CHROM. 5972

A rapid low voltage ionophoretic separation of 3',5'-cyclic adenosine monophosphate

Procedures for the separation of 3',5'-cyclic adenosine monophosphate from other adenine derivatives involve ion-exchange resin columns[1,2], paper chromatography[3], thin-layer chromatography[4,5] and high-voltage ionophoresis[6]. Here we report a quick and satisfactory separation by low voltage ionophoresis on paper.

Separations were made on Whatman paper Nos. 1 and 3MM, using several buffer solutions to see the effect of possible ion pair and/or complex formation[7], and at various pH's to take advantage of the nucleotides' different acidities. Both kinds of papers have given similar results, although a little better resolution has been obtained with 3MM paper, and this was therefore used in all subsequent runs.

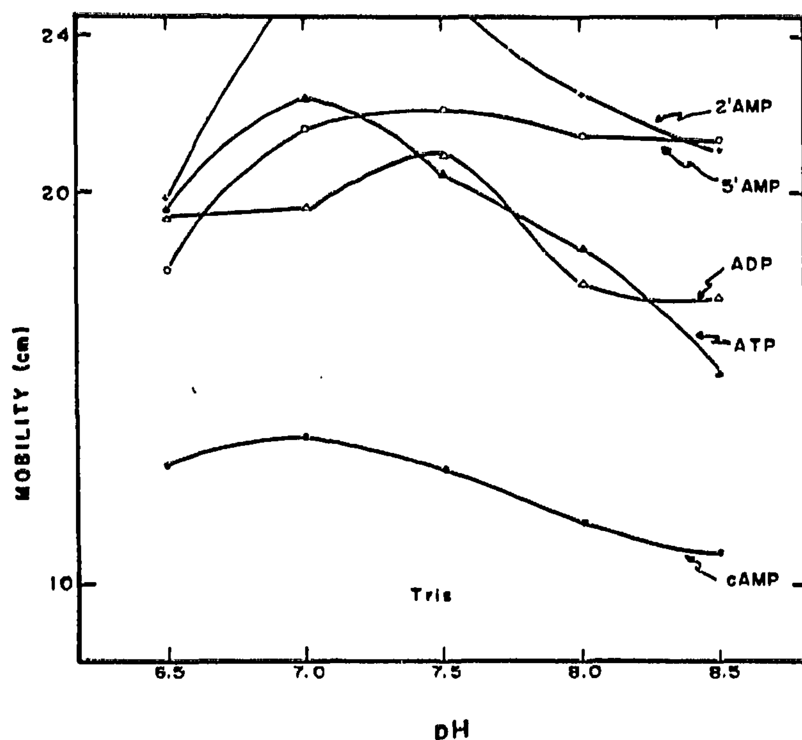

Fig. 1. Mobility of adenine nucleotides in Tris buffer at various pH's. Runs were made on 3MM Whatman paper, for 2.5 h at room temperature in a Gelman electrophoresis chamber Model 51 211, at 500 V, and cooled by running tap water. Buffer concentrations in this and other figures and Table I, at 10 mmolar. UV absorbing spots were detected by a UV lamp.

Figs. 1, 2, and 3 show the mobilities of the compounds in buffers of Tris, phosphate, and HEPES*, respectively. Wider separations are obtained towards alkalinity in phosphate (Fig. 2) and HEPES (Fig. 3), while an optimum around pH 7.0–7.5 is observed in Tris (Fig. 1), in which mobility diminishes above pH 7.5. This retention may be due to a more efficient interaction between Tris and nucleotides at high pH

