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Prediction of migration behavior of oligonucleotides in capillary gel electrophoresis

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ABSTRACT

The influence of the primary structure (base composition) on the electrophoretic migration properties of single-stranded oligodeoxyribonucleotides in capillary polyacrylamide gel electrophoresis was investigated using homo- and heterooligomers under denaturing and non-denaturing conditions. Homooligodeoxyribonucleotides of equal chain lengths but of different base composition showed significant differences in mobility. In addition, the migration properties of heterooligomers were found to be highly dependent on their base composition. A simple equation is presented for predicting relative migration times using denaturing and non-denaturing polyacrylamide capillary gel electrophoresis. Orange-G was used as an internal standard and as the basis of the relative migration time calculations. Examples are presented using homo- and heterooligomers in the 10–20-mer range to show the correlation of the primary structure and their predicted and observed migration rates.

INTRODUCTION

High-performance capillary electrophoresis (HPCE) is rapidly becoming an important separation tool in analytical biochemistry and molecular biology [1–5]. Capillary polyacrylamide gel electrophoresis of oligonucleotides and DNA and RNA molecules under denaturing and non-denaturing conditions have been shown to provide separations of very high efficiency [6–8]. As an instrumental approach to electrophoresis, the method offers the ability to do multiple injections on the same gel-filled capillary column, with on-column UV detection and on-line data processing [9].

In this work, the influence of the primary structure (base composition) on the migration properties of homo- and heterooligodeoxyribonucleotides in capillary polyacrylamide gel electrophoresis was studied. Previous reports have described slab [10] and capillary [11] polyacrylamide gel electrophoresis under denaturing conditions as an accurate method for the determination of the chain length and molecular weight of small DNA and RNA molecules. However, in our experiments with capillary gel electrophoresis, the direct correlation between the chain length of the homooligomers and their migration times was found to be unreliable. It was observed that under both denaturing and non-denaturing electrophoresis conditions, oligonucleotides were not separated according to their chain lengths alone. In fact, base composition plays a significant role in oligonucleotide migration in polyacrylamide gels. A reliable model has been developed to predict the electrophoretic migration times of any oligonucleotide with a known sequence relative to homooligomers having the same chain lengths.

EXPERIMENTAL

Apparatus

In all these studies, the P/ACE System 2100 capillary electrophoresis apparatus (Beckman Instruments, Palo Alto, CA, USA) was used with reversed polarity (cathode on the injection side and anode on the detection side). The separations were monitored on-column at 254 nm. The temperature of the gel-filled capillary columns was controlled by the liquid

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cooling system of the P/ACE instrument at 25°C. The electropherograms were acquired and stored on an Everex 386/33 computer using System Gold software (Beckman Instruments).

Procedures

Polymerization of the non-denaturing linear (non-cross-linked) polyacrylamide was accomplished within fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) in 100 m*M* Tris–borate–2 m*M* EDTA (pH 8.5) buffer. Polymerization was initiated by ammonium peroxodisulfate and catalyzed by tetramethylethylenediamine (TEMED). The denaturing gel column employed was the eCAP gel U100P (Beckman Instruments). To obtain a similar pore structure, both the non-denaturing and the denaturing polyacrylamide gels were prepared at the same concentration. The samples were injected electrokinetically into the gel-filled capillary columns, typically using 0.1 W s. Samples were boiled for 5 min and then cooled for 30 s in ice–water before injection.

