Chromatography of calf-thymus nucleoprotein on hydroxyapatite columns

It is well known that nucleoproteins can be dissociated into their protein and DNA components by exposure to strong salt solutions. Fractionation of histones has been obtained, based on selective extraction of nucleoprotein, by increasing salt concentration (see ref. 1 for a review). The stepwise removal of histones from nucleoprotein has the advantage of providing at the same time histone fractions and partially deproteinized nucleoproteins; the latter may, in turn, be used in investigations concerning their macromolecular configuration, their template activity in RNA synthesis etc. The separation of dissociated histones from the partial nucleoproteins has been accomplished so far by preparative ultracentrifugation[2] or, alternatively, by gel filtration on Sephadex G-100 columns[3]. Both methods have serious drawbacks, however, as far as both separations and recoveries are concerned; furthermore, the sedimentation procedure is very time-consuming.

We have found that hydroxyapatite chromatography, a technique extensively used in this laboratory during the past few years in the area of nucleic acid fractionation[4,5] is a much better method for separating histones from nucleoprotein partially or totally dissociated by salt.

Calf thymus nucleoprotein was prepared according to ZUBAY AND DOTY[6]; the soluble fraction only was used in the experiments described here. The nucleoprotein preparation used in most of the experiments reported here had $A_{235 m\mu} = 5.3$; $A_{260 m\mu} = 9.0$; $A_{280 m\mu} = 5.1$. Its DNA content was 0.43 mg/ml on the basis of phosphorus analysis; its protein content was 0.60 mg/ml as determined by a microbiuret reaction[7], using casein (Hammarsten) as a reference protein; absorbance readings were done at 330 mμ; at this wavelength DNA does not interfere with the reaction.

Lysine and arginine were determined on samples of nucleoprotein desalted by dialysis, or on chromatographic histone fractions; these were first gel-filtered on Sephadex G-10 (Pharmacia, Uppsala) columns using 0.001 M HCl as the eluant. Hydrolysis was performed in evacuated sealed test tubes for 24 h at 110° with constant-boiling, redistilled HCl. Lysine and arginine were separated by high-voltage electrophoresis on Whatman 3MM paper and quantitatively estimated using a cadmium–ninhydrin reagent[8].

The soluble nucleoprotein fraction in 0.7 mM potassium phosphate buffer (pH 6.8) was adjusted to different KCl concentrations by adding, under stirring, either crystals or a saturated solution of the salt. Stirring was continued for 16 h at 4°; a small amount of precipitate which formed during the addition of salt redissolved in less than 1 h. The final nucleoprotein concentration was about 1 mg/ml.

Nucleoprotein solutions in 0.7 mM potassium phosphate buffer and different KCl concentrations, ranging from 0 to 3 M, were loaded on hydroxyapatite columns (prepared according to TISELIUS, HJERTÈN AND LEVIN[9]) equilibrated with the same solvents. After washing the columns with the equilibration solvent, elution was performed with a linear molarity gradient of potassium phosphate buffer, the KCl concentration being kept constant and equal to that used in the dissociation step. Chromatograms were run at room temperature.

As shown in Figs. 1 and 2, nucleoprotein solutions in increasing KCl concen-

trations show increasing amounts of a protein component eluting at 0.07–0.10 M potassium phosphate buffer; the main component, formed by partially or totally dissociated nucleoprotein, is eluted at a molarity between 0.2 and 0.3 M potassium phosphate buffer. The eluting potassium phosphate buffer molarity was higher when the KCl concentration was higher; this phenomenon is probably due to a repression of potassium phosphate dissociation by KCl (ref. 5). NaCl was not used as the dissociating salt because of the limited solubility of Na_2HPO_4. The protein components eluting at 0.07–0.10 M potassium phosphate buffer had an $A_{280\,m\mu}/A_{260\,m\mu}$ ratio equal to 1.2–1.3. The lysine/arginine molar ratios which were determined for undissociated

TABLE I

MOLAR LYSINE/ARGININE RATIOS IN CALF THYMUS NUCLEOPROTEIN AND DISSOCIATED HISTONES AS OBTAINED FROM HYDROXYAPATITE CHROMATOGRAPHY

Analyses were performed, in triplicate, on 40–100 μg protein samples.

	Undissociated nucleoprotein	0.75 M	1.0 M	2.0 M	3.0 M
Lys/Arg	1.4	4.0	3.4	1.5	1.0
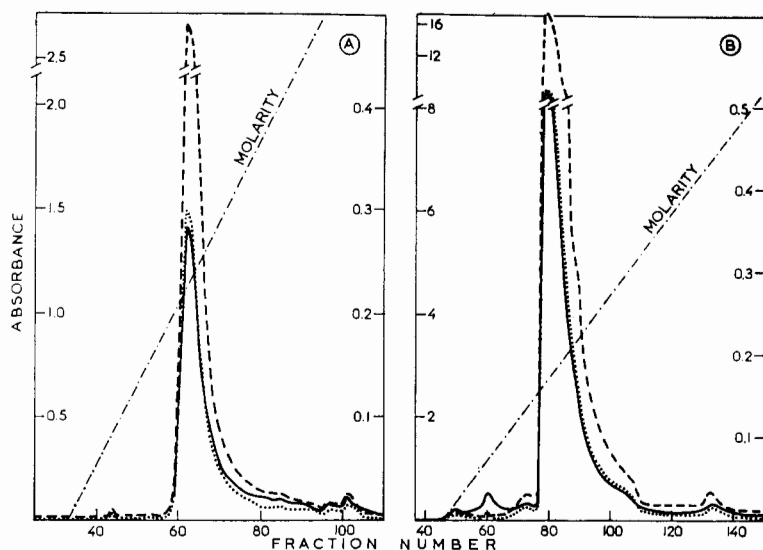
Figs. 1 and 2. Chromatography experiments were carried out on 2.3 cm × 19 cm columns. Elution was performed with a linear molarity gradient of potassium phosphate buffer (450 ml); the limiting concentrations were 0.001 and 0.5 M KCl concentration and the eluting buffers varied from 0 to 3 M in the different experiments, according to the KCl concentration in the nucleoprotein solution. The curves indicate the absorbances at 260 mμ (broken line), at 280 mμ (dotted line) and at 235 mμ (continuous line).

