CHROMATOGRAPHY OF NATIVE DEOXYRIBONUCLEIC ACID ON CALCIUM PHOSPHATE

by
Giorgio Bernardi
Centre de Recherches sur les Macromolecules
Strasbourg, France

Received July 31, 1961

In view of the results recently obtained from the chromatography of phosphoproteins on calcium phosphate (Bernardi and Cook, 1960), it seemed profitable to investigate the chromatographic behavior of deoxyribonucleic acid (DNA) on this material. Chromatographic fractionation of DNA on calcium phosphate has already been reported (Main and Cole, 1957; Semenza, 1957; Main, Wilkins and Cole, 1959). However, its meaning is not yet clearly understood, since rechromatography experiments were not unequivocal and adequate physical characterization of the starting material and the fractions was not done. Furthermore, no fractionation was achieved on the basis of DNA composition (Semenza, 1957). It is the purpose of the present communication to report some observations on the chromatography of DNA on calcium phosphate. A detailed report, also dealing with the chromatography of enzymatically and heat degraded DNA, is in preparation.

As it is not known whether any changes in the physical properties of DNA occur upon passage through calcium phosphate, this question was examined first, and the results form the subject of this communication. DNA samples (as a rule 25 mg in about 50 ml of 0.005 M potassium phosphate buffer, pH = 6.8) were adsorbed on hydroxyapatite prepared according to Tiselius, Hjertèn and Levin (1955) and packed into 2 x 15 cm columns. The column was washed with the starting buffer, which eluted up to 2% of the loaded material in some samples (this material was acid-soluble). Then DNA was desorbed with

0.5 M phosphate buffer, elution being quantitative (on the basis of ultraviolet absorption). In the case of native DNA samples, the eluted material displayed no significant differences from the starting DNA in any of the following properties: light scattering envelope (and therefore weight average molecular weight and radius of gyration; see, however the limitations discussed by Sadron (1960)), sedimentation coefficient, ultraviolet spectrum and $\mathcal{E}(P)$. This behavior was consistently found with several native DNA samples both from calf thymus and chicken erythrocytes, all of them displaying molecular weights of about 6×10^6 and radii of gyration of about 3000 Å.

A remarkably different behavior was obtained with DNA samples of higher molecular weights ($10\text{-}30 \times 10^6$), some of them having been prepared by purposely using steps which lead to an aggregation of DNA (e.g., alcohol precipitation at an early stage of the deproteinization procedure). These samples also were totally eluted from the columns at 0.5 M phosphate buffer, but their molecular weights after desorption were found to be close to 6×10^6 (by light scattering).

The second point investigated concerned fractionation of native DNA on calcium phosphate. All DNA preparations tested were eluted in two fractions, at 0.20 and 0.25 M phosphate, respectively, when eluted stepwise according to the scheme shown in Fig. 1. Occasionally, minor additional fractions were eluted when the molarity of the phosphate buffer was raised to 0.30 and 0.50. Rechromatography experiments showed that the 0.20 M fraction was again eluted at 0.20 M phosphate buffer, whereas the 0.25 M fraction was mainly eluted as 0.20 M fraction, a minor peak coming off at 0.25 M (Fig. 1). The 0.30 and 0.50 M fractions, when rechromatographed, were eluted at 0.20 and 0.25 M. By carrying out experiments on a larger scale and examining the two fractions by the physical techniques mentioned above, it was possible to show that no important differences were detectable between the two fractions, although the first one frequently showed a slightly lower molecular weight than the second.

It would appear therefore that native DNA cannot be fractionated on calcium phosphate, at least as far as the physical properties investigated in

the present work are concerned. The above data also seem to indicate that large aggregates of DNA (possibly linked through the protein still present in the preparation) can be disaggregated by calcium phosphate, but the disaggregation does not proceed to species with molecular weights lower than about 6×10^6.

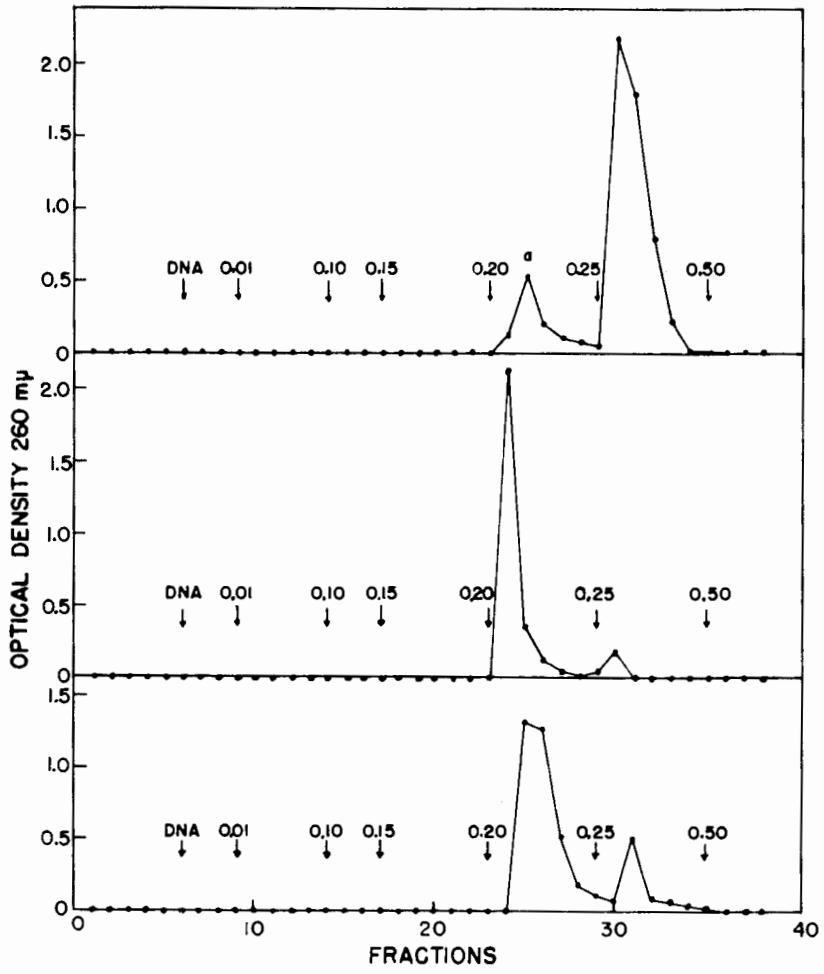

Fig. 1 Top: chromatography of calf thymus DNA on hydroxyapatite. 1.28 mg DNA A1; 1.3 x 5 cm hydroxyapatite; 3 ml/fraction. Middle and bottom: rechromatography experiments on fraction a and b (pooled from two runs), respectively. 1.3 x 3 cm hydroxyapatite; 3 ml/fraction. Middle: fraction a; bottom: fraction b. The stepwise increase in buffer molarity is indicated by the vertical arrows.

REFERENCES

Bernardi, G. and Cook, W. H., Biochim. Biophys. Acta 44, 96 (1960).
Main, R. and Cole, L. J., Arch. Biochem. Biophys. 68, 181 (1957).
Main, R., Wilkins, M. J. and Cole, L. J., J. Amer. Chem. Soc. 81, 6490 (1959).
Sadron, C., in the Nucleic Acids, Ed. Chargaff, E., Davidson, J. N., New York Academic Press, Vol. III (1960) p. 1.
Semenza, G., Ark. Kemi 11, 89 (1957).
Tiselius, A., Hjertèn, S. and Levin, O., Arch. Biochem. Biophys. 65, 132 (1955).