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Split DNA Enzyme for Visual Single Nucleotide Polymorphism Typing

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Single nucleotide polymorphisms (SNPs) are the most abundant forms of genetic variations in the human genome. Large-scale sequence analysis is needed for a population-based genetic risk assessment and diagnostic tests once a mutation has been identified. However, most of the methods for SNP screening require enzymatic manipulations such as endonuclease digestion, ligation or primer extension, and often separation of the resultant products.¹ These labor intensive and time-consuming procedures are some of the biggest impediments to moving SNP typing techniques to point-of-care settings, which require straightforward, inexpensive, and disposable detection formats. Toward the fulfilling of these requirements a probe for visual SNP detection was developed in this study.

Binary probes for fluorimetric analysis of single nucleotide substitutions were developed earlier.² The probes demonstrate improved selectivity in comparison with conventional hybridization-based approaches, since the two parts of the probes form relatively short (7–10 nucleotide) duplexes with target sequences. These short hybrids are extremely sensitive to single nucleotide substitutions at room temperature and generate a high fluorescent signal only in the presence of the fully complementary targets. Binary probes do not require precise temperature control for SNP typing.^{2d,e} However, a fluorimeter is required for signal registration. To avoid the need for instruments for both SNP typing and signal readout, a binary probe that generates a visual output after hybridization to the target was designed in this study based on a peroxidase-like DNA enzyme.

A hemin binding DNA aptamer (Figure 1A) was obtained earlier by in vitro selection.³ It was shown that in the presence of hemin it forms a guanine quartet, which demonstrates hydrogen peroxidase-like activity ~250 times greater than hemin alone.⁴ This DNA enzyme was used for the design of allosterically regulated sensors for nucleic acids, AMP, and lysozyme that allow colorimetric or luminescent readouts.⁵ To construct a binary probe, the sequence of the peroxidase-like DNA enzyme (Figure 1A) was split into two halves, the deoxycytidine was removed, and the analyte binding arms were added to each half via triethylene glycol linkers (Figure 1B). In the absence of nucleic acid analyte strands α and β exist predominantly in the dissociated form (at certain concentrations and buffer conditions), while assembling in a G-quadruplex structure and acquiring peroxidase activity when hybridized to the adjacent positions of the analyte (Figure 1C). The active peroxidase catalyzes the oxidation of a colorless substrate to a colored product, which can be detected both visually and spectrophotometrically. DNA that is a part of the coding sequence for microtubule associated protein tau (MAPT) was chosen as a model analyte for this study. Hypotype H1c carrying SNP rs242557 G to A

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Supporting Information Available: Details of the experimental procedure and the detection format using ABTS as an oxidizable substrate as well as the results with dithymidine linker containing probes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

substitution at the MAPT locus was shown to be associated with the risk of Alzheimer's disease.⁶ Therefore, the analyte binding arms of the probe were tailored to recognize the major allele rs242557-G (Figure 1B,C).

Figure 2 demonstrates the change of light absorption of the solution containing binary DNA peroxidase probe when 3-3'-diaminobenzidine tetrahydrochloride (DAB) was used as an oxidizable substrate. The solution turned brown in the presence of 1 μ M rs242557-G but not in the presence of mismatched rs242557-A (compare 4 with 3 in Figure 2A). The light absorption in the presence of mismatched target was as low as in the absence of any analyte (compare 3 with 2 in panels B and C). At the same time, high absorption was observed in the presence of complementary target (sample 4, B and C). The signal-to-background ratio (S/B) was ~ 10 after 30 min of incubation. This signal was only $\sim 38\%$ lower than that of the solution containing parent peroxidase-like DNA enzyme (sample 1).

To further support the suggested mechanism of probe activation upon hybridization to a fully complementary target (Figure 1), samples 1-4 were analyzed by native PAGE (Figure 3, lanes 1-4). In the absence of an analyte strands α and β migrate as two separate bands (lane 2). This proves the earlier suggestion that the two components of peroxidase-like DNA enzyme exist predominantly in the dissociated form in the absence of analyte. At the same time a low mobility band, which migrates above the 100 base pair (bp) DNA marker, was observed only in the presence of rs242557-G (lane 4) but not in the presence of rs242557-A (lane 3). This band can be attributed to the tripartite complex depicted in Figure 1C. The unexpectedly low mobility of this 68-nucleotide associate can be explained by the binding of the positively charged hemin, which increases the molecular weight and reduces the negative charge of the complex. The significant gel retardation of parent 17-mer DNA peroxidase, which also binds hemin (lane 1), supports this suggestion. It should also be mentioned that strand α hybridizes both to rs242557-G and rs242557-A even in the absence of strand β , thus forming the 52-nucleotide DNA associate, which migrates between 20 and 30 bp markers (lanes 3, 4, 6, and 7). The faint band below 20 bp marker in lanes 3, 6, and 7 corresponds to the mobility of the 34-nucleotide rs242557-G or rs242557-A.

