CHROMATOGRAPHY OF EHRLICH ASCITES TUMOR CELL HIGH MOLECULAR WEIGHT RIBONUCLEIC ACID ON CALCIUM PHOSPHATE

by
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The results reported in a recent paper on the chromatography of deoxyribonucleic acid on calcium phosphate (Bernardi, 1961) encouraged us to investigate the chromatographic behavior of high molecular weight ribonucleic acid (RNA) on this material. In the present communication we will describe results bearing on two points, namely the effect of passage through a calcium phosphate gel on the physical properties of RNA and the possibility of fractionating RNA on calcium phosphate columns. The RNA sample used in the present work was extracted from Ehrlich ascites tumor cells by the method of Colter and Brown (1956). Its weight average molecular weight was found to be 1.4×10^6 by light scattering; the sample showed two peaks in the analytical ultracentrifuge, the slower one with s = 19 S, the faster one with s = 32 S. The slower component accounted for 37%, the faster one for 63% of the material. These properties are in very good agreement with those reported by Brown et al. for similar samples of RNA (Colter and Brown, 1956; Kronman, Timasheff, Colter and Brown, 1960).

Chromatographic experiments were carried out at room temperature on hydroxyapatite prepared according to the method of Tiselius, Hjertèn and Levin (1955). The duration of each experiment never exceeded one hour.

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In a first series of experiments, 1-4 mg of RNA were adsorbed on the calcium phosphate columns (1.3 x 6 cm) as a 0.05% solution in 0.005 M potassium phosphate buffer, pH = 6.8. After washing the column with the starting solvent, RNA was desorbed with 0.20 M phosphate buffer, pH = 6.8. Elution was quantitative on the basis of ultraviolet absorption. As a control sample, an aliquot of the starting solution was made 0.20 M in phosphate buffer at the same time as elution of RNA from the column started. By ultracentrifugal analysis, it was possible to show that the control sample sedimented in two peaks with s = 20 S and s = 29 S, respectively, whereas the sample adsorbed on and eluted from calcium phosphate showed only one peak with s = 14 S.

Using larger scale experiments (25 mg RNA on 9x2 cm columns) sufficiently large samples were obtained to permit the performance of light scattering measurements. The weight average molecular weight of eluted RNA was found to be 0.7×10^6, whereas the control sample (prepared as described above) showed a molecular weight of 1.4×10^6. The disymmetry was 1.4 in the control sample and 1.1 in the eluted sample. These results show unequivocally that the molecular weight of the RNA decreases as a result of the adsorption-desorption procedure. It is important to point out that all the reported data deal with the state of the RNA as soon as possible (generally within 1 hour) after chromatography. Time effects, similar to those already reported (Kronman, Timasheff, Colter and Brown, 1960), were found when the solutions were stored. These effects, however, were remarkably different in the control and the chromatographed samples. While the former showed a drop of only a few percent (1-5%) in its value of $\overline{M_w}$ and s after 24 hours at 20° (in 0.20 M phosphate buffer), the latter showed in the same conditions a much stronger decrease in $\overline{M_w}$ (20-25%). Aggregation phenomena which invariably took place after about 24 hours prevented us from seeing whether the drop in molecular weight continued.

The observed degradation of RNA on the calcium phosphate column under quite mild conditions (pH 6.8, 012 M phosphate buffer) and, in particular, the continued degradation after passage through the column are in sharp

contrast with the behavior of DNA on similar colums (Bernardi, 1961). In connection with the present observation, it is of interest to recall the recent reports of Brown et al. (Brown, Ellem and Colter, 1960; Brown, 1961) on the reversible dissociation of similar samples of RNA under quite mild conditions.

Fractionation of RNA was attempted by stepwise elution according to the scheme shown in Fig. 1. Two peaks can be obtained, at 0.15 and 0.20 M phosphate (Fig. 1). The two fractions, however, were found to have identical sedimentation coefficients, and no further work was carried out along that line. This result is similar to that obtained in the fractionation of native DNA (Bernardi, 1961). In both instances it is quite possible that the peaks obtained are "false" peaks (Hjertèn, 1959).

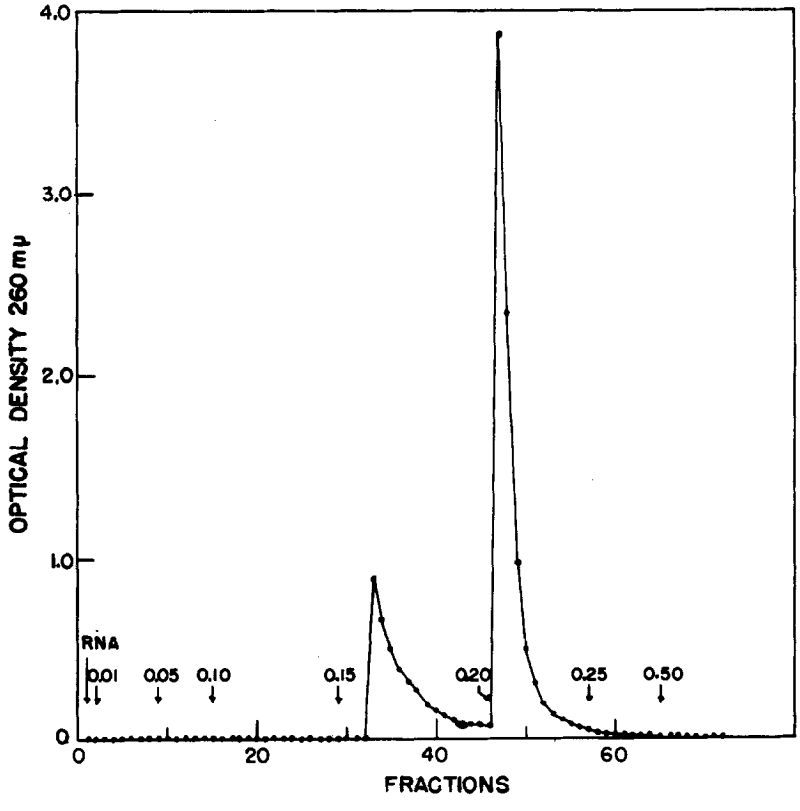

Fig. 1 Chromatography of Ehrlich Ascites Tumor RNA on calcium phosphate. The stepwise increases in buffer molarity are indicated by the vertical arrows.

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