs (m.wt. above 5×10^6) eluted in 0.8 M NaCl only 1% of the low molecular DNA (m.wt. 0.8×10^6) was removed from the column in that NaCl concentration range.

Repeated runs of fractions obtained from 29×10^6 m.wt. DNA in 0.52 and 0.56 M NaCl showed that the majority of those fractions was recovered at the same ionic strengths (fig. 3 and 4).

Thus, the fractionation was not due to the modifications of the histone KAP molecules, which could be formed when histone KAP was attached to CNBr-Sepharose, but it was caused by the differences in the properties of DNA molecules.

In each repeated run, however, ca 15-23% of the applied DNA fraction was eluted in 0.8 M NaCl which was introduced directly after the 0.6 M NaCl step as during first fractionations of all DNAs of m.wt. above 5×10^6 (fig. 3). The possible interpretation of this observation is discussed later.

Differences among melting temperatures of the subsequent fractions, not only from different peaks but also within a single peak, indicate that these fractions differ in GC pair content (fig. 1 and 2). Fractions obtained from

Fig. 1. — DNA (m.wt. 29×10^6) elution profile obtained from a KAP-HS column (1.5 x 27 cm, 12 ml/h, 6 ml fraction volume, step concentration gradient in the range of 0.4-0.8 M NaCl solution in 0.02 M phosphate buffer, pH 6.8, concentration step: 0.04 M). A 4.7 mg sample of DNA was applied in 0.4 M NaCl and 0.02 M phosphate buffer, pH 6.8, on the column equilibrated by means of this buffer.

..... Tm values for DNA from the particular fractions.

— — — Melting interval (ΔT) values for DNA from the particular fractions.

----- Corresponding Tm and ΔT values for the initial DNA preparation.

Fig. 2. — DNA (m.wt. 0.8×10^6) elution profile obtained from a KAP-HS column. Separation conditions and the meaning of marks - see legend to fig. 1.

Fig. 3. The amounts of DNA (mean % values) removed from KAP-HS columns by NaCl solution of lower ionic strengths in following types of separations:

+--+ DNA, m.wt. 29×10^6 ,

— · — · DNA, m.wt. 0.8×10^6 ,

▽ — · — ▽ DNA from the fraction eluted in 0.52 M NaCl, fractionation during re-chromatography,

Δ — · — Δ DNA from the fraction eluted in 0.56 M NaCl, fractionation during re-chromatography.

Fig. 4. Examples of thermal denaturation curves.

x—x—x DNA from the fraction No. 61

Δ—Δ—Δ DNA from the fraction No. 70

o—o—o initial DNA preparation.

The above fraction were obtained during separation of DNA of m.wt. 0.8×10^6 (see fig. 2).

Fig. 5. Rechromatography of DNA fractions obtained in two parallel separations analogous to separation shown on fig. 1. Conditions of rechromatography are the same as in former separations (see legend to fig. 1).

A. — rechromatography of the fraction eluted in 0.52 M NaCl. 51 O.D. units of DNA were applied on the column.

B. — rechromatography of the fraction eluted in 0.56 M NaCl 20 O.D. units (at 260 nm) of DNA were applied on the column.

..... elution profile of initial DNA preparation.

0.8×10^6 m.wt. DNA showed much more pronounced differences than those obtained from any DNA of m.wt. above 5×10^6 .

In principle, DNA fractions eluted at lower ionic strength were characterized by higher melting temperatures (fig. 2). The maximal differences in T_m between DNAs from individual fractions and the corresponding unfractionated DNA amounted to 3.5°C for DNA of m.wt. 0.8×10^6 but only to 0.8°C for DNA of m.wt. 29×10^6 (fig. 1, 2 and 5). Thus, the differences in GC pair content amounted to 8.5 and 2.0 mol % respectively and the richest fraction obtained from sonicated DNA (m.wt. 0.8×10^6) contained ca 50.5 mol % GC pairs - an evident proof of a considerable enrichment in satellite DNA. This conclusion is supported by the size and shape of steps on the melting curve of this fraction (fig. 5).

