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CHROMATOGRAPHIC DESALTING, DEPROTEINIZATION AND CONCENTRATION OF NUCLEIC ACIDS ON COLUMNS OF POLYTETRAFLUOROETHYLENE

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SUMMARY

A chromatographic method is described for the concentration of nucleic acids and simultaneous removal of proteins and low-molecular-weight substances, such as salts, urea and small nucleotides. Polytetrafluoroethylene is used as adsorbent.

INTRODUCTION

A study of the adsorption of proteins and tRNA on to polytetrafluoroethylene (PTFE)[1] indicated that this adsorbent should be useful for desalting and concentration of tRNA —two common steps in studies of nucleic acids.

Later experiments have shown that columns of PTFE can also be used for the same purpose for other nucleic acids (DNA, rRNA). Further, under appropriate conditions PTFE differentiates between proteins and nucleic acids and can thus be utilized to remove proteins from solutions of nucleic acids. In this paper we describe in detail how desalting, concentration and deproteinization of nucleic acids can be performed rapidly and simply in one operation with the aid of a chromatographic bed of PTFE. This method has been used routinely in connection with the development of a new method for tRNA fractionation[2].

EXPERIMENTAL AND RESULTS

Materials and equipment

PTFE (Fluon, grade L 169) was a gift from Imperial Chemical Industries (Welwyn Garden City, Great Britain). Celite 545 was obtained from Johns-Manville (London, Great Britain), DEAE-cellulose (DE-52) from Whatman (Springfield Mill, Great Britain) and RNase Tl from Sigma (St. Louis, MO, U.S.A.). DNA from calf thymus, tRNA and rRNA from *E. coli*, and tRNA from yeast were prepared as described elsewhere[3–5]. A sufficiently high flow-rate through the column could be achieved only with the aid of a pump.

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Practical performance of a run

We have found that desalting, deproteinization and concentration of nucleic acids can be performed in many different buffers and at various pHs (see Table I). The following description of a typical run should therefore be considered only as a guide for the design of an experiment.

TABLE I

CAPACITY OF PTFE FOR tRNA

Adsorbing medium	*Capacity (mg of tRNA/g of PTFE)*
0.01 *M* Tris-HCl, pH 7.5 + 0.5 *M* $(NH_4)_2SO_4$	1.1 (*E. coli* tRNA)
0.01 *M* NaOAc, pH 4.5 + 0.5 *M* $(NH_4)_2SO_4$	0.8 (*E. coli* tRNA)
0.01 *M* (NH_4)OAc, pH 6.9 + 1 *M* $(NH_4)_2SO_4$	1.0 (yeast tRNA)

A suspension of PTFE in ethanol was poured into a glass column (27 cm × 1.0 cm I.D.), the bottom of which was covered with a 5–6-mm thick layer of acid-washed coarse Celite 545. The bed was equilibrated with 0.01 *M* Tris–HCl (pH 7.5) + 0.01 *M* magnesium acetate by pumping this buffer into the column. At this stage the bed shrunk. About 77 ml of a 0.01 *M* Tris–HCl buffer (pH 7.5) + 0.5 *M* ammonium sulphate containing tRNA (A_{260nm} = 0.30) was pumped into the column, followed by 50 ml of the equilibration buffer to wash out traces of salt; the tRNA was then displaced with the same buffer containing 10% of *n*-propanol. No more material was eluted upon increasing the *n*-propanol concentration to 25%. The flow-rate was adjusted to 12 ml/h with a peristaltic pump. The fraction volumes were 2.2 ml. The distributions of ammonium sulphate and nucleic acid in the effluent were determined by measurements of conductivity and the absorption at 260 nm, respectively. The chromatogram is shown in Fig. 1a. The recovery, based

