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Note

Paper electrophoretic behaviour of cyclic-3',5'-AMP, 5'-AMP and 5'-ATP

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Adenosine-cyclic-3',5'-monophosphoric acid (c-AMP) plays a very important role in physiological reactions. The paper[1,2] and thin-layer[3] chromatographic separations of adenosines have been reported. However, in order to reduce the separation time, a more rapid method is required.

Although the chemical structure of c-AMP is very similar to that of adenosine-5'-monophosphate (AMP), c-AMP has only one dissociable proton in the phosphate group while AMP has two such protons. This suggests that good electrophoretic separation conditions should be obtained by changing the pH values of the background buffer solutions. Using the usual techniques and apparatus[4,5], we found good separation conditions and some separations are reported in this paper.

EXPERIMENTAL

Materials

Adenosine-5'-triphosphate, disodium salt, (ATP) was purchased from Kokoku Rayon and Pulp Co. (Tokyo, Japan), and adenosine-5'-monophosphoric acid and adenosine-cyclic-3',5'-monophosphoric acid from Sigma, St. Louis, Mo., U.S.A.

Procedures

The techniques and apparatus used were similar to those described in previous papers[4,5]. Background buffer solutions of different pH were prepared by mixing 0.1 *M* acetic acid, 0.1 *M* sodium acetate, 0.1 *M* sodium hydrogen carbonate and 0.1 *M* sodium hydroxide solutions. Thus, the ionic strength (μ) of the solution was maintained at 0.1 throughout this work. A filter-paper (Toyo Roshi No. 50, 1 × 40 cm) was dipped into a buffer solution, excess of which was removed with another filter-paper. A 5-μl volume of a sample solution was placed at a position 5 cm to the cathodic side from the centre of the filter-paper. The strips of filter-paper were immersed in *n*-hexane in the migration chamber and placed in the electrode cells, and then a constant stabilized voltage was applied, keeping the temperature of the chamber constant. After migration, the strips were removed from the chamber and dried in a dryer. The positions of the adenosines were detected by means of the absorption band at 253 nm.

RESULTS AND DISCUSSION

The paper electropherogram is shown in Fig. 1. In acidic media, the electro-

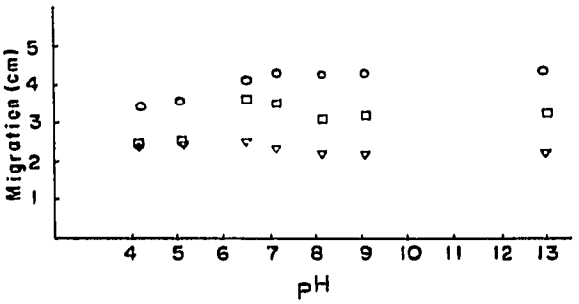

Fig. 1. Relationship between observed zone mobility of adenosines and pH of background buffer solution. Migration conditions: 400 V per 30 cm; $-1 \pm 1°$; $\mu = 0.1$; 60 min. A 5-μl volume of 10^{-2} *M* sample solution was spotted at a position 5 cm from the centre of the filter-paper. ○, ATP; ▽, c-AMP; □, AMP.

phoretic behaviours of c-AMP and AMP were very similar to each other, while significant differences in the migration distance were found in the neutral and basic regions. These results show that the second dissociable proton in the phosphate group of the AMP molecule began to dissociate and became completely dissociated[4,5]. The same effect occurred in the paper electrophoretic behaviour of ATP, which was easily separated from both AMP and c-AMP in the acidic region. In the basic region, the behaviour of ATP is similar to that of AMP but not that of c-AMP. When we used a filter-paper washed with 2 *N* acetic acid, the separability of the electrophoresis was improved and the three adenosines were separated with increasing migration distance in the order c-AMP, AMP and ATP. This separability was tested by spotting 5 μl of a mixture that was $3.3 \cdot 10^{-3}$ *M* in AMP, c-AMP and ATP. Strongly acidic or basic media are not desirable because of the decomposition of the sample and/or the evolution of heat during the migration. The results obtained from the migration of a mixture of AMP and c-AMP for 30 min are shown in Fig. 2.

