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DNA Hole Transport on an Electrode: Application to Effective Photoelectrochemical SNP Typing

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Abstract: A useful feature of DNA is that long-range hole transport through DNA is readily achieved. Photostimulated long-range hole transport through DNA has prospective use in the development of a conceptually new electrochemical single-nucleotide polymorphism (SNP) typing method for use as a versatile platform for gene diagnostics and pharmacogenetics. We have applied artificial DNAs designed for photostimulated long-range hole transport through DNA to SNP typing. By hybridizing photosensitizer-equipped DNA probes, immobilized on gold working electrodes, with a target DNA strand containing an SNP site, we observed a cathodic photocurrent, which markedly changed depending on the nature of the base at the specific site. The use of a combination of hole-transporting bases constitutes a very powerful method for a single-step electrochemical assay applicable to SNP typing of all types of sequences.

Introduction

The most significant genetic diversity in human genes is in the single-nucleotide polymorphisms (SNPs), which are being investigated to gain greater insight into the basis for disease.[1,2] An accurate and sensitive analysis of SNPs will play a central role in future genetic diagnostics. Several different techniques for SNP genotyping have been developed in the past few years, each of which has specific advantages and/or limitations.[3−7] Several electrochemical DNA detection methods using various redox-active labels have also been reported as versatile platforms for molecular diagnostics and pharmacogenetics.[8,9] These methods typically require posthybridization treatment with exogenous redox-active reporter groups that bind preferentially to a duplex structure.[10,11] Several researchers have demonstrated electrochemical methods using probes prepared through the derivatization of oligodeoxynucleotides with redox-active labels.[12,13] Despite these advances, there are still many problems for practical electrochemical SNP genotyping, such as high hybridization error, a high signal response from nonhybridized probes, low sensitivity, and difficulties in the design of probe sequences.

In the development of a new generation of electrochemical SNP typing methods, we focused on long-range hole transport through DNA. A number of mechanistic and physical studies on DNA hole transport have been reported.[14−16] DNA hole transport is mediated by an extended and well-defined π stack and can promote oxidative damage to guanine bases from a remote site. We have previously examined photostimulated long-range hole transport through DNA by modifying a gold electrode with a DNA duplex consisting of a photosensitizer-labeled strand and a thiolated strand and observed a cathodic photocurrent when an appropriate sequence was selected.[17] This phenomenon has bright prospects in the development of a conceptually new electrochemical SNP typing method. If this method were applicable to SNP typing assay, we would not require the modification of target DNA, supplementary redox-active additives, or stringent washing processes to eliminate the hybridization error. This will provide opportunities for new types of SNP sensing based on microelectronics as well as be important in the development of DNA-based molecular electronics.

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(1) McCarthy, J. J.; Hilfiker, R. *Nat. Biotechnol.* **2000**, *18*, 505−508.
(2) Risch, N.; Merikangas, K. *Science* **1996**, *273*, 1516−1517.
(3) Kwok, P.-Y. *Annu. Rev. Genomics Hum. Genet.* **2001**, *2*, 235−258.
(4) Hall, J. G.; Eis, P. S.; Law, S. M.; Reynaldo, L. P.; Prudent, J. R.; Marshall, D. J.; Allawi, H. T.; Mast, A. L.; Dahlberg, J. E.; Kwiatkowski, R. W.; de Arruda, M.; Neri, B. P.; Lyamichev, V. I. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8272−8277.
(5) Pickering, J.; Bamford, A.; Godbole, V.; Briggs, J.; Scozzafava, G.; Roe, P.; Wheeler, C.; Ghouze, F.; Cuss, S. *Nucleic Acids Res.* **2002**, *30*, e60.
(6) Ronaghi, M. *Genome Res.* **2001**, *11*, 3−11.
(7) Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820−4827.
(8) Umek, R. M.; Lin, S. W.; Vielmetter, J.; Terbrueggen, R. H.; Irvine, B.; Yu, C. J.; Kayyem, J. F.; Yowanto, H.; Blackburn, G. F.; Farkas, D. H.; Chen, Y. P. *J. Mol. Diagn.* **2001**, *3*, 74−84.
(9) Wang, J. *Anal. Chim. Acta* **2002**, *469*, 63−71.
(10) Wakai, J.; Takagi, A.; Nakayama, M.; Miya, T.; Miyahara, T.; Iwanaga, T.; Takenaka, S.; Ikeda, Y.; Amano, M. *Nucleic Acids Res.* **2004**, *32*, e141.
(11) Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2003**, *21*, 1192−1199.
(12) Immoos, C. E.; Lee, S. J.; Grinstaff, M. W. *ChemBioChem* **2004**, *5*, 1100−1103.
(13) Fan, H.; Plaxco, K. W.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9134−9137.
(14) Núñez, M. E.; Barton, J. K. *Curr. Opin. Chem. Biol.* **2000**, *4*, 199−206.
(15) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631−636.
(16) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159−170.
(17) Okamoto, A.; Kamei, T.; Tanaka, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 14732−14733.

