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Recognition of Single Nucleotide Polymorphisms using Scanning Potential Hairpin Denaturation

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Single nucleotide polymorphism (SNP) is a rich and important phenomenon in biological evolution. Most of the techniques for SNP recognition require a probe or target labeling, which limits the application of effective detection.[1] In a conventional assay of SNP, the distinction between the signal and background is based on the stringency or temperature of the washing buffers. Typically, a single base mismatch (SM) possesses about 3−6 kcal/mol difference in hybridization free energy (ΔG^H) than that of a perfect match (PM), correlating to a difference in melting temperature of merely 5 °C or so for a double-stranded DNA (dsDNA) of about 20−30 bp. Thus, a sufficient signal-versus-noise ratio is rarely reached.

Electric potential has been demonstrated to control DNA hybridization and facilitate the recognition of mutants,[2−4] but its sensitivity to detect SNP is not satisfactory.[3,4] Tyagi and Kramer have demonstrated that hairpin (stem-loop) DNA probes offer highly specific SNP recognition.[5] Theoretical studies have shown that with a suitable design, structurally constrained probes can distinguish mismatches over a wider range of temperatures than can unstructured probes.[6,7] Several groups have studied the characteristics of surface-immobilized hairpin structures,[2,8−11] and a variety of advantages have been shown. However, its capacity on SNP detection has not been fully explored.

We hypothesize that scanning surface electric potential, in a combination with hairpin probes, will significantly enhance the specificity of DNA hybridization on surfaces and provide a reliable method to detect SNP. This method is designated as "scanning potential hairpin denaturation" (SPHD). In this study, we find a new parameter, "melting potential" (V_m), and demonstrate that our SPHD method detects a marked difference in V_m between PM and SM, more than 20-fold higher than the noise. Furthermore, the principle of this method can be easily applied to various types of DNA hybridization based assays.

The experimental details are described in the Supporting Information. Silicon (111) surface was used to immobilize DNA probes, as described by Strother et al.[12,13] and Wei et al.[2] When hybridized with the target, the hairpin probe changes its configuration from closed form to extended duplex, which has been verified by various techniques.[2,8−11] Upon application of an electric field, a positive potential forces dsDNA to lie down on the surface, while a negative potential pushes the duplex to stand up from the surface because DNA is negatively charged.[14] When the voltage is varied to the negative direction, the duplex dissociates due to the drop of the free energy of dehybridization.[4] PicoGreen, a dsDNA specific intercalating dye, was used to manifest the formation and dissociation of dsDNA. Binding with the λDNA enhances the fluorescent intensity of PicoGreen 1000 times. The fluorescence intensity was normalized between 1 at the maximum intensity and 0 at the lowest background, which is the fraction of duplex with respect to the initial duplex at the most positive potential. The voltage reading

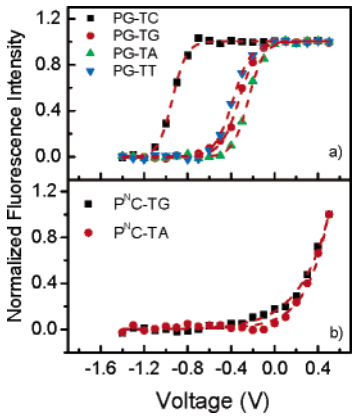

Figure 1. SPHD curves of probe with its SNP targets. (a) Probe with a stem structure; (b) probe without a stem structure.

was the potential difference between the Si(111) electrode and a Pt wire, a counter electrode. To get the potential of the Si(111) surface versus SHE, 0.49 ± 0.05 V should be added. As the voltage scanned from positive to negative, the process of duplex dissociation was clearly visualized via the decrease in fluorescent intensity of PicoGreen.

We assessed the sensitivity of SPHD to detect SNP. Figure 1a shows the SPHD curves for probe PG with four targets scanned from 0.5 to −1.5 V at 10 mV/min. The duplex of PG and its PM target TC could be clearly distinguished from those of PG and its three SMs. A hairpin probe generated a signal, $\Delta V_m = V_m(\text{SM}) - V_m(\text{PM})$, of about 600 mV. The dsDNA of PM was significantly more stable against the negative electric potential than that of SMs. However, a probe without stem structure did not generate a comparable difference (Figure 1b). Additional SNP tests with other DNA sequences showed that our observations were general (data not shown). Referring to the concept of melting temperature (T_m), we define the potential in the SPHD curve where the normalized fluorescence intensity is 0.5 as the melting potential (V_m).

On the basis of the observations in solution,[5−7] it was surprising to observe that the dissociation of a duplex with a hairpin probe required more negative voltage than that of a linear probe. We propose the following model to interpret our observation. Theoretical estimation on the thermodynamics of DNA denaturation in our solution by the MFold software[15,16] indicates that enthalpy and entropy are equally important in determining the free energy of reaction, and their contributions are opposite ($\Delta G = \Delta H - T\Delta S$), which leads to delicate balances (data not shown). Now, on the surface, the dsDNA is ordered,[14] and likely so is the hairpin probe, while the linear probe is, more or less, a random coil (the target is unaffected because it remains in solution). Such an entropic factor shifts the balances in a way that the dissociation of dsDNA with a linear probe becomes more favorable, so that it possesses less negative melting potential than that of hairpin. This seemingly

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Table 1. Sequences of Probes and Targets with Different Single-Base Mutations

