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Note

Separation of nucleic acid bases and nucleosides by high-performance affinity chromatography

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It is well known that nucleic acid bases interact with each other and there are specific interactions, especially between adenine and thymine or uracil and between guanine and cytosine. Liquid chromatographic separations of nucleic acid bases[1], nucleosides[1,2] or polynucleotides[3] based on the differences in the strengths of these interactions have been tried by using resins containing nucleic acid bases as column-packing materials, and the possibility of separating related compounds of nucleic acid bases was shown. The resolution was unsatisfactory, however, because the resins used were not appropriate for column-packing materials.

This paper describes the separation of nucleic acid bases and nucleosides employing a column packed with porous spherical resins of diameter 12–15 μm coupled with thymine. The porous spherical resins were synthesized by suspension polymerization of glycidyl methacrylate and ethylene glycol dimethacrylate in the presence of diluent. The attachment of thymine to the glycidyl methacrylate in the resin so formed was conducted in dimethylformamide at 65° in the presence of potassium carbonate. The glycidyl group reacts with nitrogen in position 1 of thymine under these conditions according to Seita *et al.*[4]. The thymine content in the final product determined by elemental analysis of nitrogen was 8.0%. These resins were packed in a stainless-steel column of length 61 cm and I.D. 7.6 mm. The chromatographic measurements were carried out at 25° on a high-performance liquid chromatograph (HLC-802U, Toyo Soda Manufacturing Co.) using distilled water as solvent. The eluate from the column was monitored with a UV detector at 254 nm. The flow-rate was 3.5 ml/min and the pressure drop was 45 kg/cm^2.

Retention volumes of nucleic acid bases and nucleosides are given in Table I. It can be seen that adenine and adenosine were retained the most strongly, which is attributable to the strong association of adenine and thymine. Table I also shows that guanine and guanosine were more retarded than three pyrimidine bases or three nucleosides of pyrimidine bases. This is consistent with the observation by Ts'o *et al.*[5] that the purine–pyrimidine interaction is stronger than the pyrimidine–pyrimidine interaction. Moreover, the retention volumes of the three pyrimidine bases or three nucleosides of pyrimidine bases were conveniently different and five nucleic acid bases or five nucleosides could be separated in a short time, as shown in Figs. 1 and 2. Five nucleic acid bases were completely separated within 25 min and the peaks of

TABLE I

RETENTION VOLUMES OF NUCLEIC ACID BASES AND NUCLEOSIDES

Sample	*Retention volume (ml)* *			
	A	*B*	*C*	*D*
Adenine	85.9	87.1	65.2	43.6
Cytosine	27.5	27.6	26.4	26.4
Guanine	53.5	53.1	43.8	30.4
Thymine	39.2	39.3	34.6	29.5
Uracyl	31.1	30.8	28.6	25.6
Adenosine	59.4	61.1	44.8	29.2
Cytidine	25.5	25.5	24.2	24.3
Guanosine	38.1	37.8	32.1	23.9
Thymidine	36.2	36.3	31.3	24.9
Uridine	27.8	27.5	25.5	22.8

* A: column-packing material, resin combined with thymine; solvent, distilled water. B: column-packing material, resin combined with thymine; solvent, 0.1 *M* NaCl solution. C: column-packing material, resin combined with thymine; solvent, 1 *M* urea solution. D: column-packing material, resin not combined with thymine; solvent, distilled water.

five nucleosides appeared to be separately although the resolution of guanosine and thymidine was not satisfactory. However, their satisfactory resolution could be achieved with a column packed with resins containing 16.4% thymine, as shown in Fig. 3. It can be expected, therefore, that the differences in retention volumes of nucleosides or nucleic acid bases will increase with increasing thymine content. It should be noted that these separations were achieved with distilled water as solvent. Related compounds of nucleic acid bases are usually separated by ion-exchange chromatography employing a buffer solution.

Table I also summarizes the results of measurements performed on the same column with 0.1 *M* sodium chloride and 1 *M* urea solutions as solvents and on a column packed with resins uncombined with thymine with distilled water as a solvent. Nucleic acid bases and nucleosides were eluted faster and at similar retention volumes when using the column packed with resins uncombined with thymine. It is evident, therefore, that the retardation of nucleic acid bases and nucleosides in the column packed with resins containing thymine is due mainly to interactions between thymine

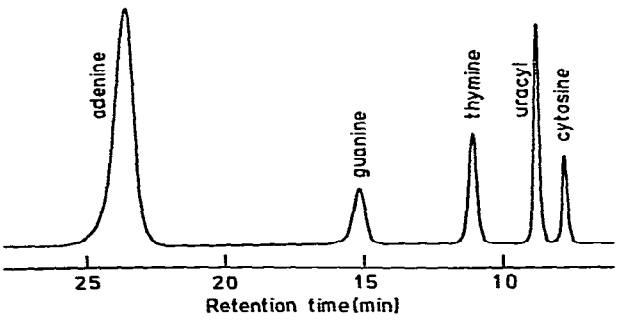

Fig. 1. Chromatogram of a mixture of five nucleic acid bases obtained by using resins coupled with 8.0% thymine.

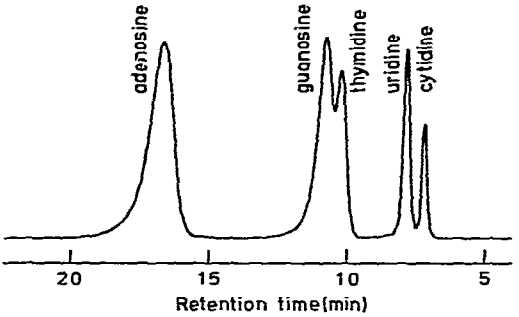

Fig. 2. Chromatogram of a mixture of five nucleosides obtained by using resins coupled with 8.0% thymine.

and nucleic acid bases. Retention volumes with 0.1 *M* sodium chloride solution were almost the same as those with distilled water, which shows that the elution behaviour is not affected by the ionic strength of the solvent in the separation with the resins coupled with thymine. It therefore seems that the interactions between thymine and nucleic acid bases described above are not due to ionic bonds. On the other hand, the retention volumes decreased appreciable for adynine, adenosine, guanine, etc., when 1 *M* urea solution, which is thought to break hydrogen bonds, was used as a solvent, suggesting that the hydrogen bond is related to the interaction between thymine in the resin and nucleic acid bases. This is consistent with the idea that the hydrogen bond is related to the specific interactions between nucleic acid bases.

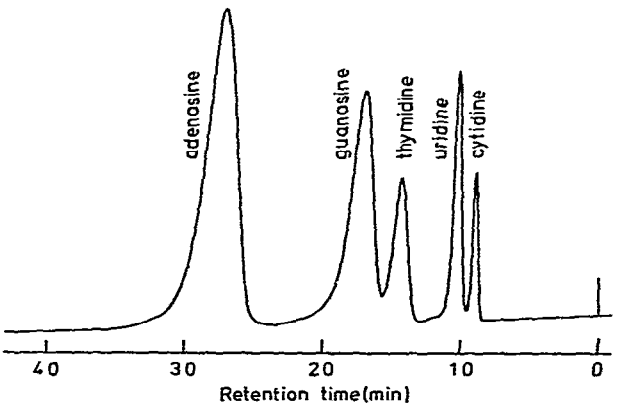

Fig. 3. Chromatogram of a mixture of five nucleosides obtained by using resins coupled with 16.4% thymine. Resin particle size, 15–20 μm; flow-rate, 3.1 ml/min; temperature, 30°.

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