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Note

Fractionation of Sendai virus RNA by polylysine-Kieselguhr column chromatography

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Poly-L-lysine–Kieselguhr (PLK) column chromatography has been shown to be an advantageous method for the separation of nucleic acids from small amounts of crude bacterial[1] and mammalian cell[2] lysates. In this paper, the applicability of PLK chromatography to the fractionation of viral ribonucleic acid in small amounts is reported.

EXPERIMENTAL

Preparation of ^{14}C-labelled Sendai virus[3]

A 5-μg amount of actinomycin D, 10 Units of penicillin, 10 μg of streptomycin and 100 μCi of [2-^{14}C]uracil in 0.1 ml of sterile phosphate-buffered saline, pH 7.4, were added to 10-day-old fertilized eggs, 4 h prior to inoculation with the virus sample; 2×10^6 infectious Units of Sendai virus in 0.1 ml of phosphate-buffered saline, pH 7.4, were used. The eggs were incubated at 37° for 48 h, then chilled at 4° overnight and harvested to yield approximately 8 ml of fluid with a virus titre of $3.2 \cdot 10^{10}$ infectious Units per millilitre, corresponding to 32,768 HAU (Haemagglutination Units) per millilitre.

Protease digestion of viral envelope

The suspension of the virus was centrifuged at *ca.* 60,000 *g* for 6 h and the supernatant removed. A 1-ml volume of 0.4 *M* NaCl solution buffered with 0.02 *M* KH_2PO_4, pH 6.7, containing 1 mg/ml of pancreatic protease (Sigma, St. Louis, Mo., U.S.A.; substantially free of DNase and RNase) was added and the viral pellet was suspended by pipetting. The suspension was then incubated at 37° for 20 h. Portions of this crude viral RNA preparation, stored at —20° and thawed before use, were applied to 1.5-g PLK columns and chromatographed under standard conditions.

Fractionation on PLK columns

The preparation of the PLK columns and the elution conditions were as described previously[2].

Treatment of the fractions

The absorbance at 260 nm was determined using a Unicam SP600 spectrophotometer.

The refractive index was determined using a Bellingham & Stanley refractometer and the corresponding sodium chloride molarity was found from a standard graph.

The radioactivity was assayed by precipitating RNA present in 0.15 ml of each fraction on Whatman grade 3 paper discs with 10% ice-cold trichloroacetic acid (TCA) solution for 25 min. The discs were washed with 10% TCA solution for 25 min, cold 95% ethanol for 25 min (twice) and diethyl ether for 15 min (twice), and then drying it under an infrared lamp. The discs were placed in 10 ml of scintillator (10 g of 2,5-diphenyloxazone, 1 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene in 2.5 l of xylene), and counted in a Packard Tricarb 3320 liquid scintillation spectrometer, using three channels with a gain of 55% and a window setting of 50–1000.

RESULTS

Fig. 1 shows the elution profile of Sendai virus RNA, previously labelled with [2-^{14}C]uracil as described above. In all of the experiments, Sendai virus [^{14}C]RNA

