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Cation-exchange chromatography of nucleotides, nucleosides and nucleic bases

Various methods for the separation of nucleotides, nucleosides and nucleic bases have been reported using adsorption chromatography[1,2] and anion-[3–6] or cation-exchange[7–10] column chromatography. BLATTNER AND ERICKSON[7] rapidly separated nucleotides on an AG 50W X4 column using an ammonium formate elution buffer (pH 3.2). BONNELYCKE *et al.*[8] reported a simultaneous analysis of purines, pyrimidines and amino acids using a modified amino acid analyzer.

In this note we report that nucleotides, nucleosides and nucleic bases were successfully separated on a cation-exchange resin (Aminex A-4) column using citrate and acetate buffers.

Materials and methods

A mixture* of nucleotides (5′-UMP, 5′-GMP, 5′-CMP and 5′-AMP), nucleosides (Guo, Ado, Ino and Urd) and nucleic bases (Ura, Gua, Ade, Cyt and Hyp) was used at a concentration of 0.25 μmole/ml in 0.05 *M* hydrochloric acid. Twelve additional compounds (5′-IMP, 2′- and 3′-CMP, 2′- and 3′-UMP, 2′- and 3′-GMP, 2′- and 3′-AMP, ATP, ADP and Thy) were also dissolved in distilled water to a concentration of 5.0 μmole/ml. All compounds were obtained from Kojin Co., Tokyo, and Sigma Chemical Co., St. Louis, Mo.

A Hitachi liquid chromatograph model 034 with a 2-mm flow cell and a three wavelength detection system was employed. In this experiment optical densities at 260, 270 and 280 mμ were measured and recorded on a three-point current recorder which prints one dot per 4 sec. A water-jacketed column contained 0.9 × 50 cm of Aminex A-4 (Bio Rad Laboratories, Richmond, Calif.). A 1-ml aliquot of the authentic

TABLE I

COMPOSITION OF BUFFER SYSTEMS

Buffer	*Autograd chamber*[a]	*Sodium citrate concn. (M)*	*Sodium acetate concn. (M)*	*pH*	*Sodium ion concn. (M)*[b]
I		0.20		3.00	0.25
II	1		0.25	6.40	0.25
	2		0.50	6.40	0.50
	3		1.00	6.40	1.00
	4		1.00	6.40	1.00

[a] Each chamber contained 50 ml of buffer solutions.
[b] Adjusted with sodium chloride.

* The abbreviations used are: UMP, uridine monophosphate; GMP, guanosine monophosphate; CMP, cytidine monophosphate; AMP, adenosine monophosphate; Guo, guanosine; Ado, adenosine; Ino, inosine; Urd, uridine; Ura, uracil; Ade, adenine; Cyt, cytosine; Hyp, hypoxanthine; IMP, inosine monophosphate; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; Thy, thymine.

mixture was applied to the column bed equilibrated with citrate buffer (pH 3.0, buffer I). After using the first buffer for 40 min, the buffer selection valve was changed to a four-component gradient elution from a Technicon Autograd. The buffers were pumped out at a flow rate of 60 ml/h and the column was operated at 30° throughout the chromatography. The elution program and buffer compositions employed in this system are summarized in Table I.

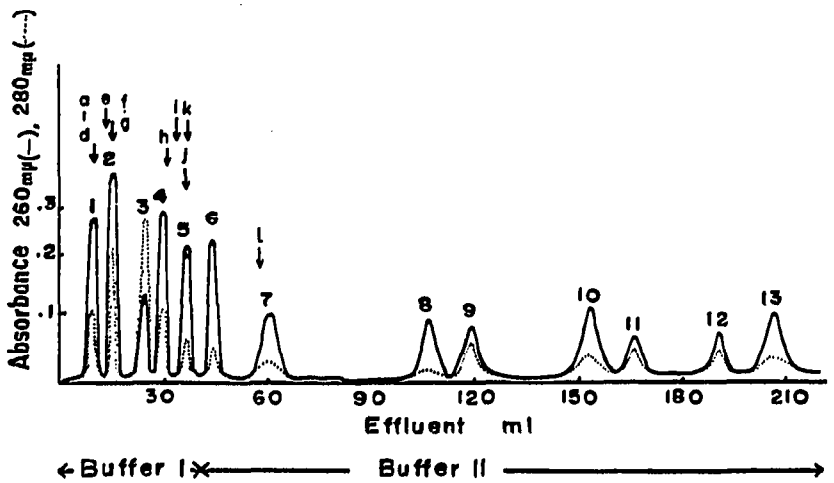

Fig. 1. Chromatogram of nucleotides, nucleosides and nucleic bases. 1 = 5′-UMP, 2 = 5′-GMP, 3 = 5′-CMP, 4 = Urd, 5 = 5′-AMP, 6 = Ura, 7 = Ino, 8 = Hyp, 9 = Guo, 10 = Ado, 11 = Cyt, 12 = Gua, 13 = Ade. a = 5′-IMP, b = 2′-UMP, c = 3′-UMP, d = ATP, e = ADP, f = 2′-GMP, g = 3′-GMP, h = 2′-AMP, i = 2′-CMP, j = 3′-CMP, k = 3′-AMP, l = Thy.

