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Poly(acrylic acid)-grafted magnetite nanoparticle conjugated with pyrrolidinyl peptide nucleic acid for specific adsorption with real DNA

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ABSTRACT

Magnetite nanoparticle conjugated with pyrrolidinyl peptide nucleic acid (MNP@PNA) was synthesized for use as both a magnetic nano-support and a probe for specific adsorption with complementary deoxyribonucleic acid (DNA). MNP@PNA with the size ranging between 120 and 170 nm in diameter was prepared *via* a free radical polymerization of acrylic acid in the presence of acrylamide-grafted MNP to obtain negatively charged magnetic nanoclusters, followed by ionic adsorption with PNA. According to fluorescence spectrophotometry and gel electrophoresis, this MNP@PNA can differentiate between fully matched, single-base mismatched and fully mismatched synthetic DNAs tagged with different fluorophores. UV–vis spectrophotometry and gel electrophoresis indicated that MNP@PNA can be used for specific adsorption with real DNA (zein gene of maize) having complementary sequence with the PNA probe. This novel anionic MNP conjugated with the PNA probe might be potentially applicable for use as a magnetic support for DNA base discrimination and might be a promising tool for testing genetic modification.

1. Introduction

The applications of magnetite nanoparticle (MNP) is now of particular interest in both scientific and technological aspects because of its good magnetic responsiveness, good stability and good biocompatibility [1]. In particular, it also shows great potential applications in biomedical field such as targeted drug delivery [2–4], magnetic hyperthermia [5–7], magnetic separation and diagnosis of biomolecules such as deoxyribonucleic acid (DNA), antibodies [8–11]. However, without stabilization, the particle tended to aggregate to each other when dispersed in the media, resulting in the loss in its nanoscale-related properties. Coating MNP with long chain polymers has thus gained a great attention in an attempt to improve its stability and dispersibility through steric and/or electrostatic repulsion stabilizations [4,12]. In addition, functional polymers coated on MNP surface also provided a platform for conjugation with bio-entities of interest such as DNA, ribonucleic acid (RNA), peptide and protein [12–15].

Separation of DNA using MNP as a support is of great interest because this can simplify the purification process through the use of a permanent magnet. Magnetic beads were successfully employed as immobilizing supports for targeting biomolecules with the use of fluorescence labels for DNA quantification [16,17]. Silica-coated MNP functionalized with amino groups has been used as a support for magnetic separation of DNA [18,19]. In addition, the use of MNP/magnetic beads as supports for DNA pre-concentration applications by taking advantage of specific streptavidin-biotin specific interaction has prevalently been reported [16,20–23]. However, the studies in the application of MNP for DNA sequence analysis were quite limited.

Much attention has been paid on the application of peptide nucleic acid (PNA) as a probe for detecting the sequence of DNA target [26–31]. It has been of great interest in this application since it has high specificity toward complementary DNA, high Watson-Crick specificity in recognition of DNA, strong binding affinity and allows for the formation of PNA/DNA or PNA/RNA duplex with good thermal stability and unique ionic strength effect [32–34]. Another great advantage of PNA/DNA strand as opposed to those of DNA/DNA strand is its strong binding interaction owing to the absence of negatively charged repulsion [35]. The utilization of MNP conjugated with PNA as a probe for sequence analysis of DNA

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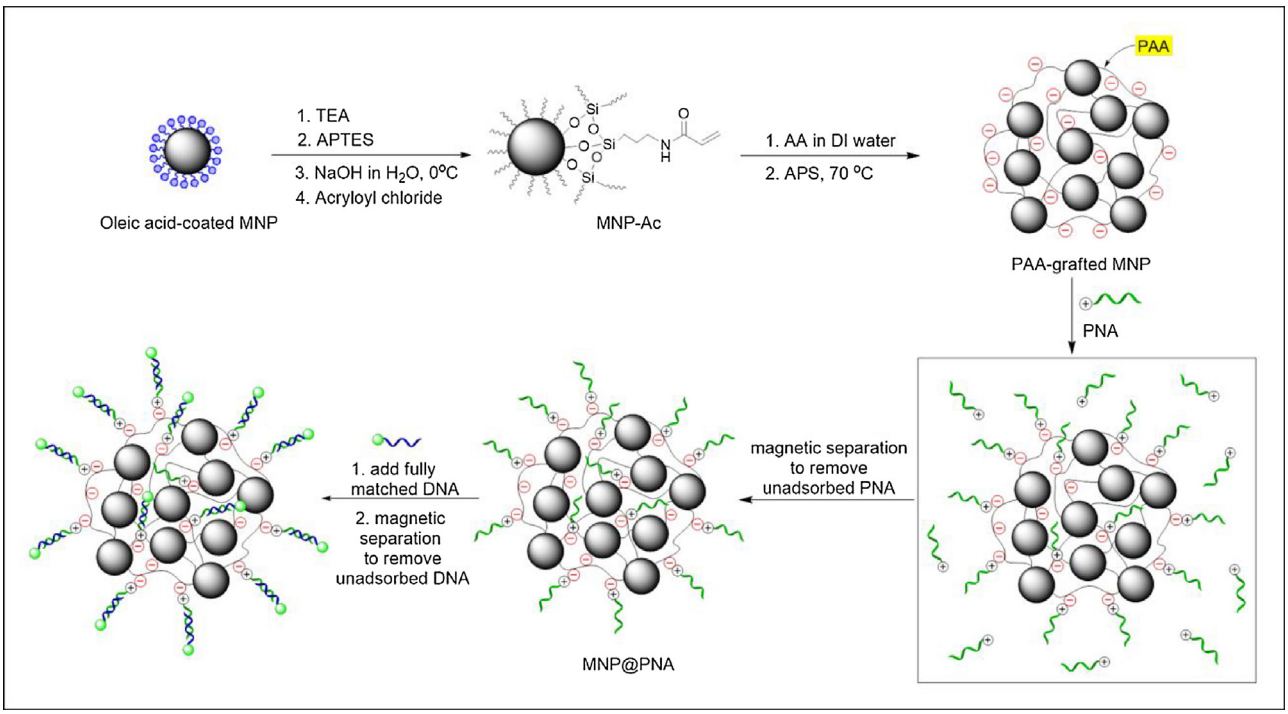

