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Effect of Neighbor Base Sequences on the Base Pair Stabilities at d(CXG) and d(GXC) in Human ε-globin Promoter

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Abstract

Human ε-globin DNA fragment was used to determine the thermal stabilities of base pairs at d(CXG) and d(GXC) by Temperature Gradient Gel Electrophoresis(TGGE). The base pair stability depends on the hydrogen bonding interaction and base stacking interaction of neighbor base sequence. The orders of base pair stabilities were T · A < A · T < C · G < G · C for d(CXG) · d(CYG) and A · T < T · A < C · G < G · C for d(GXC) · d(GYC). And also heteroduplex TGGE suggested that the mismatch bases stabilities were G · T = T · G > G · A = A · G > C · T > T · C > C · A > A · C for d(GXC) · d(GYC).

Key words – base pair stability; Mutation detection; Temperature Gradient Gel Electrophoresis(TGGE).

Introduction

The mutations happen to be occurred in the process of DNA replication or to the exposure of ultraviolet light and chemical mutagens[4]. The human diseases related to changes in gene have been reported since the first definition of altered haemoglobin gene. And also a variety of human tumors have been shown to be associated with mutations in the p53 gene[1,3,10,11]. Thus there is a need for an accurate and rapid screening technique for these mutations. One of the efficient techniques developed is TGGE (Temperature Gradient Gel Electrophoresis). This method employs a gradient of temperature instead of denaturing solvent on a polyacrylamide gel. TGGE utilizes DNA melting properties to separate DNA fragments differing by as little as one base pair substitution or mismatch. A duplex DNA migrates in a polyacrylamide gel until it reaches a temperature which induces the least stable domain to unwind. A partially melted (denatured) DNA results in a large decrease in gel mobility. The homologous DNA fragments with different base pair stability unwind at different depths in the gel. The base pair stability of DNA fragments depends on the base pair type (AT or GC) and the stacking interactions of the neighboring base pairs surrounding the base pair of interest. In this study, we measured the base pair stabilities of several base sequences in DNA fragment. These results will help to understand the effect of the neighboring base sequences on the base pair stability and to provide the valuable information for the efficient detection of mutation.

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Materials and Methods

Materials

Ex Taq polymerase and dNTP were purchased from TaKaRa (Japan). Acrylamide, bisacrylamide, Trizma base,

and Urea were obtained from Sigma (USA). Formamide was purchased from USB (USA). Gelstar was purchased from FMC BioProducts. The primers were synthesized from Bioneer (Korea).

Methods

DNA preparation

The DNA fragment used in this study was selected from human ε-globin promoter[8] by the result calculated with Meltscan program[2]. The DNA fragment was amplified by polymerase chain reaction (PCR) with primers substituted at -181(-CXG-, X= A, T, G, or C) and -185(-GXC-, X= A, T, G, or C)(shown at Fig. 1). Reaction mixture contains 100ng of plasmid DNA, 50pmol of each primers, and 400 μM of each dNTP in reaction buffer. The temperature cycles were 94°C for 1 min, 58°C for 1 min and 72°C for 1 min. 5 μL of reaction mixture was checked for size and purified on a 12% polyacrylamide gel.

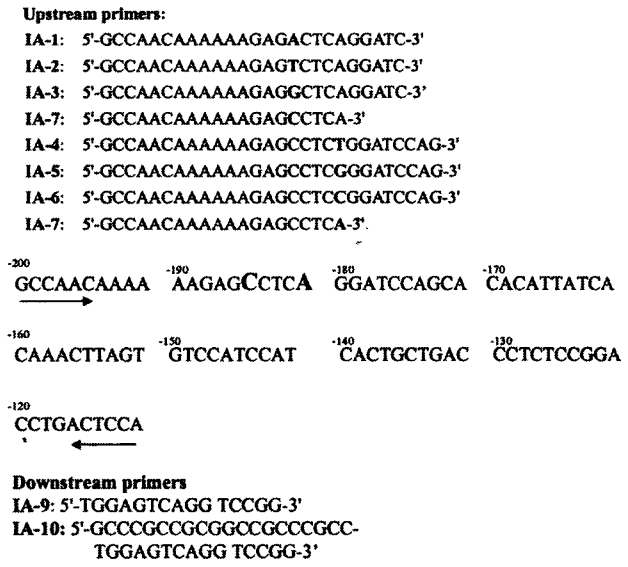

Fig. 1. Human ε-globin promoter region from -120 to -200 and primers used in this study.
The substituted bases are indicated by capital letters. The sequence shown above is the coding strand and directed from 5′ to 3′.

