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Potential binding of borate ions to mono- and oligonucleotides: a capillary electrophoresis investigation

Maria Rosa D'Acunto[a], Cecilia Gelfi[b], Alexandre Stoyanov[c], Victor Andreev[d], Pier Giorgio Righetti[a,*]

[a]*Department of Agricultural and Industrial Biotechnologies, University of Verona, Strada Le Grazie No. 15, 37134 Verona, Italy*
[b]*IBFM, CNR, LITA, Via Fratelli Cervi 93, Segrate 20090, Milan, Italy*
[c]*Faculty of Pure and Applied Science, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3*
[d]*Barnett Institute of Chemical and Biological Analysis, Northeastern University, 341 Mugar Hall, 360 Huntington Avenue, Boston, MA 02115, USA*

Abstract

The potential binding of borate to oligonucleotides and DNA fragments is here investigated. In case of free nucleotides, such as AMP, there appears to be a weak binding, although no free versus complexed species could ever be separated under any experimental condition. The binding was suggested by the strong peak asymmetry and by the fact that, at progressively lower borate molarities in the background electrolyte, the peak shape suddenly switched from fronting to tailing. This indicated, as also confirmed by theoretical simulations, that the AMP–borate complex was the slow, not the fast moving species. On the contrary, in the case of free adenosine, strong binding ensued, since in Tris–acetate buffer this compound was only eluted with the electroendoosmotic flux, being neutral, whereas in Tris–borate it had a much higher mobility, comparable to, although lower than, that of AMP. When running oligonucleotides, at standard borate molarities (ca. 45 m*M*), and under strict iso-ionic strength conditions, no binding to borate could be demonstrated, since the free mobility of a 24-mer DNA was identical in TA and TB buffers. However, at very high borate molarities (200 m*M*) and high pH values (pH 8.92), some binding to oligonucleotides could occur, since in these latter conditions the mobility of a 24-mer was seen to be ca. 20% lower than at pH 7.69, a pH value that should discourage any complex formation.

Keywords: Mononucleotides; Oligonucleotides; Borate; DNA

1. Introduction

Capillary zone electrophoresis (CZE) has recently become a powerful tool for studying the biophysical parameters of analyte solutions. In the case of DNAs, up to recent times, CZE has been used almost essentially in the sieving mode, due to the very nearly constant charge/mass ratio of such macromolecules, in order to elicit and optimize their separations [1]. Thus, most of the published work has concentrated on the properties and physicochemical parameters of the sieving liquid polymers adopted for DNA analysis [2–5]. Nevertheless, recently, a few research groups have adopted free solution electrophoresis as a tool for characterizing the behaviour of oligonucleotides and DNA fragments and deriving important information on potential interactions of such macromolecules with the buffer components, as well as measuring some

*Corresponding author. Tel./fax: +39-45-802-7901.
E-mail address: righetti@sci.univr.it (P.G. Righetti).

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inherent biophysical properties of such macroions. An important result of such analyses is that DNA species have been found to form complexes with a wide variety of organic cations commonly used as buffering groups, such as Tris [6], His [7] or Good's buffers [8–10].

The advent of DNA gel electrophoresis has sparked an interest also in the free solution mobility of DNA, because Ferguson plots (log mobility versus gel concentration) are expected to extrapolate to the free solution mobility of DNA at zero gel concentration [11]. However, it has been noted that such plots, for DNA molecules with different molecular size, do not, in reality, extrapolate to a constant mobility at zero gel concentration. Instead, the apparent (extrapolated) free solution mobilities decrease monotonically with increasing DNA molecular mass. In addition, there is a fairly wide disparity in the literature values of the free solution mobility of DNA. For all these reasons, Stellwagen et al. [12] set out to measure the free solution mobility of DNA by CZE and found that its value, in Tris–acetate–EDTA buffers (40 m*M*, pH 8.1), is $3.75 \pm 0.04 \times 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ at 25 °C, independent of DNA concentration, sample size, electric field strength and capillary coating and in good agreement with other values in the literature. The free solution mobility was independent of DNA molecular mass, from ca. 400 base pair (bp) to 48.5 kbp, but decreased monotonically with decreasing molecular mass for smaller fragments [12].

Another important physico-chemical parameter that could be measured with precision by free-solution CZE is the diffusion coefficient (D). Measuring D values of small molecules and macromolecules in capillaries was first attempted by Bello et al. [13], by exploiting the Taylor–Aris dispersion theory. Stellwagen et al. [14] adopted a different protocol for measuring D of DNA fragments, based on the stopped migration procedure, by which, after transporting the peak to the middle of the capillary, migration was stopped and the zone allowed to diffuse for different periods of time. The peak variance could then be correlated to the translational diffusion coefficient via the Einstein equation. In another approach, Nkodo et al. [15] suggested the Zimm's expression [16] for deriving diffusion coefficients under non-electrophoretic conditions.

The present report aims at settling some controversial data still existing in the literature. According to Stellwagen et al. [8], DNA fragments also bind to borate, when the latter compound is used as a background electrolyte. Apparently, such a binding would increment the DNA mobility, to the point at which the apparent free solution mobility of DNAs in such a buffer would be 20% higher than in conventional Tris–acetate buffer. By and large, borate is assumed to generate complexes with *cis*-diols [17], to the point at which such a buffer is used for electrophoretic separation of neutral sugars. The free nucleotides constituting DNA contain, in fact only one –OH group, which, however, is fully consumed in forming the phosphodiester bridge in the DNA filament. Thus, it is not quite clear how borate could form such complexes, if one assumes the binding mechanisms well-ingrained in current literature [17].

The data here presented suggest that, indeed, such a binding can only be measured in free nucleosides, i.e., in the free purines and pyrimidines bound to a sugar ring, but not esterified with phosphate. In free nucleotides, though, the very weak binding observed leads to species exhibiting lower, not higher mobilities than the borate-free species. Finally, and paradoxically, it would appear that the only possible way by which borate can bind to a DNA double filament is by acting as a cross-linker, bridging two DNA species together via binding to the free –OH group available at the free 3′ extremity of each filament.