Fig. 1A. Chromatography of nucleoprotein in the absence of KCl. The total load was 160 $A_{260\,m\mu}$ units; the fraction volume was 6.7 ml; the recovery of $A_{260\,m\mu}$ was 93 %. The absorbance ratios of the eluted material were: $A_{235\,m\mu}/A_{260\,m\mu} = 0.532$; $A_{280\,m\mu}/A_{260\,m\mu} = 0.553$.
Fig. 1B. Chromatography of nucleoprotein in the presence of 0.75 M KCl. The total load was 900 $A_{260\,m\mu}$ units; the fraction volume was 4.4 ml; the recovery of $A_{260\,m\mu}$ was 97 %. The absorbance ratios of the main fraction were: $A_{235\,m\mu}/A_{260\,m\mu} = 0.525$; $A_{280\,m\mu}/A_{260\,m\mu} = 0.555$.

nucleoprotein and the dissociated histone fractions are given in Table I. They show that lysine-rich fractions are dissociated at lower salt concentrations, as expected from results by previous authors. Differences in the lysine/arginine ratios of the different fractions would certainly be larger if the same DNA preparation had been dissociated by subsequent stepwise increases in KCl concentration. Instead, as indicated, total nucleoprotein was used in each case.

The DNA peak showed $A_{280\,m\mu}/A_{260\,m\mu}$ and $A_{235\,m\mu}/A_{260\,m\mu}$ ratios which approached the values obtained with pure DNA as increasing KCl concentrations were used. The ratios obtained with the material dissociated by 3 M KCl were the same as those for pure DNA. In some cases, a minor nucleic acid peak preceded the main peak; this minor component might be RNA or denatured DNA on the basis of its elution molarity[4,5], but has not been identified. As shown in Fig. 1A, undissociated nucleoprotein can be chromatographed on hydroxyapatite in the absence of KCl; in this case, the capacity of the column seems to be smaller than when salt is present.
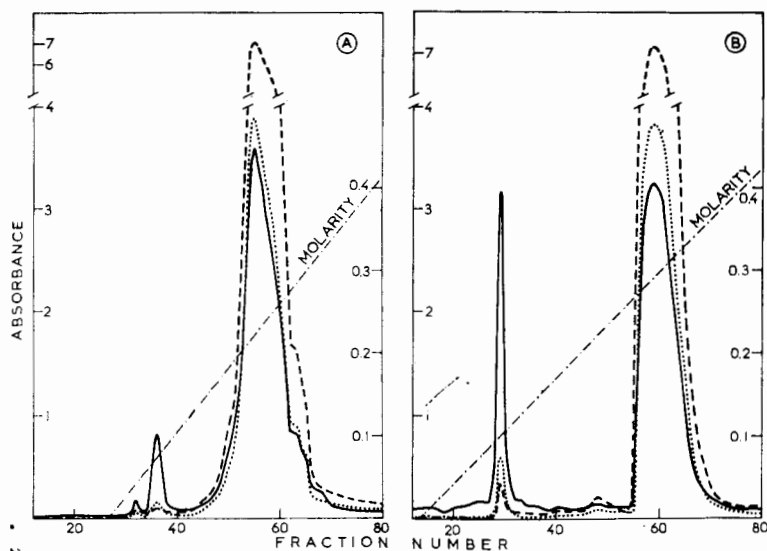

Fig. 2A. Chromatography of nucleoprotein in the presence of 1 M KCl. The total load was 450 $A_{260\,m\mu}$ units; the fraction volume was 6.8 ml; the recovery of $A_{260\,m\mu}$ was 96 %. The absorbance ratios of the main fraction were $A_{235\,m\mu}/A_{260\,m\mu} = 0.500$; $A_{280\,m\mu}/A_{260\,m\mu} = 0.548$.

Fig. 2B. Chromatography of nucleoprotein in the presence of 3 M KCl. The total load was 450 $A_{260\,m\mu}$ units; the fraction volume was 6.8 ml; the recovery of $A_{260\,m\mu}$ was 95 %. The absorbance ratios of the main peak were $A_{235\,m\mu}/A_{260\,m\mu} = 0.455$; $A_{280\,m\mu}/A_{260\,m\mu} = 0.532$.

In conclusion, hydroxyapatite chromatography can be used to separate histone and nucleoprotein dissociated by high salt. The main advantages of this technique seem to be its simplicity and the essentially quantitative recovery of material absorbing at 235, 260 and 280 mμ. It is pertinent to recall that the present results were obtained with a low-resolution technique[5], and that improved separations within the two main fractions may be expected with longer columns and shallower molarity gradients.

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