Short Communications

The Electrophoretic Mobility of Peptides on Paper at pH 1.9

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The relationship between the electrophoretic mobility, the molecular weight and the charge of peptides at pH 1.9 was re-investigated. It was shown that the relationship at pH 1.9 is quantitatively similar to that which holds at pH 6.5.

Because of the theoretical complexity of electrophoretic processes there have been few attempts to relate electrophoretic mobility of molecules quantitatively to the properties of mass, charge and size. An exception to this statement is provided by the work of Offord (1966), who showed that relative electrophoretic mobilities (m) of peptides, their charges (ϵ) and molecular weights (M) fitted the relationship:

$$m = k\epsilon M^{-3} \quad (1)$$

where k is a constant. The relationship was derived from measurements of electrophoretic mobility relative to aspartic acid in a pyridine–acetate buffer at pH 6.5. It has been widely used to detect amide groups in peptides and is therefore extensively verified. A similar relationship was found for peptide mobility at pH 1.9, but the exponent of the molecular-weight term was approx. -0.44 . This relationship is less useful than that at pH 6.5 and it has therefore not been extensively used. Nevertheless the relationship at pH 1.9 is particularly useful for checking molecular weights of peptides produced in sequence or synthetic studies.

The theoretical background of the relationships was discussed by Offord (1966), but differences in the molecular-weight exponent at the different pH values inhibit a satisfactory explanation. The present work started when some of our observations could not be fitted to the relationship at pH 1.9, although that at pH 6.5 was satisfactory. Re-investigation has shown that the relationships at pH 1.9 and pH 6.5 are quite similar, the molecular-weight exponent being -0.62 at pH 1.9 compared with the value of close to -0.67 at pH 6.5 claimed by Offord (1966).

Two independent sets of measurements have been made. In one of these the peptides were prepared from standard proteins: tryptic peptides of lysozyme were purified by ion-exchange chromatography and high-

voltage electrophoresis at pH 1.9; tryptic peptides of insulin, pepsin and glucagon, and chymotryptic peptides of insulin, were separated by high-voltage electrophoresis at pH 1.9; chymotryptic peptides of lysozyme were separated by high-voltage electrophoresis at pH 1.9 followed by paper chromatography in butan-1-ol–acetic acid–pyridine–water (15:3:12:10, by vol.) The peptides were characterized by specific stains (Easley, 1965) and by quantitative amino acid analysis. In all cases amino acid compositions of the peptides corresponded to part of the known amino acid sequence of the standard proteins and in most cases these peptides had been found during the definitive sequence studies. This set of peptides was completed by a number of commercially prepared dipeptides, all of which were electrophoretically pure. Mobilities of this set of peptides were measured after electrophoresis at pH 1.9 in formic acid–acetic acid–water (9:12:179, by vol.). Electrophoresis at 50 V/cm was carried out in a white-spirit-cooled Gilson model D Electrophorator for 1–2 h.

The other set of data was of peptides obtained by either tryptic and chymotryptic digestion of *Ginkgo biloba* cytochrome *c* (Ramshaw *et al.*, 1971), or by paper electrophoresis of thermolytic digests of plant plastocyanin (J. A. M. Ramshaw, M. D. Scawen, C. J. Bailey & D. Boulter, unpublished work). These peptides have been characterized by amino acid composition and sequence. Mobilities of this set of peptides were measured after electrophoresis at pH 1.9 in formic acid–acetic acid–water (1:4:45, by vol.) by using Locarte cooled-plate electrophoresis equipment operated at 90 V/cm for 1–3 h.

All the measurements were made with Whatman 3MM paper as a support. Measurements were made from the centre of the Dns-OH neutral marker to the front of the Dns-Arg reference and to the front of the ninhydrin-positive sample position.

