Development of a Magnetic Nanoparticle-Based Artificial Cleavage Reagent for Site-Selective Cleavage of Single-Stranded DNA

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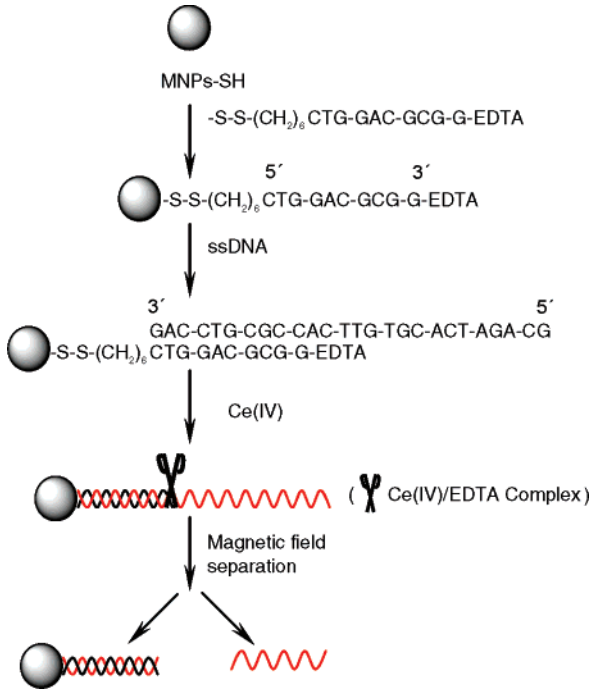
Received November 4, 2006
Revised Manuscript Received January 18, 2007

Site-selective cleavage of DNA has become one of the most attractive and challenging subjects in gene research.[1] In principle, two functional units are required for fabricating an artificial site-selective cleavage reagent, that is, a molecular "scissor" and sequence-recognizing moiety.[2] Until now, various types of site-selective cleavage reagents for DNA scission have been synthesized on the basis of metal complexes or organic molecules as "scissors", and oligodeoxynucleotide (ODN) or its analogues as the sequence-recognizing moiety.[3] Nevertheless, a number of difficulties remain to be overcome with respect to their practical applications. First, almost all DNA cleavage reactions using the above-mentioned cleavage reagents were performed in homogeneous systems; consequently, the expected cleaved product was mixed with the cleavage reagents besides the unreacted DNA. As a result, complicated purifying and isolating procedures were required prior to the use of the cleaved product.[4] Second, even though the sequence-recognizing moieties offer the cleavage reagents a targeting ability for in vivo applications, functional carriers could be useful for guiding the cleavage reagents to specific regions. In this context, to bind the artificial site-selective cleavage reagents with magnetic nanoparticles (MNPs) may greatly expand the practical potentials for the artificial cleavage reagents.

Over the past years, MNPs have been demonstrated to be useful in the collection and isolation of biomolecules as well as bacteria,[5] drug-delivery, magnetic resonance imaging,[6] and so forth. Therefore, to couple the artificial cleavage reagents with MNPs will not only facilitate the subsequent isolations of the cleaved DNA but also endow them targeting ability enabled by external magnetic fields, as well as additional functions as magnetic resonance imaging contrast agents for tracking the biological and biomedical process at the cell level. Furthermore, the interactions between nanoparticles with ODN, that is, the sequence-recognizing moieties, may also offer protections to ODN from enzymatic digestion.[7]

Scheme 1. General Designing Strategies for the MNPs-Based Artificial Cleavage Reagent and Its Applications in the Scission of a Model ssDNA and the Subsequent Magnetic Isolation of the Cleaved Product



Herein, we report our investigations on a new type of artificial cleavage reagent composed of MNPs, 5′>Y CTG GAC GCG G X<3′ (R-S-S-10mer ODN; X = PO_3^-; Y = $-(PO_3)-O-(CH_2)_6-S-S-(CH_2)_6-OH$) as the recognition unit, and a cerium(IV)/ethylenediaminetetraacetic acid (EDTA) complex as the molecular "scissor". The general designing strategies for the MNPs-based artificial cleavage reagent and its application in subsequent isolation procedures are illustrated in Scheme 1. In general, the whole preparative procedures for the MNPs-based artificial cleavage reagent started from 60 nm SiO_2-coated MNPs. Prior to the coupling reaction with ODN−EDTA, a precursor of molecular scissors for single-stranded DNA (ssDNA), the surface of the

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10.1021/cm062632b CCC: $37.00
Published on Web 06/06/2007

