An Affinity Capillary Electrophoresis for the Separation of Sequence Isomers of Oligonucleotide

Yoshiki Katayama*, Takumi Arisawa, Yoshihisa Ozaki, and Mizuo Maeda*
Department of Materials Physics and Chemistry, Graduate School of Engineering, Kyushu University, 6-10-1 Hakozaki , Higashi-ku, Fukuoka 812-8581

(Received September 20, 1999; CL-990800)

An affinity capillary electrophoresis for gene mutation assay was developed using oligonucleotide-polyacrylamide conjugate as pseudo-immobilized affinity ligand. In the system, magnesium ion concentration was found to be a key factor to achieve efficient separation of sequential isomers of oligonucleotide with the same chain length.

Affinity capillary electrophoresis is a useful method for the separation of substances which participate in specific interactions. The method is used for the study of molecular interactions as well as microscale analysis.[1] In fact, there have been many examples of studies on protein-drug and protein-small ligand interactions.[2] On the other hand, DNA analysis can be another important subject of affinity capillary electrophoresis. An introduction of affinity mode into the separation mode of DNAs by capillary electrophoresis would make possible their sequence-dependent separations while conventional capillary gel electrophoresis only allows the chain length-based separation by molecular-sieving mode. In the design of affinity capillary electrophoresis, however, the affinity ligand, which should be a single-stranded DNA complementary to target sequence, has to be immobilized in a capillary. Otherwise the analytes, the affinity ligand, and their complexes would migrate with the same mobility.

Recently, Baba et al. accomplished the affinity capillary electrophoresis using poly(9-vinyladenine),[3] which was immobilized into polyacrylamide gel as an affinity ligand, to separate some DNA sequence isomers containing A and T bases. We previously reported an affinity capillary electrophoresis, in which oligonucleotide was immobilized onto capillary inner surface, as a more versatile system for the DNA sequence-dependent separation.[4] We now report more successful and sophisticated separation of DNAs based on their sequences. The method relies on oligonucleotide-pendant polyacrylamide which serves as an pseudo-immobilized affinity ligand. Capillary which is filled with the DNA-vinyl polymer conjugate would be promising for gene analysis recognizing the DNA base sequence.

The vinyl derivative of oligonucleotides (**1**) was synthesized by the coupling of 5'-aminohexyl-$(dT)_{12}$ with methacryloyloxy succinimide according to the previous report.[4] **1** (0.13 mM) and acrylamide (450 mM) were copolymerized in water at 25 °C for 2 h using ammonium persulfate (2.9 mM) and N,N,N',N'-tetramethylethylenediamine (5.8 mM) as a redox initiator couple under nitrogen atmosphere to give a polyacrylamide-DNA conjugate with the structure illustrated in Figure 1. The conversions of **1** and acrylamide were determined to be 73 and 66%, respectively, by monitoring the peak area of the monomers on a reversed phase-HPLC, indicating that the amount of $(dT)_{12}$ incorporated to the resulting copolymer is 0.03 mol-% as the ratio of **1** to acrylamide unit. After the polymerization mixture was diluted twice with 10 mM Tris-borate buffer (pH 8.3), it was introduced into a capillary without any purification.

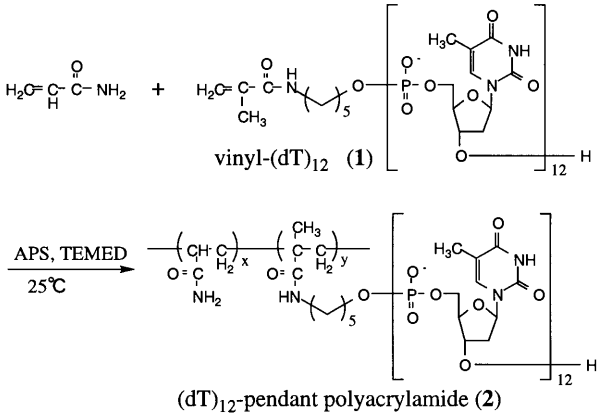

Figure 1. Synthetic scheme of $(dT)_{12}$ -pendant polyacrylamide conjugate.

Free zone capillary electrophoresis of the resulting DNA-pendant polymer (**2**) indicated that the conjugate behaved similarly to neutral (non-ionic) polyacrylamide in terms of migration rate. It means that **2** does not migrate substantially by electrophoresis, if the electroosmotic flow is fully suppressed. Therefore, we should be able to use **2** as pseudo-immobilized affinity ligand in a polyacrylamide-coated capillary.

