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Oligoglycines and oligoalanines as tests for modelling mobility of peptides in capillary electrophoresis

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ABSTRACT

Peptides in homologous series of oligoglycines and oligoalanines with degree of polymerisation n = 2 to 6 are baseline separated in 20 mM citric acid-lithium citrate buffers in the pH range 2.51 to 3.02. Mobilities determined as a function of pH allow calculation of the mobility μ_+ for the fully protonated peptide. Values of μ_+ for oligoglycines are systematically 0.6 $\cdot 10^{-4}$ cm^2 V^{-1} s^{-1} higher than those for oligoalanines, consistent with the 40% greater partial molar volume of alanine than glycine. Since these mobilities are those of peptides with a constant charge q = 1, semi-empirical models can be systematically tested for their predictions on how peptide mobilities vary with chain length or molecular mass. Within each homologous series the equation introduced by Grossman et al. *[Anal. Biochem., 179 (1989) 28]* for scaling peptide mobility with chain length, $\mu^* \propto n^{-0.43}$, accounts for variation in μ_+ with excellent correlation coefficients ($r > 0.996$). Scaling $\mu^* \propto M_r^{-2-3}$ according to Offord *[Nature,* 211 (1966) 591] also gives excellent correlation coefficients ($r > 0.996$). Since a wide range of exponents can be used to describe the data with good precision, no scaling relationship can be considered uniquely suitable for peptide mobility modelling.

INTRODUCTION

Capillary electrophoresis (CE) is an established electroseparation technique used in the analysis of a wide range of compounds including peptides and proteins [1,2]. To make use of the fast, high efficiency separations the technique has to offer, optimum conditions need to be developed rapidly. Two models have been proposed to account for the variation of peptide mobility with size and charge. A semi-empirical model developed by Grossman *et al.* [3], using as a starting point Stokes' law and the treatment of a peptide as a freely jointed chain, was apllied to 40 synthetic peptides varying in size between 3 and 39 amino acids and in calculated charge, q, between 0.3 and 14.0. The second model was originally introduced by Offord [4]. From paper electrophoretic data on more than 100 peptides mobility was shown to scale with $q/M_r^{2/3}$, where M_r is the molecular mass, suggesting that frictional drag is proportional to the surface area of the peptide treated as a spherical molecule. This has been successfully used in CE by several groups [5–7].

The present study tests the applicability of these generalised models to oligoglycines and oligoalanines, well characterised homologous series of peptides for which mobilities can be determined at a fixed charge q = 1 using known pK_a values [8].

EXPERIMENTAL

Reagents and chemicals

Compounds used were oligoglycine and oligoalanine di-, tri-, tetra-, penta-, hexapeptides, sodium hydroxide, citric acid (Sigma, Poole, UK), lithium hydroxide (Fisons, Loughborough, UK) and mesityl

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oxide (Aldrich, Dorset, UK). Solutions of all compounds were prepared in deionised water (Elgastat UHQ, High Wycombe, UK) at a concentration of 1 mg ml-' with mesityl oxide as neutral marker. When injected as mixtures concentrations were normally 0.1 mg ml-' of each component. Citrate buffer was prepared from 20 m*M* citric acid and adjusted to a range of pH values (from 2.5 1 to 4.15) using 2 A4 lithium hydroxide. pH was measured using a Ross combination electrode (Orion Research, East Sussex, UK) an pH meter (Corning ion analyzer 150, Ciba Corning, Halsted, UK) standardised with phthalate and phosphate buffers at pH 4.01 and 6.97, respectively. All samples and buffers were filtered through a 0.2-μm filter (Millipore, Watford, UK) prior to analysis.

Electrophoretic conditions

All experiments were performed using an automated CE apparatus (Beckman P/ACE with System Gold software, Beckman, High Wycombe, UK) thermostatted at 25°C, with UV absorbance detection at 200 nm. The capillary (Composite Metal Services, Hallow, UK) was conditioned using 0.1 *M* NaOH and background electrolyte prior to analysis. Samples were injected (2.2 nl) for 1 s under vacuum onto a 27 cm x 50 μm I.D. capillary and separated using an applied potential of 7.5 kV. Operating current was 4-6 μA, dependent on pH.

