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Effect of urea concentration on the base-specific separation of oligodeoxynucleotides in capillary affinity gel electrophoresis

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ABSTRACT

Base-specific separations of oligodeoxynucleotides were achieved with high resolution by electrophoresis, using a urea-gel capillary, in which poly(9-vinyladenine) (PVAd) was utilized as an affinity ligand. The migration behaviour and the plate number of oligodeoxynucleotides were investigated as a function of urea concentration between 2 and 10 *M* in capillary gel electrophoresis (CGE) as well as capillary affinity gel electrophoresis (CAGE). The migration time in CGE separation increases as urea concentration increases. An increase in the viscosity of the gel buffer medium as well as a change in the conformation of oligodeoxynucleotides is found to be a predominant factor for an increase in the migration time. The migration time and the plate number of oligothymidylic acids, which interact with PVAd in CAGE, is manipulated by differing urea concentration, which leads to the change in the dissociation process of a specific hydrogen bonding between oligothymidylic acids and PVAd. The migration time of oligodeoxyadenylic acids, which do not interact with PVAd in CAGE, increases with an increase in urea concentration as in CGE separation. The plate number of oligodeoxyadenylic acid was not affected by the urea concentration.

INTRODUCTION

Over the past decade, capillary electrophoresis (CE) has become an important technique for the separation of complex mixtures in the area of analytical biotechnology [1–9]. CE comprises several modes, including capillary zone electrophoresis (CZE) [2,3], micellar electrokinetic chromatography (MEKC) [4,5], capillary gel electrophoresis (CGE) [6–8] and capillary isoelectric focusing [9]. More recently capillary affinity gel electrophoresis (CAGE), which is another important mode of CE, has been introduced for the biospecific separation of oligodeoxynucleotides [10–14], DNA restriction fragments [15] and optical isomers [16]. CAGE, in which an affinity ligand is incorporated within a gel, provides a general means of manipulating the selectivity of CGE separations for biologically important molecules.

We demonstrated the selective and sensitive base-specific recognition of oligodeoxynucleotides with high efficiencies by using the CAGE system, in which poly(9-vinyladenine) (PVAd) was used as an affinity ligand [10–14]. PVAd is a non-degradable biomaterial that can interact to form complexes with naturally occurring nucleic acids by complementary hydrogen bonding in an aqueous solution [17,18] and is also an efficient

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affinity ligand in high-performance affinity chromatography [19,20] and affinity gel electrophoresis [21,22] as well as CAGE for the recognition of nucleobases of naturally occurring or synthetic nucleosides, oligonucleotides and polynucleotides.

Oligothymidylic acids were selectively separated from a mixture of oligodeoxynucleotides by CAGE using PVAd as an affinity ligand [10–12]. Additionally, the sequence isomers of hexadeoxynucleotides (TTATTT, TTTATT, TTTTAT and TTTTTA) that could not be separated by CGE [13] were completely separated by CAGE [13,14]. The electrophoretic behaviour of oligodeoxynucleotides in CAGE was strongly affected by several parameters, such as size of PVAd, capillary temperature and concentration of PVAd and urea [10–12]. Preliminary experiments [10] showed some relationship between the migration time in CAGE and the concentration of urea. In this study, the details of the effect of urea concentration on the migration behaviour of oligodeoxynucleotides were investigated in CAGE and CGE separations. We demonstrate that CAGE in the presence of excess urea is effective for the high-performance base-specific separation of oligodeoxynucleotides.

THEORY

PVAd, as an affinity ligand (L), interacts with oligodeoxynucleotide (N) as expressed by eqn. 1.

$$N + L \rightleftharpoons N \cdot L \quad K_a = \frac{[N \cdot L]}{[N][L]} \tag{1}$$

where K_a is the apparent association constant between oligodeoxynucleotide and an affinity ligand. The migration time, t, of an oligodeoxynucleotides in CAGE is expressed as follows [10]:

$$t = t_0(1 + K_a[L]_t) \tag{2}$$

where t_0 is the migration time of oligodeoxynucleotide in the absence of affinity ligand, *i.e.* CGE migration time, and $[L]_t$ is the total L concentration.

A change in urea concentration leads to changes in the conformation of oligonucleotides [23], the viscosity of the surrounding gel–buffer medium and the association process, as expressed in eqn. 1. The first two effects are related to t_0 as formulated in eqn. 3 [24], and the last effect is related to K_a.

$$t_0 = l \cdot 6\pi r\eta / EQ \tag{3}$$

where l is the effective length of the capillary up to the detection point, η is the viscosity of the surrounding gel–buffer medium, E is the applied field, Q is the net charge of oligonucleotides and r, which is the root mean square radius of the oligonucleotide, is related to the conformational change. The relationship between urea concentration and K_a has not been formulated.