Serine, the substance used by Offord (1966), has some disadvantages as a reference standard for peptide electrophoresis at pH1.9. The pK of its carboxylate group is very close to the pH of the buffer and thus its mobility must be very sensitive to the slight changes in buffer conditions that can occur in experiments over a lengthy period. Secondly, detection of both peptide sample and serine reference by the ninhydrin reaction is potentially confusing. For these reasons we have preferred to use the dansyl marker mixture of Brown & Hartley (1966). The mixture can also be used at pH6.5. It can be shown readily that use of different electrophoretic references causes few problems in interpretation. The mobility of one standard relative to another provides a factor for interconversion of sample measurement. In agreement with this statement, measurements relative to Dns-OH at pH6.5 can be used with the Offord (1966) chart of that pH relative to aspartic acid, after multiplication by a constant. In terms of eqn. (2), the reference marker contributes only to the intercept of a $\log m$ - $\log M$ plot. However, when our mobilities at pH1.9 relative to Dns-Arg were corrected to mobilities relative to serine, the results would not fit the Offord (1966) pH1.9 chart.

Our data are shown in Fig. 1. The lines are those calculated by unweighted least-squares fit of the data to eqn. (2), which is an alternate expression of eqn. (1):

$$\log m - \log \epsilon = -s \cdot \log M + k' \quad (2)$$

The calculated parameters for the total data expressed as means $\pm$ standard errors, for the numbers of observations in parentheses, are:

$$s = -0.622 \pm 0.009 \quad (104)$$

$$k' = 1.31 \pm 0.02 \quad (104)$$

Two sets of data were combined to give the above result. The separated values are as follows.

Solvent-cooled electrophoresis, formic acid - acetic acid-water (9:12:179, by vol.), pH1.9:

$$s = -0.626 \pm 0.011 \quad (53)$$

$$k' = 1.31 \pm 0.03 \quad (53)$$

Cooled-plate electrophoresis, formic acid - acetic acid-water (1:4:45, by vol.), pH1.9:

$$s = -0.604 \pm 0.008 \quad (51)$$

$$k' = 1.28 \pm 0.02 \quad (51)$$

The agreement between the two sets of data is sufficient to allow the presentation of combined data and shows that mobilities at pH1.9 can only be slightly sensitive to buffer composition and experimental arrangement.

Fig. 1. *Logarithmic plot of electrophoretic mobility at pH1.9 versus molecular weight*

■, Data obtained from the solvent-cooled system; ●, data obtained from the plate-cooled system. The lines drawn are those calculated from the best fit of all the data to eqn. (2). Large copies of Fig. 1 may be obtained from the authors.

The slope of our pH1.9 plot is very different from the value of about -0.44 for Offord's (1966) pH1.9 plot but quite close to the claimed slope of the Offord (1966) pH6.5 plot. The discrepancy between the values at pH1.9 can be explained by the treatment of electroendosmotic corrections. Our values are corrected for this effect by use of the neutral marker. Offord (1966) makes no mention of a correction of the data for pH1.9, although that for pH6.5 is corrected. It can be shown that neglect of the effect distorts the linear relationship between $\log m$ and $\log M$ and decreases the apparent slope of the $\log$ - $\log$ plot. A correction is also practically necessary because electroendosmosis can be variable and because some migration of the sample zone can occur when buffer is added to the paper support. The electroendosmotic origin is marked by Dns-OH in our work. Urea and the products of a lysozyme digest of glycol chitin (Senju & Okimasu, 1950) ran coincidentally with Dns-OH, confirming that the latter is a true neutral-position marker.

It thus appears that the molecular-weight exponent of eqn. (1) is about $-\frac{2}{3}$ for peptide electrophoresis at pH1.9 and pH6.5. We have extracted the Offord (1966) data for pH6.5 from graphs and fitted them to eqn. (2). The calculated values are:

$$s = -0.693 \pm 0.012 \quad (106)$$

$$k' = 1.48 \pm 0.03 \quad (106)$$

Dns-peptides also follow a similar relationship; Gray (1967) states that a $\log m$ - $\log M$ plot has a

slope of -0.6 . Thus none of the data fits an exact value of -0.67 .

Why do the slope terms fall within the range $0.6-0.7$? Offord (1966) outlines a theory appropriate to electrophoresis in insulating media, which leads to a value of -0.33 . A value of -0.67 can be derived by approximation of Henry's law, which applies to electrophoresis in electrolytes. It is hoped that this communication, which illustrates that a similar relationship holds for peptide electrophoresis at different pH values, will lead to more useful theory.

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