It should be pointed out that the curves of the migration distance plotted against the migration time did not intersect the y-axis at the origin (see Fig. 3).

In a control experiment in which the spotted filter-papers were allowed to stand for 60 min under conditions similar to those of the electrophoresis except for the application of a voltage, the movements of adenosines were *ca.* 1.4 cm (Fig. 4), which seemed to be due mainly to capillary action at the spotting position[6-9]. As the point of intersection of the curve in Fig. 3 with the y-axis is *ca.* 1.0 cm, the difference

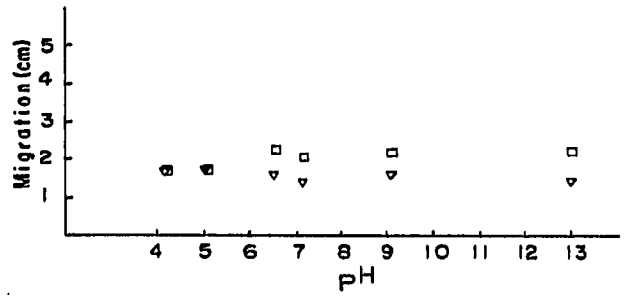

Fig. 2. Relationship between observed zone mobility of adenosines and pH of background buffer solution. Migration conditions as in Fig. 1, except for the migration time (30 min). ▽, c-AMP; □, AMP.

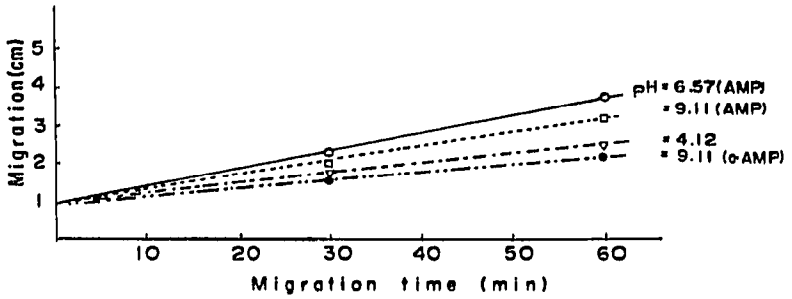

Fig. 3. Relationship between observed migration distance and migration time. Figures show the pH values of the background buffer solutions.

of 4 mm seems to be due to electroendoosmosis[10,11] and/or other causes. We know that the capillary action was related to the spotting position and that the electroendoosmosis was related to the pH of the background buffer solution. When we corrected the observed migration distances for the values from the control experiment and the electroendoosmosis, the corrected migration distances were exactly proportional to the migration time and the voltage gradient.

A separation time of less than 30 min is easily attained by applying a higher voltage to a buffer solution similar to this experimental solution.

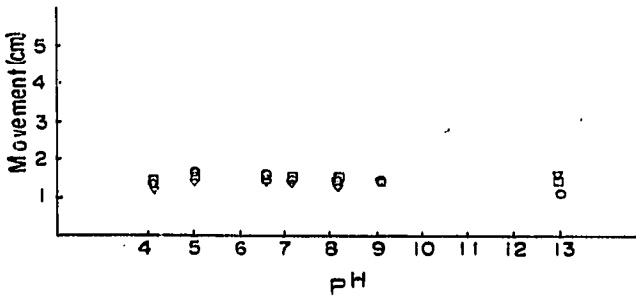

Fig. 4. Relationship between observed movement of adenosines and pH of the buffer solution. A 5-μl volume of 10^{-3} *M* sample solution was allowed to stand for 60 min at $-1 \pm 1°$ under conditions similar to those in the electrophoretic separation but without a voltage. ○, ATP; ▽, c-AMP; □, AMP.

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