Electrophoretic Behavior of Adenine, Adenosine, and Adenosine Nucleotides in Ion Exchange Cellulose Paper as a Support

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Synopsis. Studies have been carried out on the effects of cationizable and anionizable groups of ion exchange cellulose papers on an electroosmotic flow over a range of pH=5—10 and on the electrophoretic migrations of adenine, adenosine, and adenosine nucleotides. The ionizable groups strongly affected the mobilities of adenosines.

An electrophoretic migration using a support is affected by many factors,[1,2] one of which is the chemical nature of the support. To improve the separability of the electrophoresis, many kinds of substances have been tried as a support. A filter paper having ionizable groups is one of them.

In this paper, we would like to report the electrophoretic behavior of adenine, adenosine, and adenosine 5′-mono, 5′-di-, 5′-tri-, and cyclic 3′,5′-monophosphates (AMP, ADP, ATP, and cAMP, respectively) in ion exchange cellulose papers at different pH-values. An electroosmotic flow is closely related to the ionic groups of a paper and affects the migration of a migrant. Thus, the flows in the papers were measured by movement of carbohydrates.

Reagents and Methods

Adenine and adenosine were purchased from Wako (Osaka, Japan), and the sodium salts of the nucleotides from Sigma (St. Louis, Mo., U.S.A.). Carbohydrates of guaranteed grade were used without further purification. Three different kinds of papers were used as a support: Toyoroshi No. 51A (=51A, pure cellulose paper), Whatman DEAE cellulose paper (=DE81, anion exchange cellulose paper), Whatman phosphate paper (=P81, cation exchange cellulose paper). Four kinds of supporting solutions were prepared; 0.1 M (1 M=1 mol dm^{-3}) NaCl solution (pH≈7.0, buffering capacity (β)=4.6×10^{-7}), citrate solution (pH=5.0, β=0.031, 0.1 M citric acid +0.2 M NaOH), phosphate solution (pH=7.0, β=0.027, 0.025 M Na_2HPO_4+0.025 M NaH_2PO_4) and glycine solution (pH=10, β=0.028, 0.06 M glycine+0.06 M NaCl+0.04 NaOH). The procedures and apparatus used were similar to those described previously.[3] A 5 μl volume of sample solution (5×10^{-2} M) was spotted at the position (see a footnote of Fig. 1) of a strip (1×40 cm) wetted with a supporting solution. It was then dipped in hexane in the migration chamber which was kept at constant temperature (*ca.* 25 °C). A d.c. voltage (1000 V per 30 cm) was applied for 30 min. The paper after migration was then dried and the position of adenines on the paper was detected under the irradiation of ultraviolet light (253 nm). The position of carbohydrates on the paper was detected an *o*-aminophenol solution.[4]

Results and Discussion

The movements of glucose, maltose, and maltotriose in three kinds of papers are shown in Fig. 1. The observed migrations are affected by capillary action, electroosmotic flow, and other physicochemical interactions in the system.[1,2] The differences of migration among spotting positions in a given paper are due to a capillary action, which is towards the center of a paper from both sides.[2,5] The movement due to the capillary action increases with increasing distances of the spotting position from the center of a paper.[1,2] The movement of carbohydrate spotted at the center is thought to be mainly due to an electroosmotic flow because the movement due to the capillary action is zero at the center. Three different carbohydrates showed similar movements in a given paper within experimental errors, showing that the effect of molecular weight on the capillary action and on the electroosmotic flow is small in this experimental range of molecular weight (mol wt: 180—504).

The movements of glucose spotted at the center of a paper dipped in four kinds of supporting solutions are shown in Fig. 2. The differences of migration among the pH-values in a given paper were small, while those among papers were large. The fact that the movement of glucose along with the supporting solution in DE81 paper was found in the anodic side of the paper indicates that the electroosmotic flow was towards the anode by the cationizable (diethylaminoethyl-) groups of the paper. The negative movements in 51A and P81 papers are due to an electroosmotic flow towards the cathode by the anionizable (–COOH and $-OPO_3H_2$, respectively) groups of the papers.

The observed migrations of adenine, adenosine, and adenosine nucleotides in three different papers are shown in Fig. 3. The largest migration of the nucleotides was seen in 51A paper, the next in DE81, and the smallest in P81.

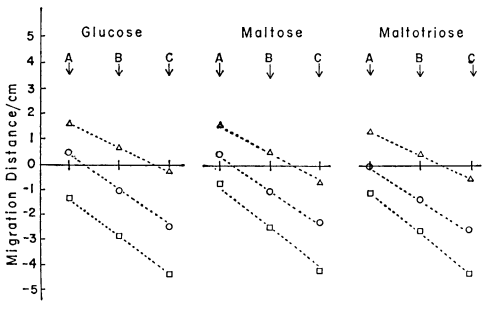

Fig. 1. Movements of carbohydrates at different spotting positions. Conditions: voltage gradient, 1000 V per 30 cm; migration time, 30 min; migration temperature, *ca.* 25 °C; supporting solution, 0.1 M NaCl. Spotting positions: A, 5 cm to the cathodic side from the center of the paper; B, the center of the paper; C, 5 cm to the anodic side from the center. Positive movement is towards the anode, negative movement towards the cathode. Supporting paper: △, DE81; ○, 51A; □, P81.