2. Theory

In order to assess any potential binding of borate to DNA, it is important to be able to explore an interval of pH values of the background electrolyte, by arranging also for rather alkaline pH values, where borate (pK 9.3) will be progressively more ionized and hopefully modulate more strongly the DNA mobility. However, during titration of borate with the Tris cation, the ionic strength will change substantially. One can try to arrange for iso-ionic strength conditions by adding appropriate concentrations of a neutral salt, such as NaCl. In the specific case of borate, intuitively, one can assume that the

salt concentration should be increased as the pH is lowered towards neutrality. The appropriate amount of salt (C_s) to be added can be obtained with the help of the following expression:

$$C_s = 1/2\,[C_b^{(2)}\,(H^{(2)}/H^{(2)} + K_b) - C_b^{(1)}\,(H^{(1)}/H^{(1)} + K_b) + C_a\,(K_a/H^{(2)} + K_a) - K_a/(H^{(1)} + K_a) + K_w(1/H^{(2)} - 1/H^{(1)})] \quad (1)$$

where C_b is the concentration of the titrant (base) corresponding to two arbitrary values ($C_b^{(2)} > C_b^{(1)}$), while the acid concentration is assumed to be the same. $H^{(1)}$ and $H^{(2)}$ are the appropriate hydrogen concentration values ($pH_2 > pH_1$); K_a and K_b are the dissociation constants of acid and base, respectively.

Fig. 1A, for instance, shows the pH variation when a constant concentration of borate (200 m*M*) is titrated with increasing molarities of Tris (C_b) in the pH 8.3–9.0 interval. During this titration, though, there is a huge variation of ionic strength, as shown in Fig. 1B (at constant 200 m*M* borate molarity, same levels of Tris titrant in the same pH interval). If one had to keep iso-ionic strength conditions, in the same pH range (always at constant 200 m*M* borate), one should have to add increasing amounts of NaCl

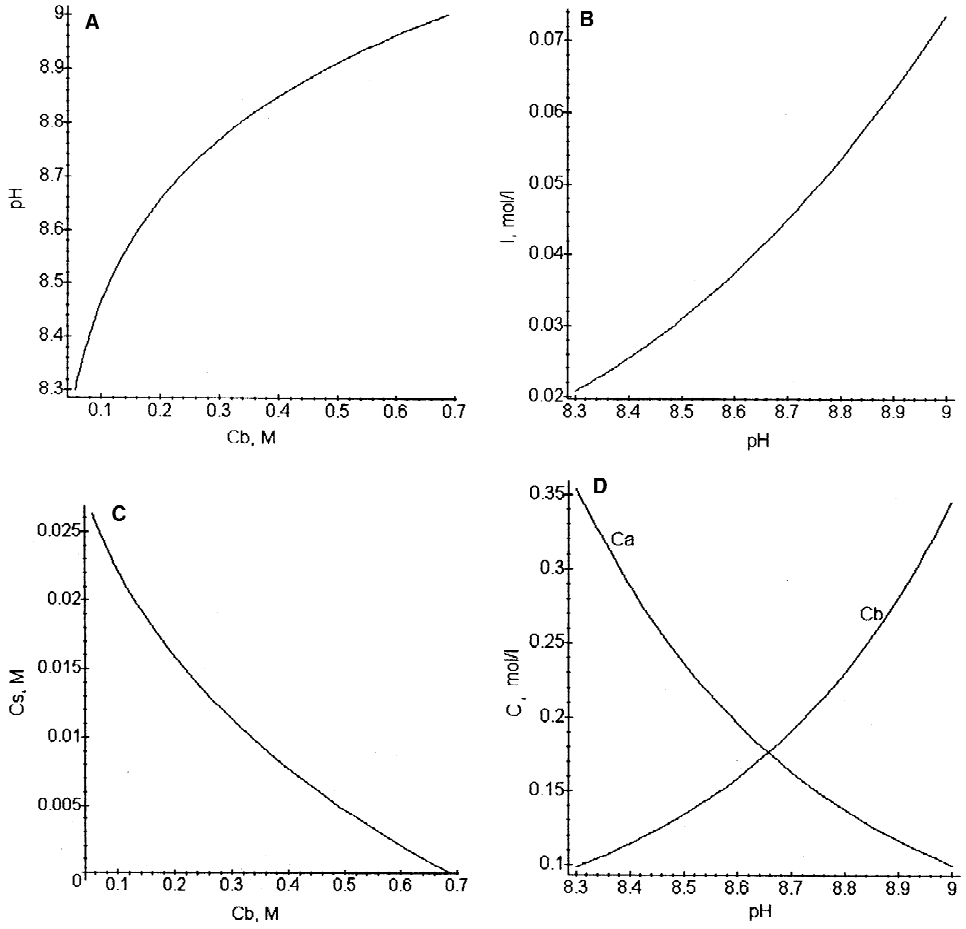

Fig. 1. (A) Titration of 200 m*M* borate with increasing molarities of Tris (C_b) in the pH 8.3–9.0 interval. (B) Variation of ionic strength (I) in the same pH 8.3–9.0 interval corresponding to the titration of a constant 200 m*M* borate with Tris. (C) Amounts of neutral salt (NaCl) to be added in the same titration interval (pH 8.3–9.0) to the increasing amounts of Tris (C_b) in order to keep a constant I value of 70 mequiv. l^{-1} all throughout the given pH range. (D) Reciprocal variation of the molarities of borate (C_a) and of Tris (C_b) for keeping constant ionic strength in the pH 8.3–9.0 titration interval.

towards the lower pH extreme and vanishing levels of NaCl towards the opposite pH extreme, as shown in Fig. 1C. There could also be an alternative approach. A constant ionic strength level could also be maintained without any addition of salt, simply by varying the respective concentrations of acid (borate, C_a) and base (Tris, C_b) according to:

$$C_a = \text{Const}(H + K_a)/K_a \quad (2)$$

where the constant is defined by the required ionic strength level, and the concentration of base is calculated by using the electroneutrality relationship:

$$C_b = (C_a K_a/(H + K_b) - K_w/H)\,(H + K_b)/H \quad (3)$$

An example of this approach can be seen in Fig. 1D, where the composition of the Tris–borate buffer in the pH range 8.3–9.0 is given, by simultaneously varying the relative concentrations of both ions, while keeping a constant, pre-defined ionic strength (I) level, here selected as $I = 70 \times 10^{-3}$ equiv. l^{-1}. We have, nevertheless, chosen the first approach (i.e., maintenance of constant ionic strength levels by addition of neutral salts), so as to be able to operate at the maximum possible levels of borate (kept at 200 m*M* all throughout the experiments) in order to see if we could elicit stronger binding of DNAs to this buffering ion.

