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Nucleoside Triphosphates as Promoters to Enhance Nanoceria Enzyme-like Activity and for Single-Nucleotide Polymorphism Typing

*Can Xu, Zhen Liu, Li Wu, Jinsong Ren, and Xiaogang Qu**

Nucleoside triphosphates (NTPs) can improve the oxidase-like activity of nanoceria and the enhancement is correlated with the type of NTP. This effect is demonstrated to be as a result of the coupling of the oxidative reaction with the NTP hydrolysis reactions, as the nanoceria has both oxidase-like and phosphatase-like activities. The differences reflect the different dephosphorylation catalytic activities of nanoceria to the NTP used. Furthermore, based on the NTP-promoted oxidase-like activity of nanoceria and the differences among the different types of NTPs, series effective and high-throughput colorimetric assays for single-nucleotide polymorphism (SNP) typing are developed.

1. Introduction

Biological coenzymes such as adenosine triphosphate (ATP), flavin adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide (NAD^+), are present in all known forms of life and play a key role in metabolism.[1] They can be considered "helper molecules" in biochemical transformations. ATP and the other nucleoside triphosphates (NTPs), including guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP), as coenzymes, have participated in many enzymatic catalysis reactions.[2] They play crucial roles for energy transfer in biology. The three high-energy phosphate bonds enable them to store energy and then release it as the bonds are broken according to the following equation: $NTP + H_2O \rightarrow NDP + Pi$, $\Delta G^{\circ\prime} = -30.5$ kJ/mol for all NTPs.[2f] The hydrolysis step, with a large change in Gibbs free energy, could couple with the biological reactions and accelerate the reactions.[2] Herein, we found that these "energy currencies" could not only be used as coenzymes for biological enzymes, but also for nano-catalysis. This is significant since NTPs can perform exceptionally efficient energy transfers at low cost.

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DOI: 10.1002/adfm.201301649

Nanoceria, which exists in a mixed valence state (Ce^{3+}, Ce^{4+}), possesses many unique properties that have proven to be of high utility in biomedical,[3] bioanalysis,[4] and catalytic applications.[5] Firstly, it shows excellent antioxidant properties at physiological pH values.[3,4e,6] Furthermore, the intrinsic oxidase-like activity of nanoceria at acidic pH values was demonstrated recently.[4a] Additionally, nanoceria and various lanthanide ions and/or their complexes have been employed to mimic phosphatase functions involved in the dephosphorylation/hydrolysis of small organic molecules, nucleotides, and peptides.[7] In the present study, we report that the NTPs could improve the oxidase-like activity of the nanoceria and, moreover, that the improved efficiencies are related to the type of NTPs. The roles of NTPs played for the nanoceria seem like that of a coenzyme. The NTPs could bind to nanoceria and the hydrolysis process, with large energy released, could couple with the oxidation reaction of nanoceria and accelerate the reaction. Intriguingly, unlike when NTPs—as coenzymes for biological enzymes—undergo hydrolysis assisted by metal ions as catalysts,[2] here the hydrolysis of the NTPs could be catalyzed in-situ by nanoceria itself, attributed to its inherent phosphatase-like activity. The energies released from the NTPs hydrolysis can further improve the oxidase-like activity of nanoceria in return. Moreover, the catalytic ability of nanoceria in NTP hydrolysis is correlated with the type of NTP, which then leads to different improving efficiencies of NTPs for the oxidase activity of nanoceria.