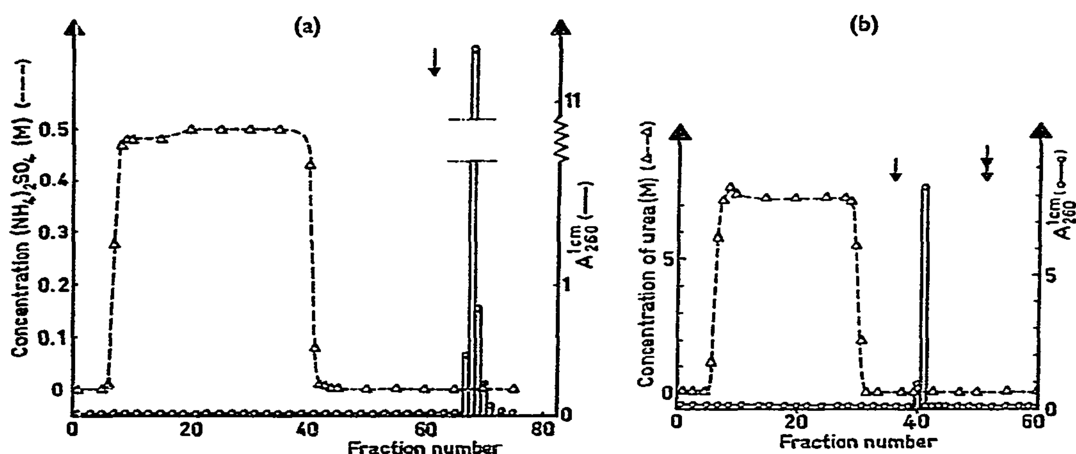

Fig. 1. Concentration of tRNA from *E. coli* and simultaneous removal of salts (a) or urea (b) on a column of PTFE. Buffers [0.01 *M* Tris-HCl, pH 7.5 + 0.01 *M* Mg $(Ac)_2$] containing 10% and 25% of *n*-propanol were applied at the arrow and the double arrow, respectively, to displace tRNA.

on the UV measurements, was 93%. Similar chromatograms were obtained using DNA from calf thymus and rRNA from *E. coli*. However, mono-, di- and triphosphates of adenosine, guanosine, cytidine and uridine passed through the column unretarded.

The experimental conditions for the desalting experiment shown in Fig. 1a were chosen so that they might also permit the separation of proteins from nucleic acids. To test this possibility for deproteinization the following run was made. A mixture of 29 ml of the starting buffer containing tRNA (A_{260nm} = 0.53) and 0.2 ml of normal human plasma, dialysed against the same buffer, was applied on a column of PTFE. The buffers, column dimensions, flow-rate, etc., were the same as in the above desalting experiment. The result obtained was similar to that shown in Fig. 1a; 25% of the proteins applied could be eluted with 25% propanol. The remainder of the serum proteins was strongly adsorbed to the column, perhaps owing to denaturation and precipitation by the alcohol. Using the method described we have also deproteinized a sample of tRNA (1 mg) and cytoplasmic proteins (2.2 mg) from *E. coli*.

The presence of denaturing agents such as urea does not influence significantly the adsorption of tRNA (Fig. 1b).

The PTFE column can be used repeatedly if it is thoroughly washed and equilibrated with the starting buffer following the elution with *n*-propanol, provided that the sample does not contain large amounts of proteins, as these cannot be desorbed efficiently by *n*-propanol or even with detergents such as G 3707[6] and SDS, and therefore may change the properties of the column. It should be stressed that ethanol can also be used for displacement of tRNA. *n*-Propanol is more efficient than ethanol, however, in the sense that a lower concentration is required for complete desorption of tRNA.