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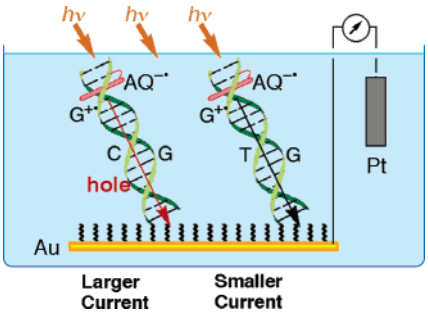

Figure 1. Measurement of a photocurrent using a gold electrode modified with an anthraquinone-modified DNA duplex.

In this study, we have applied artificial DNAs designed for photostimulated long-range hole transport through DNA to SNP typing. By hybridizing photosensitizer-equipped DNA probes immobilized on gold working electrodes with a target DNA strand containing an SNP site, we observed a cathodic photocurrent, which markedly changes depending on the nature of the base at the specific site. The use of a combination of hole-transporting bases constitutes a very powerful method for a single-step electrochemical assay applicable to SNP typing of all types of sequence.

Results and Discussion

For the DNA probes, we prepared artificial DNAs incorporating both a photosensitizer and a thiol anchor in a single strand. An anthraquinone was incorporated as a photosensitizer (^{AQ}U), mediated by an amide bond from a uracil C5,[17] and a mercaptohexanol linker was added as a thiol anchor at the 3′ end using a conventional phosphoramidite method. DNA-modified gold electrodes were prepared by immersing an electrode (2 mm^2 in area) in a thiolated DNA solution (9.9 ± 0.8 pmol cm^{-2}), followed by exposure to 1 mM mercaptohexanol to minimize nonspecific adsorption of DNA. The DNA to be examined was then hybridized with the probes on the electrode to give a modified electrode for electrochemical assays, as shown in Figure 1.

We initially performed an electrochemical experiment with the 12-mer DNA probe 5′-T^{AQ}UCACTTCAGTG-(CH$_2$)$_6$-S-Au-3′ on a gold electrode. The DNA sequence was designed on the basis of the antisense sequence of the human aldehyde dehydrogenase 2 (ALDH2) gene.[18] Photoelectrochemical measurements after hybridization with the strand 5′-CACTGAAGTGAA-3′ were carried out in a 10 mM sodium cacodylate solution (pH 7.0) using 365 ± 5 nm light at a power density of 13.0 ± 0.3 mW cm^{-2} with an applied potential of 500 mV versus SCE. A stable cathodic current appeared immediately upon irradiation of the modified gold electrode (Figure 2). The current density was −299 ± 21 nA cm^{-2} (Figure 3, i). In contrast, the current dropped instantly when the illumination ceased. These results indicate that the current density is governed by photostimulated hole transport through the DNA initiated by the photoexcitation of anthraquinone.

We next examined the photocurrent using the target sequence, 5′-CACTAAAGTGAA-3′, where an A base replaced a G base of an ALDH2 short fragment, corresponding to a G1459A SNP (Figure 3, ii). The photocurrent density was −153 ± 32 nA cm^{-2}, much smaller than that using 5′-CACTGAAGTGAA-3′. The decrease in current density shows that a G base is essential as a bridge for efficient G-hopping hole transport. The distinction between pyrimidine bases in the hybridized strand was also tested, using an electrochemical probe based on the sense sequence of the ALDH2 gene (Figure 3, iii and iv). Although the duplexes examined contain the appropriate numbers of Gs for efficient G-hopping hole transport, the photocurrent observed for the duplex containing a G/T mismatched base pair was much