Chemicals

The homodecamers of adenylic [$p(dA)_{10}$], cytidylic [$p(dC)_{10}$], guanylic [$p(dG)_{10}$] and thymidylic [$p(dT)_{10}$] acids, the homooligomer mixtures, $p(dA)_{12-18}$, $p(dC)_{12-18}$, $p(dG)_{12-18}$ and $p(dT)_{12-18}$, and the human K-ras oncogenes (dGTTGGAGCT–C–GTGGCGTAG, dGTTGGAGCT–G–GTGGCGTAG and dGTTGGAGCT–T–GTGGCGTAG) were purchased for Pharmacia (Piscataway, NJ, USA). The samples were diluted to 0.5 absorbance unit/ml (*ca.* 20 μg/ml) with water before injection and were stored at −20°C when not in use. Ultra-pure electrophoresis-grade acrylamide, Tris, boric acid, EDTA, urea, ammonium peroxodisulfate and TEMED were employed (Schwartz/Mann Biotech, Cambridge, MA, USA). Orange G (Sigma, St. Louis, MO, USA) was used in the electrophoretic separations as an internal standard at 0.001% concentration. All buffer solutions were filtered through a 0.2-μm pore size filter (Schleicher & Schüll, Keene, NH, USA) and carefully vacuum degassed.

RESULTS AND DISCUSSION

Initial efforts were focused on achieving high-resolution separations of homooligodeoxyribonucleotides using high-performance capillary gel electrophoresis under non-denaturing and denaturing conditions. As reported earlier [12], the pH of the buffer system used in capillary gel electrophoresis has a remarkable effect on the migration properties of different homooligomers; therefore, the pH in all experiments reported (denaturing and the non-denaturing) was maintained at 8.5.

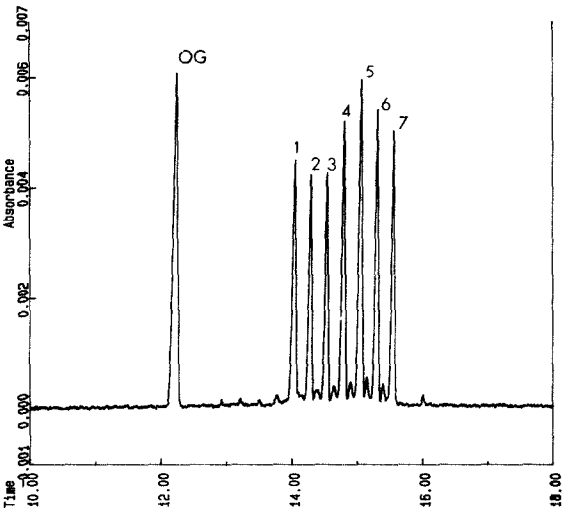

Fig. 1. Non-denaturing capillary polyacrylamide gel electrophoretic separation of $p(dT)_{12-18}$ oligodeoxythymidilic acid mixture with the internal standard Orange G. Peaks: OG = Orange G; 1 = $p(dT)_{12}$; 2 = $p(dT)_{13}$; 3 = $p(dT)_{14}$; 4 = $p(dT)_{15}$; 5 = $p(dT)_{16}$; 6 = $p(dT)_{17}$; 7 = $p(dT)_{18}$. Conditions: isoelectrostatic (constant applied electric field), 400 V/cm; prepacked non-denaturing polyacrylamide gel column, effective length 40 cm, total length 47 cm; buffer, 100 m*M* Tris–boric acid–2 m*M* EDTA (pH 8.5); injection, 0.1 W s. Time in min.

Non-denaturing capillary polyacrylamide gel columns

Fig. 1 shows the baseline resolution of one of the four homooligomer mixtures [$p(dT)_{12-18}$] separated on a non-denaturing polyacrylamide gel-filled capillary column. The peak marked OG corresponds to the internal standard Orange G, which was selected because of its rapid migration relative to the short oligonucleotides. The other three homooligomer mixtures, $p(dA)_{12-18}$, $p(dC)_{12-18}$ and $p(dG)_{12-18}$ were also separated on the same column, again using Orange G as the internal standard. The relative migration times were calculated

from the ratio of the migration time of the actual oligomer to that of the internal standard. A linear relationship existed between the relative migration times (t') and the chain lengths of the homooligomers (n) in the size range examined, as shown in Fig. 2, according to the following equations:

$$p(dA)_n\text{: } t'(A_n) = 0.0161n + 0.7979 \quad \text{(R.S.D. 0.999\%)} \tag{1a}$$

