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Note

Application of uracil-bonded silica gel to the separation of adenine and its derivatives, and to poly(A)-containing RNA

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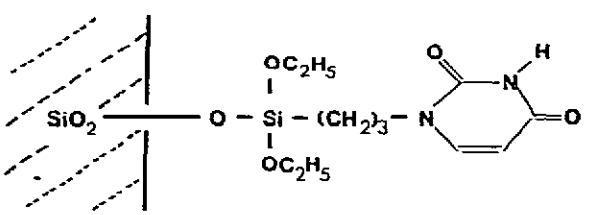
Polyadenylic sequences have been found in several mammalian messenger RNAs (mRNA) and heterogeneous nuclear RNAs (hnRNA). Their occurrence, biosynthesis and possible biological function were reviewed by Brawerman[1,2].

Because of their biological significance, several methods have been proposed for the detection of poly(A)-containing RNA [poly(A)$^+$-RNA][3,4] and its separation from other RNA species. These methods are based mainly on the ability of adenylic acid residues to pair with complementary nitrogen bases. Oligo(dT)-cellulose[5], poly(U)-Sepharose[6] and poly(U)-Sephadex[7] have been proposed as chromatographic carriers with this object in mind. Nevertheless, the use of non-specific chromatographic media[8,9], nitrocellulose filters[10] and immunological techniques[4] have also been suggested.

During our studies on the synthesis of modified nucleosides and their analogues[11], silica gel was bonded with uracil, giving a support with the potential ability to complex with adenine and its derivatives[12]. The structure of the monomer unit of the support is shown below.



This paper describes the chromatographic properties of such a modified silica gel and its application in an alternative method for the purification of poly(A)$^+$-RNA.

EXPERIMENTAL

Purine and pyrimidine nucleosides, nucleotides and polyadenylic acid were purchased from Sigma (St. Louis, Mo., U.S.A.) and oligo(dT)-cellulose from

P-L Biochemicals (Milwaukee, Wisc., U.S.A.). Rabbit reticulocyte RNA (kindly supplied by Dr. M. S. Campo) was extracted according to Loening *et al.*[13]. Other chemicals were purchased from POCh, Gliwice, Poland.

The preparation of the uracil-bonded silica gel has been published previously[12]. A sample of 50 g of silica gel (100–200 mesh) and 100 g of (3-chloropropyl)-trichlorosilane was heated at 120° for 20 h. The product was washed with benzene and methanol (or ethanol) and then was filtered and dried at 85° for 20 h. A sample of 5 g of colourless solid and 20 cm^3 of the trimethylsilyl derivative of uracil was heated at 120° for 24 h. The excess of the trimethylsilyl derivative was evaporated *in vacuo* and the residue was extracted with 20 cm^3 of hot methanol (or ethanol). The uracil-bonded silica gel was filtered and washed with hot water. It is characterized by the following parameters: 25 m*M* uracil per 100 g of silica gel; specific surface area, 100 m^2 (*i.e.*, 1 molecule of uracil per 0.55 nm^2).

Because of its strong hydrophobic character, uracil-bonded silica gel was suspended in methanol, leaded on to a column and then washed stepwise with 0.01 *M* Tris (pH 7.5) until all of the methanol had been removed. Other details concerned with the chromatographic procedures are given in the figure legends.

RNA electrophoresis in agarose gels was performed according to Loening[14].

RESULTS

The separation of the four main nucleosides in water was studied. Simple one-step elution with water allows cytidine, uridine and guanosine to be separated from adenosine, which is retained on the column (Fig. 1). However, this simple separation procedure is not applicable to mixtures of nucleotides and oligonucleotides, the separation of which could be achieved only by altering the ionic strength of the eluting buffers in a similar manner to the procedure described by Aviv and Leder[5].

