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Note

Separation of cAMP from adenine nucleotides and nucleosides by electrophoresis on cellulose acetate membranes

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cAMP is involved in many biological and biochemical events and its metabolism has been intensively studied.

The separation of cAMP from other adenine nucleotides, nucleosides and bases is essential for the determination of cAMP and the activities of its metabolizing enzymes, adenylate cyclase and phosphodiesterase. It has been achieved by specific radioimmunoassay[1], ion-exchange column chromatography[2,3], paper chromatography[4] and chemical methods[5]. The evaluation of the changing levels of cAMP and its metabolites, adenine nucleotides and nucleosides, is required for studies of cAMP metabolism, particularly in crude systems. This paper describes a simple procedure that permits the simultaneous separation of cAMP, ATP, ADP, AMP, adenosine and inosine from each other by electrophoresis on a cellulose acetate membrane at low voltage. The proposed method takes less time than paper electrophoresis and, in contrast to paper electrophoresis, can be performed at 150 V and at room temperature.

EXPERIMENTAL

The cellulose acetate membrane, Cellogel (18 × 5 cm), was a product of Chemetron (Milan, Italy). Nucleotides, nucleosides and bases were purchased from Sigma (St. Louis, MO, U.S.A.). [8-^{14}C]Adenosine, [8-^{3}H]cAMP, [8-^{14}C]AMP and [8-^{3}H]ATP were obtained from the Radiochemical Centre (Amersham, Great Britain). Other chemicals were of analytical-reagent grade.

Electrophoresis

The electrophoresis was carried out in a Type 238 electrophoresis apparatus (Jokyo Sangyo, Tokyo, Japan). The electrophoresis buffer contained 12 m*M* citric acid, 14 m*M* potassium dihydrogen orthophosphate, 14 m*M* boric acid and 2% glycerol, and was adjusted to pH 7.0 with potassium hydroxide solution.

A strip measuring 2.5 by 9 cm was cut from a sheet of Cellogel and soaked in the electrophoresis buffer at least overnight. The strip was placed on an electrophoresis cell after removing excess of buffer with filter-paper. A 3-μl volume of solution containing 4 m*M* each of ATP, ADP, AMP, cAMP, adenosine, inosine and adenine

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was applied on the cathode side of the strip as a 2-cm streak. Electrophoretic runs were carried out at 150 V for 40 min at room temperature.

To ensure the separation of each compound, labelled ATP (8800 cpm) and cAMP (10,000 cpm), or AMP (12,000 cpm) and adenosine (8900 cpm) were applied together with the carrier solution. After electrophoresis, the marker bands and the areas between each band were located under UV light, dried under hot air, cut out and counted in 5 ml of scintillation fluid (OCS; Radiochemical Centre) with a Beckman scintillation counter.

RESULTS AND DISCUSSION

We reported previously the separation of purine derivatives by electrophoresis on a cellulose acetate membrane and its application to enzyme assays[6,7]. Ribonucleotides were readily separated from the corresponding nucleosides and bases in 0.1 *M* borate buffer (pH 9.0)[6], in which the *cis*-hydroxyl groups in the ribose moiety form the chelated derivatives with orthoboric acid. In this system, cAMP was separated from AMP, but it moved together with adenosine and inosine.

In Tris–hydrochloric acid buffer (pH 8.0), cAMP was clearly separated from AMP and nucleosides (Fig. 1A). This system had been used for the determination of phosphodiesterase activity[8]. However, ATP and ADP had the same mobility and the labelled ATP showed trailing and overlapped cAMP. ATP might interact with the Tris cation. This system could not be used for the determination of the formation of cAMP from ATP. In citrate buffer (pH 6.0), ATP, ADP and AMP were separated from each other and cAMP was separated from other adenine nucleotides and nucleosides (Fig. 1B). However, the distances between cAMP and AMP and between cAMP and inosine were too short to measure the radioactivity of cAMP without contamination of the neighbouring compounds.

Complete separation of cAMP from adenine nucleotides and nucleosides was obtained in the specified buffer (Fig. 2). ADP moved close to ATP, and inosine to adenosine and adenine, but the radioactivity of ATP and AMP showed no trailing.

