

Target-triggered DNA three-way junction superstructure and cascade isothermal signal amplification enable subtyping of influenza A virus

Ying-Feng Chang^{1, #}, Ting-Yu Ke^{1, #}, Amily Fang-Ju Jou¹, Ying Li¹, Li-chem Wu², Ja-an Annie Ho^{1, *}

¹ BioAnalytical Chemistry and Nanobiomedicine Laboratory, Department of Biochemical Science and Technology, National Taiwan University, Taipei 10617, Taiwan

² Biochemistry Laboratory, Department of Applied Chemistry, National Chi-Nan University, Puli, Natou 54561, Taiwan

[#]Equal contribution in this research; ^{*}Corresponding author

Subtyping of influenza is critical for its treatment, diagnosis and surveillance. Due to the emergence of new subtypes and highly pathogenic avian influenza viruses, novel analytical approaches for monitoring their appearance and further facilitating the development of vaccine are in urgent need [1]. We herein report a sensor design that integrates DNA three-way junction and catalytic activities of deoxyribozyme (DNAzyme) for sensitive and specific identification of dual target sequences [genes encoding hemagglutinin (HA) and neuraminidase (NA)] simultaneously. By implementation of a simple concept as logic gates, output signal can only be obtained upon the coexistence of both HA and NA genes. Such design enables us to derive a rapid result exclusive of potential personal error made in data interpretation. To detect viral HA gene, two synthetic DNA primers were first mixed with viral cDNA to form DNA three-way junction, followed by the initiation of strand displacement amplification reaction upon addition of appropriate enzymes. As a consequence, a functioning DNAzyme was formed, that can hydrolyze stem-loop substrates, resulting in two separated single-stranded DNA (one of which was pre-modified with a fluorophore). Parallely, the same procedure was carried out for detection of viral NA gene. Accordingly, the Cy3- and Cy5-labeled single-stranded DNA, generated from the reactions triggered by HA and NA genes, respectively, hybridized to form duplexes. Due to the proximity of the two dye molecules, fluorescence signal could be detected as a result of Förster resonance energy transfer (FRET). Optimization of the assay performance has also been conducted in this study, in which the optimal amount of enzymes participated in strand displacement amplification, rational design of 3-way junction templates and primer conformation (linear vs. hairpin) on non-specific signal amplification were thoroughly investigated. Finally it was confirmed that FRET signal can only be acquired upon hybridization of two fluorophore-labeled sequences, products of DNAzyme-catalyzed reactions triggered by HA and NA genes, respectively. The limit of detection (LOD) of this system was calculated as 3 nM. Features of our newly designed sensor include (i) it can be operated isothermally, expensive temperature-controlling equipment is no longer needed, (ii) the entire time needed for detection is ~1 hour, which dramatically improve the assay turnover rate, (iii) the chemical and thermal stability of DNAzyme is superior than conventional protein-based enzymes, and (iv) the design for signal amplification and outputting is universal, that can be readily extended to other potential target sequences. In the light of the sophisticated signal amplification scheme and comprehensive bio-applicability, this platform technology is expected to be a promising diagnostic tool for subtyping of variable influenza viruses.

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[1] Y.-H. Lai, *Chemical Science* 5 (2014) 4082-4090.