* HEPES = N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

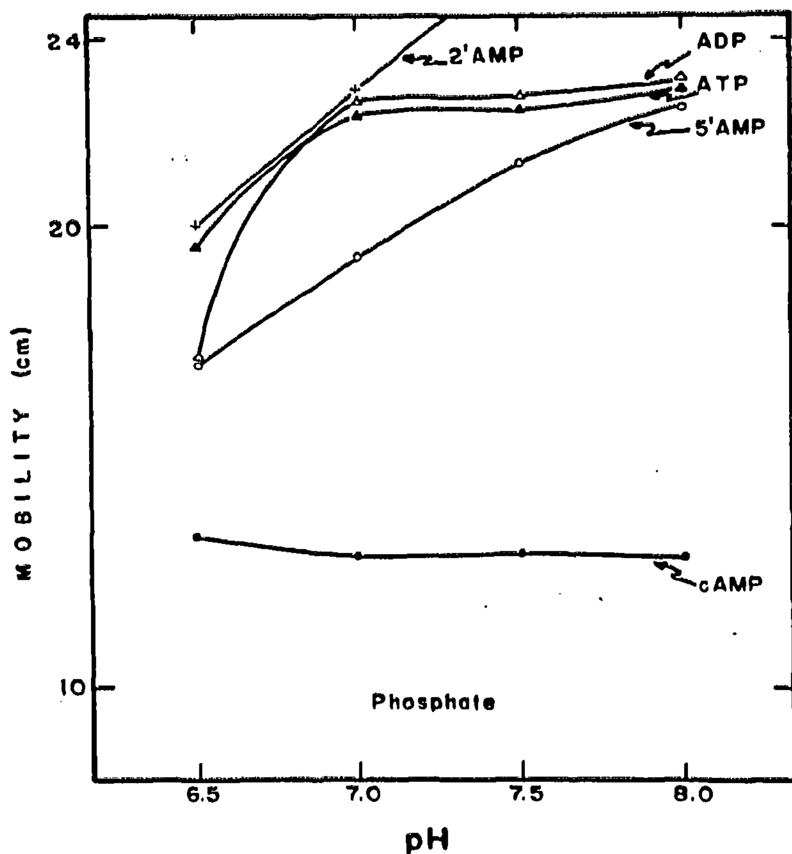

Fig. 2. Mobility of adenine nucleotides in phosphate buffer at various pH's. Conditions as in Fig. 1.

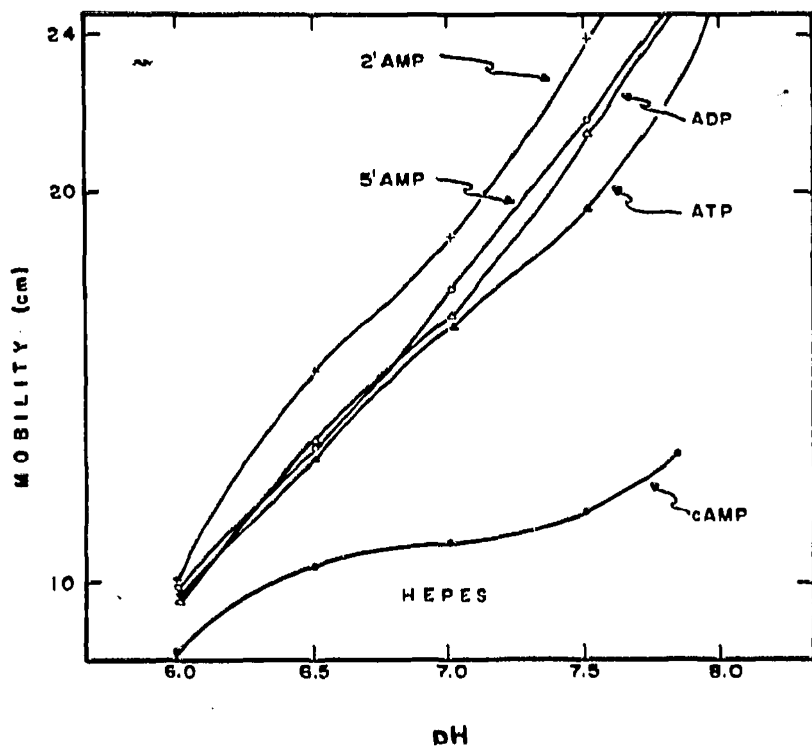

Fig. 3. Mobility of adenine nucleotides in HEPES buffer at various pH's. Conditions as in Fig. 1.