RESULTS AND DISCUSSION

Electrophoretic mobilities of fully protonated peptides

Fig. 1 shows typical separations achieved when the oligoglycines and oligoalanines were injected as mixtures in a 20 m*M* citric acid-lithium citrate buffer. Lithium was chosen as the cation in the background electrolyte because of its low mobility. This ensures buffer compatibility with analyte mobility to minimise peak distortion and a low current to minimise heating. Detection of monoglycine and monoalanine using UV detection at 200 nm was not possible because of the lack of a peptide chromophore. Baseline resolution for all analytes was obtained in the pH range 2.51 to 3.02. The advantage of using a short capillary (27 cm) in these experiments at low pH is that the migration time of the neutral marker is reasonably short, a maximum run time of 15 min being required at the lowest pH of 2.51.

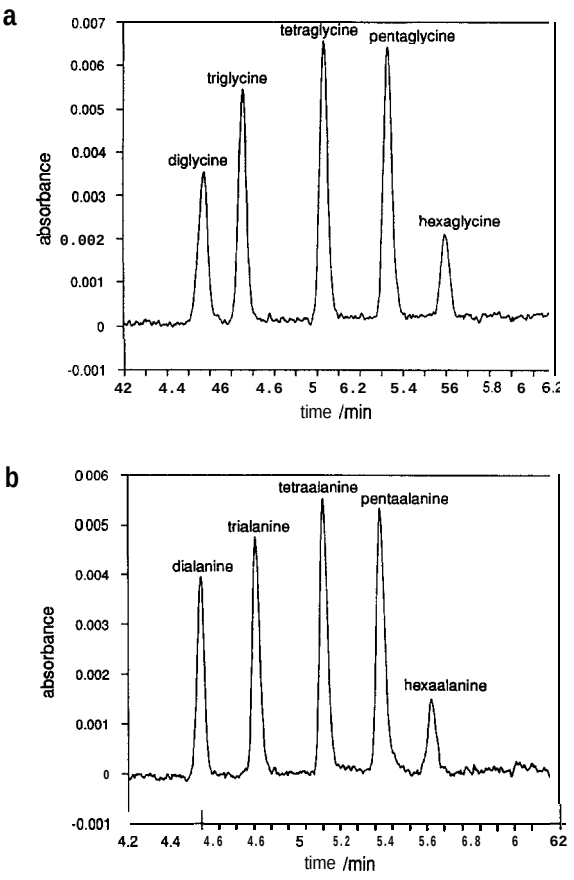

Fig. 1. Separation of (a) oligoglycines and (b) oligoalanines by CE. Buffer: 20 m*M* citrate, pH 3.02; order of elution: di-, tri-, tetra-, penta-, hexapeptides, neutral marker; neutral marker: mesityl oxide; capillary: 27 cm ×50 μm I.D.; current: 5 μA; applied potential: 7.5 kV; absorbance detection at 200 nm; sample concentration: 0.1 mg ml^{-1}.

From migration times of neutral marker and analyte injected as single components electrophoretic mobilities, μ, were determined [I] for each peptide at six pH values: 2.51, 2.75, 3.02, 3.54, 3.79 and 4.15, respectively. Degrees of dissociation, α, were calculated from pK_a values given in the literature [8] (Table I) using the equation [9]

$$\alpha = \frac{1}{10^{pK_a - pH} + 1} \quad (1)$$

and average charge, q, from

$$q = 1 - \alpha \quad (2)$$

TABLE I

p*K* DATA FOR MODEL COMPOUNDS [8]

Peptide	pK_1	pK_2
Diglycine	3.12	8.17
Triglycine	3.26	7.91
Tetraglycine	3.05	7.75
Pentaglycine	3.05	7.70
Hexaglycine	3.05	7.60
Dialanine	3.30	8.14
Trialanine	3.39	8.03
Tetraalanine	3.42	7.94
Pentaalanine	3.42[a]	7.94[a]
Hexaalanine	3.42	7.94[a]

[a] Estimated using tetraalanine values.