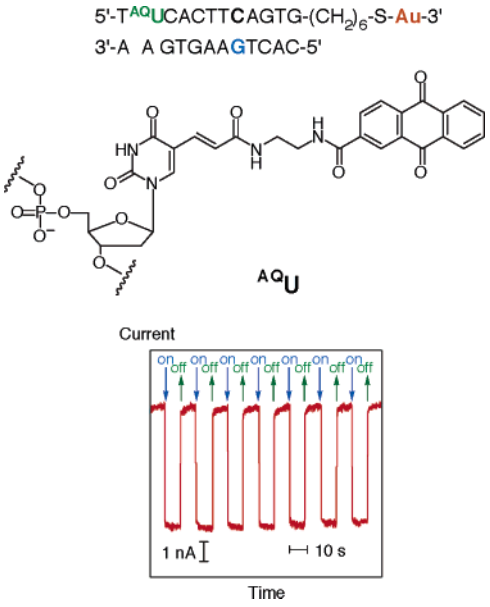

Figure 2. Amperometric $i-t$ curve of the ALDH2 duplex-modified gold electrode, 5′-T^{AQ}UCACTTCAGTG-(CH$_2$)$_6$-S-Au-3′/5′-CACTGAAGTGAA-3′, 9.9 ± 0.8 pmol cm^{-2}, under a 500 mV bias voltage versus SCE. The duplexes in 10 mM sodium cacodylate solution (pH = 7.0) were irradiated (365 ± 5 nm light at 13.0 ± 0.3 mW cm^{-2}) at 25 °C. The sampling interval was 0.1 s.

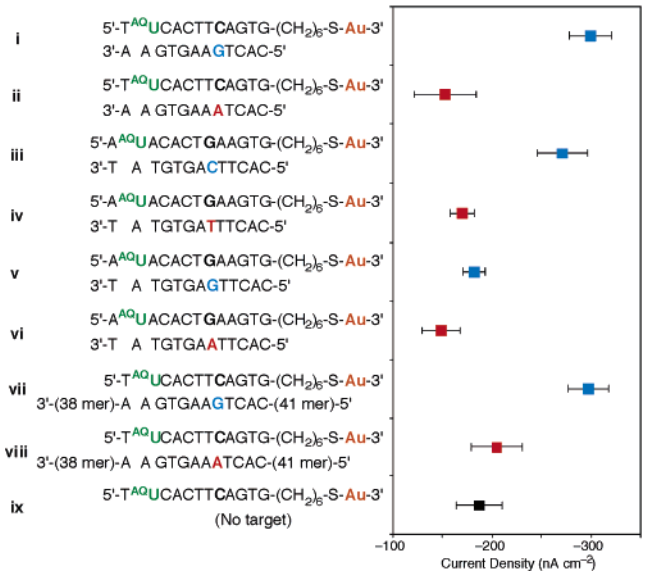

Figure 3. Photocurrent densities of the gold electrode modified by the short ALDH2 fragments containing an SNP site (G1459A). The protocol described in Figure 2 was used for the experiment. (i) and (ii): Antisense strand as probe and G/A sense strand as target; (iii) and (iv): sense strand as probe and C/T antisense strand as target; (v) and (vi): sense strand as probe and unnatural G/A strand as target; (vii) and (viii): antisense strand as probe and 91-mer G/A sense strand as target; (ix): antisense strand as probe and no target strand. Fifteen experimental results obtained using different gold electrodes are plotted for each duplex.

(18) Yoshimura, K.; Hanaoka, T.; Ohnami, S.; Ohnami, S.; Kohno, T.; Liu, Y.; Yoshida, T.; Sakamoto, H.; Tsugane, S. *J. Hum. Genet.* **2003**, *48*, 654−658.

smaller (-170 ± 12 nA cm^{-2}) compared with that of the matched duplex (-271 ± 25 nA cm^{-2}). Weak photocurrents were also observed for "unnatural" ALDH2 sequences that contained a G/G or G/A mismatched base pair, which were -182 ± 11 and -149 ± 19 nA cm^{-2}, respectively (Figure 3, v and vi). The disruption of the π-stacking array[19,20] and the inefficiency of G$^{\bullet+}$ deprotonation[21] by mismatched base pairs strongly suppressed the hole transport efficiency.