TABLE II

HW VALUES AND RETENTION VOLUMES AND THE COEFFICIENTS OF VARIATION

Compound	*HW value*[a] (*0.25 μmole*)	*C.V. of HW value* (%)	*Retention volume*[a] (*ml*)	*C.V. of retention volume* (%)
5′-UMP	1.31	2.3	9.1	0
5′-GMP	1.95	1.6	14.7	1.1
5′-CMP	2.50	1.9	24.5	0.9
Urd	2.51	2.0	29.9	0.7
5′-AMP	2.99	2.9	36.0	1.6
Ura	2.03	2.6	43.3	0.7
Ino	1.62	1.7	59.9	1.1
Hyp	1.58	2.0	107.0	0.6
Guo	2.52	0.9	118.6	0.8
Ado	3.90	1.5	153.3	0.9
Cyt	1.51	3.0	167.0	0.8
Gua	1.77	2.1	189.8	1.4
Ade	3.66[b]	1.2	206.1	2.1

[a] Average of 10 experiments.
[b] Average of 5 experiments.

Results

A chromatogram of 13 nucleotides, nucleosides and nucleic bases is shown in Fig. 1, and the retention volumes of 12 additional compounds are indicated with

arrows. The total analysis time was 210 min. Each peak area on the chromatogram, obtained using 0.25 μmole of the compound, was estimated at 260 mμ (except for CMP at 280 mμ) height–width (*HW*) method. The *HW* values, the retention volumes of each compound, and their coefficients of variation are given in Table II. Quantitative accuracy of this analysis was found to be within ± 3.0 %, and the most suitable range of sample concentration was from 0.1 to 0.8 μmole. Reproducibility of retention volumes of 13 compounds was obtained within ± 2.1 % as shown in Table II.

Discussion

By this procedure 13 nucleotides, nucleosides and nucleic bases were eluted with enough resolution for quantitative and qualitative analysis; however, some additional compounds were eluted so close together that not every compound could be found as a well-separated peak. In such cases, identification can be carried out by comparing the optical density obtained at three different wavelengths.

Careful preparation of the buffers was required for excellent separation, because the retention volumes of nucleotides were very sensitive to pH when buffers ranging from pH 2 to 4 are employed. Cyt and Ade were sensitive to the Na^+ concentration of the buffers.

Sample solutions were freshly prepared every week to prevent the reduction of the peak area that was observed when the mixture was stored in a refrigerator for some weeks.

Department of Chemistry,
Faculty of Science, Kyoto University,
Kyoto (Japan)

FUMIKO MURAKAMI
SOUJI ROKUSHIKA
HIROYUKI HATANO

1 F. J. KULL AND M. SOODAK, *Anal. Biochem.*, 32 (1969) 10.
2 J. L. HOFFMAN, *Anal. Biochem.*, 33 (1970) 209.
3 M. HORI AND E. KONISHI, *J. Biochem.*, 56 (1964) 375.
4 N. G. ANDERSON, J. G. GREEN, M. L. BARBEN AND F. C. LADD, Sr., *Anal. Biochem.*, 6 (1963) 153.
5 T. SHIMIZU, H. UDA, H. MATUMIYA, K. MIYAUCHI AND S. GANNO, *Japan Analyst*, 18 (1969) 632.
6 T. ROSETT, J. G. SMITH, Jr., I. MATSUO, P. A. BAILEY, D. B. SMITH AND S. SURAKIAT, *J. Chromatog.*, 49 (1970) 308
7 R. E. BLATTNER AND H. P. ERICKSON, *Anal. Biochem.*, 18 (1967) 220.
8 B. E. BONNELYCKE, K. DUS AND S. L. MILLER, *Anal. Biochem.*, 27 (1969) 262.
9 M. UZIEL, C. K. KOH AND W. E. COHN, *Anal. Biochem.*, 77 (1968) 25.
10 Z. ŽENÍŠEK, J. LAŠTOVKOVÁ AND J. VARHANÍK, *Sci. Tools*, 39 (1969) 16.

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