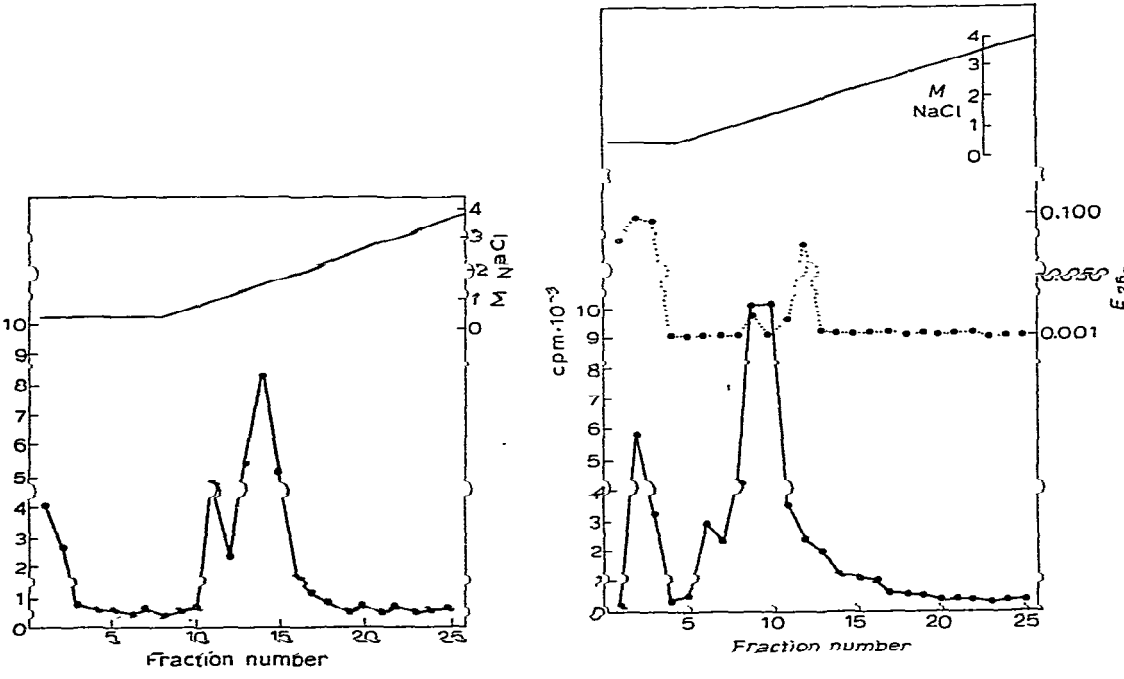

Fig. 1. Elution profile of Sendai virus RNA on a 1.5-g PLK column, using a linear gradient of 0.40–4.00 *M* buffered saline. The viral RNA was labelled with [^{14}C]uracil and RNA corresponding to $8 \cdot 10^3$ HAU was applied on the column; 32-drop fractions were collected.

Fig. 2. Simultaneous elution profiles of Sendai virus RNA and calf thymus DNA (10 μg), obtained as described in Fig. 1. Solid line, radioactivity; broken line, absorbance at 260 nm.

gave one weak peak at 0.8–1.0 *M* NaCl and one stronger peak at 1.4–1.6 *M* NaCl.

Fig. 2 shows the results of simultaneous chromatography of Sendai [^{14}C]RNA and calf thymus DNA (Sigma).

Under the same standard chromatographic conditions, the elution profile remained stable in terms of the molarity of sodium chloride at which the RNAs and DNA were eluted and the broadness of each peak.

DISCUSSION

The interference of several chemical reagents (*e.g.*, sodium dodecyl sulphate and phenol) with nucleic acid preparations[4] gives rise to questions regarding the alteration of the structure and the biological activities of the separated nucleic acids.

The PLK method provides the following advantages with nucleic acid preparation. (a) Very small samples can easily be fractionated in relatively short times. Larger columns can be used in larger-scale preparations. (b) No chemicals interfere with the process. Protein digestion is needed in order to avoid the antagonism of nucleic acid binding proteins with poly-L-lysine and to avoid blockage of the column from very dense preparations. (c) The recovery of pure nucleic acids is very satisfactory[2]. (d) RNA species and DNA are adequately separated at different sodium chloride molarities. Nucleotides and other small molecules are eluted first[2].

In this work, we have demonstrated the use of PLK chromatography for the isolation of viral nucleic acids and the separation of two RNA types from Sendai virus RNA. Further research to elucidate and evaluate the properties and the biological function of these two RNA fractions is needed, particularly in relation to the fate of the viral RNA molecules after the membrane fusion effect, which the viral envelope causes on mammalian (cellular–cellular and cellular–nuclear) membranes[5,6], as well as during RNA–viral cell infections[7].

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