EXPERIMENTAL

Chemicals

Tris(hydroxymethyl)aminomethane (Tris) from Sigma (St. Louis, MO, USA), boric acid, ammonium peroxodisulphate and urea from Nakarai Tesque (Kyoto, Japan) were of reagent grade. Electrophoretic grade acrylamide, N,N′-ethylenebis(acrylamide) (Bis) and N,N,N′,N′-tetramethylethylenediamide (TEMED) were purchased from Nakarai Tesque. Oligodeoxyadenylic acids, pdA_{12-18}, and oligothymidylic acids, pdT_{12-18}, were obtained from Pharmacia (Uppsala, Sweden). Other oligodeoxynucleotides were chemically synthesized using an Applied Biosystems (ABI, Foster City, CA, USA) Model 391 DNA synthesizer. Samples were diluted to 2.5 units per 500 μl with distilled water and stored at −15°C until use. PVAd was prepared by the method reported previously [10,17,18]. The PVAd samples thus obtained had molecular masses ranging from 10 000 to 30 000.

Apparatus

CAGE and CGE separations were carried out by using a CE-800 capillary electrophoretic system (Jasco, Tokyo, Japan). A Shimadzu (Kyoto, Japan) CR-3A was used as a data processor for CAGE and CGE separations. Polyimide-coated fused-silica capillaries (375 μm O.D. and 100 μm I.D., GL Sciences, Tokyo, Japan) were used, with an effective length of 30 cm and a total length of 50 cm. The buffer was a

mixture of 0.1 *M* Tris and 0.1 *M* boric acid with 2–10 *M* urea (pH 8.6) for the preparation of the gel-filled capillaries, as well as the running buffer. Capillaries filled with polyacrylamide–PVAd-conjugated gel (8% T, 5% C and 0.02–0.03% PVAd)[a] and polyacrylamide gel (8% T and 5% CA)-filled capillaries were prepared by the method previously reported [10–12, 25–27]. The percentage of PVAd was calculated by the equation, 100 × PVAd (g)/[acrylamide (g) + Bis (g) + PVAd (g)]. Gel-filled capillaries were mounted in the CE-800 system and run with buffer solution at 10 kV (200 V/cm) for 30 min prior to actual measurements in order to remove non-reacted monomer, peoxosulphate and TEMED. Samples were electrophoretically injected into the capillary by applying a voltage of 10 kV for 0.1–1 s. The applied voltage was 10 kV during the measurement. Oligonucleotides were detected at 260 nm. The viscosity of the buffer solution was measured by using an Ostwald viscometer.

RESULTS AND DISCUSSION

The base specificity of CAGE, using capillary filled with polyacrylamide gel entrapping PVAd as an affinity ligand, was based on the formation of complementary hydrogen bonding between PVAd and oligodeoxynucleotides [10–14]. Although urea is extensively used in gel electrophoresis as a denaturant to abolish the secondary structure of DNA and RNA [23], significant interaction between oligothymidylic acids and PVAd is observed in the presence of an excess of urea such as 7 *M* [10]. This indicates that the PVAd–DNA hybrid is more stable than normal double-stranded DNA, because the repulsion between anionic charges on the sugar–phosphate backbones in double-stranded DNA is significantly diminished in the hybrid of DNA and PVAd, having a non-charged polyvinyl backbone.

[a] C = g Bis/% T; T = (g acrylamide + g Bis)/100 ml solution.

Effect of urea concentration in the capillary gel electrophoretic separations

We first measured the migration time, t_0 in eqn. 3, of oligodeoxyadenylic acids and oligothymidylic acids using polyacrylamide gel (8% T and 5% C)-filled capillaries without an affinity ligand to examine the effect of urea concentration on the electrophoretic mobility (Fig. 1). Fig. 1 illustrates that an increase in the