$$p(dT)_n\text{: } t'(T_n) = 0.0205n + 0.9023 \quad \text{(R.S.D. 0.999\%)} \tag{1b}$$

$$p(dC)_n\text{: } t'(C_n) = 0.0182n + 0.7961 \quad \text{(R.S.D. 0.998\%)} \tag{1c}$$

$$p(dG)_n\text{: } t'(G_n) = 0.0071n + 0.9590 \quad \text{(R.S.D. 0.999\%)} \tag{1d}$$

where $p(dA)_n$, $p(dT)_n$, $p(dC)_n$ and $p(dG)_n$ are the individual homo-n-mers of adenylic, thymidylic, cytidylic and guanylic acid, respectively, and R.S.D. is the relative standard deviation.

The plots have similar slopes for the $p(dA)_{12-18}$, $p(dC)_{12-18}$ and $p(dT)_{12-18}$ samples (eqns. 1a–c), but a different slope for $p(dG)_{12-18}$ (eqn. 1d). This last slope is much lower, resulting in a different migration order depending on the base number for a mixture of the four homooligomers. For example, the migration order below 14 bases is A > C > G > T, between 14 and 18 bases A > G > C > T and above 18 bases G > A > C > T. This anomalous migration behavior may be due to the strong self-association tendency of guanosine under non-denaturing conditions, which migth cause conformational changes such as bending [10].

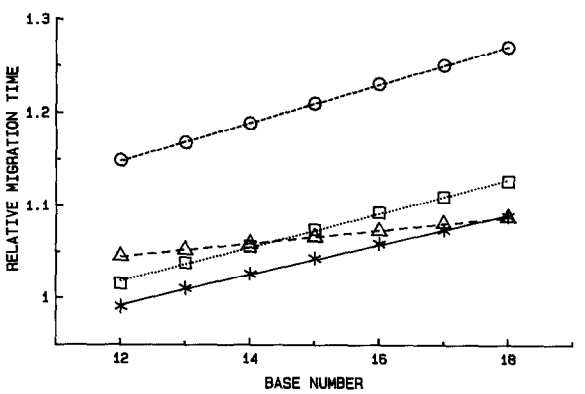

Fig. 2. Relationship between the chain length and the relative migration time of the homodeoxyribooligomer mixtures on non-denaturing polyacrylamide gel-filled capillary. Conditons as in Fig. 1. The calculation of relative migration times was based on the migration time of Orange G. ✳ = $p(dA)_{12-18}$; ○ = $p(dT)_{12-18}$; □ = $p(dC)_{12-18}$; △ = $p(dG)_{12-18}$.

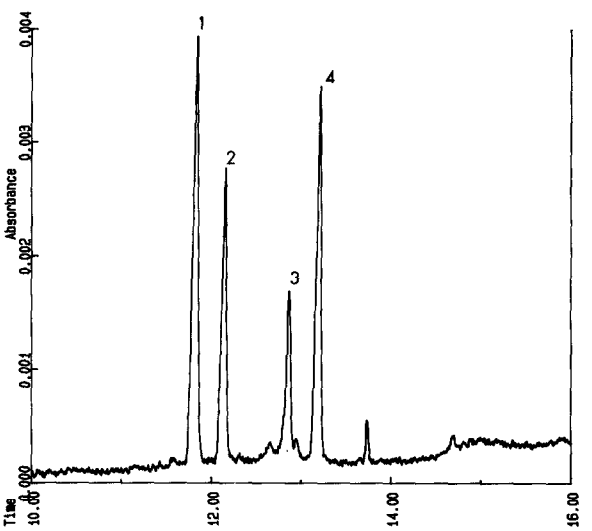

Fig. 3. Capillary polyacrylamide gel electrophoretic separation of a homodecamer mixture on non-denaturing gel. Peaks: 1 = $p(dA)_{10}$; 2 = $p(dC)_{10}$; 3 = $p(dG)_{10}$; 4 = $p(dT)_{10}$. Conditions as in Fig. 1. Migration time of Orange G (determined in the immediately following run): 12.386 min.