TGGE

A parallel TGGE apparatus[13] was employed in this study. Two aluminum heating blocks sandwiching the glass plates were used to establish a temperature gradient parallel to the electric field. The gel temperature at different depths was measured with a thermocouple probe connected to a digital thermometer (DP465, Omega). It was confirmed that the temperature gradient was linear and uniform within the region covered by the heating blocks. The 10% polyacrylamide gel (37.5:1, acrylamide/bisacrylamide) contains 4.2 M urea and 24% vol/vol formamide corresponding to 60% denaturant in 0.5×TBE (0.045 M Tris + 0.045 M sodium borate + 1 mM EDTA, pH 8.2). Electrophoresis was generally conducted at 70 volt and run overnight. Temperature gradient from 33 to 39°C was set to optimize separation of DNA fragments with single base pair substitution. The gels were stained in ethidium bromide or Gelstar and photographed.

Results and Discussion

The seven DNA fragments used in this study were prepared with PCR using primers as indicated in Fig. 1. The DNA fragments differ from each other by single base pair at sites -181 and -185. All DNA fragments showed the same mobility on native gel(not shown).

Fig. 2. shows the plot of melting temperature against the base sequence with or without GC clamp. It was calculated from Meltscan program, which was developed by Dr. Roger M. Wartell at Georgia Tech[2]. It was suggested that the attachment of GC clamp(5′-GCCCG-CCGCGGCCGCCCGCC-3′)[12] increases melting temperature dramatically around 90 bp position in DNA fragment, where it is opposite to positions -181 and -185. Thus DNA fragments with base pair substitutions at these positions are melted at slightly different temperatures (migrated at different depths of gel) without melting the GC clamped region of DNA fragment during temperature gradient gel electrophoresis.

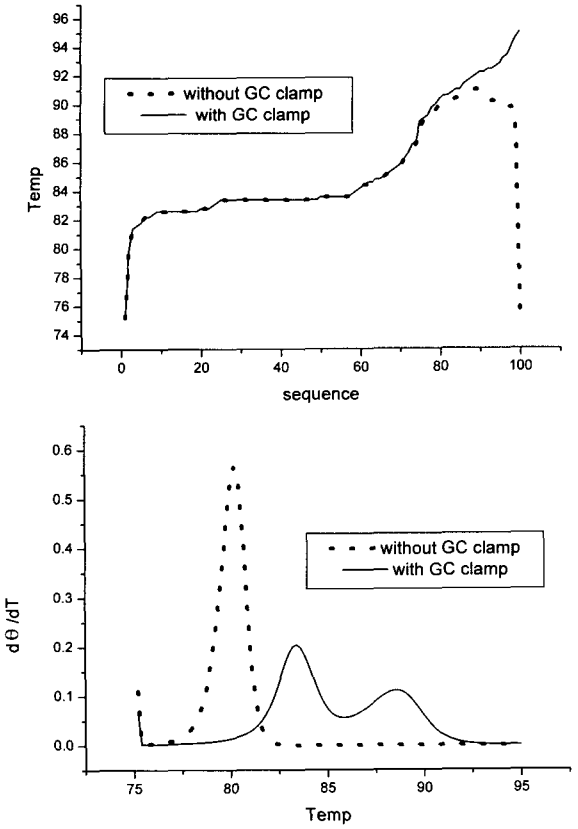

Fig. 2. Melting profiles and derivative melting curves. The melting map for 90 bp DNA fragment is calculated from Meltscan program (Brossette and Wartell, 1994) either with (solid) or without (dot) GC clamp, respectively, in above figure. The derivative melting curve (bottom) is a plot of the temperature derivative of the fraction of intact base pairs, $d\theta/dT$, against temperature (T). the solid and dot line in bottom figure represent the derivative melting curve either with or without GC clamp, respectively.

Fig. 3. shows TGGE experiment of 90 bp DNAs with four possible paired bases at 181 in the absence of GC clamp. As expected, result shows the same mobility of DNA fragments without GC clamp. That is because the region surrounding -181 is not located at the first melting domain in the DNA fragment. GC clamp should be attached for the region to become the first melting domain[9].

Fig. 4. shows TGGE experiment with single base substituted DNA fragments at positions -181 and -185. The nearest neighbor base pairs are d(CXG) · d(CYG) for -181

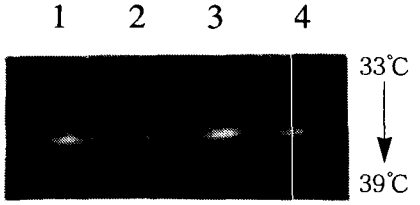

Fig. 3. Temperature gradient gel of 90 bp DNAs substituted at d(CXG) · d(CYG) without GC clamp. Temperature gradient was from 33 to 39°C. Samples were run for 17hrs at 70volts. Lane 1, 2, 3, and 4 contain A · T, T · A, G · C, and C · G, respectively.