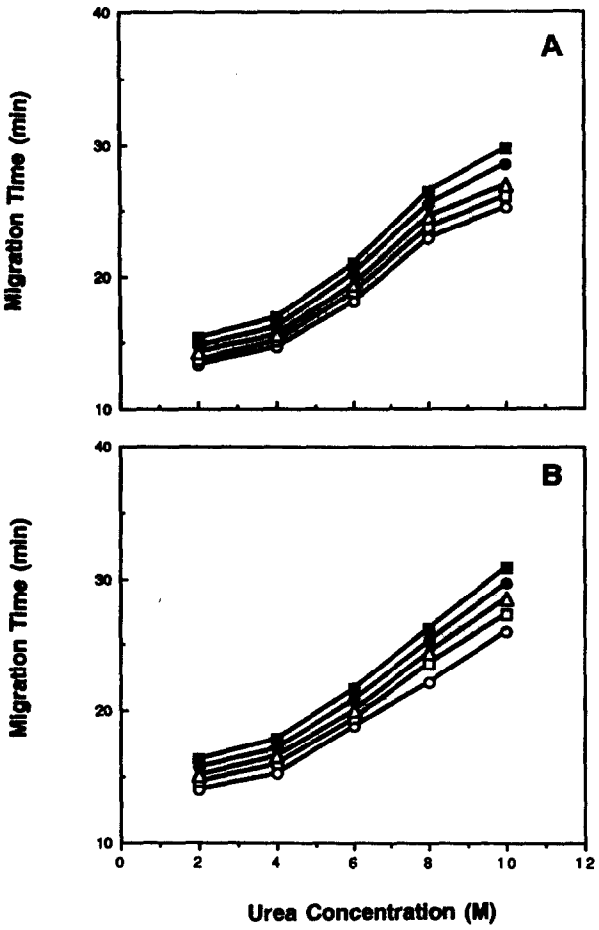

Fig. 1. Effect of the concentration of urea on the migration time of oligodeoxyadenylic acids (A) and oligothymidylic acids (B) in capillary gel electrophoresis. Conditions: capillary 100 μm I.D., 375 μm O.D., 50 cm length, 30 cm effective length; running buffer, 0.1 *M* Tris–borate and urea, pH 8.6; gel, 8% T and 5% C; capillary temperature, room temperature; field, 200 V/cm; current, 10 μA; injection, 10 kV for 1 s; detection, 260 nm. ○ = 8mer; □ = 10mer; △ = 12mer; ● = 14mer; ■ = 16mer.

concentration of urea results in an increase in the migration time of both oligodeoxyadenylic and oligothymidylic acids. This effect is caused by the change in the viscosity of the gel buffer medium and the root mean square radius of oligodeoxynucleotides, *i.e.* the conformational change, as expressed in eqn. 3. The root mean square radius increases with an increase in urea concentration, because self-associated oligonucleotides under non-denaturing conditions are transformed to the expanded conformation in the presence of an excess of urea. The viscosity of the buffer solution was measured by Ostwald viscometer at different urea concentrations and shown to increase with an increase in the urea concentration, as depicted in Fig. 2. It can be seen in Figs. 1 and 2 that the relationship between the migration time and the urea concentration is similar to that between the viscosity and the urea concentration. The viscosity, to which little attention has been paid, is proved to be a significant factor in this instance. Consequently, the migration time in CGE separation increases as the urea concentration increases in accordance with the relationship between the migration time and both the viscosity and the root mean square radius of oligodeoxynucleotides (eqn. 3).

Effect of urea concentration in the capillary affinity gel electrophoretic separations

The migration time, t in eqn. 2, of oligodeoxyadenylic and oligothymidylic acids was measured by using polyacrylamide–PVAd-conjugated gel-filled capillary (8% T, 5% C and 0.02% PVAd). Preliminary experiments [10] gave some relationship and qualitative considerations, because we measured t at only three urea concentrations. Therefore, the measurements were expanded to the wider range of the urea concentration to examine the details of the effect of the urea concentration and obtain some quantitative interpretations. Fig. 3 shows the relationship between the migration time and the urea concentration in CAGE separations. The migration time of oligodeoxyadenylic acid increases as urea

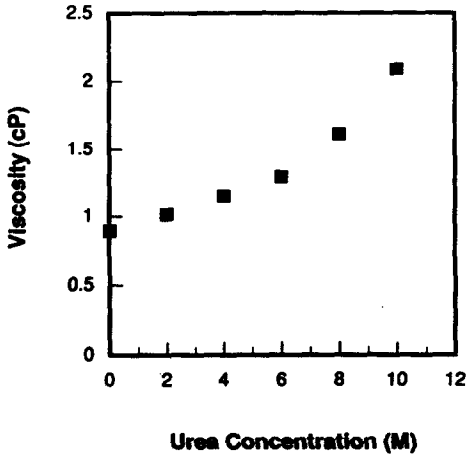

Fig. 2. The plot of the viscosity of buffer (0.1 *M* Tris–borate and urea) at 25°C *vs.* the concentration of urea.