Furthermore, based on the NTP-promoted oxidase-like activity of nanoceria and the differences among the various NTPs, series effective colorimetric assays for genotyping (identification the types of mismatched bases) of single-nucleotide polymorphisms (SNPs) are developed. SNPs are the most common variations in human genomes.[8] Studies have found close associations between SNPs and tumor development or progression, thus SNP typing has been considered a promising tool for early diagnosis and risk assessment of malignancy.[9] Recent advancements in biotechnologies have offered a wide variety of choices for SNP typing.[9] Among these strategies, colorimetric methods are very attractive because they can be easily read with the naked eye. Unfortunately, despite this advantage, only a few works have been reported and, what's more, in these works, the sequence of the oligonucleotide and the position

of the mismatched bases must be provided before typing.[9g-j] Herein, we develop series colorimetric assays for SNP typing based on NTP–nanoceria systems which do not need the properties of oligonucleotide pre-typing. The results show that the types of mismatched base pairs in SNPs can be identified by the direct visualization of a 96-well screening plate.

2. Results and Discussion

Nanoceria was synthesized by a previously reported in situ procedure[6a] and studied by high resolution transmission electron microscopy (HTEM) imaging, X-ray powder diffraction (XRD), and X-ray photoelectron spectroscopy (XPS) (Figure S1). The XPS spectra (Figure S1C) showed the existence of a mixed valence state.[6c] The effects of NTPs on nanoceria-mediated oxidizing reactions were investigated using 2,2′-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) as a chromogenic substrate.[4a] Since the oxidase-like activity of nanoceria was pH-dependent and showed the optimization activity at acidic conditions (Figure S2),[4a] our experiments were carried out at pH 4.0. As shown in Figure S3, all of the NTPs could improve the reaction activities of the nanoceria–ABTS system. The activity of the reaction increased with increasing NTP concentration, reaching a plateau at about 0.2 mM (Figure S3E). The results also showed the differences among the different types of NTPs used, GTP being the most effective, followed by ATP, UTP, and CTP. The effects of deoxynucleoside triphosphates (dNTPs) were also investigated and showed similar results as that of NTPs (Figure S3). The only difference was that at high dNTPs concentrations, the improving efficiencies decreased. Control experiments showed that neither NTPs nor dNTPs could react with ABTS in the absence of nanoceria (data not shown). Under our experimental conditions, the improving efficiencies and the differences of NTPs were significant even at very low concentration (**Figure 1**A). The oxidase activities of nanoceria in the absence or presence of 8 μM NTPs were shown in Figure 1B. The improving effects and their differences could also be observed directly by the color intensities of the products (Figure 1C).

The improving effect is supposed to be as a result of the coupling of the oxidative reaction with the NTP hydrolysis, as it is known that the nanoceria has both oxidase and phosphatase-like activity.[4,7] When being used as coenzymes for biological enzymes, the NTPs also play the role of "coenzyme" for nanoceria. It is known that the redox reactions mediated by nanoceria are carried out with the process of transfer of electrons, while the hydrolysis of NTPs can shift the reactant redox potentials and increase the electron transfer rate to make the redox reaction more effective.[10] Different from the requirement of assisted metal ions to catalyze the hydrolysis of NTPs when they are used as coenzymes for the biological enzymes,[2] the hydrolysis of NTPs can be catalyzed in-situ by nanoceria itself attributed to the phosphatase-like activity of nanoceria. That is to say, the energy released from the NTP hydrolysis can further improve the oxidase-like activity of nanoceria in return.

To test our hypothesis, NTP hydrolysis was carried out in the presence of nanoceria. The amount of inorganic phosphate liberated into the solution during hydrolysis was measured by malachite green-ammonium molybdate assays.[7a],[11] The results (**Figure 2**) showed that the nanoceria had catalytic hydrolysis activities in response to all of the NTP types, and the activities were different for each NTP species as a result of the different dephosphorylation catalytic activities of nanoceria to each type, with the most efficient being GTP, followed by ATP, UTP, and CTP. The results were consistent with the tendency of improving efficiencies of NTPs (Figure 1A), which supports our hypothesis.