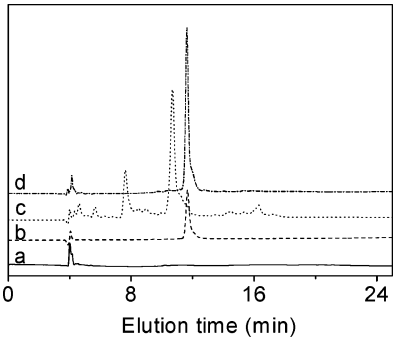

Figure 1. IP-RP-HPLC of the cleaved products from the model ssDNA obtained upon additions of 0.01 mM Ce(IV) (trace a), 0.1 mM Ce(IV) (trace b), and 1 mM Ce(IV) (trace c), respectively. The cleavage reactions were allowed for 96 h at pH 7.4, 37 °C. Trace d is the elution curve of the standard 11mer sample.

composite MNPs was grafted with thiol groups through the hydrolysis of 3-mercaptopropyltrimethoxysilane. The surface area of the resultant particles was determined to be 71.2 m^2 g^{-1} by the Brunauer−Emmett−Teller (BET) method. More details on the characterizations the composite MNPs are given in Supporting Information.

In parallel, ODN was first reacted with ethylenediamine (EDA) via its 3′-phosphate group. The resultant ODN−EDA was then coupled with EDTA via an amidation reaction between the free amino group from EDA and one carboxyl group from EDTA according to a literature method.[8] By thiol/disulfide exchange reaction, the resultant ODN−EDTA was attached to the surface of the MNPs. The effectiveness of this coupling reaction was further confirmed by surface enhanced Raman spectroscopy (SERS). By measuring the ODN−EDTA left in the supernatant after a magnetic isolation of the resultant ODN−EDTA-coated MNPs, the surface coverage of the ODN−EDTA adduct on the SiO_2-coated MNPs was quantitatively estimated to be 15.6 pmol cm^{-2}, corresponding to 1071 ODN−EDTA per MNP. In a similar way, the number of ssDNA attached to each ODN−EDTA-coated MNP during the following hybridization reaction was estimated to be 655.

The cleavage reaction was initiated upon further introduction of Ce(IV) into the aqueous dispersion of the MNPs carrying hybridized ssDNA in Tris-HCl buffer under physiological conditions (pH 7.4, 37 °C).[9] The initial concentration of ODN−EDTA in this reaction system was 0.036 mM. According to above-mentioned results, the concentration of ssDNA hybridized with ODN was around 0.022 mM. The cleavage reaction was allowed for 48−120 h before the whole reaction mixture was subjected to external magnetic fields for collecting the MNPs. Subsequently, the cleaved ssDNA fragments in the resultant supernatant was analyzed by ion-pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC).

The experimental results shown in Figure 1 strongly suggest that the efficiency of the cleavage reaction was

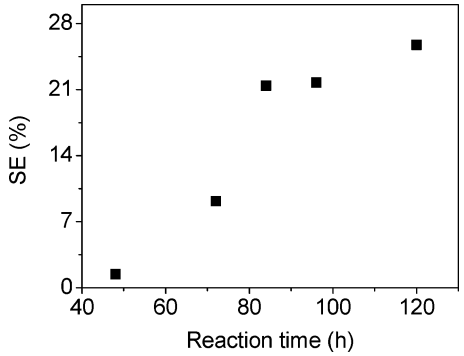

Figure 2. Correlation between the scission efficiency and the cleavage reaction time. The cleavage reactions took place at pH 7.4, 37 °C, in 0.1 mM Ce^{IV}. (SE: scission efficiency.)

dependent on the initial concentration of Ce(IV). Compared with trace *a* for 0.01 mM Ce(IV), trace *b* for 0.1 mM Ce(IV) presents a retention peak at 11.6 min apart from the retention peak that appearing at around 4 min for Tris. To further increase the concentration of Ce(IV) to 1 mM greatly decreases the cleavage site selectivity, leading to a number of additional peaks in trace *c*. Nonetheless, the strongest retention peak remains quite close to that in trace *b*, indicating that the Ce(IV) ions captured by EDTA had a spatial priority in the cleavage reaction even though random cleavages by excessive Ce(IV) had already taken place. By comparing with the elution curve of a standard sample of 11mer, shown as trace *d*, it can be concluded that a specific cleavage took place in 0.1 mM Ce(IV) and led to single product of 11mer. It is worthy of mentioning that the primary 10mer ODN attached on the surface of MNPs may also present a retention time similar to that for 11mer if it fell off the surface of the MNPs. To further confirm that 11mer was the product of the cleavage reaction, a control experiment was performed under exactly the same conditions except that no Ce(IV) was introduced into the reaction system. The IP-RP-HPLC results proved that no ODN fell off after the MNPs were magnetically isolated. Details are provided in Supporting Information.

The scission efficiency against the reaction time was also investigated by IP-RP-HPLC. The experimental results shown in Figure 2 reveal that the scission efficiency is strongly dependent on the reaction time and presents a sharp increment before 96 h followed by a slow-down increasing for prolonged reaction time. The scission efficiency at 96 h was estimated to be 22% by measuring the amount of 11mer left in the supernatant.