It was found that DAB can be substituted with another peroxidase substrate, ABTS (2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) (Figure 1S, Supporting Information). However, the S/B ratio was reduced to ~ 6 in this case, making the color difference less contrasting. Substitution of the triethylene glycol linkers in the structure of strands α and β with dithymidine linkers substantially reduced the intensity of the positive signal in the presence of complementary target (Figure 2S). However, if one of the strands contained dithymidine, while another triethylene glycol linker, the staining was intensive enough to be visualized. This observation suggests that at least one strand of the probe can be composed of purely natural deoxyribonucleotides.

Earlier, gold nanoparticle (GNP)-based approaches were suggested for SNP typing with a colorimetric/optical outcome.⁷ These methods involve attaching of noncomplementary DNA oligonucleotides capped with thiol groups to the surface of two batches of 13-nm GNPs. When DNA, which is complementary to the two engrafted sequences, is added to the solution, a polymer network is formed. This condensed network brought the conjugated GNPs to self-assemble into aggregates with a concomitant red-to-purple color change. Alternatively, a non-cross-linking DNA-GNP aggregation method takes advantage of blunt end stacking interactions of the DNA double helixes.^{7b} All these approaches demand conjugation of DNA probes with colloid gold. In addition, some of the techniques require precise temperature control for allele discrimination. These procedures complicate both the probe preparation and the assay per se. Unlike gold nanoparticle-based approaches, a binary DNA peroxidase probe requires neither postsynthetic modification of the probe oligonucleotides nor precise