Then the question came down to the different catalytic activities of nanoceria for NTPs hydrolysis. The interactions between lanthanides and nucleotides or their phosphonate derivatives have been studied for a long time.[12] Nanoceria is a lanthanide nanoparticle. The mechanism of lanthanide catalytic dephosphorylation of phosphonate-nucleotide ester derivatives is known: the coordination of the phosphate to lanthanide ions

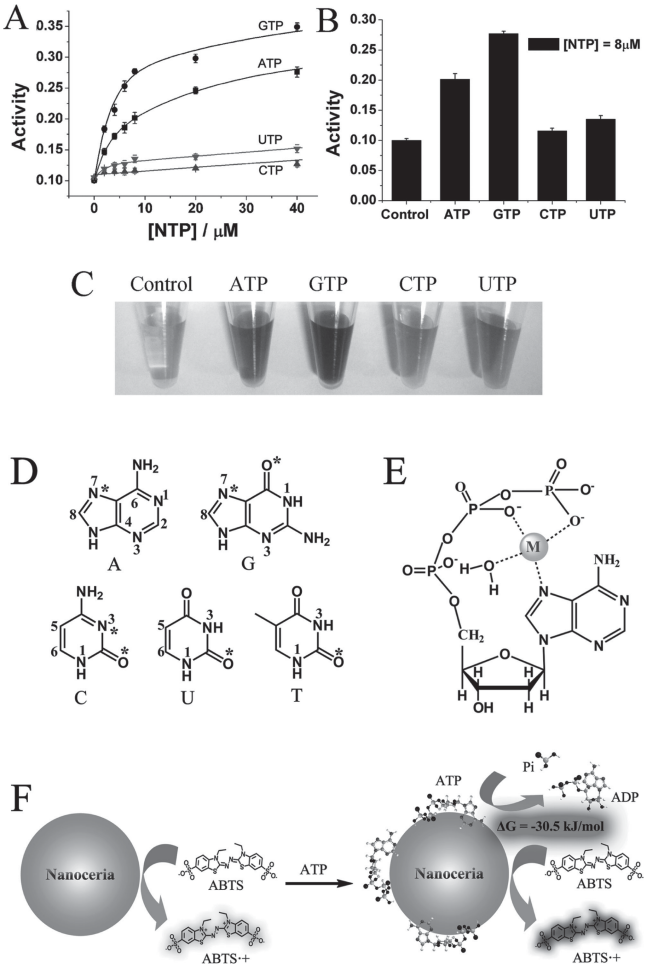

Figure 1. A) The improved efficiency of oxidase-like activity of nanoceria in the presence of low NTP concentrations. B) The oxidase activity of nanoceria in the absence (Control) or presence of 8 μM NTP (reaction for 100 s). C) Photograph of the nanoceria–ABTS system in the absence (Control) and presence of 8 μM NTP after incubation for 0.5 h. D) Chemical structures of various nucleobases. E) Illustration of ATP coordinated to the metal ion. F) Illustration of the coupling of the oxidative reaction with the ATP hydrolysis reaction.

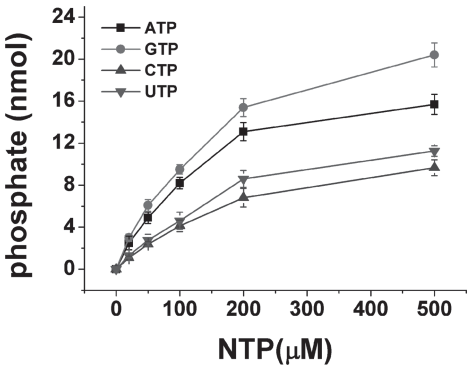

Figure 2. Comparison of the free-phosphate productions from hydrolysis of NTPs catalyzed by nanoceria (10 μg/mL).

