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Separation of oligonucleotides with single-base mutation by capillary electrophoresis using specific interaction of metal ion with nucleotide

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Received 18th February 2009, Accepted 21st April 2009
First published as an Advance Article on the web 28th April 2009
DOI: 10.1039/b903440d

A unique tactic for the separation of single-base sequential isomers of oligomeric single-stranded DNA by a CE separation system employing the specific interaction of metal ion with nucleotide was demonstrated, enabling the separation of the mixture of a 12-mer oligonucleotide and its single-base mutants, as well as their positional isomers.

The separation of sequential isomers of DNA with the same chain length by capillary electrophoresis (CE) is still one of the most challenging tasks since there is only a small difference in the electrophoretic mobility of each isomer. A size-based separation technique using a molecular-sieving medium alone, such as a gel or a polymer, has been employed in the CE separation of DNA,[1] but this does not work for the separation of sequential isomers of DNA with the same chain length. A more effective tactic might be to use suitable interaction reagents that can control the electrophoretic mobility of DNA according to the difference of their affinity to each isomer. There has been a report about the separation of DNA sequential isomers of a single-base difference by affinity CE using an oligonucleotide–polyacrylamide conjugate as the pseudo-immobilized affinity ligand for single-stranded DNA (ssDNA) with a partial complementary sequence to the sequence of the target DNA.[2] Many of the methods for the detection of single nucleotide polymorphisms (SNPs),[3–6] including the above CE-based method, are based on the formation of a duplex of the target DNA with probe DNA except for some other electrophoresis-based approaches.[7,8] These methods inevitably require the preparation of a probe DNA for limited use for every target DNA, which is troublesome, time-consuming and costly. The technological requirements in the field of genotyping can be summarized as a need for new, fast, simple, and cost-effective methods.[9] The development of a new methodology which requires neither probe DNA nor labeling, therefore, has great significance.

It has been known for some time that metal ions and DNA interact, and that the binding abilities of nucleotides with metal ions differ according to the kind of metal ion or nucleotide.[10–12] This suggests the possibility of another approach for the CE separation of the sequential isomers of DNA by an affinity CE-like technique where the metal ion–DNA interaction is employed to control the electrophoretic mobility of DNA. We describe here a unique tactic for the separation of single-base sequential isomers of oligomeric ssDNA by a CE separation system employing the specific interaction of Ni(II) to guanosine monophosphate. The separation of the mixture of a 12-mer oligonucleotide and its single-base mutants, as well as their positional isomers, was successfully demonstrated by the proposed method. The CE system is quite a simple technique in which a metal ion is added to the electrophoretic buffer solution, and does not include any complicated procedures. Our approach, which employs neither a gel nor a polymer solution as the separation medium, is innovative as a CE separation technique for sequential isomers of ssDNA, and also is a new tactic to employ in single-base gene mutation assay.

The interaction of the divalent metal ions, Ca(II), Mg(II), Co(II), Mn(II), Ni(II), and Zn(II), with nucleotides was estimated *via* the effect of the addition of metal ions to the electrophoretic buffer solutions on the relative electrophoretic mobilities (μ_{ep}) of four-types of ssDNA composed with one kind of deoxynucleotide, 5′-aaaa-3′, 5′-cccc-3′, 5′-gggg-3′, and 5′-tttt-3″, to 4-n-octylbenzenesulfonate (OBS), which has no specific interaction with any metal ions.[13] The variation in μ_{ep} from zero metal ion concentration, $\Delta\mu_{ep}$, was calculated for the evaluation of the interaction of the metal ion to ssDNA since the values of μ_{ep} for each ssDNA differ inherently.[14] In all cases, the addition of a metal ion to the electrophoretic buffer solutions resulted in an increase of μ_{ep} for each ssDNA as the concentration of each metal ion increased. In the case of the addition of Ca(II) or Mg(II), typically as seen in Fig. 1a, the degrees of the increase of $\Delta\mu_{ep}$ of each oligomeric ssDNA were almost same. On the contrary, the $\Delta\mu_{ep}$ of 5′-gggg-3′ has greatly increased compared with that of other oligomeric ssDNA in the addition of transition metal ions. In particular, the degree of the increase of $\Delta\mu_{ep}$ of 5′-gggg-3′ was remarkable in the addition of Ni(II). As shown in Fig. 1b, the $\Delta\mu_{ep}$ of 5′-gggg-3′ increased significantly compared to that of other ssDNA though the amount of increase of $\Delta\mu_{ep}$ for 5′-aaaa-3′, 5′-cccc-3′, and 5′-tttt-3′ were almost the same. When more than 3 mM of Ni(II) was added to the electrophoretic buffer solution, the peak of 5′-gggg-3′ disappeared and its $\Delta\mu_{ep}$ value could not be evaluated because the apparent charge of 5′-gggg-3′ becomes positive or zero due to complexation with the Ni(II) ion. The details about the specific interaction of the Ni(II) ion with 5′-gggg-3′ is not clear. However, the N atoms in the base, which is classified as a borderline base in the hard and soft acid and base (HSAB),[16] essentially have high coordination ability to divalent transition metal ions, such as the Ni(II) ion, classified as borderline acids in the HSAB. In addition, the high coordination ability of the N7 atom of guanine, which is the strongest metal binding site among the base of purine nucleotides,[12,17,18] to Ni(II) ion also contributes to the interaction in part. The strong interaction of Ni(II) ion with guanosine indicates that one can control the electrophoretic mobility of ssDNA according to the amount of guanosine in that sequence using Ni(II) ion as an electrophoretic buffer additive.