Our electrochemical probes are applicable for SNP typing in a long gene sequence. We prepared 91-mer ALDH2 sense strands containing a G1459A SNP site and hybridized them with a probe DNA immobilized on the electrode, 5′-T^{AQ}UCACT-TCAGTG-(CH$_2$)$_6$-S-Au-3′ (Figure 3, vii−ix). A high photocurrent density was observed for the DNA possessing a G base at 1459 (-297 ± 20 nA cm^{-2}), whereas the photocurrent for DNA in which the G base was replaced by an A base was much smaller (-205 ± 25 nA cm^{-2}). The photocurrent for a nonhybridized probe was also weak (-187 ± 23 nA cm^{-2}). This low current density should be due to energy transfer caused by the spatial proximity between the gold surface and an excited anthraquinone in a coiled single-stranded probe. Our electrochemical system can reduce the need for exclusion of the signals from hybridization errors and nonhybridization probes, which is essential for conventional assays.

Our assay depends on effective hole transport via the G-hopping mechanism. However, SNP sites are not always located within G/C-rich sequences. Obtaining positive electrochemical signals for SNPs related to A or T bases or typing SNPs in strands containing successive A/T sequences with low hole-transporting ability will not be easy with the present system. Therefore, the method was modified for SNP typing of all types of sequences. We recently developed an artificial nucleobase, methoxybenzodeazaadenine (^{MD}A), for efficient hole transport through DNA (Figure 4a).[22,23] The oxidation potential of ^{MD}A (^{MD}A/^{MD}A$^{\bullet+}$ = 1.10 V vs SCE) is similar to that of G (G/G$^{\bullet+}$ = 1.15 V vs SCE), and an ^{MD}A/T base pair behaves as a good mediator for hole transport (Figure 4b). By combining the hole-transporting bases, G and ^{MD}A, the design of the electrochemical probes has become applicable to a variety of SNP sequences. We observed effective photostimulated hole transport with an ^{MD}A-modified DNA on gold electrodes (Figure 5a). The observed photocurrent of the electrode modified by a duplex containing an ^{MD}A/T base pair was -282 ± 21 nA cm^{-2}. In contrast, the photocurrents of duplexes where the T base opposite an ^{MD}A base was replaced by C, G, and A bases were -198 ± 15, -145 ± 21, and -144 ± 22 nA cm^{-2}, respectively, and the hole transport efficiency was suppressed. This T-selective current for ^{MD}A is in marked contrast to the C-selective current for G (Figure 3). These hole transport properties increase the alternatives for probe design and are useful in improving the reliability of the SNP typing data.

Thiopurine S-methyltransferase (TPMT) is known to play an important role in catabolic inactivation of widely used anticancer and anti-inflammatory drugs.[24] TPMT*3C (A719G) is one of the important SNPs in the interindividual differences in response

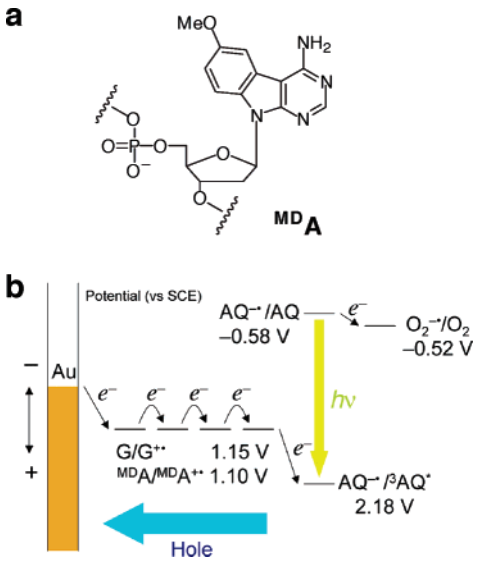

Figure 4. Photostimulated hole transport through an ^{MD}A-containing DNA duplex immobilized on a gold electrode. (a) Hole transporting nucleotide, ^{MD}A. (b) Energy diagram.

