INVESTIGATION OF MIGRATION BEHAVIORS OF PEPTIDES AND PROTEINS BY CAPILLARY ZONE ELECTROPHORESIS

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Abstract In this study, using a single solvent system (30 mM sodium phosphate buffer at four different pH values: 2.0, 5.0, 7.0 and 10.0), we have investigated the migration behaviors for not only small molecules (more than 35 peptides varying in size from 2 to 42 amino acid residues and varying in charge from -1.44 to 6.93) but also large ones (more than 20 proteins varying in molecular weight from 5 kDa to 480 kDa and varying in isoelectric point from 4.1 to 11.0). Though the size (radius) of a molecule has been difficult to evaluate, it showed best linear correlation at pH 2.0 ($r = 0.981$), with respect to $Mw^{2/3}$ as radius of a peptide. Furthermore, it was observed that a good correlation between observed and calculated mobility was only up to approximately 20 kDa, using $Mw^{2/3}$ for R and Z (calculated from the pKa of amino acid residues) at pH 2.0.

Key words capillary, capillary electrophoresis peptide, protein

In the last few years, high performance capillary electrophoresis (HPCE) has attracted much interests among researchers, especially in the field of protein chemistry, because it gives higher separation efficiency, faster separation and high sensitivity for mass detection [1,2] compared with high performance liquid chromatography (HPLC). The mass determination of peptides and proteins using HPCE, however, has not been reported by many workers. One of the reasons is that it is difficult to control and predict the migration behaviors which are complicated by the electrical double layer in the capillary [3], the extent of hydration, and the rigidity of the structure, as in the case of proteins. However, for a given set of solvent condition, the apparent charge of a solute should be proportional to its actual charge [4]. The effect of size and charge on a peptide's electrophoretic mobility has already been explored by P.D. Grossman et al in a single solvent system (20 mM monosodium citrate buffer at pH 2.5) [4]. In this study, using other buffer system (30 mM sodium phosphate buffer at four different pH values: 2.0, 5.0, 7.0 and 10.0), we have investigated migration behaviors for not only small molecules (more than 35 peptides varying in size from 2 to 42 amino acid residues and varying in charge from -1.44 to 6.93) but also large ones (more than 20 proteins varying in molecular weight from 5 kDa to 480 kDa and varying in isoelectric point (pI) from 4.1 to 11.0). In order to relate the electrophoretic mobility of a molecule to its physicochemical property, we used the classical relation:

$$\mu = v / E = Z / 6 \pi R \eta = C \cdot Z / Mw^{2/3} \eta \quad (1)$$

where μ is the electrophoretic mobility, v is the migration velocity, E is the electric field strength, C is a constant of proportionality, Z is the net charge on the molecule, η is the solvent viscosity, R is the apparent Stokes radius of the molecule and $Mw^{2/3}$ which has been often used as evaluation of radius of a molecule, R , is exponential of molecular weight of a molecule. Equation 1 is derived by equating the force exerted by the electric field to that caused by the viscous drag on the particle, assuming a spherical solute particle. The correlation coefficients between calculated mobility (calculation from molecular weight and amino acid residues) and observed mobility will be discussed.

EXPERIMENTAL

Apparatus The apparatus used was a JASCO CE-800 capillary electrophoresis system (modular type), consisting of 890-CE (regulated DC high-voltage power supply) 870-CE (UV/VIS detector) and separation unit [5].

Proteins and peptides All proteins (insulin, albumin, ovalbumin, lysozyme, trypsin inhibitor, trypsin, trypsinogen, α -chymotrypsinogen, transferrin, cytochrome c, β -lactoglobulin, collagenase, papain, α -amylase, catalase, ribonuclease A, myoglobin, thermolysin, calmodulin and hemoglobin) were purchased from Wako Pure Chemicals (Osaka, Japan) or Sigma Chemicals (St. Louis, MO, U.S.A.). Eighteen peptides were obtained by the tryptic degradation of calmodulin and hemoglobin (α -chain) [6]. All other peptides (substance P, ACTH(4-10), ACTH(11-24), [Leu⁵]-enkephalin, [Ala² Met⁵]-enkephalinamide, angiotensin II, [Ile⁵]-angiotensin II, angiotensin I, TRH, vasopressin, carnosine, Gly-Gly, Gly-Ala, Ala-Ala, Ala-phe, Gly-Gly-Gly, Trp-Gly, Tyr-Ala and Tyr-Phe) were purchased from Wako Pure Chemicals (Osaka, Japan) and Sigma Chemicals (St. Louis, MO, U.S.A.). Solvents for samples were water, and in the case of tryptic digest, 10-50 % acetonitrile in 0.1 % trifluoroacetic acid (pH 2).

Electrophoretic condition Untreated fused silica capillary (50 μ m I.D. x 30 cm in effective length, 50 cm in total length, was used as separation tube. Electrolyte was 30 mM sodium phosphate buffer (pH 2, 5, 7 and 10) with detection at 200 nm. Applied voltage was 300 V/cm, with forced air control at room temp. Injection method was by siphoning for 10 sec (10-cm height, about 3 nl).