Also of interest in Fig. 2 is the comigration of the 14-mers of p(dC) and p(dG) and that of the 18-mers of p(dA) and p(dG). These results indicate that the migration rate of some oligomers could be relatively insensitive to a difference in base composition resulting in co-migration in non-denaturing gels.

As the base-specific retardation of oligonucleotides is an additive effect [13], migration times can be easily predicted using linear extrapolation from the relative migration times of the homooligomers. Fig. 3 shows a non-denaturing capillary gel electrophoretic separation of a mixture of four homodecamers and Table I gives the predicted and observed relative migration times of the four sample components. As can be seen in Table I, there is excellent agreement between the extrapolated and observed relative migration time values (±0.1%). The identification of the homooligomers was accomplished by spiking with the individual compounds. The small peak after peak 4 is an impurity from $p(dT)_{10}$. As Orange G migrates too close to peak 3, its migration time was determined in the immediate-

TABLE I

OBSERVED AND PREDICTED RELATIVE MIGRATION TIMES OF VARIOUS HOMO- AND HETEROOLIGODEOXYRIBONUCLEOTIDES IN NON-DENATURING POLYACRYLAMIDE CAPILLARY GEL ELECTROPHORESIS

Relative migration times were calculated using Orange G as internal standard.

Nucleotide sequence	Relative migration time		Migration order
	Observed	Calculated	
p(dA)10	0.957	0.960	1
p(dC)10	0.983	0.981	2
p(dG)10	1.039	1.032	3
p(dT)10	1.068	1.107	4
dGTTGGAGCT-G-GTGGCGTAG	1.149	1.150	1
dGTTGGAGCT-C-GTGGCGTAG	1.156	1.155	2
dGTTGGAGCT-T-GTGGCGTAG	1.160	1.161	3

ly following run, by using peak 1 [$p(dA)_{10}$] as internal standard.

Whereas simple relative migration time extrapolation is satisfactory for homooligomers, the prediction of the relative migration time of a heterooligomer (t') is improved by using the relative migration times of the four corresponding homooligomers in the following relationship:

$$t'(A_aT_tC_cG_g) = \frac{a}{n}\,t'(A_n) + \frac{t}{n}\,t'(T_n) + \frac{c}{n}\,t'(C_n) + \frac{g}{n}\,t'(G_n) \quad (2)$$

where t' is relative migration time *vs.* the internal standard, n is the oligonucleotide chain length ($n = a + t + c + g$) and a, t, c and g are the numbers of the individual bases in the oligonucleotide. The parameters $t'(A_n)$, $t'(T_n)$, $t'(C_n)$ and $t'(G_n)$ correspond to the relative migration times of the homooligo-n-mers of adenylic, thymidylic, cytidylic and guanylic acid, respectively. The most accurate calculation requires the availability of standards with chain lengths equal to the unknown. If standards are not available, the extrapolated values from linear plots such as Figs. 2 and 5 should be used. Capillary polyacrylamide gel electrophoresis of homo- and/or heterooligomers of known chain lengths and base compositions showed excellent correlation between the observed and predicted migration times (Table I).

We have also obtained (see Fig. 4) the separation of heterooligomers with the same chain lengths and similar sequences in order to emphasize the effectiveness of the mathematical prediction of the migration order. Fig. 4 shows the non-denaturing gel

