Gene Detection Based on the Tetrakis-acridinyl Peptide (TAP) Cassette

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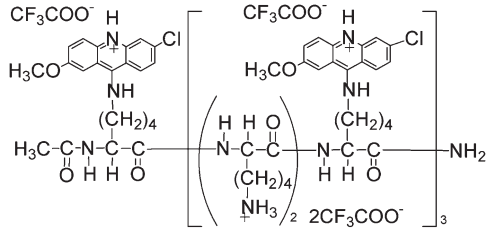
The TAP cassette, formed by the reaction of TAP with a DNA probe carrying a continuous AT sequence, was used for the detection of target DNA by the sandwich assay on the titer plate. This was achieved by the characteristics of TAP having high affinity and high preference for double stranded DNA and dramatic enhancement of fluorescence of TAP bound to [poly(dA-dT)]$_2$.

A simple gene detecting technology with high sensitivity and specificity is required in medical and areas.[1] Detection of target genes can be generally achieved by use of a fluorescence-modified DNA fragment, called DNA probe, carrying a unique DNA sequence complementary to that of the target gene.[2] Since the detection of target DNA is equivalent to detecting double stranded DNA (dsDNA) formed from target DNA with probe DNA separately from their unhybridized DNA, development of a fluorescent reagent showing high preference for dsDNA, but not for DNA sequence is essential from this point of view. In fact, fluorescent dyes such as TOTO, YOYO, and SYBR Green were developed.[3–9] However, the research on an effective fluorescent reagent showing high preference and high affinity for dsDNA was limited. We previously designed and synthesized a tetrakis-acridinyl peptide (TAP) (Figure 1) as a tetrakis-intercalating fluorescence dye.[10] TAP has a high binding affinity with a binding constant of ca. $10^8\,M^{-1}$ (0.2 M Na^+) for [poly(dA-dT)]$_2$. The fluorescence of TAP is quenched in aqueous solution but it is enhanced 1600-fold upon addition of [poly(dA-dT)]$_2$. This fluorescence enhancement of TAP hardly occurs, however, with dsDNAs carrying guanine bases such as calf thymus DNA in spite of its high affinity (ca. $10^7\,M^{-1}$, 0.2 M Na^+), since the acridine fluorescence is quenched by guanine bases through an electron-transfer mechanism.[9]

To facilitate fluorometric dsDNA detection regardless of its sequence, we devised a novel gene detecting system considering



Tetrakis-acridinyl peptide (TAP)

TAP cassette:

$$A_{18}(AT)_{51} \atop (TA)_{51}A_{18} + n \times TAP$$

Figure 1. Chemical structure of TAP and TAP cassette.

the characteristic properties of this TAP. TAP can specifically bind to an oligonucleotide carrying a continuous AT sequence to form a complex. We named such complex as a "TAP cassette" as shown in Figure 1. An oligonucleotide representing a DNA probe sequence was attached to the 5′-end of $(AT)_n$. When TAP was mixed with this probe DNA, two strands of the probe DNA form a duplex at their self-complementary $(AT)_n$ portions and TAP bound to this double stranded region with high affinity. This complex TAP cassette could hybridize with target DNA by its probe sequence, enabling the fluorometric detection of the target DNA. Figure 2 shows more versatile DNA detecting system by the sandwich assay on a titer plate based on the TAP cassette. First, two DNA probes are designed so as to be complementary to a target DNA sequence. One of them is immobilized on the well of a titer plate through a biotin–avidin conjugate. The other DNA probe is used for the combination of a TAP cassette. A solution of sample DNA is added to the well of a titer plate with the TAP cassette on it to allow hybridization reaction to proceed. After washing the titer plate with buffered solution, fluorescence is measured. When target DNA exists in this sample solution, the TAP cassette binds on the well and the fluorescence of TAP can be observed.

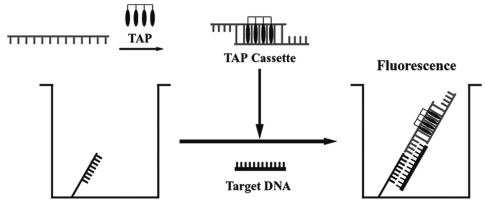


Figure 2. DNA detecting method based on the TAP cassette.

