  Bull. Chem. Soc. Jpn., 57, 1856—1858 (1984) [Vol. 57, No. 7

Comparison of the Experimental Zone Mobility-pH Curves of the Bases of Nucleic Acid on the Pure Cellulose Paper with the Calculated Ones[1)]

Yoshinori KITAOKA
Research Reactor Institute, Kyoto University, Kumatori-cho, Sennan-gun, Osaka 590-04
(Received February 2, 1984)

Comparison of the experimental zone mobility-pH curves of nucleic acid bases with the calculated ones was made over acidic and basic regions using a pure cellulose paper as a support after correction of the capillary action and the endoosmotic flow. The mobilities in the acid region agreed with the calculated ones but those in the basic region did not. Some unknown factors affect the mobilities in the basic region.

Paper electrophoretic behavior of weak acids or bases is strongly affected by the pH-value of a supporting solution, which controls the ratios of ionization of a migrant.[2)] Several workers have studied the mobilities and the pH-values of the supporting solution. Smith[3,4)] discussed the relationship between the mobility and the dissociations of amino and phosphonoxy groups of nucleotides. Wade and Morgan[5)] separated nucleoside and bases using a borate buffer. Kiso and coworkers[6)] derived an equation for the relationship between mobility and pH-value and applied it to phosphorus compounds. If we know the mobility of each species and the dissociation constant of a given compound, the zone mobility-pH curves[6)] could be drawn theoretically. However, in the actual electrophoresis using a support, the observed mobilities are affected by electroosmosis, capillary action, and so on of the support in addition to the chemical nature of the supporting solution.[7)] Thus, the good agreement between the observed and the calculated mobilities will be made only under well defined conditions. In this experiment, in order to lessen the chemical effect of a support on the mobility, a pure cellulose paper was used as a support. Although electrophoretic separation at fixed and narrow pH-range has often been reported, this is not so for the electrophoretic behavior over an extended acid and alkaline range in the same experiment. In this experiment, the pH-values were continuously varied over a range of pH=2.0—13 in the same system. The theoretical mobility-pH curves were calculated by a microcomputer.

In this report, we discuss the differences between the observed and the calculated mobilities of nucleic acid bases on pure cellulose paper (A=adenine, G=guanine, T=thymine, U=uracil, and C=cytosine). The observed mobilities of the bases were found to be in good agreement with the calculated ones in the acid region, but not in the alkaline region.

Reagent and Procedures

The procedures and apparatus used were similar to those described previously.[8)] All the nucleic acid bases (A, G, T, U, C) were purchased from Sigma (St. Louis, Mo., U. S. A.). Sørensen's buffer solutions[9)] were used as a supporting solution: citrate buffer; pH=2.0—6.0, phosphate buffer; pH6.0—8.0, glycine buffer; pH=9.0—13.0. A 5 μl volume of sample solution (10^{-2} M (1 M=1 mol dm^{-3})) was spotted at the center of the filter paper (Toyoroshi, No. 51A, pure cellulose paper) wetted with a supporting solution. The spotting paper was immersed in hexane and a constant voltage (1000 V/30 cm) applied to it for 30 min at 20 °C. The position of the bases on the paper was detected by irradiation with ultraviolet light (253 nm). In order to estimate the electroosmotic flow, glucose was migrated under the same electrophoretic conditions as for the bases. The glucose was detected by spraying with *o*-aminophenol solution.[10)]

Results and Discussion

The observed electrophoretic behaviors of the bases against pH-values are shown in Fig. 1. The migration distances shown were not corrected for the electroosmotic flow which was estimated from the movement of glucose under conditions similar to those of electrophoresis. Thus, the observed migration distances include the movements by the electroosmotic flow. The average electroosmotic flow (-1.0 ± 0.3 cm, pK_i=4.0) in this experiment is shown in Fig. 2. The effects of a capillary action on the migrations are negligible because the sample solutions were spotted at the center of the filter paper.[7)]

Generally, the zone mobility is the weighted mean value of the mobility of each species present in a given solution. In an ideal solution where only C_i and C_{i+1} species are present, the zone mobility U_{zi} is expressed by the Eq. 1

$$U_{zi} = (U_i[C_i] + U_{i+1}[C_{i+1}])/([C_i] + [C_{i+1}]), \quad (1)$$

where U_i and U_{i+1} are the mobility of C_i and C_{i+1} ions, and $[C_i]$ and $[C_{i+1}]$ the concentration of C_i and C_{i+1} ions in a given system. The concentration of each chemical species is related to the dissociation constant (K_i)

$$K_i = [C_{i+1}][H^+]/[C_i]. \quad (2)$$

Then, by substituting Eq. 2 into Eq. 1, we obtain

$$U_{zi} = \frac{U_i + U_{i+1} \cdot K_i/[H^+]}{1 + K_i/[H^+]}. \quad (3)$$

Thus, if we know the values of U_i, U_{i+1}, and K_i respectively, the value of U_{zi} against the pH value will be calculated. The curves in Fig. 2 are drawn by substituting the most suitable values obtained by the actual