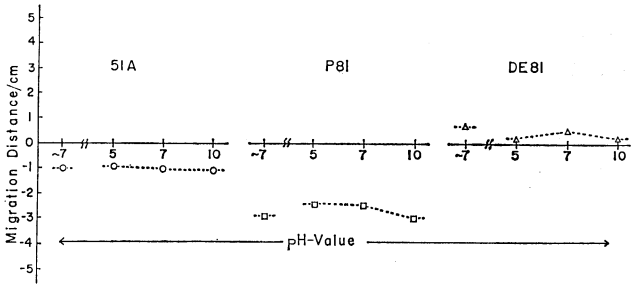

Fig. 2. Movements of glucose by different kinds of supporting solutions. Conditions: 1000 V per 30 cm; 30 min; *ca.* 25 °C. Supporting solutions: pH≈7.0, 0.1 M NaCl; pH=5.0, citrate solution; pH=7.0, phosphate solution; pH=10, glycine solution. Supporting papers: △, DE81; ○, 51A; □, P81.

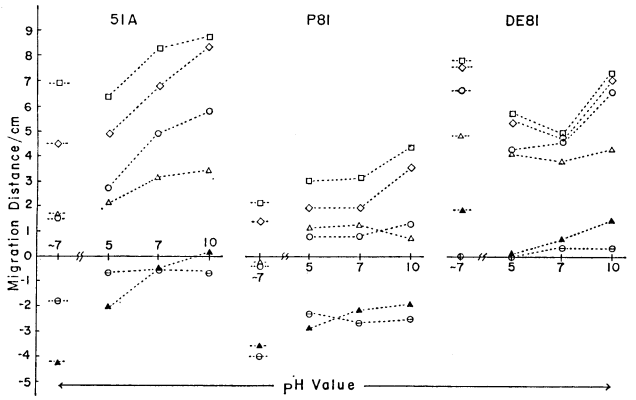

Fig. 3. Observed migrations of adenine, adenosine and adenosine nucleotides. Sample solutions: □, ATP; ◇, ADP; ○, AMP; △, cAMP; ⊖, adenosine; ▲, adenine. Conditions: 1000 V per 30 cm; 30 min; *ca.* 25 °C. Supporting solutions as in Fig. 2.

The migrations corrected for the electroosmotic flow shown in Fig. 2 are shown in Fig. 4. The migration of a weak acid or a weak base is very sensitive to the pH-value of a supporting solution.[6] When we dip a paper into a supporting solution, some ionizable groups in the paper will ionize. Thus, the pH-value of the supporting solution in the ion exchange paper becomes different from the initial pH-value. The pH change in the paper depends on the buffering capacity[7] of the supporting solution and the ion exchange capacity of the paper. The pH-value of the supporting solution in 51A paper will be not so different from the initial value because the number of ionizable (probably -COOH) groups in 51A is not sufficient to change

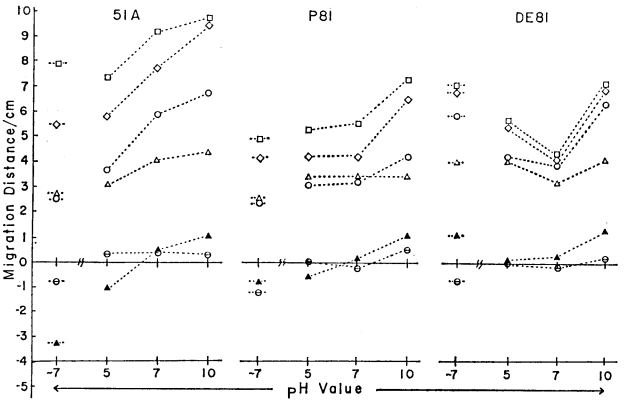

Fig. 4. Migrations corrected for electroosmotic flows. Electrophoretic conditions and symbols used are same as in Fig. 3.

the pH-value of the high buffering solution (approximately one carboxyl group per 500 glucose residues[1]). The increase[8] of the migrations due to the dissociation of the secondary-hydrogen of the phosphate group in the nucleotides is clearly observed at pH=7.0 in 51A (see Fig. 4). The migrations of the nucleotides on P81 or DE81 are strongly affected by ionizable groups of P81 or DE81 and the increase of migrations is not observed at pH=7.0. The migrations corrected for the electroosmotic flow on 51A are not identical with those on P81 or DE81. These results show that unestimated physicochemical interactions (mainly electrostatic forces) between the migrant and ionizable groups of the supporting paper are much involved in the migration of adenine, adenosine, and adenosine nucleotides, as well as an electroosmotic flow and/or a capillary action.

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