As mentioned above, the model ssDNA was a 26mer, while the primary ODN was a 10mer. However, the main product of the cleaved ssDNA fragment was 11mer rather than 16mer. This discrepancy turns out to be caused by the spacer effect of the phosphate-terminated chelating group of EDTA whose fully extended length is about 1.73 nm, imitated by the software of HyperChem7.5 as shown in Scheme 2. This length corresponds perfectly to 5 bases in ssDNA. Therefore, the specific site for cleavage was located at 15 bases from the 3′ end of the 26mer ssDNA. As a result, the cleaved fragment of 11mer with a sequence of 5′>TGC ACT AGA CG<3′ becomes the product of the cleavage reaction, as schematically illustrated in Scheme 2. Matrix-

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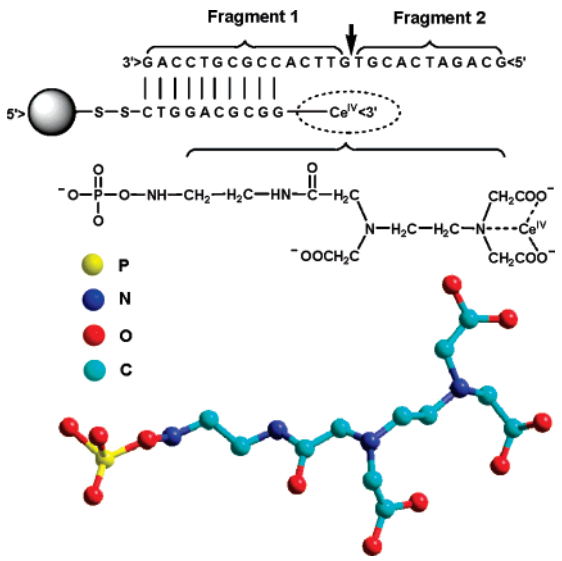
Scheme 2. Scission Profile for the Cleavage of ssDNA Together with the Molecular Model for the Chelating Group[a]



[a] The Black arrow indicates the specific cleavage site for ssDNA by the current MNPs-based artificial cleavage reagent.

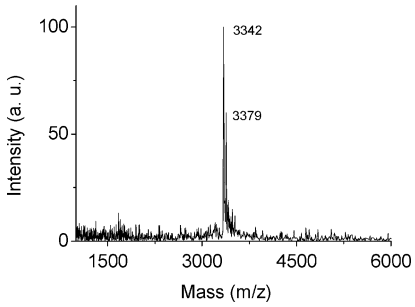


Figure 3. MALDI-TOF MS of the cleaved ssDNA fragments.

assisted laser desorption time-of-flight (MALDI-TOF) MS was further employed to analyze the cleaved ssDNA fragment, and the results are shown in Figure 3. The strongest peak at approximately 3342 Da corresponds well to 11mer of 5′>TGC ACT AGA CG<3′ whose molecular weight is 3341 Da, while the second peak at approximately 3379 Da quite possibly comes from the 11mer which absorbs some ions during the MALDI-TOF MS analysis. In addition, the experimental result obtained by denaturing polyacrylamide gel electrophoresis (PAGE) also supports that 11mer was the main product of the cleavage reaction, further supporting that the MNPs-based artificial cleavage reagent possesses an excellent site-selective cleavage specificity.

In conclusion, a novel type of MNPs-based artificial cleavage reagent is designed and successfully synthesized by coupling the molecular scissor of the Ce(IV)/EDTA complex with MNPs via a sequence-recognizing moiety, that is, the 10mer ODN. Detailed experimental results revealed that the MNPs-based artificial cleavage reagent not only was effective but also presented satisfying cleavage specificity. Most importantly, MNPs greatly facilitate the isolation of the cleaved product from the mixture of cleavage reagents and un-reacted ssDNA. Furthermore, the current investigations set the first successful example for combining the site-selective ssDNA scission reagents onto the surface of the MNPs, which can extend known DNA cleavage research from the homogeneous system to the multiphase system. Besides, considering the characteristic of a tumor cell to endocytose the nanoparticles, the carrier, that is, the MNPs, will endow the potential ability of the artificial cleavage reagent to react in the tumor cell, and this experiment is now in progress in our laboratory. All in all, this MNPs-based artificial cleavage reagent may hold great potential in gene studies.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (Nos. 20573075, 20225313), the Shanghai Science and Technology Committee (No. 0552nm037), and the Shanghai Leading Academic Discipline Project (T0402).

Supporting Information Available: TEM micrograph magnetic property of the SiO_2-coated MNPs, IP-RP-HPLC chromatographs of the ODN/EDTA coupling reaction and the control experiment, denaturing PAGE of the cleavage product, and detailed experimental procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

CM062632B