Separation of transfer-ribonucleic acid from deoxyribonucleic acid by gel filtration

In the work on the development of granulation tissue we were faced with the problem of obtaining information on all the nucleic acids simultaneously. We wished to avoid the preliminary centrifugation of the homogenate and proceed with the phenol extraction[1] with the minimum of delay in order to avoid degradation of the nucleic acids. When HMW-RNA's* were precipitated from the aqueous phase with 3 *M* sodium acetate, DNA and t-RNA remained in the supernatant. It is possible to separate DNA from t-RNA by Sephadex gel filtration.

A Sephadex (G-200, bead form) column (3 cm × 55 cm) was prepared according to manufacturer's (Pharmacia AB, Uppsala, Sweden) instructions and equilibrated with 0.1 *M* sodium acetate buffer, pH 5.1. The void volume (V_0), inner volume (V_i) and distribution coefficients (K_d) were determined according to FLODIN[2].

The mixture of DNA and t-RNA in 3 *M* sodium acetate solution was desalted by ethanol precipitation (final concentration 70%, v/v), dissolved into 0.1 *M* sodium acetate buffer, pH 5.1, and allowed to drain into the column, which was eluted with the same buffer. The U.V.-absorption in the eluate was followed with Uvicord ultraviolet absorptiometer (LKB Produkter Aktiebolag, Stockholm, Sweden) connected to a recorder. The flow rate was 30 ml/h and 5-ml fractions were collected.

A typical chromatogram is seen in Fig. 1. The first fraction has K_d-value 0, the second 0.57. Sometimes a third fraction is observed with K_d-value 0.9–1.1.

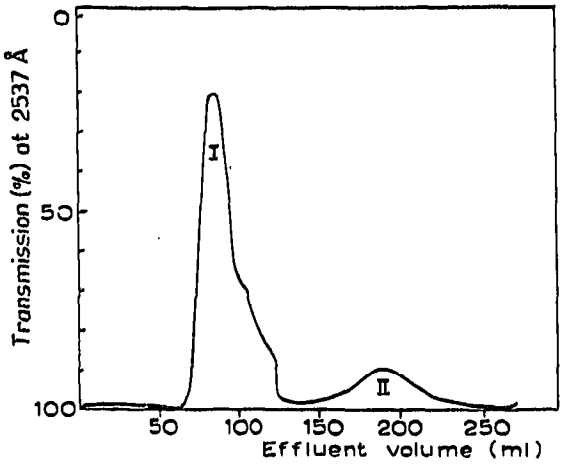

Fig. 1. Separation of DNA (fraction I) and t-RNA (fraction II) on Sephadex G-200 column. The details and the identification of the peaks are described in the text.

Nucleic acid fractions were precipitated with ethanol, washed and dried. The occasional third fraction was not precipitable with ethanol but it was soluble in ether. The base compositions of the fractions were determined after alkaline[3] and acid hydrolysis[4], the sedimentation coefficients with an analytical ultracentrifuge (Spinco Model E, schlieren optics) and the amino acid acceptor capacity was determined according to ZACHAU[5] using amino acid-activating enzymes from rat liver and ^{3}H-lysine (The Radiochemical Centre, Amersham, England).

* Abbreviations: HMW-RNA = high-molecular-weight ribonucleic acid; t-RNA = transfer-ribonucleic acid.

Fraction I was diphenylamine-positive, fraction II negative. No nucleotides were released from fraction I with alkali, but AMP, CMP, UMP, GMP and 5-ribosyluracil monophosphates were released from fraction II. The acid-released bases from fraction I were adenine, thymine, cytosine and guanine. The $S^{\circ}_{20,w}$ of fraction II was 4.6, but the sedimentation coefficient of fraction I was concentration-dependent. The sedimentation velocities agreed with those of SHOOTER AND BUTLER[6] on DNA. The amino acid acceptor-activity of fraction I was only 1.5 % when compared with fraction II.

Separation was complete if the sample volume was less than 10 % of the inner volume of the gel. Amounts up to 15 mg of nucleic acids could be separated satisfactorily.

Recovery of the U.V.-absorbing material from the column ranged from 90 to 96 %. No separation was achieved on Sephadex G-75, which is in agreement with the data by BOSCH *et al.*[7]. Phenol, if still present, is also separated from nucleic acids in Sephadex G-200 column[8].

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