3. Materials and methods

3.1. Reagents, samples and buffers

Boric acid and Tris (hydroxymethyl)-aminomethane were purchased from Merck (Darmstadt, Germany), Acetic acid was obtained from Fluka Chemie (Buchs, Switzerland). Free adenosine and adenosine monophosphate (AMP) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fused-silica capillaries (27 cm long × 75 μm I.D. × 375 μm O.D.) were from Polymicro Technologies (Phoenix, AZ, USA). In the DNA experiments the samples were 24-bp double-stranded DNA fragments, obtained from PRIMM (Milano, Italy) and dissolved in 10 m*M* Tris–HCl, pH 7.5. The nucleosides used were free adenosine and adenosine monophosphate (AMP), both at a concentration of 0.1 mg ml^{-1}. The buffers used for capillary electrophoresis (unless otherwise indicated) were Tris–acetate (TA) (20 m*M* Tris, 10 m*M* acetic acid, pH 8.24) and Tris–borate (TB) (40 m*M* Tris, 40 m*M* boric acid, pH 8.25) prepared by using doubly distilled water.

3.2. Capillary electrophoresis

Capillary zone electrophoresis was carried out by using a Beckman PACE System 2100 apparatus. The UV detection was at 254 nm. The analysis and elaboration of electropherograms was performed with the System Gold software and P/ACE Station System 5000 (Beckman). The capillary (75 μm I.D., total length 27 cm, distance to the detector 20 cm) was coated with a layer of polyacrylamide by covalent functionalization via a Grignard reaction [18]. The capillary cartridge was cooled with circulating liquid at constant temperature 25.0 + 0.1 °C. The electrophoretic mobility μ of the various DNA fragments was calculated from the equation:

$$\mu = d/Et \quad (4)$$

where d is the distance to the detector (in cm), E is the electric field strength (in V cm^{-1}), and t is the time required for the sample to travel to the detector (in seconds). Particular care was taken to equalise the liquid levels in the buffer reservoirs so as to prevent hydrodynamic flow. The rinse buffer, which was the same as the buffer used for electrophoresis, was placed in a separate vial for preventing depletion of the electrode buffer during the rinse step. The buffer in the reservoirs was typically replaced at least once during each series of measurements; the observed mobilities were independent of the frequency of replacing buffer. Between runs, the capillary was flushed with buffer for 1 min under high pressure. At the end of each day, the capillary was washed with doubly distilled water for 10–15 min under high pressure. The best conditions of injection (mode, time and voltage) were found after testing by different experiments at the same run voltage decreasing the injection's voltage. In an another set of experiments the run's voltage was changed while the

injection time and injection voltage were kept constant.

The sample injection was both by pressure (5 p.s.i. = 11 nl) and by electrokinetic means (V_{inj} = 1–5 kV, t_{inj} = 1–10 s).

4. Results

Fig. 2 shows the results of mobility assessments for a 24-mer oligonucleotide when run in TA and TB buffers of the same molarity (two upper panels) and