through the oxygen atoms strongly activates the phosphorus toward nucleophilic attack. The different binding activities of NTPs to nanoceria would lead to catalytic differences of dephosphorylation. Nucleotide phosphonate derivatives interact with lanthanide ions through both nucleic base residues and the phosphodiester backbone.[12,13] Taking ATP as an example (as illustrated in Figure 1E), the N-7 in the adenine moiety can coordinate to the metal ion as well as to the triphosphate chain to form a macrochelate.[13] For different NTPs, although the interaction of the phosphodiester backbone to nanoceria is similar, the nucleic base residues have different affinities to nanoceria. For guanine (G), the N-7 nitrogen and/or O-6 oxygen moiety could coordinate to metal ions, while the base adenine (A) has an N-7 nitrogen atom, but no O-6 oxygen atom.[12,13] The base cytosine (C) has a carbonyl oxygen atom at O-2 and a nitrogen atom at N-3, while the bases thymine (T) and uracil (U) possess carbonyl oxygens but no unprotonated nitrogen atoms at pH below 9 (Figure 1D).[12,13] The base coordination ability is dependent on pH and other factors,[13a] as nitrogen loses the ability to coordinate with the metal ions when they are protonated. The pK_a^H values of the nitrogen atom mentioned above are shown in Table S1. At pH 7.0, the following binding affinities are tentatively proposed: N-7; G > N-3; C > N-7; A.[13a] At pH 4.0, the N-3 of cytosine was protonated, accompanied by the loss of coordination ability. Thus, the base coordination ability at pH 4.0 is proposed to be G > A > C ≈ T(U). Since the interaction of metal ions with nucleic base residues plays an important role in promoting dephosphorylation of NTPs,[13] the different binding affinities of the nucleic base residues to nanoceria would result in the different dephosphorylation catalytic effects of nanoceria to various NTPs. Therefore, the values of the released energies would determine the improvement efficiencies (Figure 1F).

2.1. In-Situ Label-Free Colorimetric Assay for SNP Typing

Given the physiological functions and significances of NTPs as well as the outstanding versatility of nanoceria, and based on the differences of improvement efficiencies of NTPs to the oxidase-like activity of nanoceria, an in-situ label-free colorimetric assay for typing of SNPs was developed (**Figure 3**). The sequences of probe DNA (prDNA) and target DNA (denoted

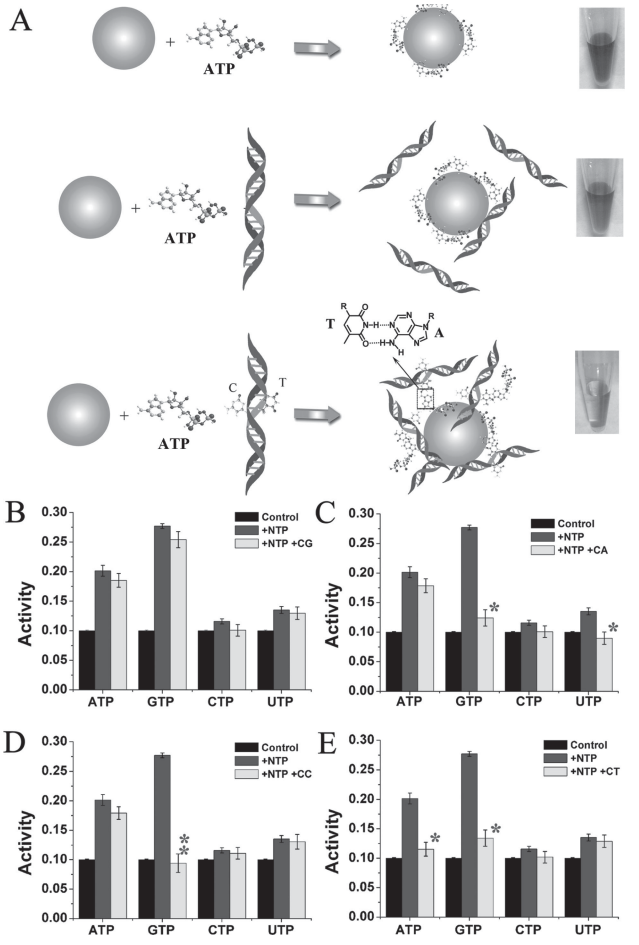

Figure 3. A) The illustration of in-situ SNP typing using ATP, CG, and CT dsDNA as models. Top: The interaction of ATP with nanoceria. ATP has a promoting effect to the oxidase activity of nanoceria. Middle: The interaction of ATP-GC with nanoceria. Bottom: The interaction of ATP-CT with nanoceria. The photographs (from top to bottom) are the color reactions of ATP-nanoceria-ABTS, ATP-CG-nanoceria-ABTS, and ATP-CT-nanoceria-ABTS systems, respectively. The spectroscopy results of in-situ label-free colorimetric assays for typing of B) CG, C) CA, D) CC, and E) CT DNA. Control: without NTPs.