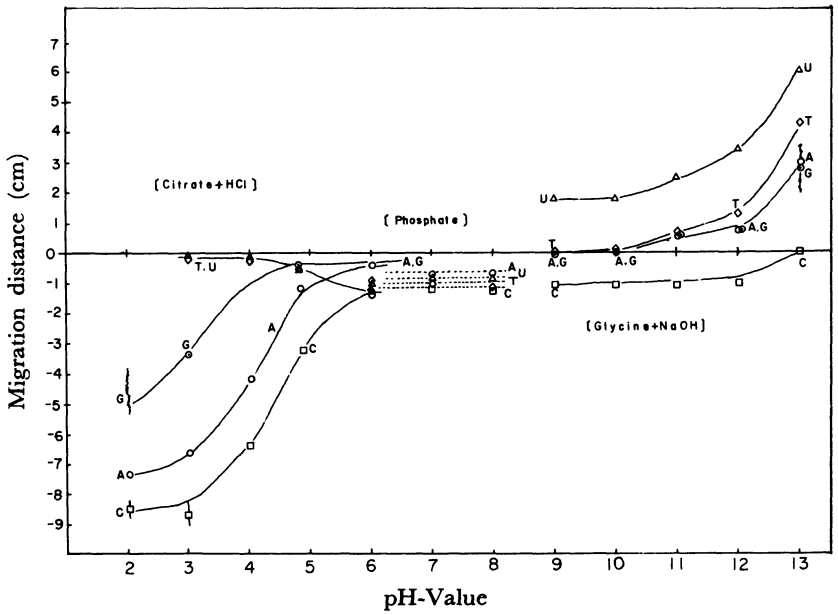

Fig. 1. Observed electrophoretic behaviors of the bases of the nucleic acid against pH-values.
Sample solutions: ○, adenine (A); ⊙, guanine (G); △, uracil (U); ◇, thymine (T); □, cytosine (C). Electrophoresis conditions: 1000 V per 30 cm, 30 min, at 20 °C. Supporting solutions, citric acid buffer (pH=2.0—6.0); phosphoric acid buffer (pH=6.0—8.0); glycine+NaOH buffer (pH=9.0—13.0). Support, pure cellulose paper (Toyoroshi No. 51 A). Spotting position, the centre of a support. Positive movement is towards the anode, negative movement towards the cathode.

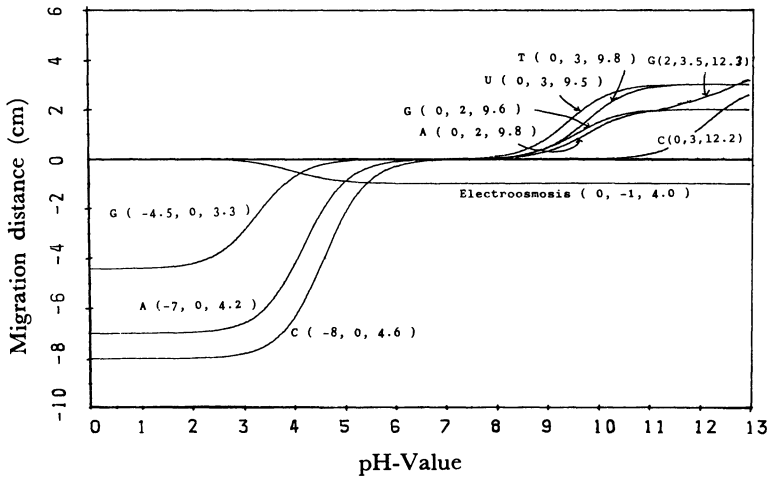

Fig. 2. Calculated migration curves of the bases of the nucleic acid.
Electrophoresis conditions and symbols used are the same as in Fig. 1. The three figures in the parenthesis are migration distances (cm) of C_i, C_{i+1}, and pK_i-value in this order.