Capacity of the PTFE columns for tRNA

The capacity of PTFE, *i.e.*, the maximal amount of tRNA (in milligrams) adsorbed per gram of PTFE was determined as follows. A PTFE column (2 g) was equilibrated with 0.01 *M* Tris–HCl (pH 7.5) + 0.05 *M* ammonium sulphate. The sample, tRNA in the same buffer, was passed through the column until the effluent had the same UV absorption as the sample (A_{260nm} = 27.2). Excess tRNA was removed by washing with the buffer. The adsorbed tRNA was displaced by the same buffer containing 10% of *n*-propanol. From the A_{260nm} values of the fractions and their volumes, the amount of tRNA adsorbed was calculated as 2.2 mg, assuming that 1 mg of tRNA dissolved in 1 ml of the buffer gives A_{260nm} = 25. The experiment was then repeated with two other buffers. As can be seen in Table I, the capacity in all of these experiments was about 1 mg of tRNA per gram of PTFE.

Behaviour of degraded tRNA on PTFE columns

The experiment shown in Fig. 1a was repeated, with the modification that the tRNA sample was first degraded (20 units of RNase T1 per milligram of tRNA were incubated for 20 h at 37°C with 6 mg of tRNA in 1 ml of 0.02 *M* Tris–HCl, pH 7.5). About half of the tRNA applied passed through the column unretarded, and half was adsorbed from the starting buffer. Desorption was accomplished by buffer containing 10% of *n*-propanol (Fig. 2). Fractions 6 and 25 in Fig. 2 were re-chroma-

tographed under identical conditions. All of the material in fraction 6 was eluted with the starting buffer, whereas all of the material in fraction 25 was adsorbed from the starting buffer and could be desorbed with buffer containing 10% of *n*-propanol. This re-chromatography proves that fractions 6 and 25 are chromatographically homogeneous in this system.

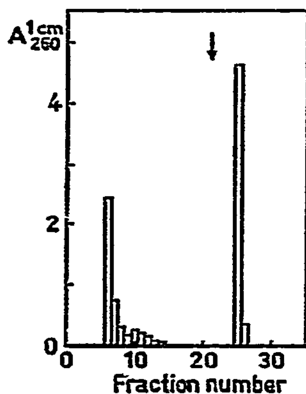

Fig. 2. Behaviour of tRNA, degraded by RNase T1, on PTFE under the desalting conditions used in the experiment shown in Fig. 1.

DISCUSSION

The nature of the adsorption of proteins to PTFE has been discussed previously[1]. There were indications that the adsorption was probably not due to electrostatic or hydrophobic interactions, or to the formation of hydrogen bonds. Nor do electrostatic and hydrophobic interactions play predominant roles in the adsorption of tRNA to PTFE, as the adsorption does not exhibit any pronounced salt dependence (unpublished results). Also, hydrogen bonds are not involved in the separation mechanism, because, as shown in Fig. 1b, tRNA adsorption is strong even in the presence of 7 *M* urea. However, one cannot exclude a combination of two or all three of these types of interaction. A charge-transfer interaction is also possible.

Apparently a multi-point attachment is required for adsorption, as mono-nucleotides and half of the degraded tRNA were not adsorbed to PTFE (Fig. 2) whereas native tRNA was completely adsorbed (Fig. 1). This is analogous to what has been found for proteins chromatographed on amphiphilic gels[7]: proteins are adsorbed but amino acids are not.

A comparison of Figs. 1 and 2 shows that also some low-molecular-weight degradation products in a tRNA sample can be removed concomitantly with desalting, concentration and deproteinization.

Removal of proteins from nucleic acids is often accomplished by ion-exchange chromatography or extraction with phenol. The chromatographic method described in this paper is an alternative method, at least for preparation on a relatively small scale. The method is probably milder than a phenol extraction. The risk of denatu-

ration of the nucleic acids is completely eliminated if the starting buffer is chosen such that proteins but not nucleic acids are adsorbed.

The capacity of the column for tRNA varies with the experimental conditions. The variations are relatively small, however, as shown in Table I, which indicates that the capacity is about 1 mg of tRNA per gram of PTFE.

We have also carried out experiments with the aim of fractionating tRNA on columns of PTFE. However, the results were inferior to those obtained on naphthoyl Sepharose CL-6B[2].

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