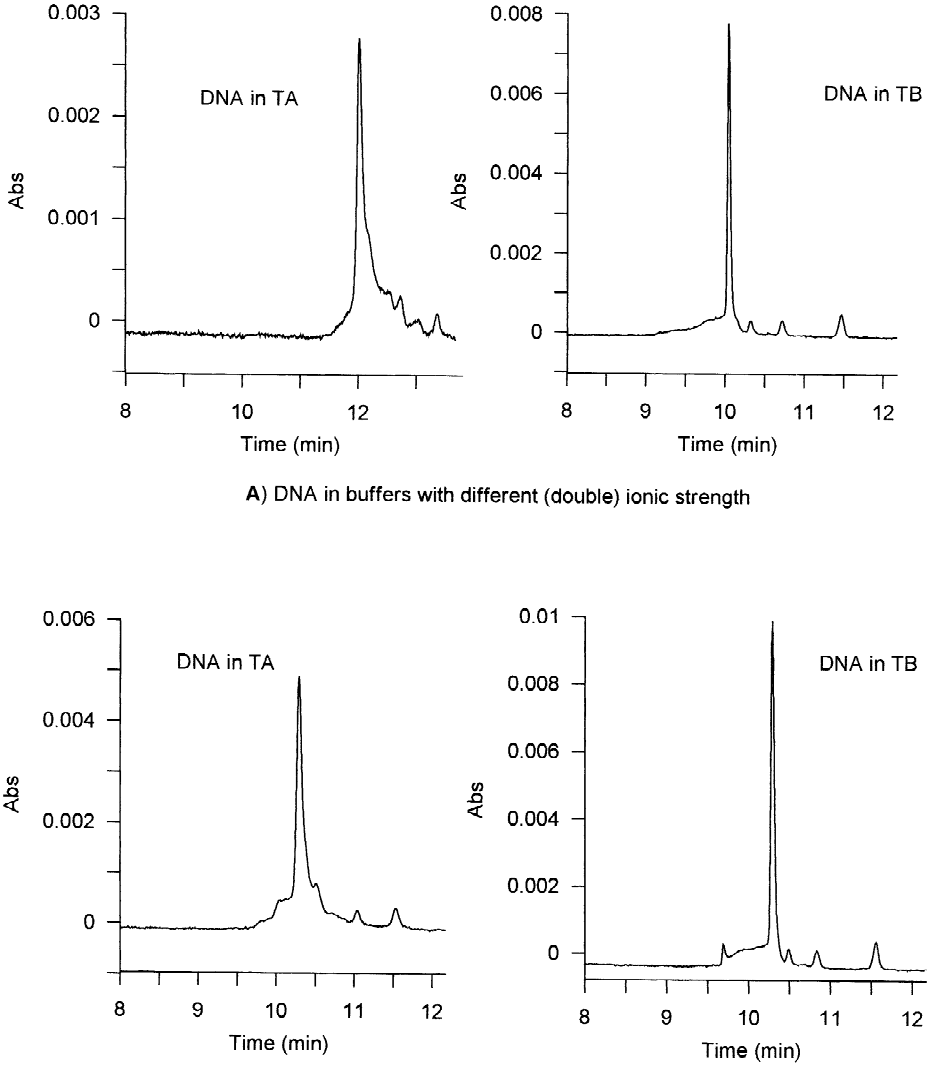

Fig. 2. CZE analysis of a 24-mer oligonucleotide in TA versus TB buffers. The two upper panels refer to buffers of equal molarities, thus different ionic strength values. TA buffer: 40 m*M* Tris, 20 m*M* acetate, pH 8.24; TB buffer: 40 m*M* Tris, 40 m*M* H_3BO_3, pH 8.25 (note that in the latter case the *I* value is 1/2 that of TA buffer). The two lower panels refer to buffers of iso-ionic strength. TA buffer: 20 m*M* Tris, 10 m*M* acetate, pH 8.24; TB buffer: 40 m*M* Tris, 40 m*M* H_3BO_3,pH 8.25. Note the identical mobilities of the 24-mer in the two last buffers.

when repeating the same experiments in TA and TB buffers endowed with the same ionic strength (two lower panels). It should be emphasized that the experiments reported in [8] dealt with equal molarity buffers, thus leading to the conclusions of an apparently higher mobility of DNAs in borate buffer. However, when assessing free mobility values, the correct experimental protocol calls for iso-ionic strength buffers, due to the strong influence of such a parameter on the diffuse Debye-Hückel layer of a polyelectrolyte [19,20]. In the latter case, the oligonucleotide shows identical mobilities in both buffers (see also Table 1).

In order to ascertain if any binding could be elicited with the free monomers, we have repeated this experiment by comparing the mobilities of AMP and of adenosine in TA versus TB buffers. The two upper panels of Fig. 3 indicate that, if there were any binding of borate to AMP, it should be quite weak, since the two mobilities are quite similar, although the peak in TB is strongly asymmetric as compared with the peak profile in TA. Conversely, when the same experiments are repeated with free adenosine (the dephosphorylated derivative of AMP), one can appreciate a strong binding of TB (lower left panel). In fact, it should be considered that adenosine is a neutral compound, so it should not migrate in the electric field; this is clearly shown in Fig. 3, lower right panel, which shows a peak of adenosine eluting in TA buffer with the residual electroendoosmotic flow ($>$108 min elution time). In addition, the broad peak of adenosine in TB buffer suggests that it could be an envelope of bound versus unbound species in fast equilibrium. The borate–adenosine complex, although being eluted in ca. 16 min, has a considerably reduced mobility as compared with the AMP derivative and other free nucleotide monophosphates, all migrating in a ca. 6-min time window. The present data, though, do not allow us to discriminate whether such a reduced mobility is due to only partial negative charge on the borate complex, or to the fact that the bound form is in equilibrium with the unbound species, or to the fact that the complex might have a higher Stokes radius, resulting in a lower mobility even in presence of a full negative charge.

The experiment shown in Fig. 4 aims at answering such a question. As shown in Fig. 3, AMP in borate buffer exhibited a strong fronting, suggesting that, perhaps, there could be partial binding of AMP to borate, although no separation of bound versus unbound species could be elicited by widely altering the experimental parameters. By assuming that, nevertheless, this asymmetry had been caused by borate binding, we have tried to ascertain which of the two species could be the fastest migrating compound. As shown in the two upper panels of Fig. 4, at standard (45 m*M*) and 1/2 borate concentrations, the peak asymmetry still exhibits strong fronting; however, at 1/4 and 1/8 borate concentrations, there is a sudden switching from fronting to tailing. It is reasonable to assume that, at standard borate values, the complexed species could represent the most populated state, whereas at the highest borate dilution the complex should be the least abundant species. On this assumption, the evident switching from fronting to tailing clearly suggests that the borate–AMP complex has a lower, not higher mobility as compared with the borate-free peak. This change in sign of asymmetry of peaks as a function of the change of ligand (buffer) concentration could be nicely simulated with the program developed by Andreev et al. [21]. As shown in the simulations of Fig. 5, the peak at high buffer

Table 1
Mobility variations of a 24-mer oligonucleotide in TA versus TB buffers of different or iso-ionic strength (the data refer to Fig. 2)

TA		TB		$\Delta\mu$%
T (min)	μ (10^{-4} cm^2/Vs)	T (min)	μ (10^{-4} cm^2/Vs)	
12.071±0.070[a]	3.064±0.017[a]	10.045±0.047[a]	3.676±0.015[a]	17±1[a]
10.047±0.013[b]	3.675±0.011[b]	10.045±0.047[b]	3.676±0.015[b]	0[b]

[a] DNA experiments with different (double) ionic strength. TA buffer (40 m*M* Tris, 20 m*M* AcOH, pH 8.24). TB buffer (40 m*M* Tris, 40 m*M* H_3BO_3, pH 8.25).

[b] DNA experiments with the same ionic strength. TA buffer (20 m*M* Tris, 10 m*M* AcOH, pH 8.24). TB buffer (40 m*M* Tris, 40 m*M* H_3BO_3, pH 8.25).