experiment for equation 3. The zone mobility-pH curves shown in Fig. 2 are drawn by a microcomputer (Model, NEC PC-9801) equipped with a plotter (Watanabe multi myplot).* The three figures in the parenthesis in Fig. 2 show U_i, U_{i+1}, and pK_i (negative logarithm of K_i) in this order. U_i and U_{i+1} are the mobilities of each species, they varies with the charge, the molecular weight, the hydration, the shape, the size etc. The U_i and U_{i+1} in Fig. 2 were taken from this experiment. The pK_i values were referred to the data[11] which are determined by the chemical methods other than the electrophoresis. The electroosmotic flows were measured by the movement of glucose. The main cause of the flows seems to be due to the carboxyl groups in cellulose paper introduced during the manufacturing of the filter paper.[12] As the inflection point of the electroosmotic flow was estimated to be around pH=4.0 from this experiment, the pK_i value are assumed to be 4.0, and the calculated curve was drawn by the microcomputer. In acid region, movements due to the protonated amino groups of the bases are expected. Three bases (A, G, C) moved towards the cathode while two bases (T, U) having no amino group did not. Compar-

* The computer program is available on application to the author.

ing the actual results with the calculated ones in the acid region, we find a good agreement. In the neutral region, as all the bases have no charge on the molecule, it is assumed that they do not migrate. The observed small migrations of all the bases towards the cathodic side were within the range of the electroosmotic flow. In alkaline region, the dissociation of the hydrogen of the phenolic hydroxyl groups or of the imino group is expected. The p$K$is of the phenolic groups of U, G, and T or of the imino group of A are reported to be within pH=9—10.[11] In this experiment, no inflextion point was found within pH=9—10. Furthermore, the movement of U at pH=9.0 is too large, comparing with that of G or T. In the strongly alkaline region (pH=12—13), the dissociation of the hydrogen atom of the imino groups of G or of phenolic hydroxyl group of C is expected. The high migration distances of U and T may be due to the dissociation (pK>13[13]) of the hydrogen of the second phenolic hydroxy group. Although we tried to select the most appropriate mobility for the anions obtained from actual experiment, agreement between observed and calculated values were never obtained. The inflection points of the experimental curves were not definite and the inflection point seemed to be shifted to a more alkaline region. The data for migration at pH=13 may not be reliable because of the possibility of partial decomposition of the sample and the support. The migration of C in the cathodic side is due to the electroosmosis. The discrepancy between the calculated and observed curves are much large in alkaline region. The cause of the discrepancy seems to be some unknown factors other than electroosmosis and capillary action. Some chemically specific interaction between a migrant and a supporting solution or the structual change of a filter paper in alkaline region might be considered. Further studies on the cause of the discrepancy in alkaline region is needed.

From the chemical isolation point of view, A, G, and C could be separated from T and U in acid region, and U and T could be separated from each other in alkaline region. The neutral region is not good for the separation of any bases.

In conclusin, it is found that the electrophoretic behavior in the acid region could be explained satisfactorily but not in the alkaline region.

References

1) Part XVII of "Study on the separation mechanism of electrophoresis." Part XVI; Lit. 7.
2) J. R. Whitaker, "Paper Chromatography and Electrophoresis," Academic Press, New York, London (1967), Vol. 1.
3) J. D. Smith, in "Nucleic Acid" (E. Chargaff and J. N. Davidson, eds.), Academic Press, New York (1955), Vol. 1, p. 267.
4) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 558 (1952).
5) H. E. Wade and D. M. Morgan, *Biochem. J.*, **60**, 264 (1955).
6) Y. Kiso, M. Kobayashi, Y. Kitaoka, K. Kawamoto, and J. Takada, *J. Chromatogr.*, **35**, 215 (1968).
7) Y. Kitaoka, *Bull. Chem. Soc. Jpn.*, **55**, 2281 (1982).
8) Y. Kitaoka, *J. Chromatogr.*, **252**, 301 (1982).
9) S. P. L. Sørensen, *Erg. Physiol.*, **12**, 393 (1912).
10) V. B. Kupressova, *Izv. Sibirsk Otd. Akad. Nauk SSSR, Ser. Biol. Med. Nauk*, **141** (1965).
11) R. M. C. Dawson, D. C. Elliott, W. H. Elliott and K. M. Jones (Editors), "Data for Biochemical Research," Clarendon Press. Oxford (1959).
12) Y. Kitaoka, *Bull. Chem. Soc. Jpn.*, **54**, 757 (1981).
13) "Handbook of Biochemistry," 2nd edition, ed by H. A. Sober and R. A. Hart, The Chemical Rubber Co., Ohio (1968).