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Do zwitterions contribute to the ionic strength of a solution?

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Abstract

Capillary electrophoresis has been used to determine whether zwitterions contribute to the ionic strength of a solution, by measuring the mobility of a double-stranded DNA oligomer in cacodylate-buffered solutions containing various concentrations of the ionic salt tetraethylammonium chloride (TEA^+Cl^-) or the zwitterion tricine^{+/-}. The mobility of the DNA decreased as the square root of ionic strength, as expected by the Debye-Hückel-Onsager theory of electrophoresis, when TEA^+Cl^- was added to the buffer. However, the mobility was independent of the concentration of added tricine^{+/-}. Hence, zwitterions do not contribute to the ionic strength of a solution.

Keywords

Zwitterions; ionic strength; DNA; capillary electrophoresis

The question of whether zwitterions contribute to the ionic strength of a solution has been a topic of confusion for many years. Some authors have assumed that zwitterions do not contribute to ionic strength [*e.g.*, ^{1–3}]. Other authors have assumed that zwitterions *do* contribute to ionic strength [⁴], acting as monoanionic ions [⁵], 1:1 electrolytes [⁶], or molecules with a fractional electronic charge [^{2,7,8}].

Very few experiments have been designed to directly test whether zwitterions contribute to the ionic strength of a solution. However, capillary electrophoresis measurements in free solution are ideal for this purpose. If an analyte, such as a small DNA oligomer, does not bind one of the ions in the background electrolyte, its electrophoretic mobility will decrease as the square root of ionic strength [⁹], as predicted by Debye-Hückel-Onsager theory of electrophoresis [¹⁰], regardless of whether the ionic strength is increased by increasing the buffer concentration or by adding a neutral salt to the buffer. The same result would be expected upon adding a zwitterion to the buffer, if zwitterions contribute to the ionic strength. By contrast, if a zwitterion does not contribute to the ionic strength (and does not bind to the analyte), the observed mobility will be independent of the concentration of the added zwitterion.

Capillary electrophoresis experiments were therefore carried out using a random-sequence, 26 base-pair double-stranded DNA oligomer with the sequence 5'-CGCTTAGTAGATACTACTAGTAG-3', called ds26 for brevity, as the analyte. The duplex, which was prepared by standard methods from single-stranded oligomers synthesized by Integrated DNA Technologies (Coralville, IA), was monodisperse when analyzed by polyacrylamide gel electrophoresis. The capillary zone electrophoresis experiments were

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carried out with a Beckman Coulter P/ACE MDQ Capillary Electrophoresis System (Fullerton, CA), run in the reverse polarity mode (anode on the detector side) with UV detection at 254 nm, as described previously [9,11]. Coated capillaries were used to minimize the electroosmotic flow of the solvent; the current in the capillary was kept below 30 μ A to prevent Joule heating. The temperature of all experiments was 20.0°C. The observed mobilities were reproducible within $\pm 0.4\%$.

The background electrolyte was 0.08 M cacodylate buffer [0.08 M cacodylate acid, $(\text{CH}_3)_2\text{As}(\text{O})\text{OH}$, titrated to its pK_a of 6.27 with tetraethylammonium hydroxide, $(\text{CH}_3\text{CH}_2)_4\text{NOH}$]. To this buffer, various concentrations of the ionic salt tetraethylammonium chloride (TEA^+Cl^- , $(\text{CH}_3\text{CH}_2)_4\text{NCl}$) or the zwitterion tricine (N-[tris-(hydroxymethyl)methyl]glycine, $(\text{HOCH}_2)_3\text{CNHCH}_2\text{CO}_2\text{H}$) were added. At the pH of 6.27 used for the experiments, tricine (carboxylate $\text{pK}_a = 2.3$, amino $\text{pK}_a = 8.15$ [12]) is essentially completely in its zwitterionic form. It is designated tricine $^{+/-}$ to indicate that it has zero net charge. TEA^+Cl^- and tricine $^{+/-}$ were chosen as the test molecules for the experiments because they are similar in size and have similar branched structures.