Fig. 1. Synthesis of PAA-grafted MNP and MNP@PNA and application of MNP@PNA as a support for specific adsorption with fully matched DNA.

target is rather limited [12,24]. The hypothesis involved the use of PNA as "anchoring strand" conjugated on the MNP surface and used it to selectively fish out its complementary DNA as "working strand" [25,36].

In the current report, MNP was coated with poly(acrylic acid) (PAA) which can serve as particle stabilizer as well as provide a function for ionic adsorption with pyrrolidinyl PNA (PNA). This PNA-functionalized MNP (MNP@PNA) was then used to differentiate between fully complementary and non-complementary/single-base mismatched DNA. In addition to serving as the particle stabilizer *via* steric and electrostatic repulsions, the negative charge of PAA on the surface of MNP@PNA also provided the negatively charged repulsion to non-complementary DNA, thus reducing the non-specific background adsorption. Pyrrolidinyl PNA (acpcPNA) is of our interest in this work because it shows several unique properties especially nucleic acid binding properties in a high binding affinity, specificity and preference for antiparallel binding mode. In addition, the specificity towards DNA target is in fact considerably better than the original PNA system [35,37,38]. Previous works in this area involve the use of MNP coated with the copolymer of poly (ethylene glycol) methyl ether methacrylate and poly(diethylamino ethyl methacrylate) as anion exchanger solid support for detection of DNA sequence using pyrrolidinyl PNA probe [12,24]. Also, MNP coated with anionic poly(*N*-acryloyl glycine) followed by pyrrolidinyl PNA probe immobilization was effectively used as solid support to differentiate between fully complementary and non-complementary/single-base mismatched DNA oligonucleotides [31].

In this report, MNP@PNA was synthesized *via* a free radical polymerization of acrylic acid monomer (AA) in the presence of acrylamide-grafted MNP to obtain PAA-grafted MNP, followed by ionic adsorption with positively-charged, lysine-modified pyrrolidinyl PNA. This MNP@PNA functioning as both a magnetic solid support and a probe would be then used for the study in base discrimination of synthetic DNA oligonucleotide (Fig. 1). In addition, the use of MNP@PNA for specific extraction of real DNA samples (zein gene of maize) having complementary sequence with the PNA probe was also presented.

2. Experimental

2.1. Materials

Iron(III) chloride anhydrous ($FeCl_3$, 98%, Acros), iron(II)chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, 99%, Acros), ammonium hydroxide (NH_4OH, 30%, Carlo Erba), sodium hydroxide (NaOH, 97%, RCI Labscan), oleic acid (Carlo Erba), (3-aminopropyl) triethoxysilane (APTES, 99%, Acros), triethylamine (TEA, AR., Fisher scientific), ethanol (AR., RCI Labscan) and ammonium persulfate (APS, Bio Basic Canada, Inc) were used as received. Acryloyl chloride was freshly prepared *via* a chloride exchange reaction between acrylic acid (98%, Acros) and benzoyl chloride (99%, Acros) at 75 °C to give a colorless liquid; 60% yield [39]. Fluorophore-tagged DNA oligonucleotides (95%, Pacific Science, Thailand) was used as received. Pyrrolidinyl peptide nucleic acids (PNA) bearing lysinamide at the C-terminus were synthesized *via* a manual Fmoc solid-phase peptide synthesis and purified by reverse phase high performance liquid chromatography (HPLC) as described earlier [35,37].

2.2. Synthesis of poly(acrylic acid)-grafted MNP (PAA-grafted MNP)

2.2.1. Synthesis of MNP and its functionalization with acrylamide (MNP-Ac)

Solutions of $FeCl_3$ (1.66 g in 20 mL deionized water) and $FeCl_2{\cdot}4H_2O$ (1.00 g, in 20 mL deionized water) (1:2 molar ratio of Fe^{3+} to Fe^{2+}) were mixed together with vigorous stirring. After addition of 30% NH_4OH solution (20 mL), a black precipitate was observed, indicating the formation of MNP. After stirring for 30 min, the particle was separated by a magnet and subsequently washed with deionized (DI) water for 3 times. To stabilize MNP in the dispersion, an oleic acid solution (2 mL in 20 mL toluene) was added to the dispersion with stirring for 30 min. The oleic acid-coated