Mobilities at unit charge, μ_+, were determined by a linear regression analysis of μ *versus* q

$$\mu = a + \mu_+ q \quad (3)$$

Fig. 2 for diglycine is a typical plot of mobility as a function of average charge. Mobility is seen to be directly proportional to the calculated charge, and there is excellent correlation between mobility and charge. This allows precise determination of μ_+, the mobility of the fully protonated peptide, which has q = 1. Table II gives values for μ_+ and correlation coeffkients from eqn. 3 for all peptides. Within the 95% confidence limits intercepts are zero and the gradient may be equated with the mobility of the fully protonated peptide. Results in Table II show that oligoglycines have systematically higher mobilities (by approximately $0.6 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}) than oligoalanines of the same degree of polymerisation. This is consistent with the greater volume of the peptide carrying methyl side chains rather than hydrogen. Alanine and glycine have partial molar volumes 60.6. 10^{-6} and 43.5. 10^{-6} m^3 mol^{-1}, respectively [10].

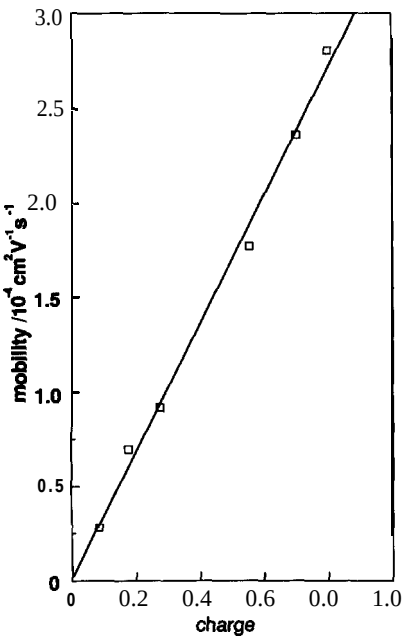

Fig. 2. Plot of mobility against charge for diglycine to determine μ_+.

Testing of models

As suggested by Grossman *et al.* [3], a plot of ln μ_+ versus ln n was obtained to establish the way μ_+ scales with n, the degree of polymerisation. Correlation coeffkients of >0.995 were obtained (Fig. 3) and slopes were -0.42 (oligoglycines) and -0.55 (oligoalanines). In the previous study using the same experimental conditions (20 m*M* citrate, pH 2.50) with a much more diverse range of peptides, slopes for six sets of peptides of approximately the same charge were in the range -0.32 to -0.54 with an average of -0.43 ± 0.08 [3]. The present data set therefore shows good agreement with the slope obtained in the study by Grossman *et al.* [3].

Data for the oligoglycines and oligoalanines are

TABLE II

SUMMARY OF PEPTIDE MOBILITY DATA, μ_+, AND CORRELATION COEFFICIENTS IN 20 m*M* CITRATE BUFFER

Peptide	μ_+ ($\cdot 10^{-4}$ cm^2 V^{-1} s^{-1})	r
Diglycine	3.37 ± 0.13	0.9968
Triglycine	2.81 ± 0.10	0.9974
Tetraglycine	2.57 ± 0.12	0.9957
Pentaglycine	2.33 ± 0.12	0.9947
Hexaglycine	2.08 ± 0.13	0.9924
Dialanine	2.87 ± 0.20	0.9907
Trialanine	2.26 ± 0.07	0.9982
Tetraalanine	1.94 ± 0.06	0.9978
Pentaalanine	1.74 ± 0.07	0.9964
Hexaalanine	1.55f0.08	0.9946

