New Strategy of Ligand-Based Assay for SNPs Typing: Highly Selective and Strong Binding of Small Ligands to a Nucleobase Opposite an Abasic Site in DNA Duplexes

○Seiichi Nishizawa (Department of Chemistry, Graduate School of Science, Tohoku University, and CREST, JST)

A new strategy of ligand–based fluorescence assay is presented for SNPs (single-nucleotide polymorphisms) typing, in combination with abasic site (AP site)-containing probe DNAs. In contrast to current assays based on hybridization or enzymatic technologies, our method is characteristic of construction of the AP site in DNA duplexes, which allows small synthetic and/or biotic ligands to bind to target nucleotides opposite the AP site, and this is accompanied by fluorescence signaling: an AP site-containing probe DNA is hybridized with a target DNA so as to place the AP site toward a target nucleotide, by which hydrophobic microenvironments are provided for ligands to recognize target nucleobases through stacking and hydrogen-bonding interactions. Highly selective bindings toward target nucleobases are indeed obtained with the binding affinity up to the micromolar range, as has been demonstrated in AMND (2-amino-7-methyl-1,8-naphthyridine)-cytosine, pterin-guanine, vitamin B_2-thymine, and amiloride-thymine bindings [1]. Our method is effectively applicable to the analysis of PCR (polymerase chain reaction) amplification products, for which a complexation-induced fluorescence quenching of these AP site-binding ligands is utilized to detect the single base mutation. In addition, our system is further developed for an SPR (surface plasmon resonance) assay by preparing sensor chips carrying ligands on their surfaces. On the basis of these results, potential of our assay is demonstrated for quick, simple and cost-effective detection of SNPs.

[1] J. Am. Chem. Soc., 125 (2003) 8982; Chem. Commun., (2003) 2960; Talanta, 63 (2004) 175; Anal. Chim. Acta, in press; Angew. Chem. Int. Ed., in press; Chem. Commun., in press.