	DNA sequence
PC	5′-*GCGA* CGG TTC ATG C*C*G CCC ATG CAG *TCGC*-3′
PG	5′-*GCGA* CGG TTC ATG C*G*G CCC ATG CAG *TCGC*-3′
PA	5′-*GCGA* CGG TTC ATG C*A*G CCC ATG CAG *TCGC*-3′
PT	5′-*GCGA* CGG TTC ATG C*T*G CCC ATG CAG *TCGC*-3′
P^NC[a]	5′-*TTT* CGG TTC ATG C*C*G CCC ATG CAG *TTT*-3′
TC	5′-CTG CAT GGG C*C*G CAT GAA CCG-3′
TG[b]	5′-CTG CAT GGG C*G*G CAT GAA CCG-3′
TA[c]	5′-CTG CAT GGG C*A*G CAT GAA CCG-3′
TT	5′-CTG CAT GGG C*T*G CAT GAA CCG-3′

[a] The superscript N stands for the probe without a hairpin design. [b] TG is a segment with the codon 245 from the wild-type p53 gene. [c] TA is the segment of the highest probable single-base mutant of the codon 245.

Table 2. Melting Potentials and Differences of Hybridization Free Energies between PM and SM

duplex pairs	V_m[a] (V)	$\Delta\Delta G^H$ (kcal/mol)	duplex pairs	V_m[a] (V)	$\Delta\Delta G^H$ (kcal/mol)
PC-TC	−0.07	5.9	PA-TC	+0.02	6.1
PC-TG	−0.99	0.0	PA-TG	−0.24	4.4
PC-TA	−0.03	6.4	PA-TA	−0.10	5.2
PC-TT	−0.13	5.6	PA-TT	−0.88	0.0
PG-TC	−0.94	0.0	PT-TC	−0.24	4.3
PG-TG	−0.31	4.1	PT-TG	−0.51	2.4
PG-TA	−0.24	4.7	PT-TA	−0.91	0.0
PG-TT	−0.37	3.7	PT-TT	−0.48	2.7

[a] V_m was dependent on the temperature, ionic strength, and target DNA concentration. In this work, the temperature was 20 °C; the detection buffer was 1 TE (10 mM Tris-EDTA, pH=7.5), and the target DNA concentration was 5×10^{-8} M.

perplexing fact might well be a good example of entropy driving effects. Furthermore, unlike a thermal dissociation, the electric field has a direction and its strength decreases gradually along the distance from the surface. As the surface potential is scanned toward negative direction, the dsDNA with a linear probe dissociates in a point-by-point manner, starting from the end close to the surface, as if a zipper were unzipped. Therefore, the SPHD curve is not sensitive to the middle point of mutation. In contrast, the cooperation between duplex dissociation and hairpin stem formation during denaturation creates a reaction barrier. The barrier is sensitive to the middle point of mutation and makes a two-state model of reaction feasible, which leads to a sigmoid SPHD curve and larger SNP differentiation. We will fully elaborate our theoretical interpretation in future publications.

We investigated the V_m values of 16 duplexes formed between the four probes and four targets (Table 1). The experimentally measured V_m values for all of the probe−target pairs are presented in Table 2. The data clearly show that the PM pairs require stronger negative electric potential to dissociate. The results also illustrate the sensitivity of our method on the sequences of ssDNA. For instance, the V_m of PC-TG is not identical to that of PG-TC due to the effect of base-pair stacking. Typically, the experimental error on V_m is within ±0.02 V, so that the signal/noise ratio is about 20−40 (400−800 mV signal/20 mV noise). The difference of hybridization free energies between SM and PM for a given probe in solution, $\Delta\Delta G^H = \Delta G^H_{SM} - \Delta G^H_{PM}$, can be estimated.[15,16] Figure 2 is the plot of ΔV_m versus $\Delta\Delta G^H$ for given probes. A very good linear relationship is observed, with a correlation coefficient of 0.998, suggesting a direct linkage between V_m and ΔG^H. We will deliver an in-depth theoretical account on the relationship between V_m and ΔG^H in future publications.

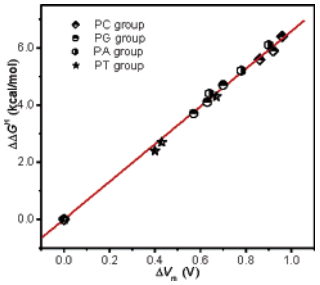

Figure 2. Relationship between $\Delta\Delta G^H$ and ΔV_m.

Under condition of 5×10^{-8} M of an oligonucleotide target concentration, single-base mutation can be perfectly detected. The new parameter, V_m, provides a good criterion for dsDNA dissociation in an electric field. Only 1 kcal/mol difference in ΔG^H between mutants will result in about 150 mV gap of voltage, indicating 400−800 mV gap in case of 3−6 kcal/mol ΔG^H for single-base mutant oligos. Our method presents a marked signal-versus-noise ratio for SNP recognition, virtually eliminating false positives and false negatives. In comparison, in a traditional SNP assay, the fluctuation in signal intensity from a surface is usually high. The introduction of V_m, which is based on the changes of intensity instead of the intensity itself, can avoid the high fluctuation in signal intensity. The SPHD method does not merely provide a powerful tool for SNP detection but it also can provide new insight into the behavior of DNA molecules on a surface in an electric field.

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Supporting Information Available: Preparations of DNA-modified silicon electrode and experimental procedures in acquisition of SPHD curve. This material is available free of charge via the Internet at http://pubs.acs.org.

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