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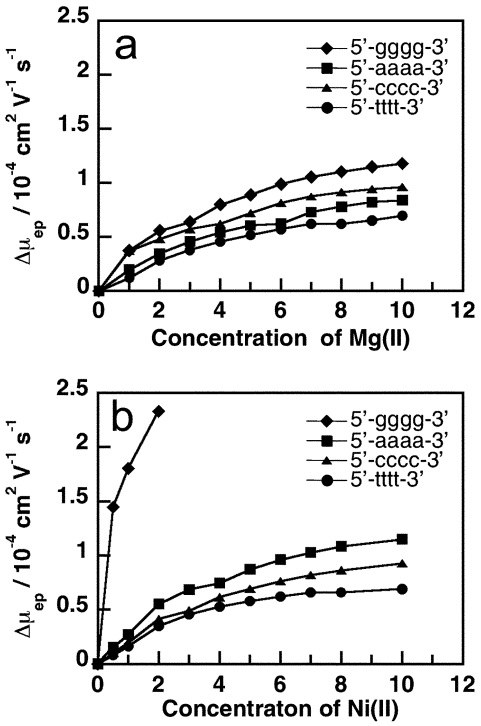

Fig. 1 The dependence of $\Delta\mu_{ep}$[13,14] of 4 kinds of ssDNA consisted of their corresponding homogeneous bases on the concentration of Mg(II) (a) and Ni(II) (b) in the electrophoretic buffer solution. $\Delta\mu_{ep}$ is the variation in the relative electrophoretic mobility of each ssDNA to OBS from zero metal ion concentration. Sample: [ssDNA] = 8 μM, [OBS] = 13 μM. Electrophoretic buffer solution: 20 mM MES (pH 6.0). The applying voltage: −25 kV. Capillary: polyacrylamide-coated,[15] L = 48.5 cm, l = 40 cm, i.d. = 50 μm.

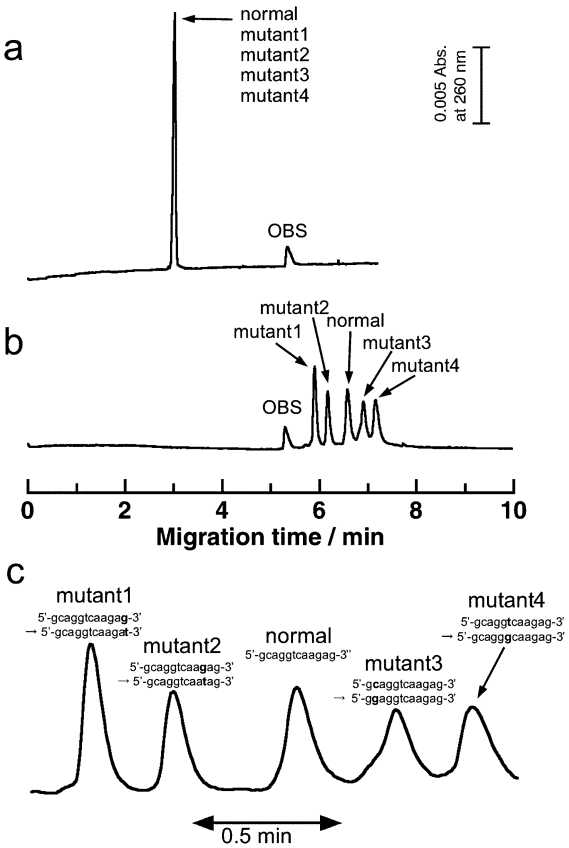

Fig. 2 Electropherograms of the mixture of five single-base isomers of 12-mer ssDNA without (a), and with addition (b, c) of 2 mM Ni(II) to the electrophoretic buffer solution. A magnified electropherogram of that in panel (b), and the type and the position of each mutation (the positions of mutation were highlighted with bold font) were shown in panel (c). The sequences of each ssDNA are as follows: normal: 5′-gcaggtcaagag-3′; mutant1: 5′-gcaggtcaaga**t**-3′; mutant2: 5′-gcaggtcaa**t**ag-3′; mutant3: 5′-**gg**aggtcaagag-3′; mutant4: 5′-gcagg**g**caagag-3′. Capillary: polyacrylamide-coated,[15] L = 47 cm, l = 38.5 cm, i.d. = 75 μm. Other CE conditions are the same as those in Fig. 1.

We decided to utilize the specific interaction of Ni(II) with guanosine monophosphate to the CE separation of the mixture of single-base-substituted 12-mer ssDNA.