A-DNA, G-DNA, C-DNA, and T-DNA) of completely complementary or with C–A, C–C, C–T mismatches to the prDNA are listed in Table S2. The thermal denaturation experiments were carried out first and the melting temperatures (T_m) are given in Table S3. For SNP typing, the pre-annealing probe-target double stranded DNA (denoted CG, CA, CC, and CT; 20 μM) was added into the solutions containing 8 μM of ATP, GTP, CTP, or UTP individually and incubated. Then, the nanoceria and ABTS were added, and the oxidase activities were measured. As shown in Figure 3, without dsDNA, the nanoceria exhibited correspondingly high oxidase activities due to the presence of NTPs. However, the activities decreased slightly for all NTPs upon addition of the complementary double-stranded DNA (GC) (Figure 3B), which was caused by a shielding effect (Figure 3A).[14] After the addition of mismatched dsDNA, the changes in the activities were dependent on the types of

mismatched bases and the NTP species. Taking CT for example (Figure 3C), the catalytic activity of nanoceria decreased sharply in GTP and ATP systems as compared with that in the absence of CT. We propose that the GTP and ATP were paired with the C and T moieties of this C–T mismatched DNA (CT) though hydrogen bonding interactions (Figure 3A, bottom). This may hinder the nucleic base residues binding to nanoceria as well as the self-stacking effect: these two factors are very important to promote dephosphorylation of NTPs.[13] In addition, because of the high coordination ability of the triphosphate group to cerium,[12,15] the DNA coupled with NTP would be more easily adsorbed onto the surface of nanoceria, which could enhance the shielding effect and further decrease the oxidase activity (Figure 3A, bottom).[14] The corresponding activity changes for the other mismatched dsDNA were also investigated and the results are shown in Figure 3. Taken together, the addition of mismatched DNA would reduce the improving activities of the corresponding NTPs and from the activity changes we could deduce the typing of the SNPs.

2.2. Magnetic Assisted Reusable Probe for SNP Typing

Although the in-situ label-free assay was low-cost, easily operated, and provided fast output, it was vulnerable to be affected by the DNA sequences, the identities of mismatched base pairs, and the pH conditions (in acidic conditions, C–C mismatch may take place). In addition, the prDNA could not be recycled. Thus, by introducing magnetic separationa reusable SNP typing probe with high sensitivity and environmentally independent property was designed. Briefly, gold nanoparticle (AuNP)-coated SiO_2@Fe_3O_4 nanoparticles (defined as AuNPs@SiO_2@Fe_3O_4 NPs) were synthesized by previously reported methods.[16] The synthesis processes are illustrated in Figure S4. The resulting NPs were examined by scanning electron microscopy (SEM) (Figure S5). The results showed that AuNPs successfully adhered to the surface of SiO_2@Fe_3O_4 with a high density (Figure S5E,F). Figure S6 shows that the resulting AuNPs@SiO_2@Fe_3O_4 nanoparticles could be successfully magnetically separated by an external magnetic field. Then the thiol-labeled prDNA (SH-prDNA) was conjugated to the AuNPs@SiO_2@Fe_3O_4 NPs through S–Au bonds, as described by Mirkin and co-workers.[17] From absorbance changes of the SH-prDNA solution before and after incubation (Figure S7), we could calculate that the density of SH-prDNA was about 1017 per AuNPs@SiO_2@Fe_3O_4 nanoparticle. The conjugation was denoted prDNA-NPs. Before typing, the prDNA-NPs were annealed with the target DNA and separated by a magnet. Then the conjugation was incubated with the four NTPs (8 μM) individually in pH 7.4 PBS. After that, prDNA-NPs were separated by a magnet and the residual NTP solutions were diluted with pH 4.0 Na_2HPO_4 buffer to the same volume as that before incubation. The oxidase activity-improvement assays were tested. Results (**Figure 4**) show that treatment with complementary dsDNA(CG)-NPs had negligible effect on the promoting activities of all of the NTPs (Figure 4A), while treatment with the mismatched dsDNA(CA, CC, and CT)-NPs had effects that were dependent on the promoting activities of NTPs. Taking CA-NPs for example (Figure 4B), the promoting

