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Note

Rapid analysis of adenosine, AMP, ADP, and ATP by anion-exchange column chromatography

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We have developed a simple conventional anion-exchange column chromatographic method for the determination of nucleotides [1, 2]. The present study demonstrates that the analysis of adenosine, AMP, ADP, and ATP can be accomplished with speed, sensitivity, and accuracy by simply modifying the elution mode and using a smaller column. This method is therefore suitable to many areas of application, such as rapid determination of adenylate energy charge [3], enzymatic activity involving adenine nucleotides, and the content of adenine nucleotides in human erythrocytes. High-performance liquid chromatography (HPLC) has been used to monitor adenine nucleotides in human blood [4], and to determine the profile in the erythrocyte [5]. The chromatographic method described in this report is simpler and more convenient than that of HPLC.

EXPERIMENTAL

Materials

Materials used were described previously [1].

Column chromatography

A column packed with AG MP-1 resin, 10 × 3 mm, was eluted with a programmed hydrochloric acid gradient established by using a GP-250 gradient programmer equipped with two P-500 pumps (Pharmacia, Piscataway, NJ, U.S.A.); one pumps distilled water, the other 0.192 *M* hydrochloric acid. The gradient programme comprised four steps: distilled water for 1 min, 6% of

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0.192 *M* hydrochloric acid for 1 min, a linear gradient from 6% to 100% of 0.192 *M* hydrochloric acid for 2 min, and then 0.192 *M* hydrochloric acid for 2 min. A column flow-rate of 2 ml/min or 1 ml/min was used. The column was washed with distilled water for 1 min at a flow-rate of 10 ml/min to regenerate the column. The effluent was monitored at 257 nm, and the peaks were integrated by a minigrator of Spectra Physics (Piscataway, NJ, U.S.A.), and the amount of solute in each peak was calculated according to the following equation:

$$\text{Amount (nmol)} = \frac{Int \cdot Av \cdot \text{a.u.f.s.} \cdot F \cdot 1066}{E_{257}}$$

The equation is derived from the equation previously described [1]. *Int* is the integration value in mV-min; *Av* is the signal output of the monitor, and is equal to 0.2 absorbance units/mV; a.u.f.s. is the sensitivity setting of the monitor in absorbance units at full scale; *F* is the flow-rate in ml/min; 1066 is the equation constant; and E_{257} is the molar extinction coefficient at 257 nm. The extinction coefficients are $15.4 \cdot 10^3$ for adenosine, $15.0 \cdot 10^3$ for AMP and ADP, and $14.7 \cdot 10^3$ l mol^{-1} cm^{-1} for ATP.

Preparation of samples

Blood or packed erythrocytes (25 µl) which were packed by centrifugation at 400 *g* for 10 min was pipetted into 225 µl of cold distilled water. After vigorous agitation on a vortex mixer, 250 µl of cold 6% trichloroacetic acid (TCA) were added and thoroughly mixed. The precipitate was cleared by centrifugation and 250 µl of the supernatant solution were neutralized with 50 µl of 2 *M* tris(hydroxymethyl)aminoethane (Tris). The solution (100 µl) was then injected into the column for chromatography.

RESULTS AND DISCUSSIONS

As shown in Fig. 1, resolution of adenosine, AMP, ADP, and ATP is accomplished rapidly using the programmed elution mode. Two chromatograms are shown in the figure, one is eluted at a constant flow-rate of 2 ml/min (A); the other at 1 ml/min (B). In A, the analysis took only 4 min; therefore, the last step of the gradient programme could be eliminated so that a cycle of analysis required only 5 min. Decreasing the flow-rate to 1 ml/min as shown in B, a total of 7 min was required to run a sample; however, the sensitivity increased two-fold.

In this study, a small column was used. This has three advantages; it reduces the retention time and speeds up the chromatography; a relatively small volume of distilled water is required to regenerate the column; and a relatively lower flow-rate can be used to increase the sensitivity.

The sensitivity of the method is about 30 pmol. However, the reliable ranges which yield a linear relationship between the amounts and the integration values were obtained at higher concentrations. These ranges are listed in the second column of Table I, and were obtained by monitoring the effluent at 0.2 a.u.f.s. and at a constant flow-rate of 2 ml/min. Table I also shows the reproducibility and accuracy of the chromatography. The results were obtained

from eight repeated runs of a mixture containing the four authentic compounds.

