The Role of Magnesium Ion in Gene Mutation Diagnosis Using DNA-nanosphere

Mizuo Maeda, *Graduate School of Engineering, Kyushu University*

Small mutations of certain genes are the definitive origin of many heritable disorders and cancers. Consequently, the development of simple and practical method for the sequence-selective DNA detection has been one of the most important subjects in analytical biochemistry. In this work, we present the novel method for the detection of DNA sequence and chain length utilizing the aggregation of DNA-carrying nanospheres. The exact discrimination of normal DNA from mutant ones by this method requires strict control of the concentration of Mg^{2+}.

The DNA-nanosphere was composed of amphiphilic copolymers of thermo-responsive poly(*N*-isopropylacrylamide) (polyNIPAAm) and oligodeoxyribonucleotide (ODN) derivative having vinyl group at its 5'-terminus. The sequence (5'-GCCACCAGC-3') is the part of K-*ras* oncogene. When the solution temperature was kept above the phase transition temperature of polyNIPAAm, the copolymers spontaneously formed DNA-nanosphere with hydrophobic core surrounded by ODNs.

The complementary ODN **I**, one base mutant ODN **II** and one base longer ODNs **III** and **IV** were employed as the target (Table 1). The increasing turbidity caused by the aggregation of nanospheres was monitored with UV-VIS spectrophotometer (Figure 1). Interestingly, the DNA-nanospheres aggregated rapidly only in the presence of the complementary ODN **I**. On the other hand, the nanospheres kept completely dispersed in the presence of other target ODNs **II**-**IV**. In order to achieve these clear-cut distinctions between ODN **I** and ODNs **II**-**IV**, the appropriate concentration of metal salt such as NaCl or $MgCl_2$ should be determined in advance.

The assembling behaviors of the DNA-nanospheres with the target DNAs are applicable for the detection of DNA sequence and chain length. The results obtained here indicate that this system is promising method for gene diagnosis.

Table 1 Sequences of target ODNs

code	sequence (3'→5')
I	CGGTGGTCG
II	CGGTAGTCG
III	TCGGTGGTCG
IV	CGGTGGTCGT

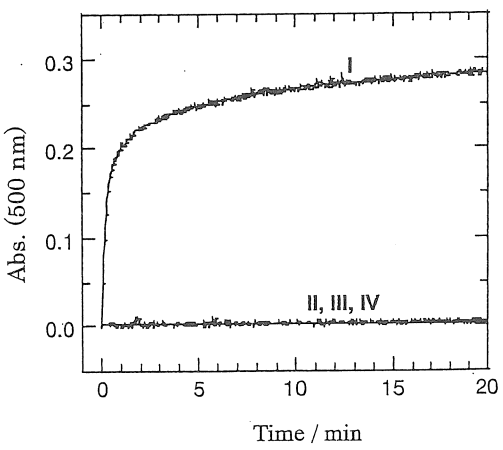

Figure 1. Time course for increasing turbidity by the aggregation of the nanoparticles induced by the addition of target ODNs shown in Table 1 in 10 mM Tris-HCl buffer (pH 7.4) containing 500 mM NaCl.