Specifically, we designed the following experiment to assess gene detection based on the TAP cassette, which was prepared by mixing 0.25 μM $A_{18}(AT)_{51}$ with 1.6 μM TAP. One hundred μL of 1.0 μM 5′-biotinyl oligonucleotide of 5′-GTT TTC CCA GTCACG ACG TT-3′ were added to the streptavidin-coated well of a titer plate (Pierce Biotech., IL, the immobilized avidin per well: 125 pmol by avidin inhibition assay), kept for 1 h at room temperature and washed with 2 × SSC (0.03 M sodium citrate and 0.3 M NaCl) three times. After blocking this well with 200 μL of casein blocking buffer (Sigma, MO) at room temperature for 0.5 h, 100 μL of 0–1.0 μM following sample DNA were allowed to hybridize with this well for 1 h at 37 °C. After washing the well with 200 μL of 2 × SSC three times, 100 μL of the TAP cassette in 2 × SSC were added to this well and allowed to stand for 1 h at room temperature. After washing this well with 2 × SSC three times, fluorescence was measured. Shown in Figure 3 are data for fully matched, mismatched, and noncomplementary sequences of 5′-T_{18}AAC GTC GTG ACT GGG AAA AC-3′, 5′-T_{18}A<u>C</u>C GTC GTG ACT GGG AAA AC-3′(mismatched base is underlined) and 5′-T_{18}CAG GAA ACA

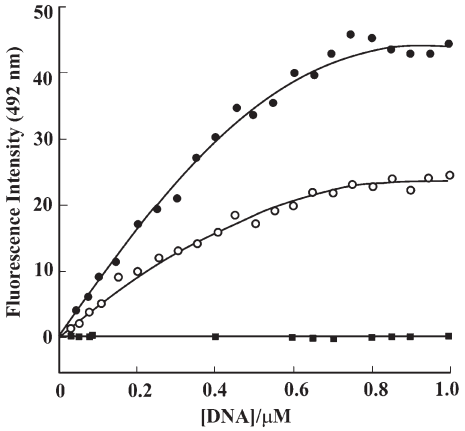


Figure 3. Gene detection based on a sandwich assay coupled with the TAP cassette. TAP cassette was prepared by 1.6 μM TAP and 0.25 μM $A_{18}(AT)_{51}$. Fluorescence intensity was monitored using a 470-nm filter with excitation at 444 nm after hybridization with fully matched (●), mismatched (○), and noncomplementary sequences (■).

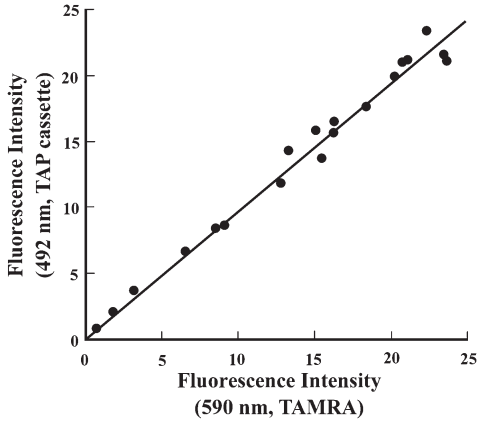


Figure 4. Correlation of the fluorescence intensities of TAP cassette and TAMRA. Similar experiment of Figure 3 was carried out for 5′-T_{18}AAC GTC GTG ACT GGG AAA ACC GAC GTT GTA-3′ carrying TAMRA at 3′-end as target DNA in the case of 5′-biotinyl oligonucleotide of GTT TTC CCA GTC ACG ACG TT-3′ immobilized on the titer plate. Fluorescence intensity of TAMRA at 590 nm was monitored using a 570-nm filter with excitation at 544 nm.

GCT ATG ACC AT-3′ (negative control), respectively. Fluorescence enhancement of TAP was observed upon addition of the matched target DNA and the intensity was proportional to its concentration. On the other hand, noncomplementary DNA sample did not give rise to any fluorescence enhancement. In the case of the one base-mismatched sequence, fluorescence enhancement was also observed but the intensity was one half that for the fully matched sequence, presumably as a result of diminished hybridization efficiency. The fluorescence intensity of the fully matched oligonucleotide sequence almost saturated over 0.8 μM (80 pmol) and this value is in agreement with the amount of avidin on the well.

Since the fluorescence intensity of TAP cassette was observed in the presence of an excess of noncomplementary DNA sample such as calf thymus DNA, it is suggested that TAP did not come off the TAP cassette of double stranded AT sequence and did not relocate to the noncomplementary DNA sample under the conditions employed. Furthermore, the fluorescence intensity did not change after washing the titer plate several times. To confirm whether the observed fluorescence was due to hybridization with target DNA or not, popular fluorescent dye TAMRA was introduced to target DNA and similar experiments were carried out with a TAMRA-labelled target DNA. The fluorescence intensity of TAMRA is plotted against the fluorescence intensity of TAP as in Figure 4 to show a good correlation of both fluorescence intensities, whereas noncomplementary DNA sample did not give rise to any fluorescence enhancement for both TAMRA and TAP. These results suggested that the system based on the TAP cassette can detect and quantify target DNA. Target DNA of as small as 10 pmol could be detected with the TAP cassette.

In conclusion, TAP enables simple detection of a target gene by a sandwich assay based on a TAP cassette without time-consuming fluorescent labeling. Where target nucleic acid has a polyA sequence like eukaryotic mRNA,[11] this system may find use for its analysis or the gene expression analysis. We are trying to optimize conditions for more effective gene detection system based on this TAP cassette.

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