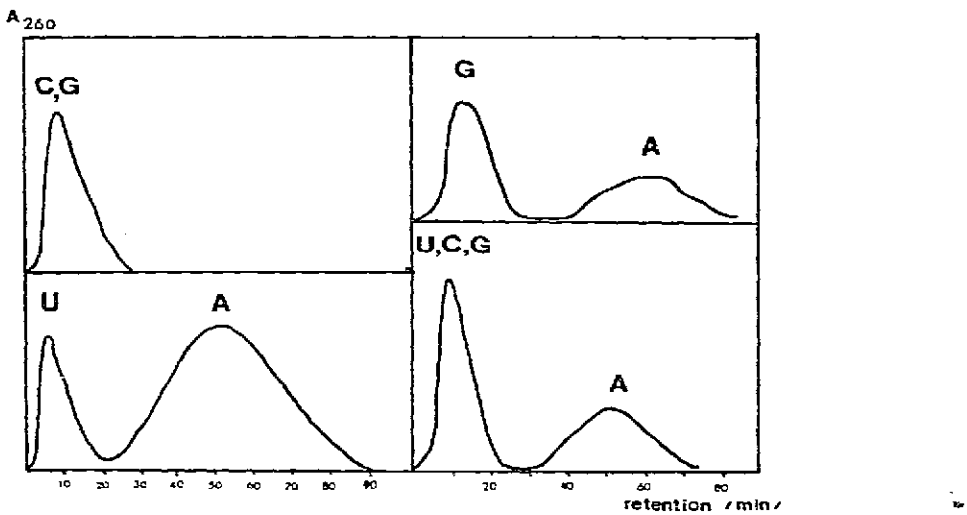


Fig. 1. Retention of adenosine on a column (7 × 0.7 cm I.D.) of uracil-bonded silica gel. Eluent: water; flow-rate, 0.6 ml/min. A = Adenosine; C = cytidine; G = guanosine; U = uridine.

A mixture of nucleotides (CMP, GMP and UMP) and poly(A) was separated by washing the column with a buffer of high ionic strength followed by a buffer of low ionic strength (curve not shown). Subsequent filtration of poly(A) alone under the same conditions gave a single peak on elution with a buffer of low ionic strength, demonstrating the complete resistance of poly(A) to elution with a buffer of high ionic strength. The modified gel remained stable in the presence of eluting buffers. A parallel control experiment using oligo(dT)-cellulose produced nearly the same profile (Fig. 2). The column can be regenerated by extensive washing with the initial buffer.

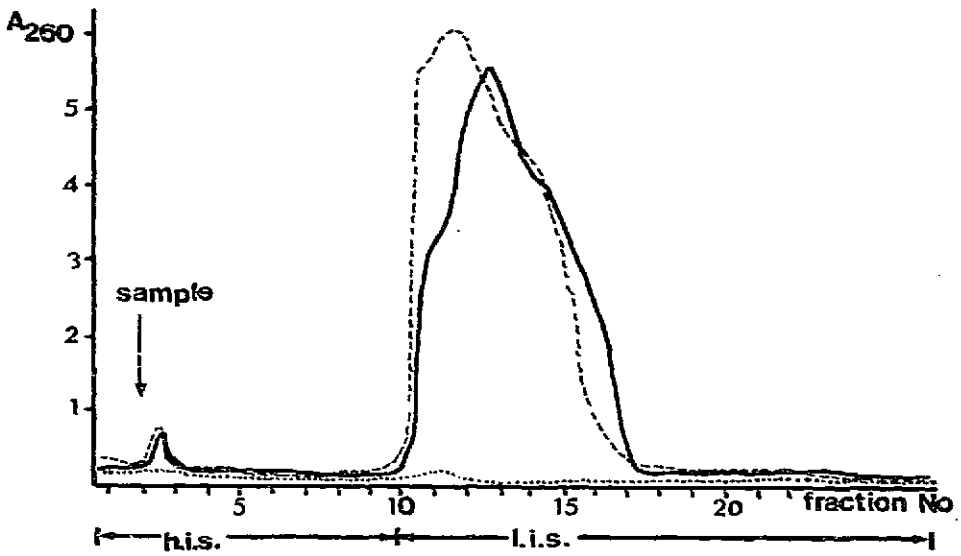


Fig. 2. Chromatography of poly(A) (20 a.u./ml) on a column of uracil-bonded silica gel (solid line) and parallel chromatography on oligo(dT)-cellulose (broken line). Sample application showed by arrow. Eluting buffers: 0.5 *M* KCl, 0.01 *M* Tris (pH 7.5) of high ionic strength (h.i.s.) and 0.01 *M* Tris (pH 7.5) of low ionic strength (l.i.s.) Dotted line: blank experiment.

