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Desalting of nucleic acid hydrolysates, nucleosides and bases by chromatography on poly-N-vinyl pyrrolidone*

It is often desirable to separate nucleic acid components from salts used in their isolation. Methods available include use of charcoal[1–3], anion exchangers[4,5], cross-linked dextrans[6], polyacrylamide gels[7–9], and extraction with an acetone–ethanol mixture[10,11]. All of these have certain merit, but also possess a number of disadvantages.

We have recently reported on the use of insoluble poly-N-vinyl pyrrolidone for the fractionation of certain nucleotide derivatives[12]. This technique has now been extended and found to be a practical substitute for the above mentioned methods of desalting nucleotides, nucleosides, purines, and pyrimidines. Distilled water is used as eluant, compounds are quantitatively recovered in small volumes, and the bases show clean spectra. No regeneration of the column is required, and it may be used repeatedly for a number of experiments.

Materials and methods

RNA (Na salt, purified from Torula) was obtained from Calbiochem**. DNA (sperm), bases, nucleosides, and nucleotides were purchased from Nutritional Biochemical Co., and were used without further purification. Insoluble PVP (GAF Corporation, New York), sold under the trade name of Polyclar AT Powder, was used in these studies.

The Polyclar AT was mixed with distilled water, and the fines were discarded by repeated decantation. The suspension was poured into a column and allowed to pack with gravity flow. The bed was supported by glass wool.

Hydrolysis of DNA was carried out by heating 20 mg DNA in 1 ml 0.1 *M* H_2SO_4 at 100° for 35 min in a sealed tube. After neutralization with NH_4OH and centrifugation, a 0.5 ml aliquot was added to 0.5 ml 10 *N* LiCl. A 0.3 ml aliquot of this (equivalent to 2.5 mg DNA) was then applied to the PVP column.

RNA was hydrolyzed by heating 4 mg RNA in 0.5 ml 1 *N* HCl at 100° for 1 h. The pH was adjusted to 7 with 1 *N* NaOH and 0.2 ml was applied to the column.

Tests of the separation of bases and nucleosides from ammonium sulfate were

* The following abbreviations will be used: CMP = cytidine monophosphate; UDP = uridine diphosphate; RNA = ribonucleic acid; DNA = deoxyribonucleic acid; PVP = poly-N-vinyl pyrrolidone.

** Mention of trade or company names does not imply endorsement by the Department over others not named.

conducted by addition of standard samples (0.06 mg each of uracil and adenosine) to saturated solutions of ammonium sulfate.

The columns were eluted with distilled water (pH 6) delivered from a one liter container at room temperature and atmospheric pressure. The eluate was collected in 0.5 or 0.6 ml fractions. The effluent volume was determined from the time of sample application and was monitored at 260 mμ in a Gilford 220 spectrophotometer. Chloride

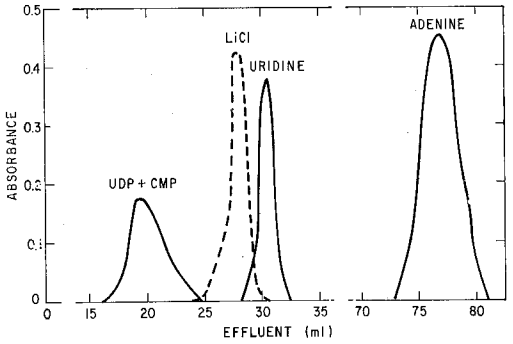

Fig. 1. Chromatogram of an UDP ,CMP, uridine and adenine mixture in 1 *M* LiCl. Column, 1.0 × 39.5 cm; CMP, UDP, adenine (0.04 mg each), uridine (0.024 mg) in 0.2 ml 1 *M* LiCl; eluted with distilled water at a flow rate of 10 ml/h. Absorbance measurements at 260 mμ except for LiCl (as AgCl) at 340 mμ.

was determined with $AgNO_3$–HNO_3 reagent and sulfate with $BaCl_2$ reagent. Both were quantitated by turbidity measurement at 340 mμ. Identification of components was made by comparison with elution volumes of known standards and from the ultraviolet absorbance spectra at neutral, acid and alkaline pH.

