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Note

Effect of various metal ions on the electrophoretic migration of adenosine and adenosine nucleotides

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Metal ions play an important role in biochemical reactions. The interaction between metal ions and adenosine nucleotides has been studied by many workers using various analytical methods[1,2]. However, little is known about the effect of metal ions on the electrophoretic migration of adenosine nucleotides. One of the difficulties in elucidating the interaction of metal ions by use of electrophoresis is the fact that the migration of low concentrations of metal ions does not give well defined migration zones. Thus, to improve the shape of the migration zone, an electrolyte must be added to the supporting solution. We have employed sodium chloride as the added electrolyte. We report the effect of Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+} and Fe^{3+} in different concentrations on the migrations of orthophosphate (P), adenosine (As) and adenosine 5′-mono- (AMP), 5′-di- (ADP), 5′-tri- (ATP) and 3′,5′-cyclic mono-phosphate (c-AMP).

EXPERIMENTAL

Materials

Adenosine and adenosine nucleotides were purchased from Sigma (St. Louis, Mo., U.S.A.). Chlorides of guaranteed grade, tetramethylammonium chloride (Me_4NCl), NaCl, $BaCl_2 \cdot 2H_2O$, $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 6H_2O$, $ZnCl_2$ and $FeCl_3$, were used without further purification.

Procedures

The techniques and apparatus used were similar to those described in a previous paper[3]. The supporting solutions of $\geqslant 0.1$ *M* metal chloride were prepared by dissolving the chloride in distilled water. A solution of 0.1 *M* $ZnCl_2$ was cloudy. Supporting solutions containing <0.1 *M* metal chloride were prepared by dissolving a known amount of metal chloride in 0.1 *M* NaCl solution. A filter-paper (Toyoroshi No. 51A, 1 × 40 cm) was dipped into a supporting solution, the excess of which was removed by another filter-paper. A 5-μl volume of sample solution ($5 \cdot 10^{-3}$ *M*) was placed 5 cm from the cathodic side or from the anodic side, or at the centre of the filter-paper. The spotting filter-paper was immersed in hexane and a constant stabilized voltage (1000 V per 30 cm) was applied to it, keeping the temperature constant. Electrophoresis in 0.5 *M* chloride was carried out at a lower voltage gradient (500 V per

30 cm) in order to decrease the amount of heat liberated. The positions of adenosine and adenosine nucleotides on a filter-paper were detected by means of the absorption band at 253 nm. Orthophosphoric acid was detected by the formation of molybdophosphate complexes.

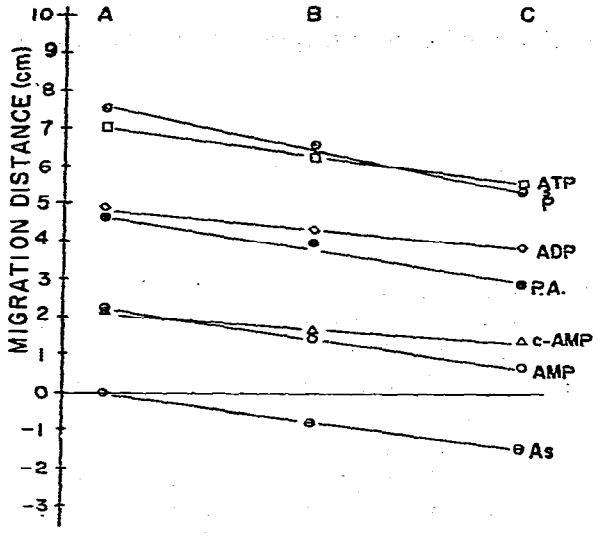

Fig. 1. Observed mobilities of adenosine nucleotides at different spotting positions. Conditions: voltage gradient, 1000 V per 30 cm; migration time, 30 min; supporting solution, 0.1 *M* NaCl; migration temperature, *cc.* 20°. Spotting positions: A, 5 cm to the cathodic side from the centre of the filter-paper; B, centre of the filter-paper; C, 5 cm to the anodic side from the centre. Positive movement is towards the anode, negative movement towards the cathode. P.A. = Picric acid.