A typical chromatogram of blood nucleotides is shown in Fig. 2B. A blank which contains no blood but TCA and Tris is shown in A. It shows two ultraviolet-absorbing peaks; one is due to Tris and the other to TCA. The latter peak co-eluted out with AMP; therefore, the amount was subtracted from that of AMP in the subsequent analyses. It should be noted that solutes were retained slightly longer than the standard samples. This is due to the presence of excess amount of Tris used to neutralize the TCA extract. The peak shown by a broken line is the position of adenosine, indicating that the presence of excess amount of Tris base causes a slight increase in the retention time but

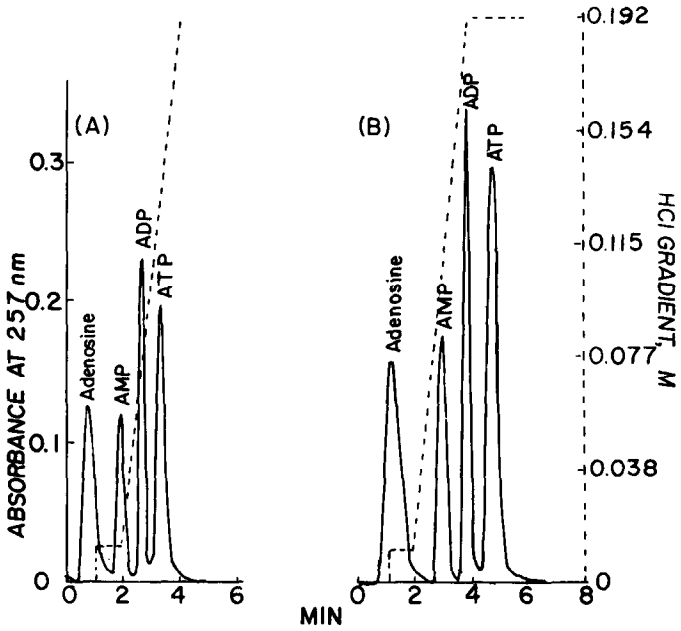

Fig. 1. Resolution of adenosine, AMP, ADP and ATP at flow-rates of 1 ml/min (A), and 2 ml/min (B). The hydrochloric acid gradients are indicated by the broken lines.

TABLE I

LINEAR RANGES, ACCURACY AND REPRODUCIBILITY OF THE CHROMATOGRAPHIC METHOD IN THE DETERMINATION OF ADENOSINE, AMP, ADP, AND ATP AT A COLUMN FLOW-RATE OF 2 ml/min

Means were obtained from eight runs of the same mixture.

Compound	Linear range (nmol)	Mean (nmol)	Standard deviation	Coefficient of variation (%)
Adenosine	0.6—155	11.81	0.17	1.44
AMP	0.5—116	10.49	0.37	3.53
ADP	0.5— 87	13.34	0.04	0.30
ATP	0.5—130	19.89	0.18	0.91

without interfering with the resolution. The levels of adenine nucleotides represented by the figure were 46 nmol of AMP, 84 nmol of ADP, and 538 nmol of ATP per ml of blood.

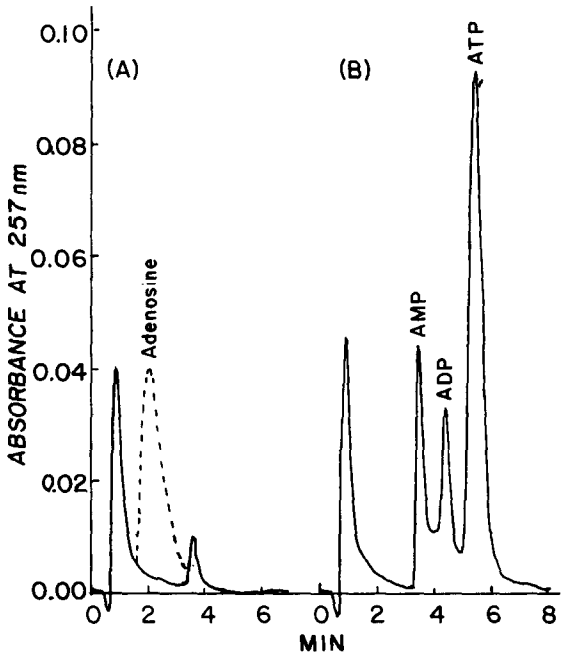

Fig. 2. Chromatogram of adenine nucleotides in human blood. The column flow-rate was 1 ml/min. A is a blank containing TCA and Tris. The position of adenosine peak obtained at another run with external adenosine is indicated by the broken line. A typical blood adenine nucleotide profile is shown in B.

Analyses of eight samples of packed human erythrocytes from hospitalized patients showed that the total adenine nucleotide concentrations varied from 1153 to 1497 nmol/ml packed erythrocytes with the ratios of ATP:ADP:AMP comparable to the values reported [5].

REFERENCES

1 D.-S. Hsu and S.S. Chen, J. Chromatogr., 192 (1980) 193—198.
2 S.S. Chen and D.-S. Hsu, J. Chromatogr., 198 (1980) 500—505.
3 D.E. Atjubsib and G.M. Walton, J. Biol. Chem., 242 (1967) 3239—3241.
4 P.R. Brown, R.E. Parks, Jr. and J. Herod, Clin. Chem., 19 (1973) 919—922.
5 P.D. Schweinsberg and T.L. Loo, J. Chromtogr., 181 (1980) 103—107.