temperature control for SNP typing. The assay is simple and inexpensive. Taking into account these advantages, binary peroxidase probe represents a promising platform for point-of-care SNP diagnostics of the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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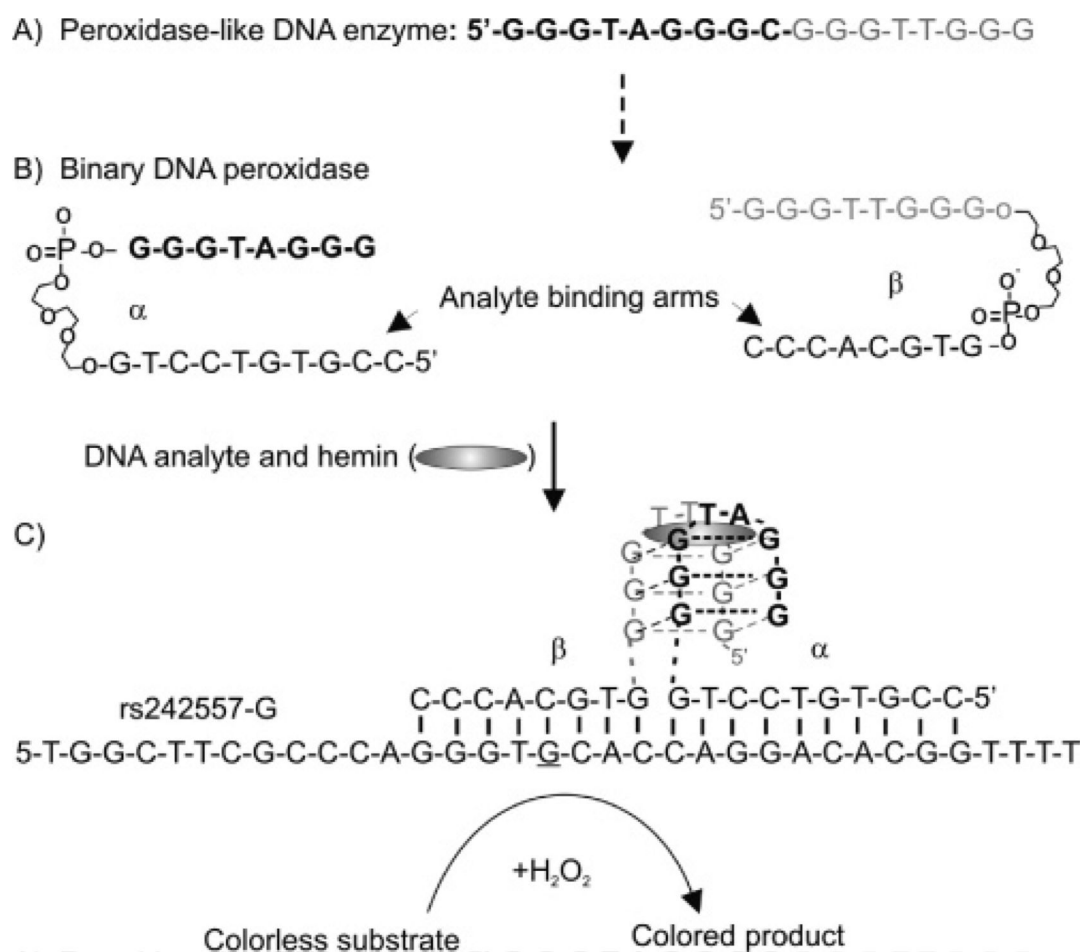
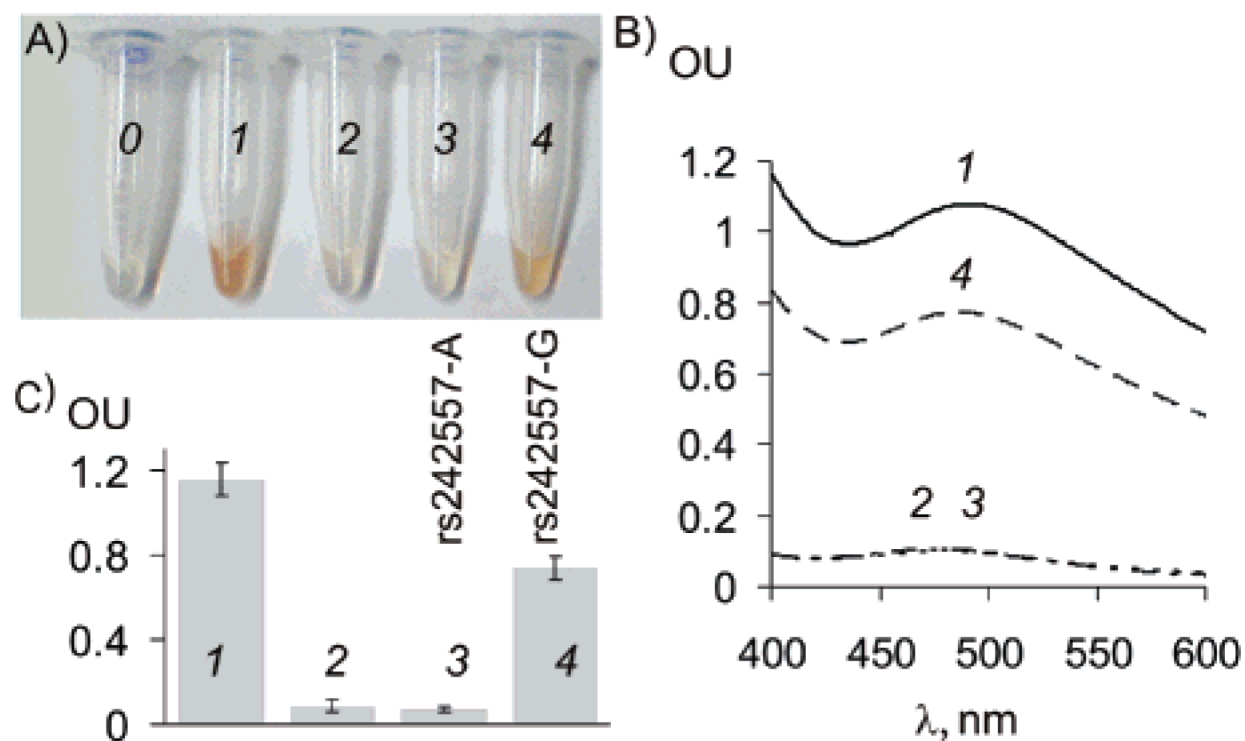


Figure 1.

Design of the binary DNA peroxidase for SNP analysis: A: parent peroxidase-like DNA enzyme; B: binary DNA peroxidase probe; and C: the probe forms active peroxidase upon hybridizing to the abutting positions of the analyte. The enzyme catalyzes oxidation of a colorless substrate to colored products. The triethylene glycol linkers are shown as dashed lines in panel C. The SNP site in the analyte sequence is underlined.