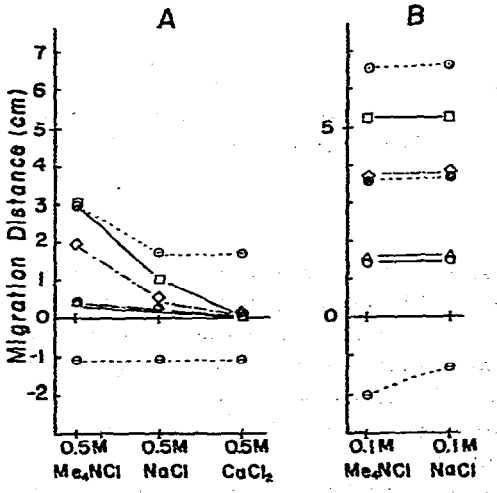

Fig. 2. Observed mobilities of adenosine nucleotides at different concentrations of the added electrolyte. Conditions A: spotting position, the centre of the filter-paper; voltage gradient, 500 V per 30 cm; migration time, 30 min; migration temperature, *ca.* 15°. Conditions B: voltage gradient, 1000 V per 30 cm; other conditions as in A.

RESULTS AND DISCUSSION

The relation between the migration distances in 0.1 *M* NaCl and the spotting positions on a filter-paper are shown in Fig. 1. The differences in the migration distances among the spotting positions are mainly due to capillary action[4]. Since the same behaviour occurred in the other supporting solutions, all the comparisons of the migration distances were made on the results obtained from spotting at the centre of a filter-paper.

Since it is known that the interactions of Me_4NCl with adenosine nucleotides are weak[5], an electrophoresis of the nucleotides was first carried out in the supporting solution containing Me_4NCl. When the migration distances of the nucleotides in 0.5 *M* Me_4NCl were compared with those in 0.5 *M* NaCl or $CaCl_2$ (Fig. 2), it was found that the migration distances in 0.5 *M* Me_4NCl were the largest. However, the

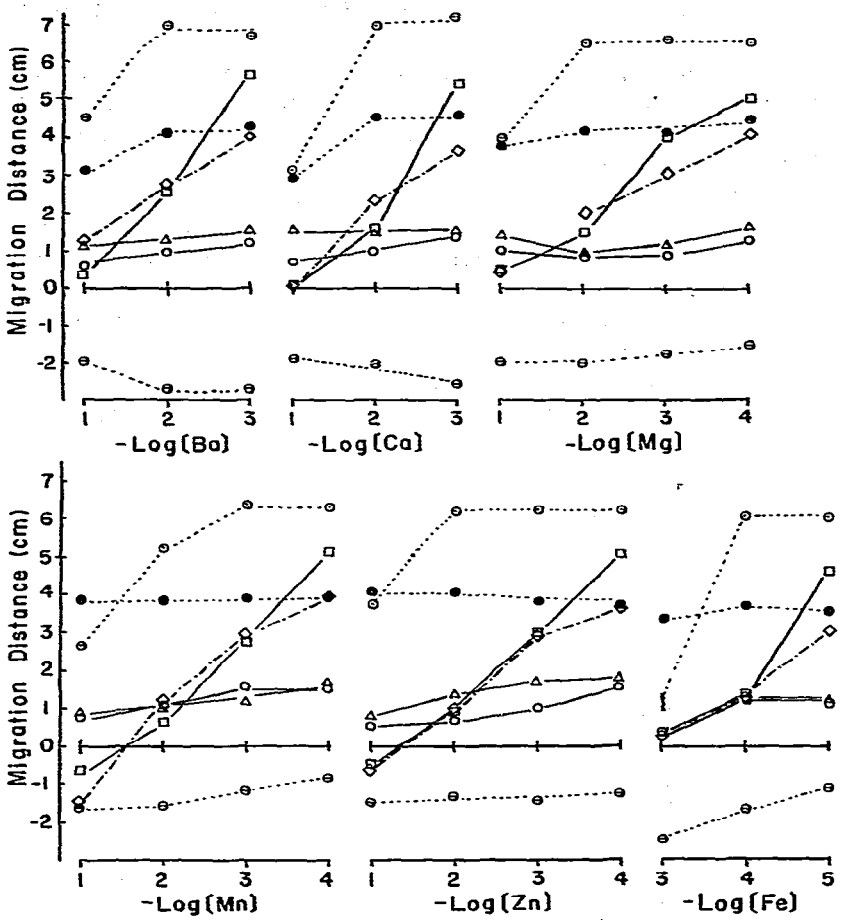

Fig. 3. Relation between migration distance and concentration of various metal ions. Conditions: spotting position the centre of the filter-paper; voltage gradient, 1000 V per 30 cm; migration time 30 min; migration temperature, *ca.* 15°. Supporting solutions: 0.1 *M* solutions contain only a metal chloride; solutions less than 0.1 *M* of a given metal chloride contain 0.1 *M* NaCl. Symbols as in Fig. 1.