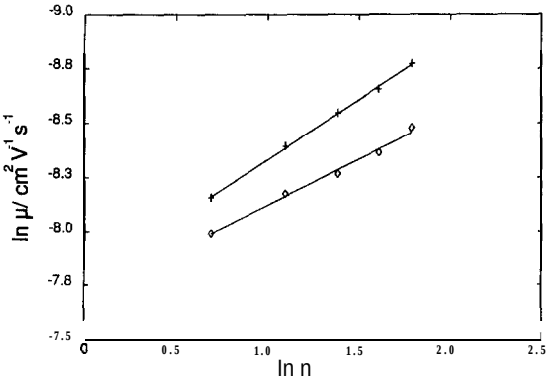

Fig. 3. Effect of oligopeptide size on μ_+. (+) Oligoalanines, $y = -(0.55 \pm 0.01)x - (7.78 \pm 0.01)$, $r = 0.999$; (0) oligoglycines, $y = -(0.42 \pm 0.02)x - (7.70 \pm 0.02)$, $r = 0.995$.

superimposed in Fig. 4 upon the regression line from the data set of 40 synthetic peptides reported by Grossman et al. [3]. The correlation developed in previous work used peptides with both neutral and positively charged side chains, R, and incomplete protonation of the C terminal carboxylate group. In the present study using oligopeptides R is either H (oligoglycines) or Me (oligoalanines), and experiments over the pH range of the COOH ionisation have allowed mobilities to be compared at a well defined charge of $q = 1$. Fig. 4 illustrates both the general utility and the limitations of a correlation of peptides of diverse type. The utility is that the present data lie within the 95% confidence limits of the Grossman data set. The limitation is that the 95% confidence limits are wide, allowing mobility to be specified only to $\pm 0.3 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} in our oligopeptide range. In contrast, the 95% confidence limits within each of the homologous series are $\pm 0.2 \cdot 10^{-4}$ for oligoglycines and $\pm 0.1 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} for oligoalanines in the middle of the ranges.

Rickard *et al.* [5] have obtained impressive correlations between free solution peptide mobilities using the Offord analysis [4]. Excellent fits are also obtained on scaling the present data for oligoglycines and oligoalanines in the form $\mu \propto M_r^{-2/3}$, the Offord correlation. Fig. 5 shows mobilities for oligoglycines, oligoalanines and for peptides studied by Grossman *et al.* [3] as a function of $q/M_r^{2/3}$. Because of the scaling with molecular mass rather than degree of polymerisation, oligopeptide data are grouped more closely in Fig. 5 than in Fig. 4. However, differences between the two series are still outside the limits of experimental error and results are systematically lower than the Grossman *et al.* [3] data.

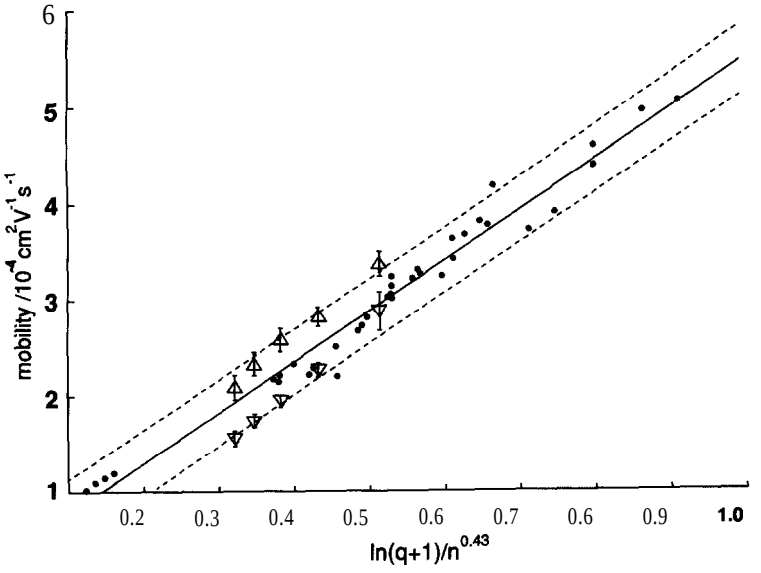

Fig. 4. Peptide mobilities μ_+ superimposed upon regression line (solid line) and 95% confidence limits (dotted lines) for data set reported by Grossman *et al.* [3]. ● = Original; △ = glycines; ▽ = alanines.