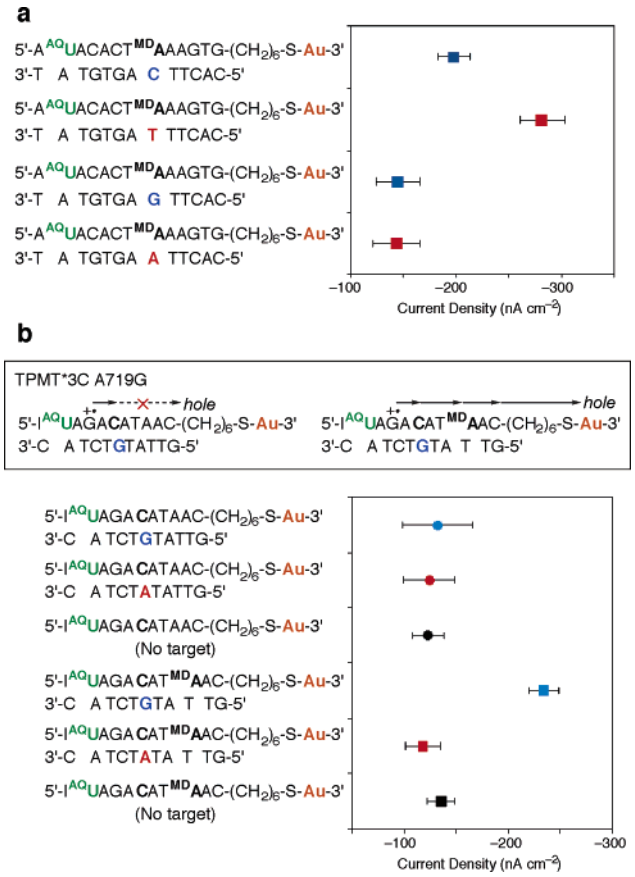

Figure 5. Photostimulated hole transport through an ^{MD}A-containing DNA duplex immobilized on a gold electrode. (a) Photocurrent densities of the gold electrode modified with the ALDH2 duplexes, which contain an ^{MD}A/N base pair (N = C, T, G, or A). Protocol described in Figure 2 was used for the experiment. Ten experimental results obtained using different gold electrodes are plotted for each duplex. (b) Photocurrent densities of the gold electrode modified with the A/T-rich TMPT*3C duplexes containing an SNP site (A719G). "I" in the probe denotes an inosine. Protocol described in Figure 2 was used for the experiment. Ten experimental results obtained using different gold electrodes are plotted for each duplex.

(19) Arkin, M. R.; Stemp, E. D. A.; Pulver, S. C.; Barton, J. K. *Chem. Biol.* **1997**, *4*, 389−400.
(20) Bhattacharya, P. K.; Barton, J. K. *J. Am. Chem. Soc.* **2001**, *123*, 8649−8656.
(21) Giese, B.; Wessely, S. *Chem. Commun.* **2001**, 2108−2109.
(22) Okamoto, A.; Tanaka, K.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 5066−5071.
(23) Okamoto, A.; Tanaka, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 9458−9463.

to thiopurine administration and is located adjacent to an A/T-rich sequence. When the A/T-rich probe 5′-I^{AQ}UAGACATAAC-$(CH_2)_6$-S-Au-3′ was used with the TPMT*3C fragment 5′-GTTAT(G/A)TCTAC-3′, the signal remained weak (−123 to −132 nA cm^{-2}) due to the difficulty in G-hopping (Figure 5b). We designed a probe containing an ^{MD}A base as a "helper base" for efficient hole transport, 5′-I^{AQ}UAGACAT^{MD}AAC-$(CH_2)_6$-S-Au-3′. In this case, a G-selective photocurrent was observed (−234 ± 14 nA cm^{-2}). The appropriate incorporation of ^{MD}A into probes facilitates the typing of SNP sequences containing A/T-rich sites. Electrochemical SNP typing of a variety of sequences becomes possible using the combination of G and ^{MD}A bases.

Having established the utility of the assay, we next tested the SNP typing of amplified samples. We determined the SNP in the antisense sequence of human ALDH2 genes containing a G1459A site, 5′-...TTCACTTYAGTGTAT...-3′ (Y = C or T), by means of hybridization with probes immobilized on gold electrodes. Twenty-five target gene fragments, amplified using an asymmetric polymerase chain reaction (PCR) method between nucleotide positions 1414 and 1504, were hybridized with 13-mer probes immobilized on electrodes, 5′-A^{AQ}UA-CACTPAAGTG-$(CH_2)_6$-S-Au-3′ (P = G or ^{MD}A). When the photocurrent densities calculated from the amperometry of each probe were plotted in the *xy* plane, all samples clearly separated into three clusters, which were identified as G-homozygotes (C/C), A-homozygotes (T/T), and heterozygotes (C/T) (Figure 6a). All SNP typing results performed by this method were in perfect agreement with those obtained by direct sequencing. To establish an easier SNP-typing procedure, the ratios of the photocurrent densities were calculated and plotted for each sample (Figure 6b). We obtained very well-defined clusters corresponding to the three possible genotypes: allelic fractions ranging from <0.78 for A-allele homozygous samples, >1.73 for G-allele homozygous samples, and 1.04−1.29 for heterozygous samples. All data points fell within one of the three distinct and nonoverlapping clusters.

