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A method for the separation of cyclic adenosine 3',5'-monophosphate from other adenine nucleotides by high voltage ionophoresis on paper

Previously published methods for the separation of adenosine 3',5'-monophosphate (cyclic AMP) from other adenine nucleotides have involved chromatography over successive ion exchange resin columns[1] or a combination of ion exchange columns and thin layer chromatography[2]. In the present communication a method is described for the rapid quantitative separation of cyclic AMP from other adenine nucleotides by high voltage ionophoresis on paper.

Separations were carried out on Whatman filter papers No. 3 MM which had been washed with 1 N nitric acid, following the procedure of SATO[3]. Two buffer solutions gave essentially similar results. These were pyridine–acetate buffer, pH 3.5 (pyridine–acetic acid–water; 1:10:292) or 20 mM lactate buffer, pH 3.6. After completely drying the papers, the nucleotides were located by visualization in ultraviolet light. The ionophoretic migration rates of AMP, cyclic AMP, ADP and ATP are illustrated in

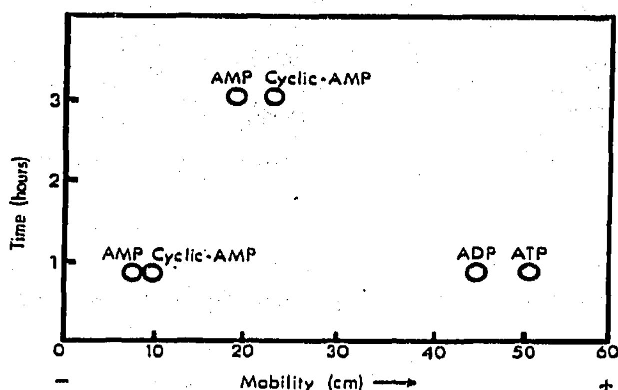

Fig. 1. Ionophoretic mobilities of ATP, ADP, cyclic AMP, and AMP in pyridine–acetate buffer, pH 3.5 at 4000 V, 4°. Ionophoresis was carried out in a high voltage electrophorator (Model D, Gilson Medical Electronics, Middleton, Wisc.) under ice water-cooled amysol.

Fig. 1. Ionophoresis for 1 h at 4000 V (4°) gave good separation of ADP and ATP but not of cyclic AMP and 5'-AMP. Ionophoresis for 3 h under these conditions gave good separation of AMP and cyclic AMP; ATP and ADP moved off the paper. Essentially the same results were obtained after ionophoresis for 6 h with 2000 V at room temperature.

For assay of the nucleotides, the areas containing the nucleotides were cut out, eluted with distilled water, diluted, and the extinction at 260 mμ measured. Lactate is the preferred buffer for this method of measurement since it gives less blank extinction as compared to pyridine–acetate buffer.

Table I shows virtually quantitative recovery of cyclic AMP and AMP in amounts of 0.5 μmoles to 2.5 μmoles after ionophoresis in 20 mM lactate buffer pH 3.6, 2000 V for 6 h at room temperature or after ionophoresis in pyridine–acetate buffer, 4000 V for 3 h at 4°.

Preliminary results indicate that this method can be applied to the assay of

TABLE I

RECOVERY OF CYCLIC AMP AND AMP AFTER IONOPHORETIC SEPARATION

		Test (O.D.)	Standard (O.D.)	Recovery (%)
A.	Cyclic AMP	0.169 ± 0.005 (4)	0.167 ± 0.001 (4)	101
	AMP	0.185 ± 0.012 (4)	0.176 ± 0.003 (4)	105
B.	Cyclic AMP	0.259 ± 0.017 (4)	0.279 ± 0.003 (4)	93
	AMP	0.283 ± 0.014 (4)	0.282 ± 0.003 (4)	100
C.	Cyclic AMP	0.301 ± 0.010 (4)	0.325 ± 0.004 (3)	93
	AMP	0.354 ± 0.024 (4)	0.358 ± 0.004 (3)	99

A. 0.5 μmole each of cyclic AMP and AMP applied as mixture. Pyridine–acetate buffer, pH 3.4; 4000 V for 3 h; 4°. Each spot eluted in 40 ml H_2O. Standards, 0.5 μmoles of cyclic AMP or AMP in 40 ml H_2O.
B. 1 μmole each of cyclic AMP and AMP applied as mixture. Lactate buffer, pH 3.6; 2000 V for 6 h; room temperature. Each spot eluted in 50 ml H_2O. Standards, 1 μmole of cyclic AMP or AMP in 50 ml H_2O.
C. 2.5 μmoles each of cyclic AMP and AMP applied as mixture. Pyridine–acetate buffer, pH 3.4; 4000 V for 3 h; 4°. Each spot eluted in 100 ml H_2O. Standards, 2.5 μmoles of cyclic AMP or AMP in 100 ml H_2O.
O.D. measured at 250 mμ. Test values are corrected for the paper blanks. Values, in order, are the means, standard errors of the means, and, in parentheses, number of observations.

adenyl cyclase activity in tissue preparations if ^{14}C- or ^{3}H-labeled ATP or ATP-α-^{32}P is used as substrate and carrier cyclic AMP is added.

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