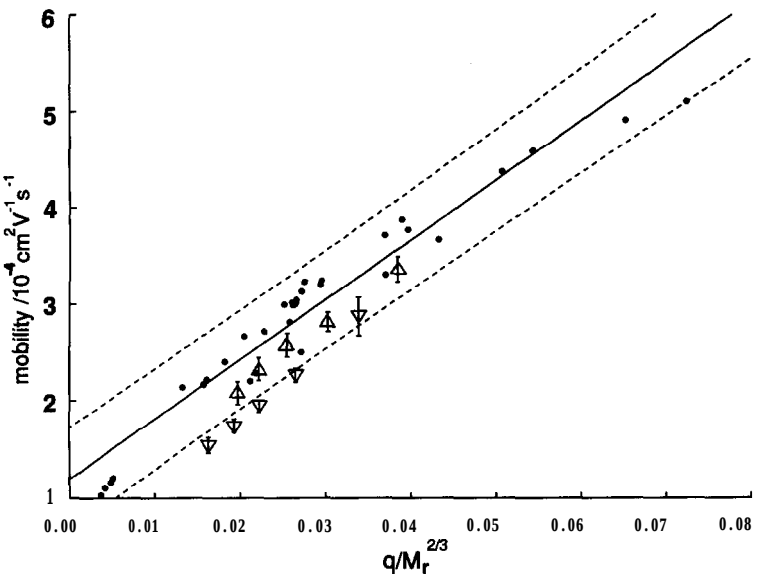

Fig. 5. Peptide mobilities μ_+ superimposed upon regression line (solid line) and 95% confidence limits (dotted lines) for Grossman et *al.* [3] data set converted to Offord [4] form. Symbols as in Fig. 4.

The effect of temperature should be considered. Previous peptide studies were carried out at 30°C, whereas present work was carried out at 25°C. Mobilities adjusted to 25°C would be lower than at 30°C by approximately 10%, since μ varies with $1/\eta$, the reciprocal viscosity, and η decreases by 2% per °C. Adjustment of the Grossman data in Fig. 5 to compensate for temperature differences would improve the tit in the Offord correlation.

Charges for the Grossman data set were calculated from a knowledge of the peptide sequence by a modification of a program devised by Skoog and Wichman [9]. When μ is scaled against the Offord parameter (Fig. 5), wider confidence limits are seen in comparison to scaling μ against the Grossman parameter. This shows the need to have an accurate measure of charge determined by experiment in order to obtain a better fit for the Offord relation.

CONCLUSIONS

CE has been used to demonstrate the baseline separation of oligoalanines and oligoglycines with degree of polymerisation n = 2 to 6 in 20 m*M* lithium citrate buffers between pH 2.51 and 3.02 at 30°C. When the oligopeptides were injected as single components with neutral marker in the pH range 2.51 to 4.15, extrapolation of calculated charge to unity, $q = 1$, enabled determination of mobility of the cationic peptide, μ_+. The mobilities for oligoglycines were found to be 0.6 $cm^2 V^{-1} s^{-1}$ higher than for oligoalanines, consistent with the 40% greater partial molar volume of alanine compared to glycine. These mobilities, μ_+ were fitted to semi-empirical models relating $\mu + a\, n^{-0.43}$ and $\mu_+ \propto M_r^{-0.67}$. Results obtained for oligopeptides were found to be in satisfactory agreement with previous correlations using a more diverse set of peptides.

It should be noted that the goodness of fit to the scaling function is relatively insensitive to the value of the exponent. Since a wide range of powers can be used to describe the data with good precision, and apparent optimum values are found to be different for the oligoglycine and oligoalanine series, none of the proposed scaling functions can be considered to be uniquely suitable for peptide mobility modelling.

At the present time extrapolation of any model to larger peptides is unwarranted. Further experimental work is in progress with longer chain oligoglycines in order to refine the correlations and narrow the range of acceptable exponents.

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