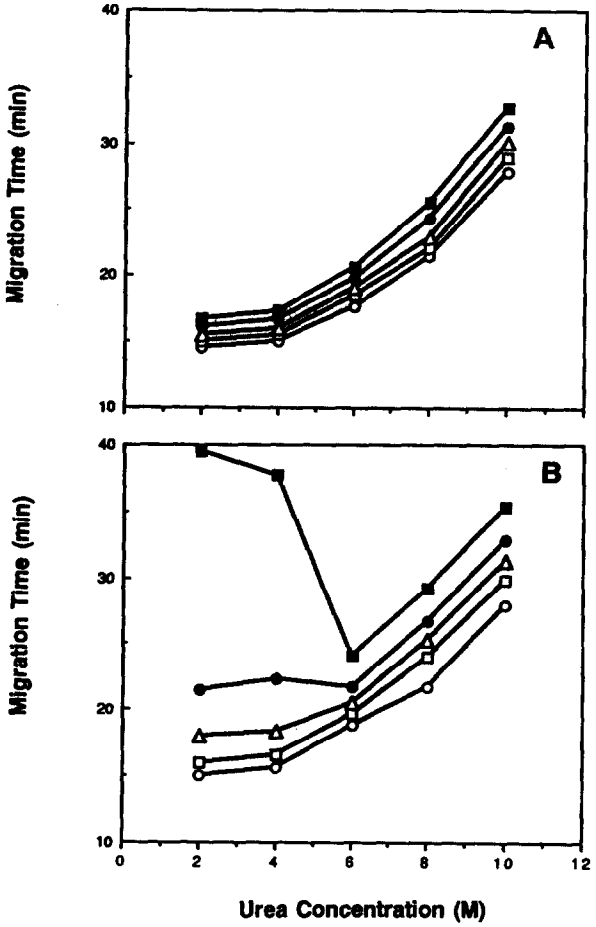

Fig. 3. Effect of the concentration of urea on the migration time of oligodeoxyadenylic acids (A) and oligothymidylic acids (B) in capillary affinity gel electrophoresis. The gel contained 8% T, 5% C and 0.02% poly(9-vinyladenine) with a molecular mass range of 10 000–30 000. Other conditions are as in Fig. 1. ○ = 8mer; □ = 10mer; △ = 12mer; ● = 14mer; ■ = 16mer.

concentration increases, as shown in Fig. 3A. The result in Fig. 3A was similar to that in Fig. 1A. This indicates that the effect of urea concentration on K_a in eqn. 2 is not taken into account in this instance, because the interaction of oligodeoxyadenylic acids with PVAd is negligible.

The effect of the urea concentration on the migration time of oligothymidylic acid as shown in Fig. 3B was different from that in CGE (Fig. 1B). Fig. 3B illustrates that an increase in the concentration of urea up to 6 *M* results in a decrease in migration time of larger oligothymidylic acids such as 14mer and 16mer, in contrast to the results in Fig. 1B. Urea will be effective in weakening the interaction between N and L, since it can break the hydrogen bonding of the base-paired complex. A decrease in the association constant (K_a in eqn. 2) caused by the dissociation of hydrogen bonding leads to a decrease in the migration time of larger oligothymidylic acid. The relationship between the migration times of shorter oligothymidylic acids such as 8–12mer and urea concentration is almost the same as that in CGE separations, as shown in Fig. 1B. The effect of the urea concentration over 6 *M* on the migration time of each oligothymidylic acid is also similar to that in Fig. 1B. Accordingly, larger oligothymidylic acids can interact with PVAd even in the presence of 2–6 *M* urea, but very weak interaction of smaller oligothymidylic acids with PVAd is exhibited. Urea at higher concentration over 6 *M* almost completely breaks the complementary hydrogen bonding of the oligothymidylic acids and PVAd hybrid.

