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Temperature-programmed capillary affinity gel electrophoresis for the sensitive base-specific separation of oligodeoxynucleotides

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ABSTRACT

High-performance base-specific separations of oligodeoxynucleotides were performed by temperature-programmed capillary affinity gel electrophoresis (CAGE), in which poly(9-vinylacenine)(PVAd) was utilized as an affinity ligand. The migration behaviour of oligodeoxynucleotides was investigated at different capillary temperatures. The migration time and resolution of oligodeoxyadenylic acids, which do not interact with PVAd, decrease with increase in temperature as in capillary gel electrophoretic separation. The migration behaviour of oligothymidylic acids, which interact with PVAd, is manipulated by varying the capillary temperature, which leads to changes in the dissociation process of specific hydrogen bonding between oligothymidylic acids and PVAd. The implementation of temperature-programmed CAGE was illustrated by the selective and sensitive base recognition of oligodeoxynucleotides with efficiencies as high as several times 10^6 plates per metre.

INTRODUCTION

Capillary gel electrophoresis (CGE) [1] is rapidly becoming an important tool for the separation of single- and double-stranded polynucleotides (DNA and RNA). Extremely high-resolution and high-speed separations could be performed by CGE. However, CGE is not applicable to the sequence-specific separation of polynucleotides, such as sequence isomer separations, as the separation by CGE is based on a molecular sieving effect. Development of new CGE columns with special selectivity extends the versatility of CGE, as in HPLC.

Recently we developed capillary affinity gel electrophoresis (CAGE) as an alternative format of CGE, incorporating an affinity ligand within a polyacrylamide gel matrix, for the specific base recognition of oligodeoxynucleotides [2–5]. The incorporation of an affinity ligand within a gel can be used to manipulate the selectivity of CGE separations. CAGE using poly(9-vinyladenine)(PVAd) as an affinity ligand was effective for the selective separation of oligothymidylic acids from a mixture of oligodeoxynucleotides [2–4]. Additionally, CAGE separated completely the sequence isomers of hexadeoxynucleotide (TTTATT, TTTTAT and TTTTTA) [5], which could not be separated by CGE [5]. PVAd has been shown to form *in vitro* the complex with the complementary strand of polynucleotide by complementary hydrogen bonding [6,7] and to be an excellent affinity ligand [8–10] for high-performance affinity chromatography and affinity gel electrophoresis. The selectivity and efficiency of

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base-specific separations by CAGE were strongly affected by several parameters, such as the size of PVAd, capillary temperature and concentrations of PVAd and urea [2–4]. In this study, the effect of temperature on the migration behaviour of oligodeoxynucleotides was investigated in CAGE separations. It is demonstrated that temperature-programmed CAGE is effective for the high-performance base-specific separation of oligodeoxynucleotides.

THEORY

The migration time, t, of an oligodeoxynucleotide in CAGE in the presence of the interaction (eqn. 1) of oligodeoxynucleotides (N) and affinity ligands (L) is expressed as eqn. 2 [2]:

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

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

where t_0 is the migration time of oligodeoxynucleotide in the absence of affinity ligand, K_a is the apparent association constant between oligodeoxynucleotide and an affinity ligand (L) and [L], is the total L concentration. Temperature affects two parameters in eqn. 2, *viz.*, t_0 and K_a. The relationship between temperature and t_0 was formulated by Guttman and Cooke [11] as follows:

$$t_0 = l \cdot 6\pi r\eta/EQ$$

$$\eta = C_1 \exp(E_a/RT)$$

$$\ln t_0 = \ln(l \cdot \text{constant}/EQ) + E_a/RT \tag{3}$$

where I is the effective length of the capillary up to the detection point, η is the viscosity of the surrounding gel-buffer medium, r is the root mean square radius of the polynucleotide, C_1 is a constant, E is the applied field, Q is the net charge of polynucleotides, E_a is the activation energy for the viscous flow, R is the universal gas constant and T is the absolute temperature. The effect of temperature on K_a was expressed as

$$\ln K_a = -\Delta G/RT \tag{4}$$

where AG is the free energy change in the equilibrium of eqn. 1.

EXPERIMENTAL

Chemicals

Unless stated otherwise, chemicals were of analytical-reagent or electrophoretic grade from Wako (Osaka, Japan). Oligodeoxyadenylic acids, pdA_{12-18}, and oligothymidylic acids, pdT_{12-18}, were obtained from Pharmacia (Uppsala, Sweden). The pdT_{15} sample was chemically synthesized using a 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 −18°C until used. PVAd was prepared by the method reported previously [2,6,7]. The PVAd sample thus obtained had a relative molecular mass ranging from 10 000 to 30 000.