Conclusions

We have demonstrated the design and development of a new photoelectrochemical SNP typing method using hole-transporting DNAs immobilized on gold electrodes. Photosensitizer-equipped DNA wires showed an SNP-specific current through photostimulated long-range hole transport. The use of a combination of hole-transporting probes constitutes an accurate and sensitive method for a single-step electrochemical SNP typing assay, which will pave the way for the further development of microbioelectronics encompassing well-regulated biosensing.

Experimental Section

Probe Preparation. 2-Anthraquinonecarboxylic acid *N*-hydroxysuccinimide ester (50 mM) in 1:1 DMF−dioxane (40 μL) was added to a 25 μM solution (total volume 100 μL) of synthetic DNA,[17] which has an amino linker and a disulfide end, in 50 mM sodium phosphate buffer (pH 8.0) and incubated at room temperature for 12 h. The reaction mixture was purified by reverse-phase HPLC and eluted with a solvent mixture of 0.1 M TEAA, pH 7.0, linear gradient over 60 min from 0% to 60% acetonitrile at a flow rate of 3.0 mL/min. Subsequently, 0.1 M dithiothreitol (50 mM sodium phosphate, pH 8.0) (300 μL) was added to a 25 μM solution (total volume 100 μL) of the modified DNA in 50 mM sodium phosphate buffer (pH 8.0) and incubated at room temperature for 1 h. The reaction mixture was purified by reverse-phase HPLC and eluted with a solvent mixture of 0.1 M TEAA, pH 7.0, linear gradient over 60 min from 0% to 60% acetonitrile at a flow rate of 3.0 mL/min.

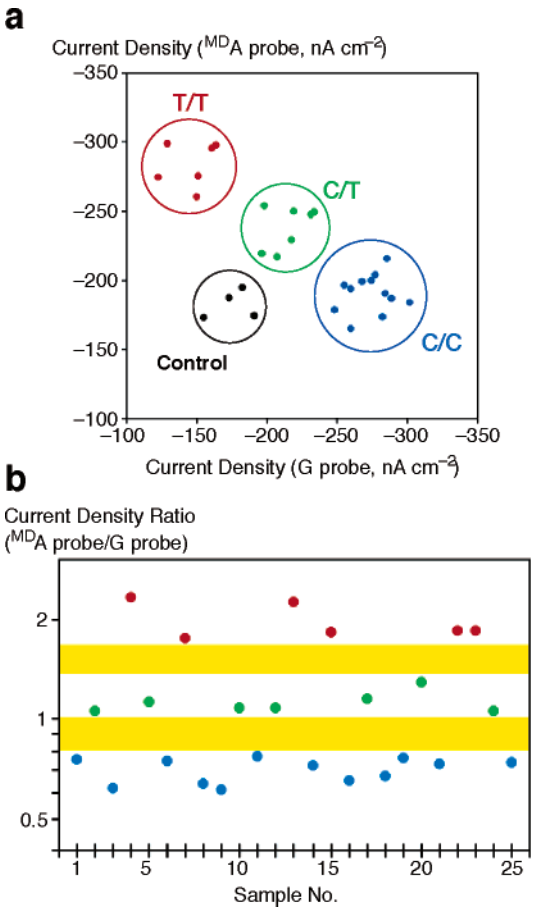

Figure 6. SNP typing using photostimulated hole transport through a DNA duplex immobilized on a gold electrode. (a) Cluster diagram showing the genotype assignment for an ALDH2 SNP in 25 individuals. The 91-mer antisense strand of the ALDH2 gene containing a G1459A site was amplified by asymmetric PCR and was hybridized with a probe strand immobilized on a gold electrode. Assay protocol described in Figure 2 was used for the experiment. The *x*-axis shows the photocurrent density of a G probe, 5′-A^{AQ}UACACTGAAGTG-$(CH_2)_6$-S-Au-3′, and the *y*-axis shows that of an ^{MD}A probe, 5′-A^{AQ}UACACT^{MD}AAAGTG-$(CH_2)_6$-S-Au-3′. (b) Ratios of the photocurrent density for an ^{MD}A probe to that for a G probe are plotted for each sample. Ratios between 1.04 and 1.29 are scored as heterozygous (C/T), while ratios <0.78 and >1.73 are scored as homozygous for the G (C/C) and A (T/T) alleles, respectively. These boundaries are represented on the chart by the yellow areas.