Results and discussion

Nucleotide fractions elute prior to NaCl and LiCl; hence, these appear to be excluded from the bed material. Nucleosides and bases exhibit interaction with PVP, resulting in delayed elution. Standard UDP, CMP, uridine, and adenine can be desalted from 1 *M* LiCl on a 1.0 × 39.5 cm PVP column (Fig. 1). NaCl in an RNA hydrolysate

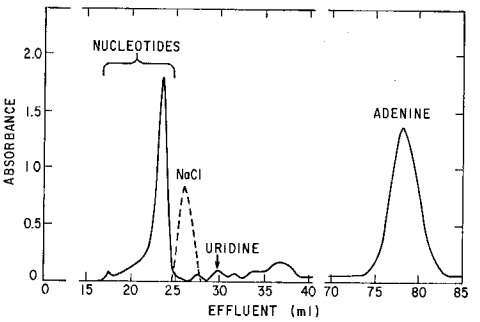

Fig. 2. Fractionation of HCl hydrolysate of RNA. 0.2 ml (equivalent to 0.8 mg RNA) neutralized hydrolysate applied to a 1.0 × 39.5 cm column. Absorbance measurements at 260 mμ except for NaCl (as AgCl) at 340 mμ.

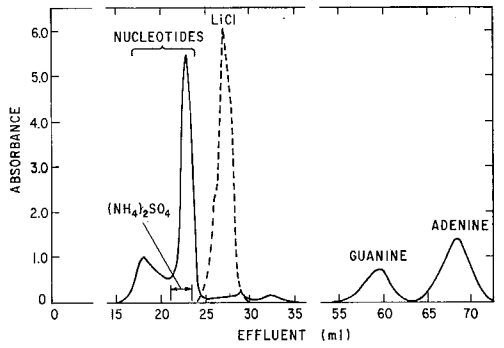

Fig. 3. Chromatography of 0.1 *M* H_2SO_4 hydrolysate of DNA in 5 *M* LiCl. 0.3 ml neutralized hydrolysate (equivalent to 2.5 mg DNA) containing 5 *M* LiCl eluted with distilled water from a 1.0 × 39.5 cm column at a flow rate of 10 ml/h. Absorbance measurements at 260 mμ except for LiCl (as AgCl) at 340 mμ.

behaves similarly (Fig. 2). Fig. 3 shows an H_2SO_4 hydrolysate of 2.5 mg DNA completely desalted from 5 *M* LiCl. Recovery of 260 mμ absorbing material was 99.2%. Hence, pyrimidine nucleotide mixtures derived from ion exchange chromatography of nucleic acid hydrolysates can be desalted and separated from purines in one step.

Desalting of bases and nucleosides on PVP is further illustrated by use of saturated ammonium sulfate. Uracil and adenosine in 0.3 ml saturated $(NH_4)_2SO_4$ can be desalted on a 0.9 × 11.3 cm column in approximately 1 h (Fig. 4). Although some overlap is noted on the short column, complete separation of $(NH_4)_2SO_4$, uracil and adenosine can be attained on a 1.0 × 38.2 cm column. Even in volumes as large as 1 ml of saturated $(NH_4)_2SO_4$ uracil can be completely desalted on such a column. It is evident from Fig. 3 that nucleotides are not separated from $(NH_4)_2SO_4$.

As indicated in a previous publication[12], uracil was eluted from PVP prior to the other bases and nucleosides studied. Since, in the present work, salts elute before uracil, desalting on PVP should be applicable to many bases and nucleosides. Any number of salts should also be separable from bases and nucleosides on PVP, since the

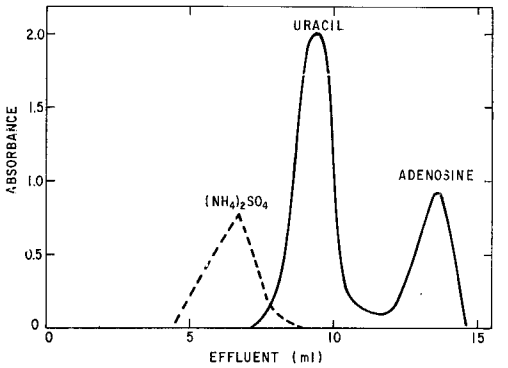

Fig. 4. Fractionation of $(NH_4)_2SO_4$, uracil and adenosine on a 0.9 × 11.3 cm column. Uracil and adenosine (0.06 mg each) in 0.3 ml saturated $(NH_4)_2SO_4$ eluted with distilled water at a flow rate of 14 ml/h. Absorbance measurements at 260 mμ except for $(NH_4)_2SO_4$ (as $BaSO_4$) at 340 mμ.

former apparently filter through the gel without interaction, while the latter are selectively retained.

In cases where slow flow rates present a problem, a bead form of PVP (now under development by GAF Corporation) should be useful.

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