

DNA-CARRYING POLYMERS AND POLYMERIC NANOPARTICLESM.Maeda^{1,2}¹ Department of Applied Chemistry, Kyushu University, Fukuoka 812-8581, Japan² Bioengineering Laboratory, The Physical and Chemical Research (RIKEN), Wako 351-0198, Japan

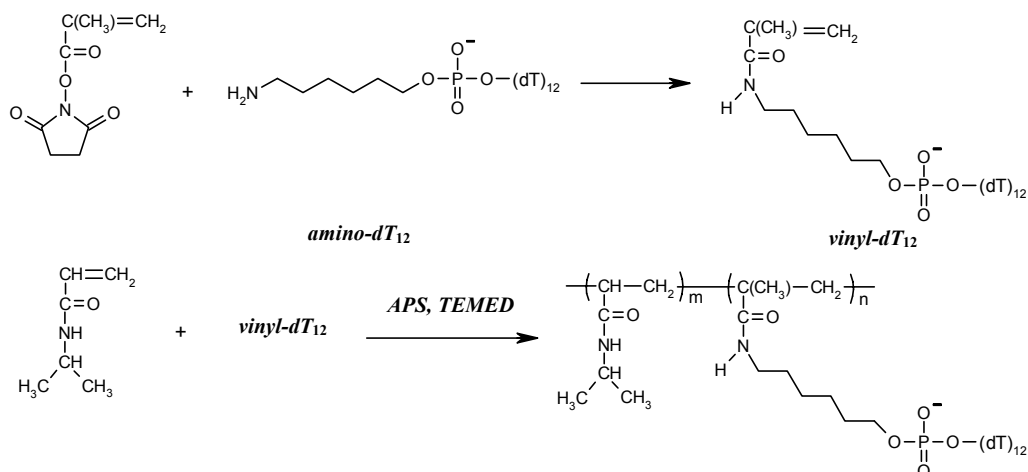
INTRODUCTION: Gene diagnosis shows tremendous promise for medical, pharmaceutical, forensic, and other applications. Recognition of DNA sequence and chain length is the basis of gene diagnosis. A number of methods for recognition of DNA sequence have been proposed based on DNA hybridisation, such as oligonucleotide micro array (DNA chip). However, it is rather difficult to detect the single nucleotide polymorphisms (SNPs) by those methods, because there is only a small difference between their melting temperatures (T_m). In other words, it is difficult to find appropriate discrimination conditions.

In this paper, I will discuss about DNA conjugate which can recognize oligodeoxynucleotide (ODN) fragments with one base specificity. The conjugate is used in its aqueous solution or transparent dispersion. Such a system is very much different from conventional solid materials on which biological components including enzymes, antibodies, and receptors are immobilized. In addition to soluble bioconjugates, dispersed colloidal nanoparticles are also promising for biological and medical diagnosis.

We have synthesized ODN-poly(N-isopropylacrylamide) (polyNIPAAm) conjugate, and found that it formed colloidal nanoparticle by heating over the phase transition temperature of polyNIPAAm. Moreover, the nanoparticles aggregated in the presence of the complementary DNA, while dispersed in the presence of the non-complementary DNA.

METHODS: The ODN (dT_{12}) modified with amino group at 5' ends (amino- dT_{12}) was reacted with N-menthacryloyloxysuccinimide (39 mmol) to give vinyl- dT_{12} , which was then copolymerised with NIPAAm with the aid of redox initiator system from ammonium persulfate and N,N,N',N'-tetramethylethylenediamine. The resulting mixture was purified with dialysis and gel filtration to yield the ODN-polyNIPAAm conjugate. The molecular weight (Mw) of the conjugate was determined by static light scattering (SLS). The solution of ODN-polyNIPAAm conjugate was incubated at 40°C for 30 min to form a stable nanoparticle. As target DNAs, we used a complementary ODN (dA_{12}), a point-mutant ODN ($(dA_6)dT(dA_5)$) and four kinds of different length ODN (dA_{16} , dA_{18} and dA_{24}). The assembling behaviour of the nanoparticles after adding the target DNA was monitored by UV spectrometer.