migration distances in 0.5 *M* NaCl were smaller than half those in 0.1 *M* NaCl, meaning that the effects of NaCl at the higher concentration were not negligible. The migration distances in 0.1 *M* NaCl were identical to those in 0.1 *M* Me_4NCl, and thus the effects of various metal ions on the mobilities of the nucleotides were compared with those in 0.1 *M* NaCl.

Alkaline-earth metal ions (Ba^{2+}, Ca^{2+} and Mg^{2+})[6–8] affected the migrations in a similar manner (Fig. 3). The migration distances in 10^{-3} *M* alkaline-earth metal ions were close to those in 0.1 *M* NaCl. Those of AMP and c-AMP were not changed as much as those of ADP or ATP with increasing concentration of metal ion.

The migration zones of ADP and ATP in 0.1 *M* $MnCl_2$ or $ZnCl_2$ were found on the cathodic side, showing that the main species were cationic species believed to contain two metal atoms in a molecule of nucleotide[9,10]. With decreasing concentration of Mn^{2+} or Zn^{2+}, anionic species became the main species. The migration distance of ATP was obtained in 10^{-4} *M* $MnCl_2$ or $ZnCl_2$ was similar to that in 0.1 *M* NaCl.

The interaction between Fe^{3+} and the nucleotide anions was so strong that the colourless spot of the migration zone was not observed in the supporting solutions containing $>10^{-3}$ *M* $FeCl_3$ (ref. 11). The migration distance of ATP in 10^{-5} *M* $FeCl_3$ was a little smaller than that in 0.1 *M* NaCl.

The effects of addition of methanol are shown in Fig. 4. The addition of methanol to 0.1 *M* NaCl solution decreased all the migration distances non-selectively, and the results obtained were very different from those obtained in the supporting solution containing metal ions. Although it is not possible to use metal ions or organic solvent for a separation of c-AMP from AMP, the separation could be easily made in 0.1 *M* sodium hydrogen carbonate[12].

Intrinsically, the observed mobility of the electrophoretic zone is the weighted mean of the corresponding mobilities of the ionic species present in the given solu-

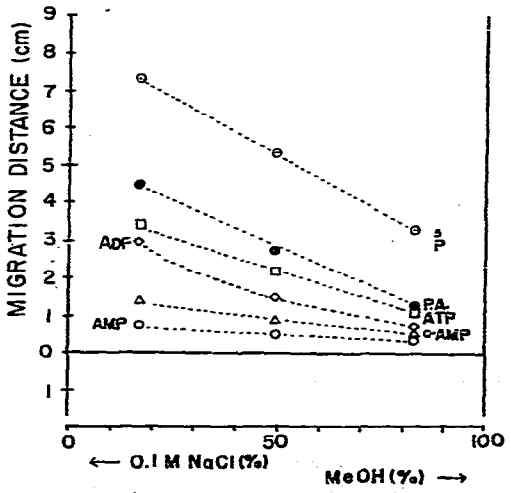

Fig. 4. Relation between migration distance and content of methanol. Conditions: spotting position, the centre of the filter-paper; voltage gradient, 1000 V per 30 cm, migration time, 30 min; migration temperature, *ca.* 20°.

tion[13]. Thus, when the composition of the complex ions or free ions is changed the observed zone mobility should also change. The larger the stability constant of the complex ions, the larger the decrease in the mobility. The effects of metal ions increased in the order $Me_4N^+ < Na^+ < Ba^{2+} < Ca^{2+} < Mg^{2+} < Mn^{2+} \approx Zn^{2+} < Fe^{3+}$, and for the nucleotides there used in the order As < c-AMP ≈ AMP < P < ADP < ATP.

We think that the effect of metal ions is closely related to the stability constants of the metal–adenosine nucleotide complexes. The estimation of the mobility of each species will be reported in future work.

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