Capillary electrophoresis

An ABI Model 270A capillary electrophoresis system was used for the CAGE 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 22 cm and a total length of 42 cm. The buffer was a mixture of 0.1 M Tris and 0.1 M boric acid with 7 M urea (pH 8.6) for the preparation of the gel-filled capillaries and as the running buffer. Capillaries filled with polyacrylamide–PVAd conjugated gel (8% T, 5% C and 0.05–0.1% PVAd)[a] were prepared by the method reported previously [2,12–14]. 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 ABI Mode1 270A system and run with buffer solution at 9 kV (214 V/cm). Samples were electrophoretically injected into the capillary by applying a voltage of 5 kV for 0.1-I s. The temperature of the agitated air surrounding the capillary was maintained constant within ± 0.1 "C. Oligonucleotides were detected at 260 nm.

RESULTS AND DISCUSSION

In the previous work [2–5], we developed a CGE column that included polyacrylamide gel entrapping PVAd as an affinity ligand for the CAGE sep-

[a] C = g N,N'-methylenebisacrylamide (Bis)/% T; T = g acrylamide + g Bis per 100 ml of solution.

arations of oligodeoxynucleotides. Oligothymidylic acids were selectively separated from a mixture of oligodeoxynucleotides by using CAGE utilizing PVAd as follows. In CGE separations, each oligodeoxynucleotide with the same chain length and difference base composition would elute with almost the same electrophoretic mobility [2]. On the other hand, the mobility of oligothymidylic acids shows strong retardation due to its specific interaction with PVAd, whereas the mobilities of other oligodeoxynucleotides that cannot interact with PVAd are unchanged. A difference in the mobilities results in the specific recognition of oligothymidylic acids over other oligodeoxynucleotides.

Effect of temperature in the capillary affinity gel electrophoretic separations

Figs. 1 and 2 compare the separations of the pdA_{12-18} and the pdT_{12-18} mixture by CAGE at different temperatures. It can be seen in Fig. 1 that

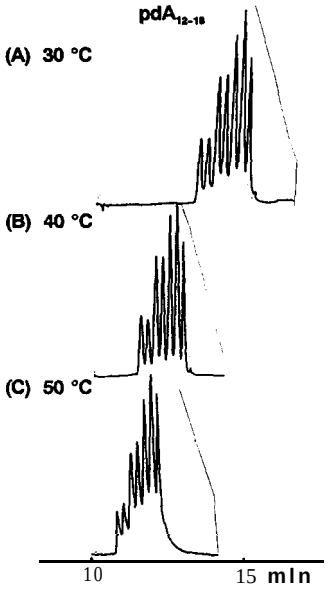

Fig. 1. Separation of pdA_{12-18} by CAGE at (A) 30, (B) 40 and (C) 50°C. Capillary, 100 μm I.D., 375 μm O.D., total length 42 cm, effective length 22 cm. Running buffer, 0.1 *M* Tris-borate and 7 *M* urea (pH 8.6). Gel contained 8% T, 5% C and 0.05% PVAd with a relative molecular mass range of 10 000-30 000. Field, 214 V/cm; current, 9 μA; injection, 5 kV for 1 s; detection, 260 nm.

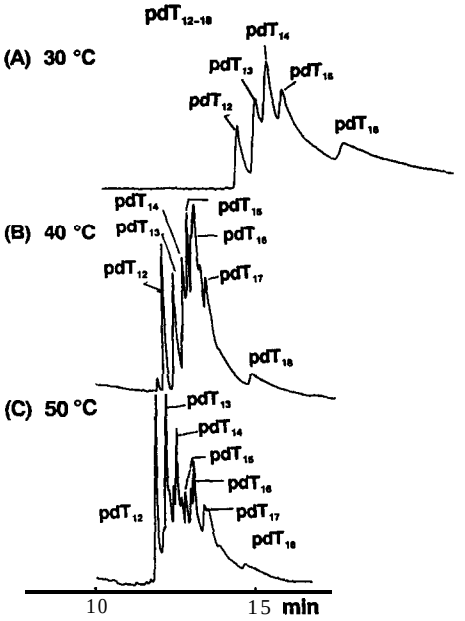

Fig. 2. Separation of pdT_{12-18} by CAGE at (A) 30, (B) 40 and (C) 50°C. Conditions as in Fig. 1.

the migration time and resolution of pdA_{12-18} decrease as the temperature increases. The results in Fig. 1 were comparable to the effect of temperature on the migration time of pdA_{12-18} in CGE without an affinity ligand [15]. This suggests that a decrease in the migration time of pdA_{12-18} is mainly caused by a decrease in the viscosity of the surrounding gel-buffer with an increase in temperature as expressed in eqn. 3 [11]. The effect of temperature on K_a in eqn. 4 is not taken into account in this instance, because the interaction of pdA_{12-18} with PVAd is negligible.