**Figure 2.**

Binary DNA peroxidase probe reports single nucleotide substitution in tau protein coding DNA. A: Visual detection. Sample 0: reaction buffer (50 mM HEPES, pH 7.4, 50 mM MgCl_2 , 20 mM KCl, 120 mM NaCl, 0.03% Triton X-100, 1% DMSO, hemin (125 nM), H_2O_2 (1 mM), and DAB (1 mM)); Sample 1: reaction buffer in the presence of 1 μM peroxidase-like DNA enzyme (positive control). Strands α and β (1 μM) in the absence (sample 2) or presence of 1 μM rs242557-A (TGG CTT CGC CCA GGG TAC ACC AGG ACA CGG TTT T) (sample 3), or 1 μM rs242557-G (TGG CTT CGC CCA GGG TGC ACC AGG ACA CGG TTT T) (sample 4). The samples were incubated 30 min at room temperature. B: Electron spectrum of the same samples; sample 0 was used as a reference. C: Average optical densities at 500 nm of five independent measurements.

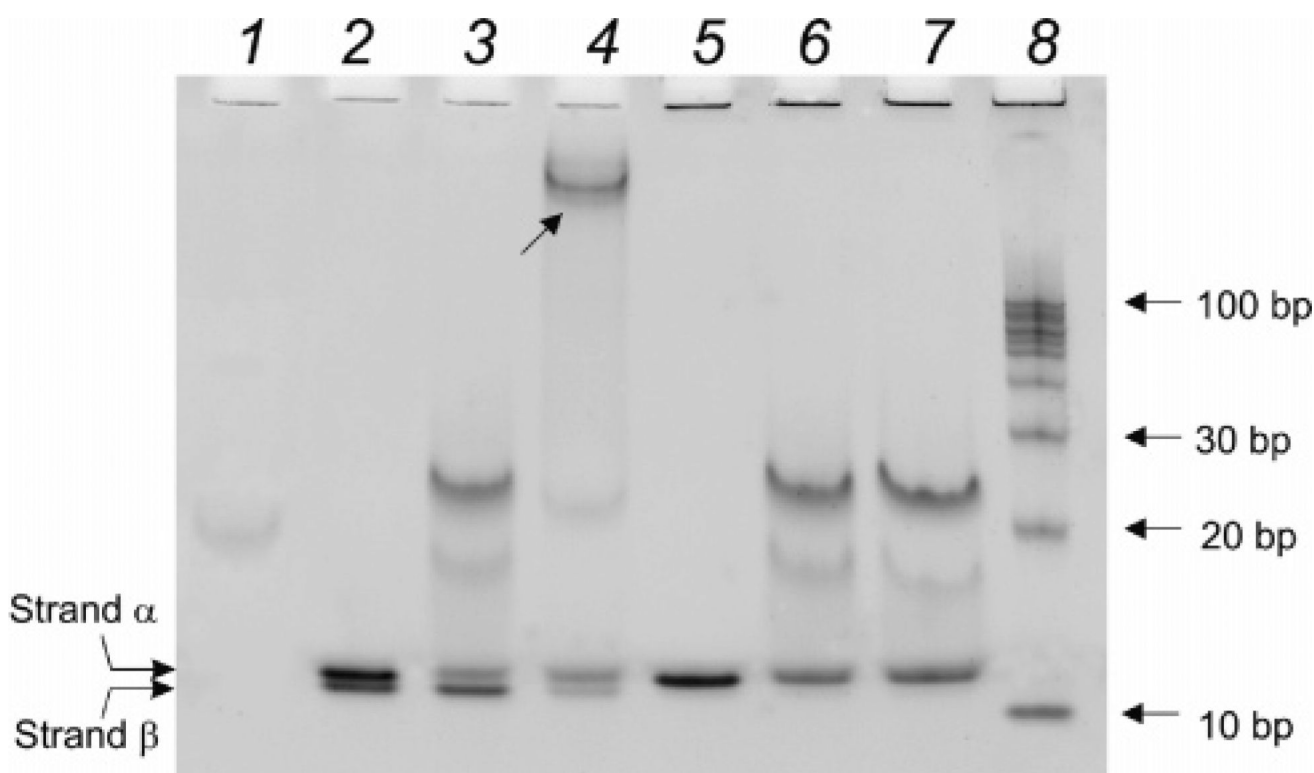


Figure 3.

Analysis of binary DNA peroxidase probe by native 12% PAGE. All samples contained the reaction buffer (see legend to Figure 2). In addition, the following oligonucleotides were added: lane 1: peroxidase-like DNA enzyme (1 μ M); lane 2: binary DNA peroxidase (1 μ M both strands); lane 3: binary DNA peroxidase (1 μ M both strands) and rs242557-A (1 μ M); lane 4: binary DNA peroxidase (1 μ M both strands) and rs242557-G (1 μ M); lane 5: strand α (1 μ M) only; lane 6: strand α (1 μ M) and rs242557-A (1 μ M); lane 7: strand α (1 μ M) and rs242557-G (1 μ M); and lane 8: 10 base pairs (10 bp) DNA ladder. The low mobility band in lane 4 is indicated by an arrow.