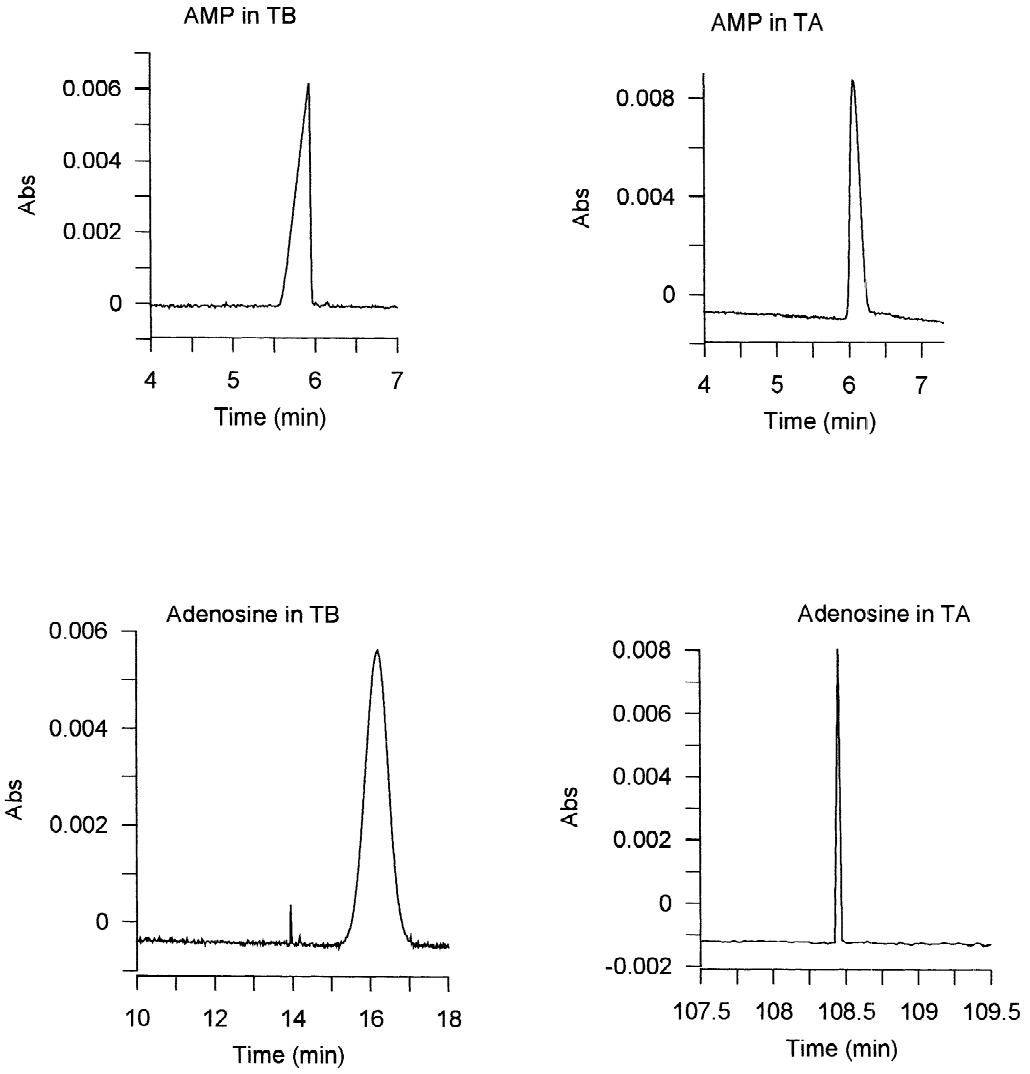

Fig. 3. CZE analysis of AMP (0.1 mg ml^{-1}) and of adenosine (0.1 mg ml^{-1}) in TA and TB buffers. Note that adenosine is an uncharged compound, thus in TA buffer (lower right panel) it migrates with the electroendoosmotic flow (ca. 108 min), whereas in TB buffer is eluted in only 16 min. Note in addition the strong fronting of AMP in TB buffer.

molarity (45 m*M*) has a strong fronting (tracing 1); as the buffer molarity is lowered, the peak becomes more symmetric (profile No. 2), till it suddenly switches to tailing at 11.25 and 5.62 m*M* borate concentration. It is quite remarkable how the simulated peak profiles, assuming fast bound versus free equilibria, closely follow the experimental ones displayed in Fig. 4.

It remains now to be seen whether such a binding could also be demonstrated in oligonucleotides, which in principle, by not having any free –OH group, should not form borate complexes. That this should be the case was already demonstrated in Fig. 2, which indicated, in TA and TB buffers of iso-ionic strength, indistinguishable mobilities. However, these experiments had been performed at low borate molarities, comparable to those adopted by Stellwagen et al. [8]. At the light of the experiments shown in Fig. 4, it would appear that varying borate molarities could elicit more or less pronounced