In the next experiment, reticulocyte RNA was chromatographed on the column using two-step elution. As shown in Fig. 3A, most of the RNA was eluted in a large peak at the front of the buffer of high ionic strength. About 2.5% of the RNA was retained on the column, which could be eluted with buffer of low ionic strength. The recovery of RNA in this 2.5% fraction was checked by re-filtering it under the same conditions (Fig. 3B). The recovery of total RNA, as checked by UV absorption, varied between 90 and 95%.

Reticulocite RNA was characterized by electrophoresis (Fig. 4). The starting preparation shows clear bands of 28S, 18S and 4S RNA and some unresolved zones between the bands. The finally purified RNA preparation shows a relatively strong band in the position where 9S mRNA is normally found. 9S mRNA is known to be rich in poly(A) sequences[2,5]. There is also a very weak band of 18S RNA, and an even weaker band of 28S RNA.

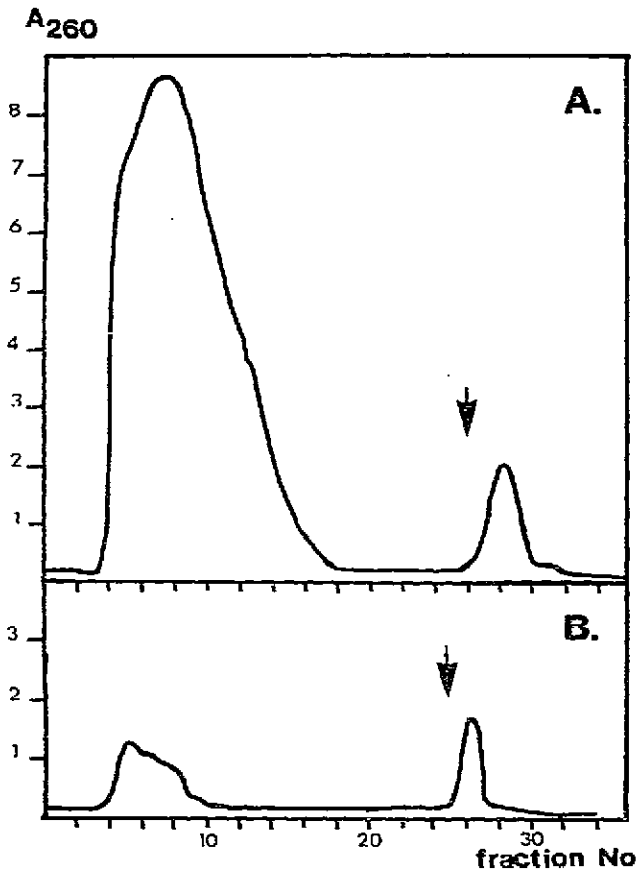


Fig. 3. Chromatography of reticulocite RNA (100 a.u.) on uracil-bonded silica gel (A) and re-chromatography of the small peak (B). For conditions see Fig. 2. Arrows show the start of elution with the buffer of low ionic strenghth.

DISCUSSION

Purification of adenosine and its derivatives is of interest both because of their role in various metabolic processes and also for studying the problems associated with the separation of synthetic mixtures.

On the basis of the above results, we conclude that uracil-bonded silica gel is a useful chromatographic medium for the purification of adenosine and related compounds. The simplicity of the method is to be stressed. Moreover, the physical parameters of the gel will allow high-pressure chromatographic techniques to be developed.