The melting interval, ΔT , is considered to be a measure of heterogeneity of DNA preparations¹⁰. Thus, ΔT of the obtained fractions should increase in parallel to the content of the satellite DNA fractions. However, if the content of the satellite DNA fractions exceeded some level (in the case of a single satellite DNA fraction this level would be 50%) ΔT should again decrease.

Our fraction obtained from DNA of 0.8×10^6 m.wt. which showed the highest melting temperature, was not that of the widest ΔT (fig. 2). Thus, this fraction seems to consist, above all, of satellite DNAs. Some fractions eluted in significant amounts after the GC-rich fraction had either lower melting temperature or narrower melting interval than unfractionated DNAs (fig. 2). It indicates that a considerable amount of satellite DNAs was removed from these fractions.

DISCUSSION

Our previous unpublished data showed that the phenomenon of concentration of the GC-rich DNA fractions in the supernatants depends on the procedure used for preparation of DNA - histone KAP complexes. If the complexes were formed by mixing DNA and histone KAP in 0.15 M NaCl, this concentration effect was much weaker than in the case of dialysis of the DNA - histone KAP mixtures in 1.5 M NaCl subsequently against 0.5, 0.3 and down to 0.15 M NaCl². Therefore, we thought that application of DNA on the KAP-HS columns should be carried in 0.4 M NaCl, i.e. the ionic strength which only slightly differs from that necessary for dissociation of the DNA-KAP histone complexes. We suppose that such a procedure prevents formation

of unspecific bonds between DNA and the immobilized histone KAP. Their formation might deteriorate the fractionation process causing the elution profiles to depend not only on the composition of DNA but also on the way of binding of DNA to the variety of capturing groups which probably exist on the KAP-HS bed.

In our experiments the majority of DNA was eluted, depending on its m.wt., in 0.52 or in 0.56 M NaCl (fig. 1, 2 and 3). These conditions are more consistent with the value of 0.6 M NaCl, known to cause dissociation of KAP histone from DNA ^{11, 12}, than those found by Ayad and Parker ³, who observed that most of calf thymus DNA was eluted from KAP-HS in 0.46 M NaCl. The above discrepancies may derive from differences in ionic strength used for application of DNA on the bed.

The dependence of the fractionation of DNA in respect of its GC content on the degree of degradation may be explained by assuming the existence of regions in DNA molecules, adapted to form specific bonds with histone KAP. Clark and Felsenfeld ¹³ proved that such regions do exist for the histone ARE.

Let us assume that: 1) both the molecules of main DNA and those of satellite DNAs contain the same specific sequences responsible for binding histone KAP; 2) DNA molecules which contain these specific areas require higher ionic strength than other DNA fragments in order to get removed from KAP-HS. With such assumptions made, there exist several possible explanations of our results: 1) specific areas may be evenly dispersed along DNA molecules - at higher frequency in main DNA than in satellite DNAs. In this case the probability of finding the specific area on a DNA molecule of a given length would be higher for main DNA, 2) specific regions may appear clustered in groups in form of repetitive sequences. In this case the process of fractionation should be influenced by the number of repetitions of specific regions in a single group. If the number of repetitions in one block were greater in satellite DNAs, the amount of specific areas per unit length being even equal or greater, the probability of finding a long fragment lacking in specific regions would be higher for satellite DNAs.

It should be stressed that Varshavsky and Georgiev ¹⁴ found the arginine rich histones GRK and ARE are clustered in groups containing about 15 protein molecules.

We considered only two out of a great number of other possible explana-

tions, like: 1) satellite DNA molecules may be lacking in specific areas, 2) specific sequences in satellite DNAs may differ from those of main DNA, 3) the lack of specific areas on DNA molecules does not necessarily mean that these fragments must be eluted from KAP-HS at lower ionic strength. The fact that about 15-23% of high m.wt. DNA, irrespective of the amount applied on the column, is removed in the range of 0.6-0.8 M NaCl is in some respect consistent with the results of Bradbury et al. 15. They found that 8% of KAP histone remained on DNA after the extraction by means of 0.6 M NaCl. One can assume that this phenomenon is caused by some rare base pair sequences able to form especially strong bonds with histone KAP. The amount of more strongly bound DNA reflects the ratio of probabilities of finding the usual or rare binding sequences by the capturing KAP-HS groups.

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