The effect of temperature on the electropherogram of pdT_{12-18} as shown in Fig. 2 was different from that in CGE, which was similar to the results in Fig. 1 [15]. The migration time of pdT_{12-18} was more affected by temperature than that of pdA_{12-18}, as shown in Fig. 1. In addition, some band broadening was observed at lower temperature. This indicates that temperature affects not only the viscosity process in eqn. 3, but also the association process expressed as eqn. 4. As the affinity ligand would bind tightly to pdT_{12-18} by hydrogen bonding at lower temperature, band broadening increased as temperature decreased. Dissociation of hydrogen bonding, which occurred at elevat-

ed temperature, resulted in a decrease in the association constant, K_a, in eqn. 4. Therefore, the migration times at 40 and 50°C (Fig. 2B and C) were almost comparable to those in CGE [15]. Some peaks that appeared in Fig. 2B and C in addition to the peaks for pdT_{12-18} would correspond to the peaks for dephosphorylated oligodeoxythymidylic acids.

We tried the base-specific separation of pdT_{12-18} from a mixture of oligodeoxynucleotides by using isothermal CAGE as shown in Fig. 3. Oligothymidylic acids were selectively separated from the mixture at 30°C, but the resolution of pdT_{12-18} decreased considerably as shown in Fig. 3A. The base selectivity of pdT_{12-18} over pdA_{12-18} was significantly diminished above 40°C (Fig. 3B). Consequently, sensitive base-specific separation was not be achieved by using isothermal CAGE. We next turned to the use of temperature-programmed CAGE for high-performance specific base recognition.

High-performance base-specific separation by temperature-programmed CAGE

As in chromatographic methods [16], the main purpose of using temperature programming in CAGE is to improve the speed and/or the efficiency of a separation. Fig. 4 demonstrates the effect of temperature programming on the electrophoretic behaviour of a mixture of pdA_{12-18} and pdT_{12-18} in CAGE. CAGE was initially performed at 30°C. After an appropriate time as shown in Fig. 4, the temperature was elevated to 50°C. The temperature programming was actually performed as a gradient in which the temperature was gradually increased from 30 to 50°C during a *ca.* 3-min interval during electrophoresis rather than as stepwise changes. An attempt to improve the resolution by temperature programming in Fig. 4A was not successful. The base-specific separation can be further improved by employing temperature programming with a longer period at the initial temperature during electrophoresis, as shown in Fig. 4B and C. The migration time of pdT_{12-16} in Fig. 4C decreased substantially in comparison with isothermal CAGE at 30°C in Figs. 2A and 3A. In addition, temperature programming resulted in sharper bands compared with

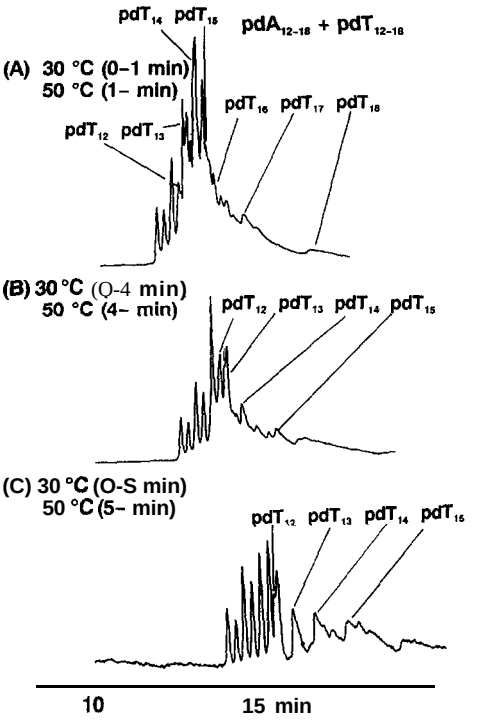

Fig. 4. Influence of temperature programming on the separation of a mixture of pdA_{12-18} and pdT_{12-18}: (A) 30°C for 1 min, then step to 50°C; (B) 30°C for 4 min, then step to 50°C; (C) 30°C for 5 min. then step to 50°C. Conditions as in Fig. I.

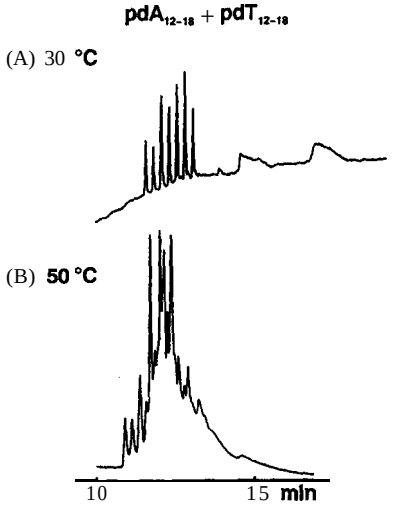

Fig. 3. Separation of a mixture of pdA_{12-18} and pdT_{12-18} by capillary affinity gel electrophoresis at (A) 30 and (B) 50°C. Conditions as in Fig. 1.