The use of uracil-bonded silica gel to isolate poly(A)$^+$-RNA has been established. The method is highly efficient in comparison with other techniques based on the pairing of poly(A) segments with complementary homopolynucleotides. Usually RNA purified with oligo(dT)-cellulose[5,15] or poly(U)-Sepharose[6,7] is enriched in mRNA, but still contains considerable amounts of 18S and 28S RNA. This contamination is due partly to an association of 9S RNA with 18S RNA, and

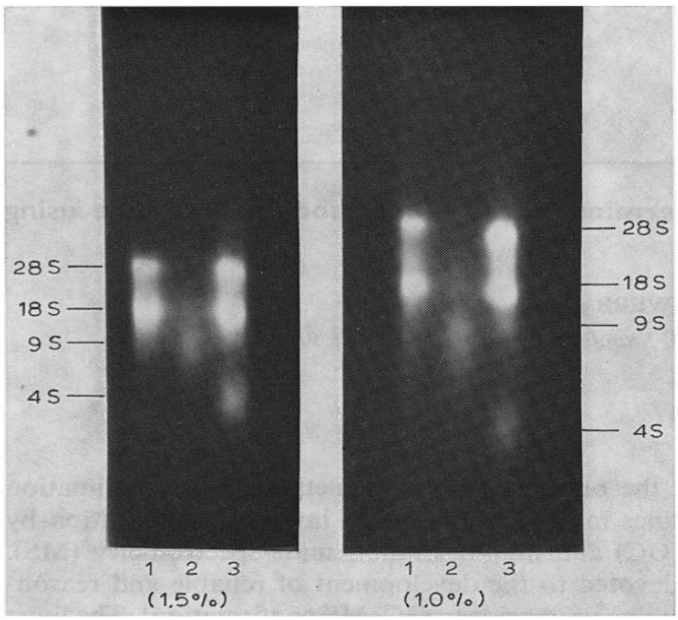


Fig. 4. Electrophoresis of reticulocite RNA in 1.5% (left) and 1% (right) agarose gels. 1, rRNA marker; 2, RNA purified with uracil-bonded silica gel; 3, unpurified reticulocite RNA.

partly to the method itself[15]. The proposed method yields relatively highly purified poly(A)$^+$-RNA. Possibly the surface of the modified gel (highly hydrophobic) can denature RNAs to some extent, releasing mRNA. An alternative method, producing highly purified preparations of specific mRNA, is affinity chromatography using complementary DNA bound to cellulose[16], but this procedure is much more complicated than that described here.

REFERENCES

1 G. Brawerman, *Ann. Rev. Biochem.*, 43 (1974) 621.
2 G. Brawerman, *Progr. Nucl. Acid. Res. Mol. Biol.*, 16 (1976) 117.
3 R. S. S. Rogers and U. E. Loening, *Eur. J. Biochem.*, 34 (1973) 153.
4 I. Schechter, *Biochemistry*, 13 (1974) 1875.
5 H. Aviv and P. Leder, *Proc. Nat. Acad. Sci. U.S.*, 69 (1972) 1408.
6 U. Lindberg and T. Persson, *Eur. J. Biochem.*, 31 (1972) 246.
7 P. Lizardi, *Cell*, 7 (1976) 239.
8 W. K. Roberts, *Biochemistry*, 13 (1974) 3677.
9 Yu. P. Zerov, *Biokhimiya*, 41 (1976) 35.
10 G. Brawerman, J. Mendecki and S. Y. Lee, *Biochemistry*, 11 (1972) 637.
11 J. Langer, *Ph.D. Thesis*, A. Mickiewicz University, Poznan, 1975.
12 J. Langer and Z. Kornetka, *Pol. J. Chem.*, 6 (1978) 1303.
13 U. E. Loening, K. W. Jones and M. L. Birnstiel, *J. Mol. Biol.*, 45 (1969) 353.
14 U. E. Loening, *Biochem. J.*, 102 (1969) 131.
15 S. S. Longacre and W. J. Rutter, *J. Biol. Chem.*, 252 (1977) 2742.
16 H. Potuzak and P. D. G. Dean, *FEBS Lett.*, 88 (1978) 161.