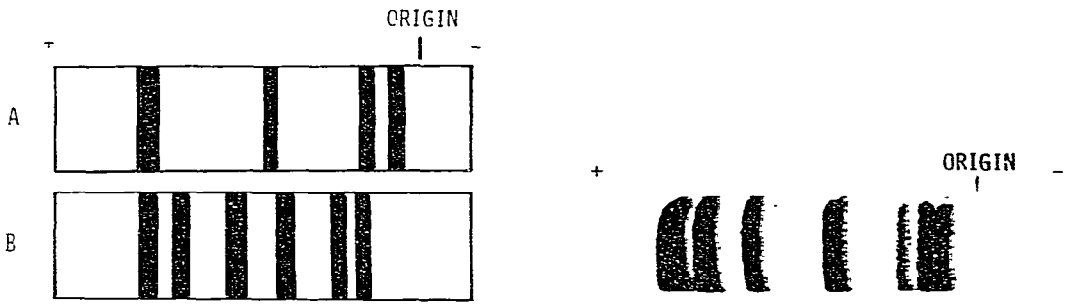

Fig. 1. Electrophoretic patterns of the carrier substances in (A) 0.1 *M* Tris–HCl buffer (pH 8.0) and (B) 0.03 *M* citrate buffer (pH 6.0). Electrophoresis was carried out at 150 V for 40 min. From the cathode side: A, adenosine, inosine, cAMP, and AMP + ADP and ATP; B, adenosine, inosine, cAMP, AMP, ADP, and ATP.

Fig. 2. Electrophoretic separation of cAMP from other adenine nucleotides, nucleosides and adenine in the buffer containing 12 m*M* citric acid, 14 m*M* KH_2PO_4, 14 m*M* boric acid and 2% glycerol (pH 7.0). From the cathode side: adenine, adenosine, inosine, cAMP, AMP, ADP and ATP.

TABLE I

RADIOACTIVE MEASUREMENT OF LABELLED NUCLEOTIDES AND NUCLEOSIDES AFTER ELECTROPHORESIS

Substance	*Radioactivity (cpm)*	
	*Experiment 1**	*Experiment 2**
Origin	22	80
Adenine	60	380
Adenosine	321	9618 (109)**
Inosine	85	215
Blank 1	53	20
cAMP	9748 (97)**	62
Blank 2	42	55
AMP	32	11,072 (92)**
Blank 3	85	352
ADP	325	122
ATP	8172 (93)**	74

* In experiment 1, [8-^{3}H]ATP (8800 cpm) and [8-^{3}H]cAMP (10,000 cpm) were applied together with the carrier solution. In experiment 2 [8-^{14}C]AMP (12,000 cpm) and [8-^{14}C]adenosine (8900 cpm) were applied. After electrophoresis, each band and the areas between the marker bands (indicated as Blank) were located under UV light, cut out in order of migration from the cathode side (origin) as shown in Fig. 2, and counted.

** Numbers in parentheses are the recoveries of the ratioactivity of the compounds (%).

The radioactivity of cAMP was measured without interferences from ATP, AMP and adenosine (Table I). This separation method could be easily performed at low voltage and at room temperature in a reasonable time. It might be applicable to the determination of adenylate cyclase and phosphodiesterase activities and for the quantification of ATP and cAMP metabolites, by using the labelled nucleotides, as ATP, ADP, AMP, cAMP and the nucleosides were simultaneously separated from each other.

REFERENCES

1 G. Brooker, J. F. Harper, W. L. Terasaki and R. D. Moylan, *Advan. Cyclic Nucleotide Res.*, 10 (1979) 1.
2 Y. Salomen, C. Londos and M. Rodbell, *Anal. Biochem.*, 58 (1974) 541.
3 W. J. Thompson, G. Brooker and M. M. Appleman, *Methods Enzymol.*, 38 (1974) 205.
4 C. Nakai and G. Brooker, *Biochim. Biophys. Acta*, 391 (1975) 222.
5 J. Forn, P. Schönhöfer, I. Skidmore and G. Krishna, *Biochim. Biophys. Acta*, 208 (1970) 304.
6 H. Kizaki and T. Sakurada, *Anal. Biochem.*, 72 (1976) 46.
7 H. Kizaki and T. Sakurada, *J. Lab. Clin. Med.*, 89 (1977) 1135.
8 M. A. Delaage, B. N. Bellon and H. L. Cailla, *Anal. Biochem.*, 62 (1974) 417.