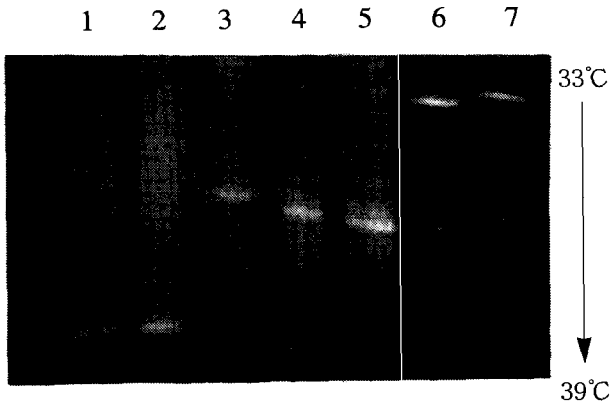

Fig. 4. Temperature gradient gel of 110 bp DNAs substituted at d(CXG) · d(CYG) and d(GXC) · d(GYC) in the presence of GC clamp. Samples were run for 17hrs at temperature gradient from 33 to 39°C. Lane 1 through 4 and lane 4 through 7 contain 110 bp DNAs substituted at d(CXG) · d(CYG) and d(GXC) · d(GYC), respectively. Lane 4 contains wild-type DNA fragment. Each lane contain the following base pair: for d(CXG) · d(CYG), 1) C · G, 2) G · C, 3) T · A and 4) A · T. for d(GXC) · d(GYC), 4) C · G, 5) G · C, 6) T · A and A · T.

and d(GXC) · d(GYC) for -185. The results from TGGE suggests that the orders of base pair stabilities are T · A< A · T < C · G < G · C for d(CXG) · d(CYG) and A · T < T · A < C · G < G · C for d(GXC) · d(GYC). As expected, DNA fragments with G · C or C · G are more stable than those with A · T or T · A base pair. The different stabilities are shown in DNAs with A · T and T · A base pairs at both positions. It suggests that base stacking interaction contributes dominantly to the base pair sta-

bility of DNA's at two positions. The base stacking interaction depends on the neighbor bases sequence surrounding the specific base pair(X · Y).

Heteroduplex TGGE experiment[5] in Fig. 5 shows that two homoduplex bands are appeared at the bottom in addition to two heteroduplex bands at the top in each lane. The heteroduplex DNA's migrate slowly due to the low melting temperature caused from the mismatched single base. The heteroduplex bands was distinguished according to the results from Ke and Wartell[6]. Then the mismatched bases stabilities are G · T = T · G > G · A = A · G > C · T > T · C > C · A > A · C for d(GXC) · d(GYC). Although the most stable mismatch bases can be different for different neighbor sequences, G · T was the most stable mismatch for d(CXA) · d(TYG) and d(TXT) · d (AYA)[6]. And also both G · T and G · A can form two hydrogen bonds and stack within a B-DNA without a structural distortion[13].

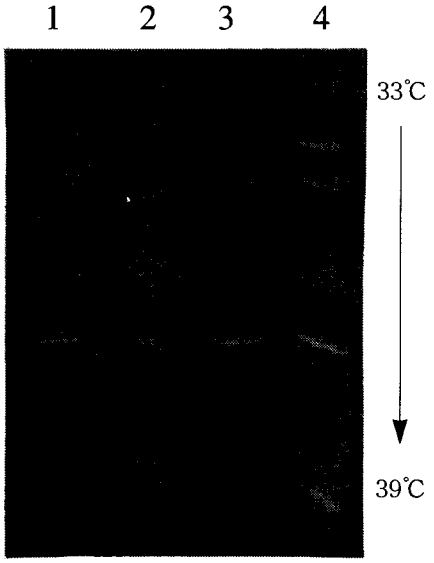

Fig. 5. Heteroduplex temperature gradient gel of 110 bp DNAs substituted at d(GXC) · d(GYC).
Temperature gradient, Run time and voltages were the same as in Fig. 4. From the top to bottom in each lane DNA bands contain the following bases: 1) A · C, G · T, A · T, G · C, 2) C · A, T · G, T · A, C · G, 3) T · C, G · A, T · A, G · C and 4) C · T, A · G, A · T, C · G. The mismatch bases stabilities were determined according to the results from Ke and Wartell(1993).

The pattern of bands produced by DNA molecules differing by a single base pair substitution depends on the type of base pairs and its nearest neighbor sequence surrounding the specific base pair (X · Y). And also this study shows the effect of the neighboring base pair sequences on the mismatch base stability. The base pairs stabilities in this study are valuable in characterizing base pair substitutions at certain base sequences for the mutation detection.

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초록 : 사람의 ε -글로빈 프로모트에서 d(CXG)와 d(GXC)의 안정성에 인접한 염기 서열들의 영향에 관한 연구

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온도 기울기 전기영동장치를 이용하여 d(CXG)와 d(GXC) 염기의 열 안정성을 결정하는데 사람의 ε-글로빈 DNA 조각을 사용하였다. 염기 쌍의 안정성은 이웃하는 염기서열에 의한 수소결합과 base stacking 상호작용에 의존한다. 염기 쌍의 안정성은 d(CXG) · d(CYG)의 경우에 T · A < A · T < C · G < G · C이고 또한 d(GXC) · d(GYC)의 경우는 A · T < T · A < C · G < G · C 이다. 그리고 d(GXC) · d(GYC).의 경우에 mismatch 염기 쌍의 안정성은 G · T = T · G > G · A = A · G > C · T > T · C > C · A > A · C 이다.