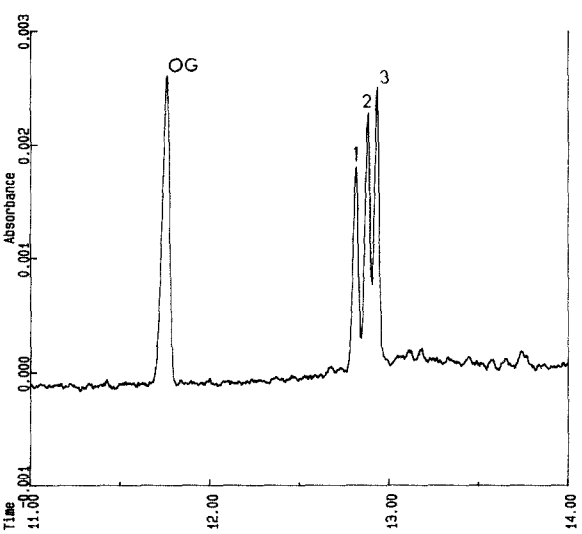

Fig. 4. Non-denaturing capillary polyacrylamide gel electrophoretic separation of a human K-ras oncogene mixture. Peaks: 1 = dGTTGGAGCT-G-GTGGCGTAG; 2 = dGTTGGAGCT-C-GTGGCGTAG; 3 = dGTTGGAGCT-T-GTGGCGTAG. Conditions as in Fig. 1.

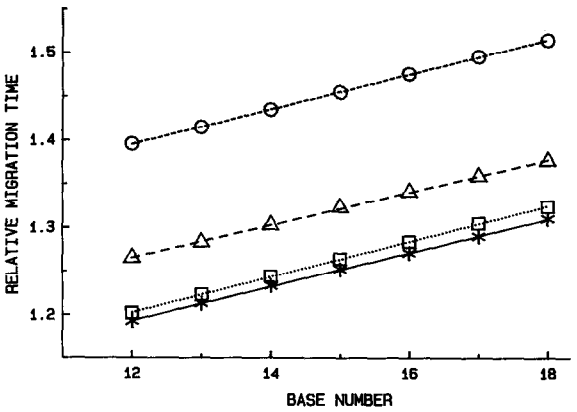

Fig. 5. Relationship between the chain length and the relative migration time of the homodeoxyribooligomer mixtures on a denaturing polyacrylamide gel-filled capillary. Conditions: isoelectrostatic (constant applied electric field), 400 V/cm; eCAP gel U100P column, effective length 40 cm, total length 47 cm; injection, 0.1 W s. The calculation of relative migration times was based on the migration time of Orange G. Symbols as in Fig. 2.

electrophoretic separation of a mixture of three human K-ras oncogenes. These oligomers have the same chain length (19-mers) and almost the same primary sequence, differing only by one base in the middle (position 10) of the chain (see primary structure in Table I). Because of the high resolving power of capillary gel electrophoresis, this method is capable of separating these closely related species. Note that the observed and predicted migration times are very similar (see Table I). The location of guanosine in the tenth position speeds up the migration rate of the heterooligomer, relative to that of cytidine or thymidine. This migration order is consistent with the data in Fig. 2. The identity of the oligomers was confirmed by spiking the mixture with the individual compounds. As no homooligo-nanodecamers were available for these experiments, the $t'(A_n)$, $t'(T_n)$, $t'(C_n)$ and $t'(G_n)$ parameters for eqn. 2 were calculated by extrapolation of the linear plots in Fig. 2.

Denaturing capillary polyacrylamide gel columns

When denaturing gels (eCAP gel U100P) were used for the separation of the same oligonucleotide mixtures, different migration properties were observed. In contrast to the behavior seen in Fig. 2, all four of the homooligomer mixtures have parallel plots of relative migration time as a function of the chain lengths, as shown in Fig. 5, according to the following equations:

$$p(dA)_n: t'(A_n) = 0.0193n + 0.9609 \quad \text{(R.S.D. 0.999\%)} \tag{3a}$$

$$p(dT)_n: t'(T_n) = 0.0202n + 1.1559 \quad \text{(R.S.D. 0.999\%)} \tag{3b}$$

$$p(dC)_n: t'(C_n) = 0.0204n + 0.9590 \quad \text{(R.S.D. 0.999\%)} \tag{3c}$$

$$p(dG)_n: t'(G_n) = 0.0188n + 1.0398 \quad \text{(R.S.D. 0.999\%)} \tag{3d}$$

TABLE II

OBSERVED AND PREDICTED RELATIVE MIGRATION TIMES OF VARIOUS HOMO- AND HETEROOLIGODEOXYRIBONUCLEOTIDES IN DENATURING POLYACRYLAMIDE CAPILLARY GEL ELECTROPHORESIS

Relative migration times were calculated using Orange G as internal standard.