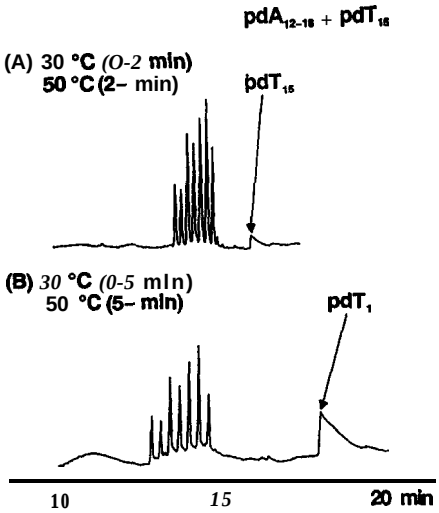

Fig. 5. Influence of temperature programming on the separation of a mixture of pdA_{12-18} and pdT_{15}: (A) 30°C for 2 min, then step to WC; (B) 30°C for 5 min, then step to 50°C. Gel contained 8% T, 5% C and 0.1% PVAd with a relative molecular mass range of 10000–30 000. Other conditions as in Fig. 1.

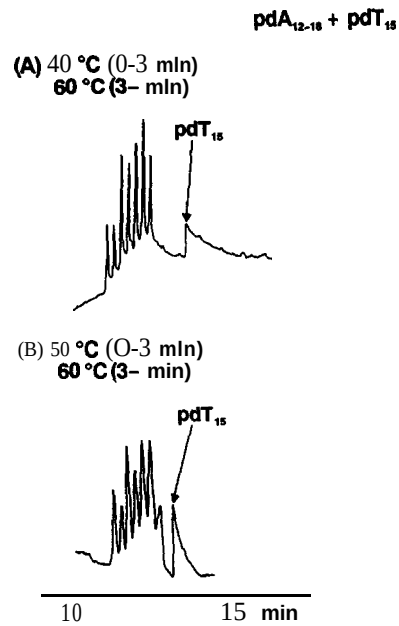

Fig. 6. Influence of initial temperature on the separation of a mixture of pdA_{12-18} and pdT_{15}: (A) 40°C for 3 min, then step to 60°C; (B) 50°C for 3 min, then step to 60°C. Other conditions as in Fig. 4.

isothermal conditions. The pdT_{13-16} bands were separated completely from pdA_{12-18}, but the pdT_{12} band partially overlapped that of pdA_{18}. The pdT_{14-16} bands were severely broadened owing to the strong interaction with PVAd. A relatively simple mixture of oligodeoxynucleotides was next separated by temperature programming to show the performance of the base-specific separation by CAGE.

Fig. 5 demonstrates that the base-specific separation of a mixture of pdA_{12-18} and pdT_{15} was achieved using CAGE with temperature programming, which was similar to that in Fig. 4, whereas that without an affinity ligand did not [2]. However, the pdT_{15} band was severely broadened and the efficiency of CAGE was not improved, probably owing to the strong interaction of pdT_{15} with PVAd. An increase in the initial temperature of the programme would be effective in achieving high-efficiency recognition of pdT_{15}.

Fig. 6 shows the effect of elevating the initital temperature on the efficiency of the base-specific separation of a mixture of pdA_{12-18} and pdT_{15}. An attempt to reduce the band broadening by raising the initial temperature to 40°C was not totally successful, although the analysis time was shortened (see Fig. 6A). The band broadening was further diminished by employing an initial temperature of 50°C, as shown in Fig. 6B. The plate number of pdT_{15} was $1 \cdot 10^5$–$3 \cdot 10^6$ per metre in Fig. 6B. This value was slightly reduced compared with that obtained by CGE [2], but much higher (by several tens of thousands) than that achieved by high-performance affinity chromatography [16]. The results in Fig. 6 clearly demonstrate that the resolving power and separation speed of temperature-programmed CAGE in base-specific separation compare favourably with those of high-performance affinity chromatography [16].

In conclusion, temperature programming was found to be effective for manipulating the selectivity and improving the speed and the efficiency of CAGE, and for achieving sensitive and high-resolution base-specific separations of oligodeoxynucleotides. This method will allow its application to the complex mixture of DNA, where inadequate separation is obtained in isothermal CAGE experiments.

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