The capillary was precoated with linear polyacrylamide according to the literature.[5] The resulting capillary was filled with **2** by introducing the twice-diluted polymerization mixture in 25cm length from the cathodic end and the rest of the capillary was filled with 3.4% polyacrylamide. Then the capillary was charged with 15 kV to remove the unpolymerized **1**. Electrophoresis of sample oligonucleotides was performed using this capillary in the presence of $MgCl_2$ with various concentration in 5 mM Tris-borate buffer (pH 7.4). Sample solution was introduced into the capillary at the cathodic side by positive pressure (0.1 kgf/cm^2·sec) and was charged with 15 kV constant voltage.

Figure 2 shows electropherograms on the separation of two oligonucleotides which have the same chain length. In the experiments, oligonucleotides examined were $(dA)_{12}$, $(dA)_6(dT)(dA)_5$, and $(dA)_8(dT)(dA)_3$. $(dA)_{12}$ is the perfect match sequence to the affinity ligand $(dT)_{12}$, while $(dA)_6(dT)(dA)_5$ and $(dA)_8(dT)(dA)_3$ have one mismatched base but the mismatch position is different from each other. We added Mg^{2+} in order to enhance the ligand-analyte interaction, since Mg^{2+} is known to stabilize double-stranded form of DNA due to the reduction of an electrostatic repulsion between the two DNA strands.[6] In Mg^{2+}-free buffer, two components could not be separated on either polyacrylamide- or **2**- loaded

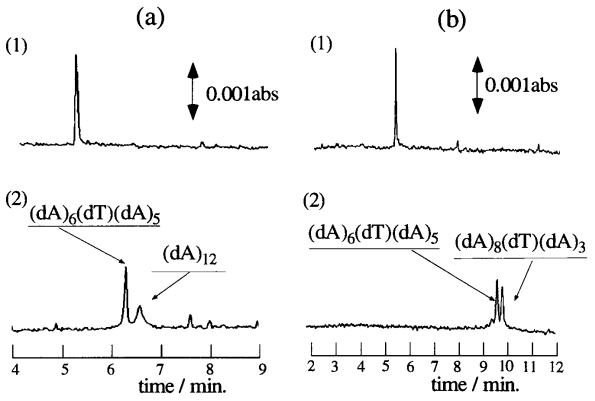

Figure 2. An effect of Mg^{2+} concentration on the separation of $(dA)_{12}$ and $(dA)_6(dT)(dA)_5$ (a) and $(dA)_6(dT)(dA)_5$ and $(dA)_8(dT)(dA)_3$ (b) in the presence of the $(dT)_{12}$ - pendant polyacrylamide (**2**). Mg^{2+} concentrations in (1) and (2) were 0 and 75 μM in (a), and 0 and 1 mM in (b), respectively. A mixture of two oligonucleotides were introduced into the affinity capillary (effective length; 38 cm) with positive pressure, and was charged at 15 kV in the presence or absence of $MgCl_2$ at 25 ℃ using 5 mM Tris-borate (pH 7.4) as working solution. The magnitude of current was 1 μA for (a)(1), (a)(2), and (b)(1), and 5 μA for (b)(2), respectively.

capillaries. However, increase of the concentration of Mg^{2+} ion improved the separation of two peaks effectively. The peaks became completely separated when concentrations of Mg^{2+} were 75μM for $(dA)_{12}$ and $(dA)_6(dT)(dA)_5$ as shown in Figure 2(a) and 1mM for $(dA)_6(dT)(dA)_5$ and $(dA)_8(dT)(dA)_3$ in Figure 2(b). On the other hand, electropherograms using the capillary filled with polyacrylamide showed only a single peak for the both cases even though Mg^{2+} was added.

In Figure 2(b), $(dA)_8(dT)(dA)_3$ which has a longer consecutive sequence of dA showed larger retardation than $(dA)_6(dT)(dA)_5$. Tm of the isomers for the duplex with $(dT)_{12}$ was determined with monitoring the temperature dependence of UV absorbance at 260 nm. Tm values of $(dA)_6(dT)(dA)_5$ and $(dA)_8(dT)(dA)_3$ with $(dT)_{12}$ were 11.3 °C and 13.6 °C, respectively in the presence of 0.5 mM of Mg^{2+}. These values increased to 15.2 °C and 16.0 °C, respectively when Mg^{2+} concentration was raised to 1 mM. These data indicate that the degree of peak retardation has a good correlation with the stability of the duplex between each analyte and the pendant affinity ligand.