Nucleotide sequence	Relative migration time		Migration order
	Observed	Calculated	
p(dA)10	1.153	1.155	1
p(dC)10	1.163	1.162	2
p(dG)10	1.237	1.233	3
p(dT)10	1.356	1.355	4
dGTTGGAGCT–G–GTGGCGTAG	1.427	1.422	2
dGTTGGAGCT–C–GTGGCGTAG	1.416	1.419	1
dGTTGGAGCT–T–GTGGCGTAG	1.430	1.429	3

The non-parallel discrepancy previously observed on non-denaturing gels does not occur in this instance, probably owing to the denaturing effect of the 7 *M* urea in the gel [14]. By means of eqn. 2, relative migration times can be calculated in a similar way as above. The relative migration data for the samples are summarized in Table II, which shows the observed and calculated migration times of the homo- and heterooligonucleotide mixtures separated on denaturing gel. Referring back to the migration behavior of the oligonucleotides on non-denaturing gels, it is important to note that the migration order has been changed among the three human K-ras oncogenes (Tables I and II). Again, this was confirmed by spiking the mixture with the individual oncogenes. As in this instance the increasing guanosine content does not have the same accelerating effect as was observed on the non-denaturing gel, the migration order is the same as that observed for the homooligomers (Table II, A > C > G > T). The accelerating effect of A- and C-rich oligomers and the retarding effect of G- and T-rich oligomers can be predicted for the chain-length range examined.

CONCLUSIONS

Investigations of the electrophoretic migration behavior of various homo-and heterooligomers of known nucleotide sequences have been presented. Using non-denaturing gels, we found that the relative migration order is not constant for homooligomers of the same chain length, but is dependent on the base number: for base numbers less than 14, it is A > C > G > T, and for base numbers larger than 18, it is G > A > C > T. This discrepancy is probably caused by the strong self-association tendency of guanosine (conformation changes such as bending). Employing denaturing gels, however, the migration order of the homooligomers is the same for the entire chain-length range examined, namely A > C > G > T. In this instance, the self-association effect of the guanosine is assumed to be negligible owing to the presence of urea, a denaturing agent, in the gel. The denaturing gel has a much higher sensitivity to guanosine content than the non-denaturing gel. There is, however, an increased ability of the non-denaturing gel to resolve A and C over the denaturing gel (compare Figs. 2 and 5). Because of the parallel slopes achieved using denaturing gels, we believe that the migration times of heterooligonucleotides are more predictable in this instance. In addition, it can be demonstrated using eqn. 2 that several combinations of sequences might be resolved in one system and not in another. Therefore, in order to increase the confidence in oligomer identity, one might need to utilize both denaturing and non-denaturing conditions.

Oligonucleotides of different sequences, but with the same chain length, are more likely to show different migration times. With respect to base composition, an equation was derived in order to predict the migration time of a known oligonucleotide sequence. Reproducibility and additivity of base-specific retardation are the basis of the calculation procedure for the relative migration times. It should be emphasized that eqn. 2 is considered to be valid only for primer-sized oligonucleotides ($n < 25$), and it should be further evaluated for longer ones.

The method opens up a new feature of capillary polyacrylamide gel electrophoresis in the identification of, and discrimination between, oligonucleotides by their mobility shift relative to an internal standard. This can be easily computed by an automated capillary electrophoretic system, such as the P/ACE 2100. A further interesting application of this equation is to calculate the migration time differences between oligonucleotides, and to design appropriate electrophoresis conditions, such as column length, necessary for their separation. This can be important for separations of oligonucleotides of the same chain length but different base composition, such as two strands of double-stranded DNA (denaturing gel), or in point mutation studies (non-denaturing gel). Whereas denaturing conditions are necessary to reduce guanosine self-association and solve compression problems, non-denaturing gels might also offer different selectivity in certain instances.

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