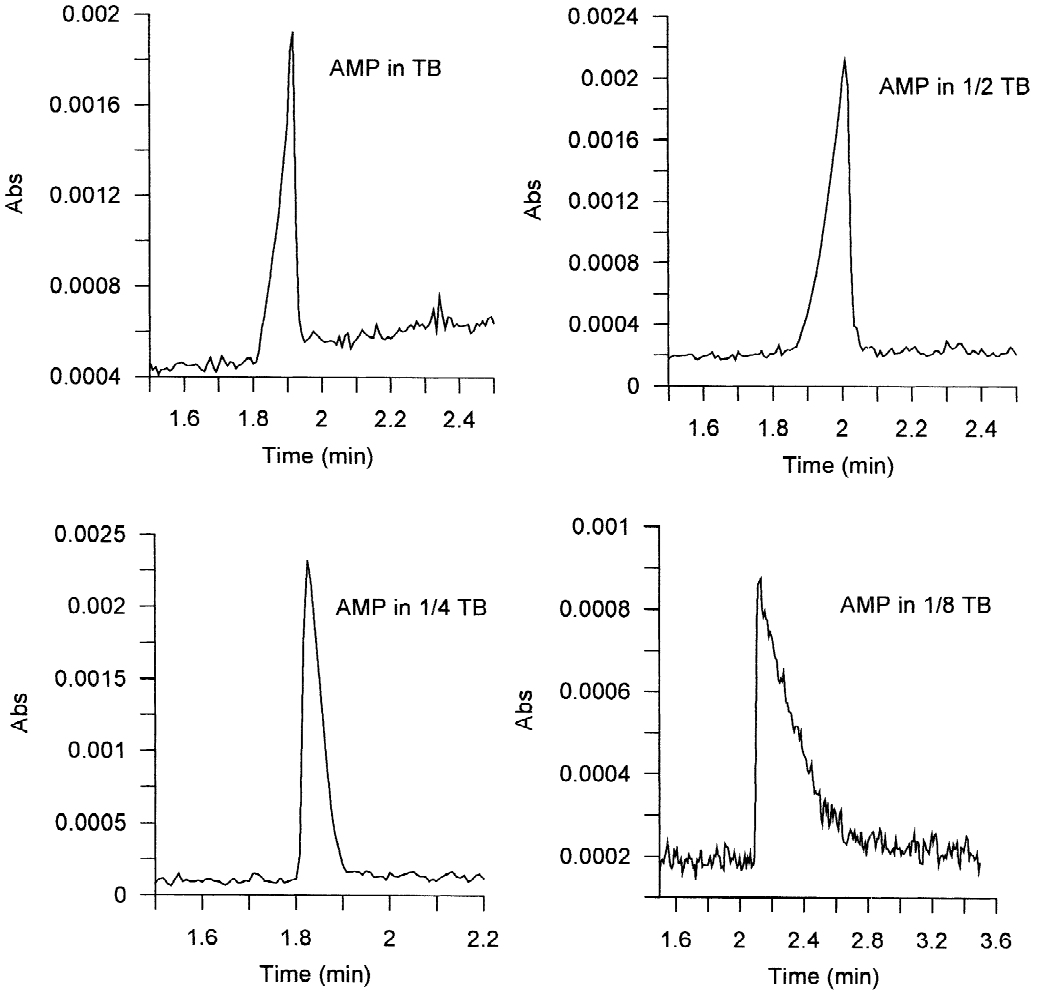

Fig. 4. CZE analysis of AMP (0.1 mg ml^{-1}) in TB buffer of different molarities. Starting borate buffer (upper left panel): 40 m*M* Tris, 40 m*M* H_3BO_3, pH 8.25. Note the strong peak fronting in 40 and 20 m*M* borate, versus the tailing in 10 and 5 m*M* borate.

binding. In addition, exploring a range of pH values, directed towards the p*K* value of borate (p*K* 9.3) should also favour complexation, since it would promote ionization of the complex. We have therefore devised the set of ternary buffers listed in Table 2, all at constant (and maximum) borate concentration (200 m*M*), all at equal ionic strength (70×10^{-3} equiv. l^{-1}), encompassing the pH 7.69–8.9 range. This was obtained by a judicious blending of Tris and NaCl, as illustrated in Fig. 1 (see also Section 2). We have taken as control mobility the one measured in 200 m*M* buffer at the highest possible pH value under the present experimental conditions (pH 8.92). As shown in the series of panels in Fig. 6, and as summarized in Fig. 7, it would appear that, at progressively lower pH values, calling for less favourable borate binding conditions, the 24-mer oligonucleotide peak shows progressively higher apparent mobility values. It would thus appear that, if any borate binding occurs, the bound species would exhibit lower, not higher, free mobility values.

5. Discussion

The present data appear to settle the controversial

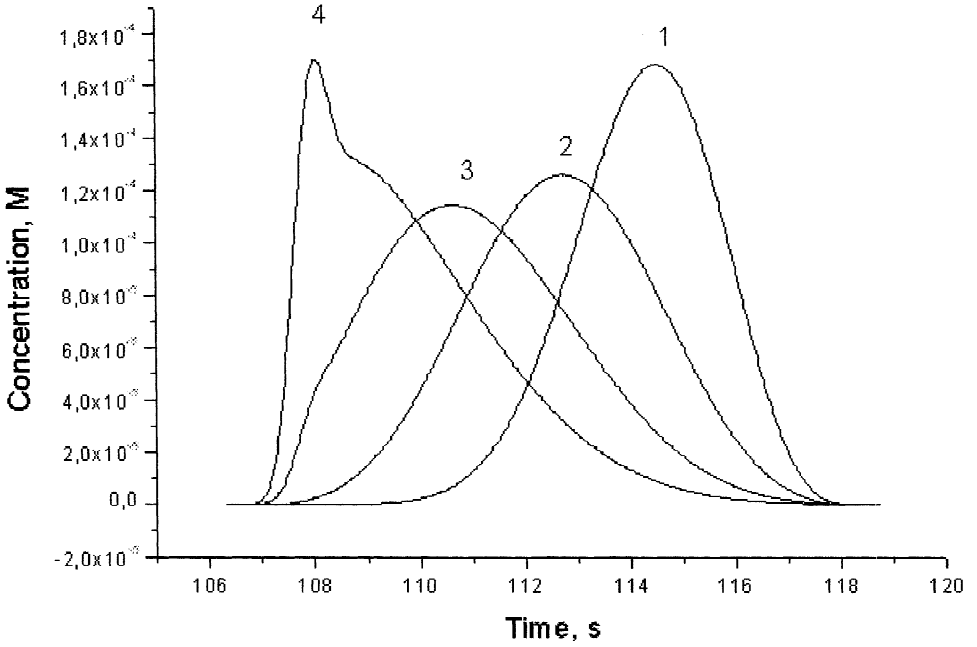

Fig. 5. Simulated peak shapes. Time dependence of the concentration of sample molecules at the detector location. Illustration of the change of the sign of asymmetry of peaks with the change of ligand (buffer) concentration. Sample initially dissolved in the running buffer. $L=20$ cm, $v_1=0.185$ cm s^{-1}, $v_2=0.17$ cm s^{-1}, $v_R=0.1$ cm s^{-1}, $k_{1+}=3.0$ M^{-1} s^{-1}, $k_2=0.06$ s^{-1}, $c_{10}=0.001$ M, $D_1=D_2=D_3=10^{-5}$ cm^2 s^{-1}, $l_s=0.1$ cm. (1) $c_{R0}=0.045$ M; (2) $c_{R0}=0.0225$ M; (3) $c_{R0}=0.01125$ M; (4) $c_{R0}=0.005625$ M.