In order to evaluate the potential of this affinity capillary electrophoresis for gene mutation assay, we applied this system to an analysis of K-ras sequence and its one base mutant on the codon 12 which is one of the major origin of cancer.[7,8] Figure 3 shows electropherograms of the mixture of both sequences. In this experiment, polyacrylamide carrying anti-sence sequence of c-K-ras codon 11-12 (5'-ACCAGC-3') as the pendant was employed as pseudo-immobilized ligand. The normal c-K-ras codon 10-13 (5'-GGAGCTGGTGGC-3'), which is complementary partner of the affinity ligand, was successfully separated from its single base mutant (5'-GGAGCTAGTGGC-3') in the presence of Mg^{2+} at a concentration of 150 μM using the affinity capillary (Figure 3(b)), whereas an ordinary polyacrylamide gel capillary could not separate them at all in the same conditions. In this case, Mg^{2+} concentration for the perfect separation was much lower than that for the case shown in Figure 2. This is explained by the fact that G-C pair interaction is stronger than A-T.

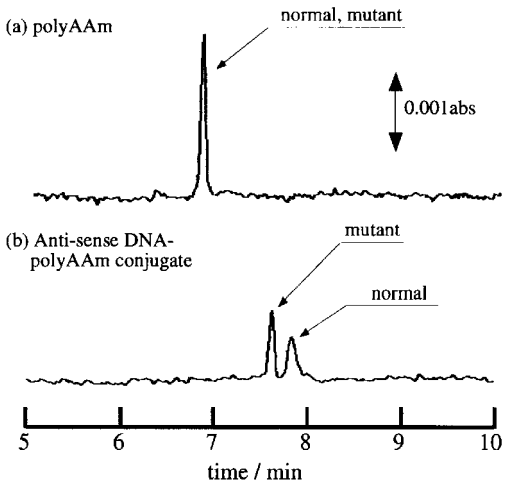

Figure 3. The separation of c-K-ras codon 10-13 (5'-GGAGCTGGTGGC-3') and its single base mutant (5'-GGAGCTAGTGGC-3') using ordinary polyacrylamide gel capillary (a) and that filled with (5'-ACCAGC-3') - pendant polyacrylamide (**b**). Mg^{2+} concentration was 150 μM in both cases. Other experimental conditions were the same as those in Figure 2. The magnitude of current was 2 μA for both cases.

Although capillary electrophoresis has been recognized to be one of the most effective tools for DNA separation analysis, it was not easy to distinguish sequence isomers which have the same chain length but have different base sequences. However, separation of such isomers is very important on gene assay. Baba et al. prepared an affinity capillary, which carried adenine bases in polyacrylamide gel, and used it for DNA separation.[9] We used here oligonucleotides as an affinity ligand with higher information and realized higher performance capillary electrophoresis with wider applicability. Recently, Muscate *et al.* reported an affinity capillary electrophoresis using oligonucleotide as an affinity ligand.[10] However, they conducted stepwise raising of the capillary temperature to separate each oligonucleotide. Thus, all components could not be separated at the same time. In contrast, we uses Mg^{2+} concentration for controlling the interaction of each analyte with affinity ligand, so that many components would be separated at the same time by choosing an appropriate Mg^{2+} concentration. The novel approach of the affinity capillary electrophoresis using oligonucleotide and Mg^{2+} can offer a powerful tool for gene analysis.

We thank the financial support by a Grant-in-Aid for Scientific Research from Ministry of Education, Science, Sports and Culture of Japan and that by The Tokuyama Science Foundation.

References

1 K. Shimura and K. Kasai, *Anal. Biochem.*, **251**, 1 (1997).
2 J.Liu, S.Abid, M.E.Hail, M.S.Lee, J.Hangeland, and N.Zein, *Analyst*, **123**, 1455 (1998).
3 Y. Baba, M. Tsuhako, T. Sawa, M. Akashi, and E. Yashima, *Anal. Chem.*, **64**, 1920 (1992).
4 Y. Ozaki, Y. Katayama, T. Ihara, and M. Maeda, *Anal. Sci.* **15**, 389 (1999).
5 S. Hjerten, *J. Chromatogr.*, **347**, 191 (1985).
6 H. Krakauer, *Biopolymers*, **10**, 2459 (1971).
7 S. Rodenhuis and R.J. Slebos, *Cancer Res.*, **52**, 2665S (1992).
8 N.R. Lemoine, S. Staddon, J. Bond, F.S. Wyllie, J.J. Shaw, and D. Wynford-Thomas, *Oncogene*, **5**, 1833 (1990).
9 Y. Baba, T. Sawa, A. Kishida, and M. Akashi, *Electrophoresis*, **19**, 433 (1998).
10 A. Muscate, F. Natt, A. Paulus, and M. Ehrat, *Anal. Chem.*, **70**, 1419 (1998).