TABLE I

IONOPHORETIC MOBILITIES OF ADENINE DERIVATIVES WITH DIFFERENT BUFFERS AT VARIOUS pH's

Adenine, adenosine and theophylline move towards the cathode, except theophylline in Tris at pH 8.5. Nucleotides move towards the anode. Conditions as in Fig. 1. Mobilities given in cm.

Buffer	*pH*	*cyclic-AMP*	*5'-AMP*	*2'-AMP*	*ADP*	*ATP*	*Adenine*	*Adenosine*	*Theophylline*
Tris	6.5	13.0	18.0	20.0	19.5	20.0	1.0	1.5	2.0
	7.0	14.0	22.0	24.5	20.0	22.0	2.0	2.0	2.0
	7.5	13.0	22.0	24.5	21.0	20.5	1.0	2.0	2.0
	8.0	11.5	21.0	22.5	18.0	19.0	1.0	2.0	1.0
	8.5	11.0	21.0	21.0	17.0	15.0	1.0	2.0	1.0
Phosphate	6.5	13.0	17.0	20.0	17.0	19.5	1.0	1.0	1.0
	7.0	13.0	19.0	23.0	23.0	22.0	1.0	1.0	1.0
	7.5	13.0	21.0	24.5	23.0	22.5	1.0	1.0	1.0
	8.0	13.0	22.5	24.5	23.0	23.0	1.0	1.0	0.0
HEPES	6.0	8.0	10.0	10.0	10.0	10.0	1.0	1.0	1.0
	6.5	10.0	13.5	15.0	14.0	13.0	2.0	2.0	3.0
	7.0	11.0	17.5	19.0	17.0	17.0	2.0	3.0	4.0
	7.5	12.0	22.0	24.0	21.5	20.0	2.0	3.0	2.0
	8.0	13.0	24.5	24.5	24.5	24.5	2.0	2.5	0.0
Tartrate	3.5	7.0	6.5	7.0	14.0	17.0	13.0	5.0	2.0
	4.0	10.0	10.0	10.5	19.0	21.0	10.0	4.0	2.0
	4.5	12.0	12.0	12.0	20.5	22.0	5.0	2.0	1.5
Borate	8.0	10.0	17.0	16.0	13.5	15.0	2.0	0.5	2.0

values. Nevertheless, there is a convenient separation in all three buffers of the main troublesome compounds, AMP and ATP.

Table I summarizes the electropherograms and shows the actual lengths of migration of relevant compounds in different buffers at pH's in the range of their pK's. Borate buffer was utilized at only one pH because of the already high pH value and poor resolution of 3',5'-cyclic adenosine monophosphate from ADP and ATP.

The compounds tested tend to form three groups as regards mobility: one remaining about the origin, a second migrating slowly, and a third migrating more rapidly. The middle group is formed solely by cyclic 3',5'-adenosine monophosphate in the first three buffers, but by cyclic 3',5'-adenosine-, adenosine 2'- and adenosine 5'-phosphates in the runs made with tartrate buffer (see Figs. 1–3 and Table I). The first and third groups contain the almost neutral and more acidic compounds, respectively. This fact makes the first three buffers really advantageous for isolating cyclic 3',5'-adenosine monophosphate from all other compounds present in adenylate cyclase assays. Because of the high mobility of adenosine 2'-phosphate and its possible labelled state resulting from ATP hydrolysis in the enzyme assay system it would be preferable to use HEPES buffer at pH 7.5 with 30-cm sheets.

This work was supported in part by a grant from the Ford Foundation (710-009).

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Received February 9th, 1972