Effect of urea concentration on the plate number

Fig. 4A and B compare the separations of a mixture of pdA_{12-18} and pdT_{15} by CAGE at different urea concentration. The relative migration time of pdT_{15} *vs.* oligodeoxyadenylic acids is reduced significantly at elevated concentration of urea. In addition, the band width of pdT_{15} decreases with an increase in urea concentration, as shown in Fig. 4, whereas that of oligodeoxyadenylic acid was insensitive to the urea concentration. Plate numbers in both the CAGE and the CGE separation were estimated and are

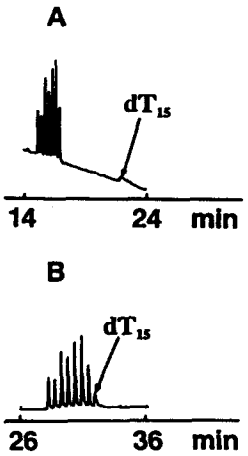

Fig. 4. Capillary affinity gel electrophoresis of a mixture of pdA_{12-18} and pdT_{15}. The gel contained 8% T, 5% C and 0.02% poly(9-vinyladenine) with a molecular mass range of 10 000–30 000. Running buffer, 0.1 *M* Tris–borate and urea, pH 8.6. Concentration of urea: 4 *M* (A) and 8 *M* (B). Other conditions are as in Fig. 1.

compiled in Table I. The plate number of oligodeoxyadenylic acid was kept almost constant during all measurements.

The plate number of oligothymidylic acid in CAGE separation was very sensitive to the urea concentration as listed in Table I. For example, the plate number of pdT_{15} at 6 *M* urea was 28 times larger than that at 4 *M* urea in CAGE separation. Since the affinity ligand would bind tightly to pdT_{15} by hydrogen bonding, the pdT_{15} band was severely broadened at lower concentrations of urea. Dissociation of hydrogen bonding, which occurred at higher concentrations of urea. Dissociation of hydrogen bonding, which occurred at higher concentrations of urea, resulted in sharper bands of oligothymidylic acid. The results show that the base-specific separation was achieved with high resolution only in the presence of an excess of urea.

High performance base-specific separation of oligodeoxynucleotides

We tried the base-specific separation of a complex mixture of pdT_{12-18} and pdA_{12-18} by using 10 *M* urea–gel capillary, as shown in Fig. 5. The pdT_{14-18} bands were selectively separated from pdA_{12-18}, whereas the pdT_{12} band co-migrated with the pdA_{16} band and the pdT_{13} band partially overlapped the pdA_{18} band. The pdT_{17}

TABLE I

PLATE NUMBER OF OLIGODEOXYNUCLEOTIDES IN CAPILLARY AFFINITY GEL ELECTROPHORESIS AND CAPILLARY GEL ELECTROPHORESIS

Sample	Concentration of PVAd (%)	Concentration of urea (*M*)	Plate number per metre
pdT_{12}	0	4.0	$5.7 \cdot 10^5$
	0.02	4.0	$2.8 \cdot 10^4$
	0.02	6.0	$4.6 \cdot 10^5$
pdT_{15}	0	4.0	$6.4 \cdot 10^5$
	0.02	4.0	$1.2 \cdot 10^3$
	0.02	6.0	$3.4 \cdot 10^4$
pdA_{12}	0	4.0	$5.1 \cdot 10^5$
	0.02	4.0	$3.2 \cdot 10^5$
	0.02	6.0	$1.2 \cdot 10^5$
pdA_{15}	0	4.0	$5.7 \cdot 10^5$
	0.02	4.0	$5.5 \cdot 10^5$
	0.0	6.0	$2.4 \cdot 10^5$

and pdT_{18} bands were severely broadened owing to the powerful interaction with PVAd. Although the temperature programming technique [12] was demonstrated to be essential to the base-specific separation of the mixture of oligodeoxynucleotides, complete resolution of such complex mixture was not realized even by using the temperature-programmed CAGE [12]. The result in Fig. 5, therefore, demonstrates that the CAGE using urea–gel capillaries as well as temperature-programmed CAGE [12] is very effective for improving the resolution in base-specific separation. In conclusion, a urea–gel capillary is indispensable to the analytical purpose of CAGE, because severe band broadening was significantly reduced and high-performance base-specific separation was accomplished.

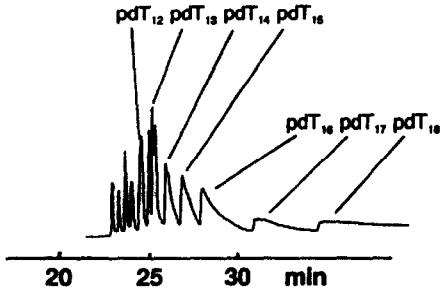

Fig. 5. Capillary affinity gel electrophoresis of a mixture of pdA_{12-18} and pdT_{12-18}. The gel contained 8% T, 5% C and 0.03% poly(9-vinyladenine) with a molecular mass range of 10 000–30 000. Running buffer, 0.1 *M* Tris–borate and 10 *M* urea, pH 8.6. Other conditions are as in Fig. 1.

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