Table 2
Buffer composition providing constant ionic strength values and constant borate (200 mM) concentration in the pH 7.69–8.92 interval

Expt no.	Buffer composition	pH	$I\times10^{-3}$ (equiv. l^{-1})	$\Delta\mu$ ($\mu_{sol}-\mu_{ctrl}$)	$\Delta\mu$ (%)
1	36 mM Tris 200 mM Borate 50 mM NaCl	7.69	70	0.653	20.7
2	50 mM Tris 200 mM Borate 47 mM NaCl	7.80	70	0.626	19.6
3	100 mM Tris 200 mM Borate 36 mM NaCl	8.20	70	0.529	17
4	200 mM Tris 200 mM Borate 20 mM NaCl	8.60	70	0.378	13.3
5	248 mM Tris 200 mM Borate 10 mM NaCl	8.78	70	0.245	9.2
Ctrl.	401 mM Tris 200 mM Borate	8.92	70	–	–

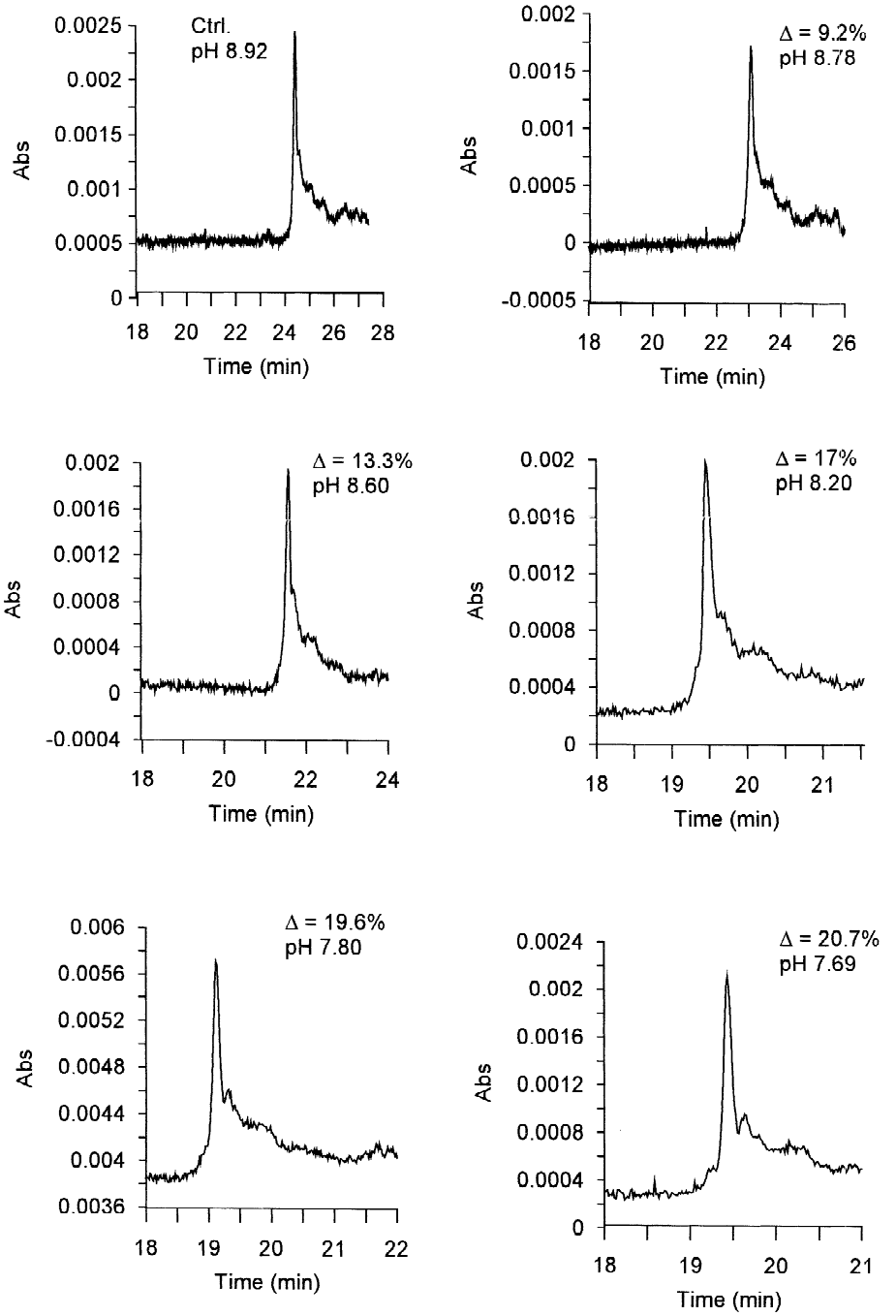

Fig. 6. Migration times of a 24-mer oligonucleotide as a function of buffer pH (from pH 8.92, uppermost left panel) down to pH 7.69, lowermost right panel), at constant borate molarity (200 m*M*) and constant ionic strength conditions ($I = 70$ mequiv. l^{-1}) all throughout the titration experiment. For buffer compositions, see Table 2.

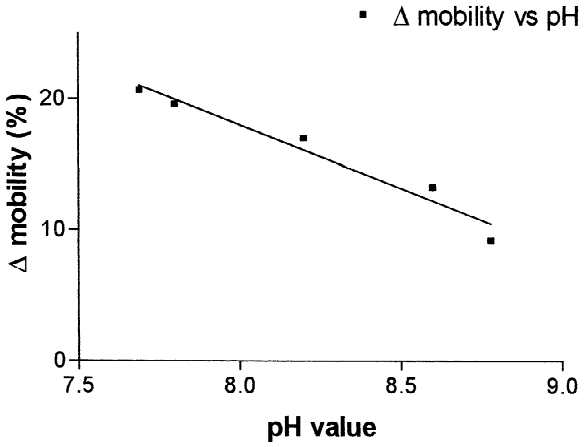

Fig. 7. Variation of mobility of the 24-mer oligonucleotide in the set of experiments of Fig. 6. The $\Delta\mu$ values have been calculated against the mobility of a reference run in the pH 8.92 borate buffer (upper left panel in Fig. 6).