RESULTS AND DISCUSSION: The apparent behaviour of phase transition of ODN-polyNIPAAm conjugate was different from that of polyNIPAAm, which has lower critical solution temperature (LCST) at around 32°C to change its conformation between coil and globule. Figure 1 shows that the transmittance of the polyNIPAAm solution decreased rapidly above LCST. This is due to the change of polyNIPAAm from hydrophilic coil to hydrophobic globule. On the other hand, the transmittance of the DNA conjugate solution scarcely decrease even above



40°C. This is probably due to the formation of nanoparticles, which disperse in an aqueous medium. In fact, the averaged radius of the nanoparticles was estimated to be 25 nm at 40°C by dynamic light scattering measurement using cumulants method. Mw of nanoparticle was determined to be 8.6×10^6 by SLS. In average, one particle is calculated to consist of ca.30 conjugate molecules. In contrast, an obvious decrease of transmittance was observed at 38 °C for the conjugate solution containing the complementary ODN (dA₁₂) due to the aggregation of the nanoparticles. However, no decrease was observed in the case of a point-mutated ODN ((dA₆)dT(dA₅)), the result being just similar to the solution containing the DNA conjugate only. The single nucleotide change of the target ODN (dA to dT) can be detected clearly by using the DNA-linked nanoparticles.

The rate of aggregation of the ODN-polyNIPAAm after adding the equimolar complementary ODN (dA₁₂) was examined (Fig. 2). A rapid decrease of transmittance was observed in the conjugate solution containing the complementary ODN (dA₁₂) at 40 °C. The nanoparticles aggregated within 5 min, showing usefulness for a rapid gene diagnosis. On the other hand, no decrease was observed at 60 °C; the nanoparticles kept dispersed. Since the melting temperature (T_m) of the duplex between native ODN (dT₁₂) and ODN (dA₁₂) was 51 °C as determined by UV measurement under the identical conditions, the duplex should almost dissociated at 60°C. These results indicate that the nanoparticles aggregate in response to the DNA hybridisation. When the same experiment was conducted at 30 °C, the transmittance did not change, indicating that aggregation takes place only when the nanoparticles are present.

Figure 3 shows the response of the nanoparticles to dA₁₆, dA₁₈ and dA₂₄. Surprisingly, the nanoparticles did not aggregate with the addition of these ODNs, which are longer than dA₁₂ but still complementary to dT₁₂ on the nanoparticles. This strongly suggests that the aggregation was not brought about by crosslinking mechanism between (dAn) oligomers (n>12) and (dT₁₂)-linked nanoparticles. In other words, it is suggested that the DNA chain length of target DNA can be detected clearly by using the DNA-linked nanoparticles.

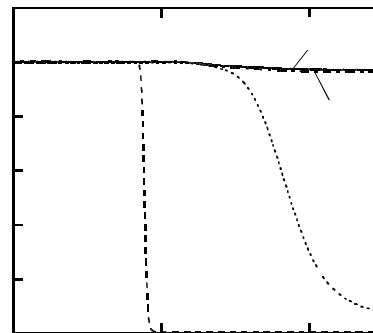


Fig. 1 Temperature-dependence of the transmittance at 500 nm of the polyNIPAAm or conjugate solution (0.05wt%) with or without target DNA (dA₁₂ or (dA₆)dT(dA₅)) equimolar to that on the nanoparticle.

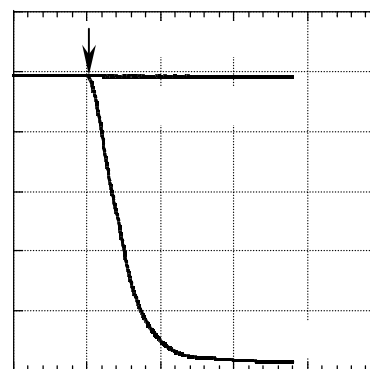


Fig. 2 Time course of the transmittance at 500 nm of the conjugate solution (0.05 wt%) after the addition of target ODN (dA₁₂) (14.0 μM) at 30°C, 40°C or 60°C.

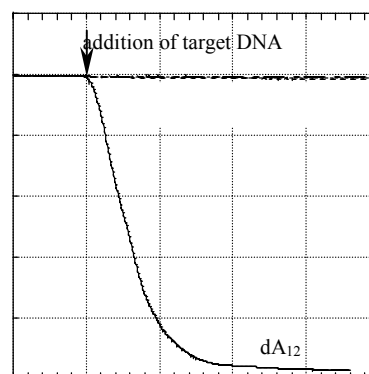


Fig. 3 Time course of the transmittance at 500 nm of the conjugate solution (0.05 wt%) after the addition of target ODN (dA₁₂, dA₁₆, dA₁₈ and dA₂₄) (14.0 μM) at 40°C.

REFERENCES: T. Mori, D. Umeno, M. Maeda (2001) *Biotech. Bioeng.*, **72**: 261-268.