Since relatively high concentrations of TEA^+Cl^- and tricine $^{+/-}$ were used in some of the experiments, the increased viscosity of solutions containing high concentrations of added salt would have decreased the observed mobilities [10,13]. Therefore, the mobilities observed for ds26 in solutions containing added TEA^+Cl^- or tricine $^{+/-}$ were corrected to the mobility that would have been observed if the solutions had had the viscosity of 0.08 M cacodylate buffer alone, using Eq. (1):

$$\mu_{\text{DNA,corr}} = \mu_{\text{DNA,obsd}} \times \eta_{\text{rel}} \quad (1)$$

where $\mu_{\text{DNA,corr}}$ is the viscosity-corrected mobility of ds26, $\mu_{\text{DNA,obsd}}$ is the observed mobility in the buffer/salt solutions, and η_{rel} is $\eta_{\text{soln}}/\eta_{\text{buf}}$. For solutions with added TEA^+Cl^- , η_{rel} was calculated from the B coefficients given for TEA^+ and Cl^- by Marcus [14]. For the tricine $^{+/-}$ -containing solutions, η_{rel} was estimated from the viscosity [15] of solutions containing an equimolar concentration of uncharged Tris base ($(\text{HOCH}_2)_3\text{CNH}_2$), which has a branched structure similar to that of tricine $^{+/-}$ [16]. It is well known that the concentration dependence of the viscosity of an electrolyte solution is determined primarily by the size and shape of the electrolyte [14,17]. The values of η_{rel} calculated for solutions containing equal concentrations of tricine $^{+/-}$ or TEA^+Cl^- were approximately equal, as expected from the similarity of their structures.

The dependence of the conductivity of cacodylate buffer solutions containing TEA^+Cl^- or tricine $^{+/-}$ on the concentration of the added salt is indicated in Fig. 1. The observed conductivities, Λ , normalized to the conductivity observed in 0.08M cacodylate buffer, Λ_0 , are plotted as a function of the square root of the total concentration of ions in the solution, $C^{0.5}$. The relative conductivities of cacodylate buffer solutions containing added TEA^+Cl^- increased linearly with $C^{0.5}$, as expected for solutions of strong electrolytes [18]. However, the relative conductivities of solutions containing the zwitterion tricine $^{+/-}$ were essentially independent of the concentration of added tricine $^{+/-}$, as expected for undissociated ion pairs [18].

The viscosity-corrected mobilities observed for ds26 in 0.08M cacodylate buffer solutions containing added TEA^+Cl^- or tricine $^{+/-}$ are illustrated in Fig. 2A. As shown by the open circles, the mobility of ds26 decreased progressively with increasing $[\text{TEA}^+\text{Cl}^-]$, in agreement with the results observed for DNA in other buffer/salt solutions [19,20]. However the mobility of ds26 was independent of the concentration of added tricine $^{+/-}$, as shown by the solid circles in Fig. 2A. The constant mobility observed in the cacodylate/tricine $^{+/-}$ solutions clearly indicates that tricine $^{+/-}$ does not contribute to the ionic strength of the solution. Similar results would be expected for other zwitterions.

Although tricine^{+/-} and other zwitterionic Good's buffers can form complexes with DNA [21], DNA-tricine^{+/-} complex formation cannot explain the constant mobility observed for ds26 in Fig. 2A, for three reasons: 1) DNA-tricine^{+/-} complex formation would decrease the mobility because of the decreased charge/mass ratio of the complex. 2) DNA-buffer complex formation is usually accompanied by peak broadening [21,22]; however, in the present case the peaks remained sharp at all tricine^{+/-} concentrations (data not shown). 3) Tricine^{+/-} was added to solutions containing 0.08 M cacodylic buffer as the background electrolyte. Neutral salts in this concentration range are known to eliminate DNA-zwitterion complex formation [22,23].

The viscosity-corrected mobility of ds26 decreased linearly with the square root of the concentration of added TEA⁺Cl⁻, as shown in Fig. 2B. Hence, the Debye-Hückel-Onsager theory is obeyed for cacodylate-buffered solutions containing this neutral salt, as expected since TEA⁺ and cacodylate do not bind to DNA [2,11].

In summary, the decrease of the mobility observed for ds26 in cacodylate buffer solutions containing added TEA⁺Cl⁻, and the constant mobility of ds26 in cacodylate buffer solutions containing added tricine^{+/-}, indicate that zwitterions do not contribute to the ionic strength of a solution. However, zwitterions have very large dipole moments [24–26] and interact electrostatically with the solvent and with other charged molecules in the solution [1,2,7,8,27,28]. Hence, electrostatic effects due to zwitterions are important, even if the zwitterions do not contribute directly to ionic strength.

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Fig. 1. Relative conductivity, Λ/Λ_0 , of cacodylate buffer solutions with (Λ) and without (Λ_0) added TEA^+Cl^- (○) or $\text{Tricine}^{+/-}$ (●), plotted as a function of the square root of the total concentration of ions in the solution.

Fig. 2.

(A) Dependence of the viscosity-corrected mobility of ds26, $\mu_{\text{DNA,corr}}$, on the concentration of TEA⁺Cl⁻ (o) or tricine^{+/-} (●) added to 0.08 M cacodylate buffer. (B) Dependence of $\mu_{\text{DNA,corr}}$ on the square root of the TEA⁺Cl⁻ concentration.