issue of borate binding to nucleic acids and how such a potential binding would affect their free mobilities. It should, first of all, be emphasized that all experiments comparing the potential effects of ions in the background electrolyte on the mobility of any analyte should be performed at rigorously constant ionic strength values. The effect of I values on free μ measurements is an important aspect, often ignored in current literature. Under dilute conditions the thickness of the ionic atmosphere surrounding a small ion could be as high as 100 times the radius of said ion. However, as the ionic strength of a solution increases, the thickness of the ionic atmosphere decreases, as described in the equation giving the Debye-Hückel reciprocal length [19]. Thus, both theoretical and empirical relationships predict that mobility will decrease with the square root of the ionic strength. A few relationships have been derived. In one approach, Reijenga and Kenndler [20] derived the following empirical expression for mobility relative to infinite dilution mobility (μ_o):

$$\mu = \mu_o \exp(0.5z^{1.78}\sqrt{I}) \quad (5)$$

where z is the charge number of the solute and I is the ionic strength of the buffer. This expression was stated to be valid for solute charge numbers from 1 to 3 and ionic strengths up to ca. 10 mM. In another approach, Friedl et al. [22], observed the following empirical relationship:

$$\mu = \mu_o \exp(0.77\sqrt{zI}) \quad (6)$$

valid, apparently, for solutes with charge numbers from 2 to 6 and ionic strengths in the 1–100 mM range.

In the light of the above considerations, in fact, when oligonucleotide mobilities were compared under strict iso-ionic strength conditions, no mobility differences could be measured in TA versus TB buffers (Fig. 2). In order to generalize these findings, we took free nucleotides, precursors of RNA (therefore with free vicinal diols in the sugar moiety) and run them in TA versus TB buffers: however, even under these conditions the free mobility differences where so minute that we could not measure significant $\Delta\mu$ values (Fig. 3). Nevertheless, when we took a free nucleoside (thus an uncharged compound), the differences in mobility were enormous: in TA buffer, the free nucleoside had zero mobility, since it was eluted with the velocity of the electroendoosmotic flow; on the contrary, in a TB milieu, the same compound had an apparent mobility about seven times higher than in TA (see Fig. 4). This suggests that, in presence of free vicinal diols, a complex with borate is made, which however possesses only 40% of the mobility of the corresponding nucleotide. This might be due to an equilibrium between the complexed and free state, as also indicated by a rather broad peak. But if a borate complex is clearly made with a free nucleoside, it should also be made with the AMP nucleotide (or any other nucleotide containing a ribose ring); therefore, why are we unable to see any mobility differences? We have thus repeated the above experiments at standard borate molarity (45 mM Tris–borate) and at progressive buffer dilutions, in order to see if we could detect any change in behaviour of the eluted peaks. Under these conditions, as shown in Fig. 4 (two upper panels), there is a strong fronting of the peak, suggesting that such a peak could be a mixture of two species, the free- and borate-bound nucleotide.

It is, however, impossible to tell if, first of all, this is a simple artefact due to conductivity differences between the analyte and the background electrolyte and, secondly, if such a complex were to exist,

which is the slow and which one the fast moving ion (both, however, with minute $\Delta\mu$ values, considering that we cannot achieve peak splitting under any circumstance). The subsequent panels in Fig. 4 give us a clue of what is happening: by progressively diluting the borate in the background electrolyte, the peak shape suddenly switches from fronting to tailing. Now, it is reasonable to assume that, at high borate values, the center of mass of the peak should be shifted towards the borate complex, whereas at low borate the prevailing peak should be the free species. Under these assumptions, it is clear that, indeed, when borate complexes are formed, the resulting adduct is not the fast moving, but the slow-moving ion, just the opposite of what hypothesized by Stellwagen et al. [8]. This also implies that, upon borate binding, the increment of Stokes radius of the complex offsets the increment of charge, rendering thus the complexed nucleotide a slow-moving, rather than a fast-moving ion. As an additional conclusion, it would appear that such complexes, while highly favoured for free nucleosides (neutral species) are rather difficult to be made in the case of nucleotides (see Fig. 3), this suggesting that the high negative charge of phosphate in the 5′ position of the sugar moiety tends to inhibit the formation of an additional neighbouring charge in the same ribose ring. If the matters are really as we depicted them, it is clear that the notion of binding of borate to oligonucleotides and DNA fragments in general is a mere chimera, considering that a dsDNA would present a cloud of negative charges along the axis of the helix.

Having established that there could hardly any borate–DNA complex formation, due to lack of free vicinal diols in the polymer, as opposed to simple nucleotide monomers, there remain some puzzling data in Stellwagen et al. [9] which need a further explanation. When running a linear pUC19 DNA and a ds20-mer in TAE buffer, they were seen to behave as single, well separated entities. However, when the two samples were run in a mixture in TBE buffer, they exhibited a single peak (see Fig. 3 in Ref. [9]), which apparently contained a mixture of the two species. This is also corroborated by our findings (see Fig. 6), which suggest that, at high borate molarities and at high pH values (conditions that should favour borate binding), such complexes could indeed be formed, since a 24-mer oligonucleotide exhibits a ca. 21% lower free mobility when run at pH 8.92 as compared to the mobility exhibited at pH 7.69. The only possible explanation is that borate, somehow, is able to act as a bridge or a cross-linker between the two DNA fragments. This appears to be the only explanation of the phenomena discussed so far: in fact, for borate, the only way to complex DNA and find two –OH groups in a sugar moiety, is to seek a free 3′ –OH, which can only be found at one extremity of a ds-DNA; the second free 3′ –OH has then to be offered by a second DNA filament. Only in this way can borate form the classical diol complex in a DNA: by finding the only two free 3′ –OH groups offered by two different DNA double filaments in solution. Thus, to our reckoning, borate might indeed form complexes with DNA, but only at the chain extremity and by seeking a free 3′ –OH partner in a neighbouring DNA double helix in solution. Such complexes, if any, though, appear to be very weak and to be elicited only by very high borate molarities and at rather alkaline pH values.

6. Notation

TA, Tris–acetate buffer
TB, Tris–borate buffer

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