Immobilization of Probes on a Gold Electrode. A gold electrode with an area of 2 mm^2 was used for this study. Prior to DNA immobilization, it was soaked in boiling 2 M potassium hydroxide for 3 h and washed with deionized water. The electrode was then soaked in concentrated nitric acid for 1 h and washed with deionized water. For chemisorption of DNA, a 1-μL solution of 10 μM thiolated probe DNA was placed on a gold electrode held upside-down, and the end of the electrode was fitted with a rubber cap to protect the solution from evaporation. The assembly was kept standing for 2 h at room temperature. Subsequently, for masking of the gold surface, a 1-μL solution of 1 mM 6-mercaptohexanol in 10 mM Tris-EDTA buffer (pH 8.0) was placed on a gold electrode held upside-down, and the end of the electrode was fitted with a rubber cap to protect the solution from evaporation. The assembly was kept standing for 1 h at room

(24) von Ahsen, N.; Armstrong, V. W.; Oellerich, M. *Clin. Chem.* **2004**, *50*, 1528−1534.

temperature. The electrode was then carefully washed with a small amount of deionized water. For hybridization of target DNA to the probe immobilized on the gold electrode, a 1-μL solution of 10 μM target DNA was placed on a gold electrode held upside-down, and the end of the electrode was fitted with a rubber cap to protect the solution from evaporation. The assembly was kept standing for 30 min at room temperature.

Photoelectrochemical Measurements. Photoelectrochemical measurements were performed in a one-compartment Pyrex cell. The cell was illuminated with monochromatic excitation light through a 365 $\pm$ 5 nm band-pass filter (ϕ 25 mm, Asahi Bunko) by a 200 W UV lamp (Sumida YLT-MX200). The photocurrent was measured in a three-electrode arrangement (ALS, model 660A), a modified Au working electrode (electrode area, 2 mm^2), a platinum counter electrode, and a SCE reference electrode at 25 °C. The light intensity was monitored by an optical power meter (Ushio UIT-150). The photocurrent measurements were performed in a 10 mM sodium cacodylate solution (pH 7.0) with $\lambda = 365 \pm 5$ nm light with a power density of 13.0 $\pm$ 0.3 mW cm^{-2} at an applied potential of +0.5 V versus SCE.

Preparation of DNA Samples by Asymmetric PCR. Amplification reactions were performed on a BioRad Mycycler thermal cycler 96-well sample loading tray. Primers and template DNA were added to a 50-μL reaction mixture containing 7 units HotStarTaq DNA polymerase (Qiagen), 2$\times$ premixed PCR buffer, 1.5 mM $MgCl_2$, and 200 μM dNTPs (Qiagen). For the hALDH2 SNP sequence, a 91-mer fragment contained at nucleotide position 1459 in the hALDH2 gene (5 nM) was prepared, and primer 1,5′-d(GGGAGTGGCCGGGAGTT)-3′ (400 nM) and primer 2,5′-d(CTTATGAGTTCTTCTGA)-3′ (400 nM) were added. The thermal cycling program consisted of an initial incubation at 95 °C for 15 min, followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C, and 60 s at 72 °C.

After thermal cycling and cooling, aliquots (2 μL) of the amplified DNA mixtures were used for asymmetric second PCR. Primer 1 (4 μM) and primer 2 (400 nM) were added to a 50-μL reaction mixture containing 7 units HotStarTaq DNA polymerase (Qiagen), 2$\times$ premixed PCR buffer, 1.5 mM $MgCl_2$, and 200 μM dNTPs (Qiagen). The thermal cycling program consisted of an initial incubation at 95 °C for 15 min, followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C, and 60 s at 72 °C.

After thermal cycling and cooling, the amplified DNA was purified through Qiagen Min Elute. The DNA was mixed with 2.5 μM BDF probes in a buffer solution (pH 7.0) of 25 mM sodium phosphate and 50 mM sodium chloride at room temperature on a 1536-well microtiter plate and then measured with a fluorescence reader.

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