Fig. 2 shows the electropherograms of the CE separation of the 12-mer ssDNA, 5′-gcaggtcaagag-3′ (normal), and its four single-base mutants, 5′-gcaggtcaagat-3′ (mutant1), 5′-gcaggtcaatag-3′ (mutant2), 5′-ggaggtcaatag-3′ (mutant3), and 5′-gcagggcaagag-3′ (mutant4). In mutant1 and mutant2, one guanosine was substituted for one thymidine. One cytidine was substituted for one guanosine in mutant3, and one thymidine was substituted for one guanosine in mutant4. Only a single peak was observed in the CE separation of the mixture of the five sequence isomers using the electrophoretic buffer solution without the addition of Ni(II), as can be seen in Fig. 2a. In contrast, the mutual separation between normal and its four mutants was completely achieved with the addition of 2 mM Ni(II) to the electrophoretic buffer solution, as shown in Fig. 2b. The type and the position of each mutatuion were shown in Fig. 2c. The detection of a single-base mutation was successful using the proposed method, which is an extremely simple technique requiring only the addition of Ni(II) ion to the electrophoretic buffer solution. In addition, it is noteworthy that the mutual separation between positional isomers with the same amount of guanosine, such as is the case between mutant 1 and mutant2 or between mutant3 and mutant4, was achieved. This indicates that the positional isomer of the single-base mutants also can be discriminated at the same time with the proposed method. To the best of our knowledge, this is the first case of a simultaneous separation of a mixture of the positional isomers of single-base mutants by CE.

Though no change in the electrophoretic mobility of OBS, which does not interact with Ni(II) ion, was observed before and after addition of Ni(II) ion, the electrophoretic mobilities of all the oligonucleotides were larger than that of OBS in the case of addition of Ni(II) ion, and they became smaller than that of OBS in the case of addition of Ni(II) ion. These are due to the decrease of the anionic charge of the oligonucleotides after complexation of Ni(II) ions with guanosine. The order of the migration time of the oligonucleotides, mutant1 < mutant 2 < normal < mutant3 < mutant4, also indicates the interaction of the oligonucleotides with Ni(II) ions *via* guanosine: the order of the decrement of the effective charge of the oligonucleotides is likely to be mutant1 = mutant2 < normal < mutant3 = mutant4, since the order of the amount of guanosine is mutant1 = mutant2 < normal < mutant3 = mutant4. At the same time, the fact that the separation between positional isomers with the same amount of guanosine was successful using the proposed method indicates that the interaction of oligonucleotides with Ni(II) ion differs according to the position of the mutation. It is therefore concluded that the

separation of single-base mutants and their positional isomers can be accomplished according to the difference of the interaction of the Ni(II) ion to ssDNA arising from the amount and the position of guanosine in their sequence in the proposed method.

We have proposed in this paper a novel concept for the separation of single-base substitution isomers of ssDNA with same chain length by CE using the specific interaction of metal ion with nucleotide. Our proposed method also may contribute the detection of single-base mutations in the field of genotyping. The most conventional methods for the detection of single-base mutations require the time-consuming and costly preparation of probe DNA for exclusive use for every target DNA.[2–6] In our proposed method, however, no extra reagent besides Ni(II) ion is required. Our proposed method using the specific interaction of Ni(II) ion to guanosine is applicable only to the guanosine-related mutation. If the proper respective metal ions or interaction reagents that show the specific interaction with each three bases other than guanosine can be found out, the multi-detection system by CE of single-base mutations can be constructed. Though the proposed method was applied to the separation of single-stranded oligonucleotide, the application to longer DNA is one of the important considerations in the next stage. There is strong demand for a faster, better, and cheaper SNP detection technique.[7] The proposed method provides a new tactic for the construction of a novel single-base mutation detection system with the following advantages: (i) ease of implementation; (ii) cost-efficiency; and (iii) no need for labeling.

Experimental

Synthesized oligomeric ssDNA, 5′-aaaa-3′, 5′-cccc-3′, 5′-gggg-3′, 5′-tttt-3′, normal, mutant1, mutant2, mutant3, and mutant4, were purchased from Nippon EGT (Toyama, Japan). Electrophoresis was performed on an Agilent (Santa Clala, USA) HP^{3D} capillary electrophoresis system with constant voltage operation mode at −25 kV using GL Science (Tokyo, Japan) fused silica capillaries (50 μm i.d., L = 48.5 cm, l = 40 cm, or 75 μm i.d., L = 47 cm, l = 38.5 cm). Direct photometric detection at 260 nm was used. The polyacrylamide-coated capillaries were prepared in the same manner as described in an earlier paper.[15] The sample solutions of ssDNA (each 4 mM) were containing 5 mM phosphate buffer (pH 6.0), and 0.13 mM sodium 4-n-octylbenzenesufonate (OBS). Metal ion standard solutions of Ca(II), Mg(II), Co(II), Cd(II), Mn(II), Ni(II), and Zn(II) (each 0.01 M) were prepared by dissolving the chlorides in *ca.* 0.01 M hydrochloric acid solution. The electrophoretic buffer solutions containing 20 mM morpholinoethanesulfonic acid (MES, pH 6.0) and metal ions were used. Sample injection was performed hydrodynamically by applying the pressure to the sample vial at cathodic side of the capillary (50 mbar for 7 s).

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