RESULTS AND DISCUSSION

The apparent electrophoretic mobility μ_e can be expressed as:

$$v = (\mu_e + \mu_o)E \quad (2)$$

where μ_o is the apparent mobility created by electroosmotic flow. The mobility, μ_o was estimated by measuring of the mobility of non-ionic substance, methanol. The resulting data, μ_e was compared with the calculated mobility, μ which can be determined from the molecular weight, Mw, the net charge, Z and the viscosity of electrolyte, η using the following equation.

$$v = C \cdot ZE / Mw^x \eta \quad (3)$$

where Mw^x is the radius of a peptide, which should be logically in proportion to $Mw^{1/3}$. The net charge, Z, was calculated from the pKa of the amino acid side residues. In the case of proteins, in addition, pI value of a protein was used in substitution for the net charge, Z, because we have often met with inconsistency for calculated net charges. In the case of peptides, at pH 2.0, the plot of relation between μ_e and μ for 1/3, 1/2, 2/3 and 1 values of x where the Mw^x was used to estimate μ was shown in Fig. 1. Assuming $Mw^{2/3}$ it showed a best linear correlation ($r = 0.981$).

Fig. 1 Plot of relation between observed mobility, μ_e and calculated mobility μ ($\propto Z / Mw^x$) for various values of exponential x for Mw^x . The electrolyte used was pH 2.0. (1) $x = 1/3$, (2) $x = 1/2$, (3) $x = 2/3$ and (4) $x = 1$. The correlation coefficients were (1) $r = 0.814$, (2) $r = 0.935$, (3) $r = 0.981$ and (4) $r = 0.676$, respectively.

Furthermore, at the other pH, the plot of correlation coefficients between observed mobility and calculated mobility vs the exponential x of Mw^x in Fig. 2.

Fig.2. Plot of correlation coefficients between observed mobility and calculated mobility vs. the exponential x of Mw^x . The electrolyte used was pH 2.0, 5.0, 7.0 and 10.0.

In acidic and basic region, the correlation coefficients were higher than in neutral on the whole. It is possible that the charge associated with silanol group on the inside wall surface of capillary tube under acidic or basic conditions is more stable than under neutral conditions. In the case of proteins, the plot of correlation coefficients between observed mobility and calculated mobility vs. the exponential x of Mw^x , was shown in Fig. 3-A, where the net charge was calculated from the amino acid residues, and in Fig. 3-B, the net charge was substituted by pI values.

Fig. 3. Plot of correlation coefficient between observed and calculated mobility vs. the exponential x for Mw^x of proteins. (A) The net charge was calculated from amino acid residues (B) The net charge was substituted by pI values.

At pH 5.0, most of proteins did not migrate through the capillary. It is considered that interactions between proteins and silanol group on capillary were probably stronger under this condition. The net charge, Z offered better correlation with Mw^x than the pI value on the whole. Compared with peptides, however, the both correlation were lower because the values for net charge and size (radius) were difficult to evaluate in the case of proteins. (Furthermore, to be exact, one should use not only net charge and radius but also its shape and its rigidity as parameters in the case of large molecules.) Though we have often used the logarithm of Mw for R , the radius in neutral pH, $Mw^{2/3}$ offered better correlation than $\log Mw$ at pH 2.0 both in peptides ($r = 0.602$ ($\log Mw$), 0.981 ($Mw^{2/3}$)) and proteins ($r = 0.290$ ($\log Mw$), 0.338 ($Mw^{2/3}$)). But at pH 7.0, the correlation coefficients were similar in values for both peptides ($r = 0.764$ for $\log Mw$, 0.792 for $Mw^{2/3}$) and proteins ($r = 0.803$ for $\log Mw$, 0.827 for $Mw^{2/3}$). Furthermore, we observed that μ_e and μ showed good correlation for proteins with molecular weight up to 20 kDa, when μ was calculated using $2/3$ as the exponential for molecular weight and Z from the amino acid residues in equation 1 (Fig. 4)

Fig. 4. Changes in the correlation coefficients between observed mobility and calculated mobility. The numbers of molecular weight on the horizontal axis indicate the most molecular weight among proteins used for calculation. The electrolyte used was pH 2.0.

In conclusion, in the case of smaller peptides or proteins below about Mw 20 kDa, the migration behavior can be predicted at low pH values of the electrolyte using the following equation:

$$Mt = C \cdot Mw^{2/3} L / ZE \quad (4)$$

where Mt is the migration time, C is a constant of proportionality and L is the effective length of capillary. If one wish to obtain better measurement of molecular weight of proteins, using electrophoretic mobility in a capillary column, different parameters for proteins need to be determined.

REFERENCES

1. B.L.Kager, A.S.Cohen and A.Guttman, *J.Chromatogr.* **492**, 585-614(1989)
2. B.L.Hogan and E.S.Yeung, *J.Chromatogr. Sci.* **28**, 15-18(1990).
3. P.C.Hiemenz, *Principles of Colloid and Surface Chemistry*, Chap.11 Dekker, New York, 1977
4. P.D.Grossman, J.C.Colburn and H.H.Lauer, *Anal.Biochem.*, **179**, 28-33(1989).
5. Y.Kurosui, K.Hibi, T.Sasaki and M.Saito, *HRC*, **14**, 200-203(1991)
6. H.Kawasaki, Y.Kurosui, T.Isobe, H.Kasai and T.Okuyama, *Anal.Sci.* **2**, 287-291(1986)