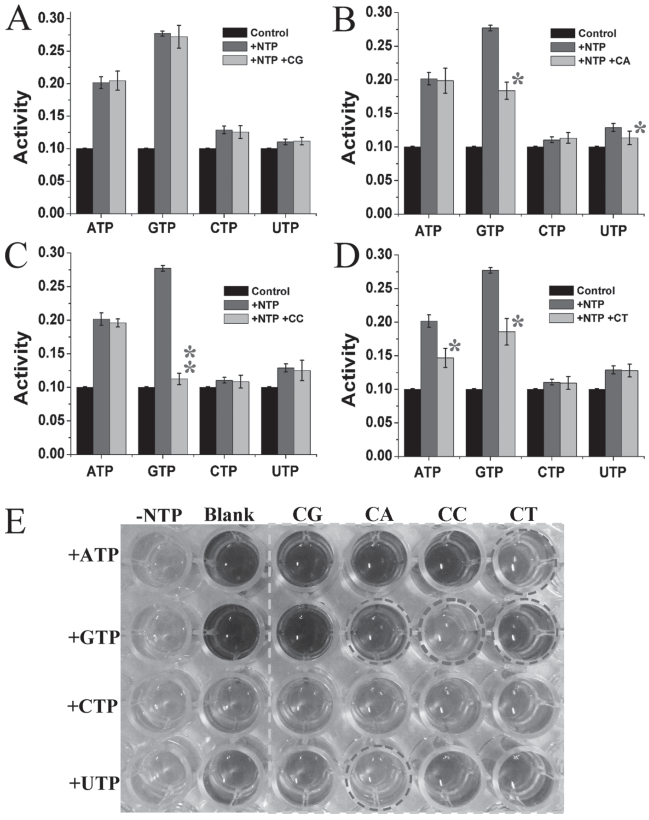

Figure 4. Reusable dsDNA-NPs assays for typing of A) CG, B) CA, C) CC, and D) CT DNA. E) SNP typing for CG, CA, CC, and CT DNA operated on a screening plate by dsDNA-NP method.

activities of GTP and UTP decreased obviously after incubation with CA-NPs, while the activities of ATP and CTP had negligible changes. This indicates that the concentrations of GTP and UTP decreased after incubation with CA-NPs. This is due to the fact that the GTP and UTP could be complementary to the C and A mismatched moieties, respectively, and be separated out along with the CA-NPs.[9f] The corresponding changes for the other mismatched dsDNA were also investigated and the results are given in Figure 4. Furthermore, taking advantage of the colorimetric properties, the types of mismatched bases could be screened out with the naked eye directly. The screening was performed with a 96-well plate analysis system (Figure 4E). ABTS was used as the chromogenic substrate. As shown in Figure 4E, compared with the first line of the plate without NTPs (-NTP), there were corresponding color intensity enhancements in the presence of NTPs (Blank). After incubation with dsDNA-NPs, color changes took place except for the complementary CG-NPs. In CA-NP case, the color intensities of GTP-nanoceria-ABTS and UTP-nanoceria-ABTS decreased compared with the Blank, while in the CC-NP case, the color intensity of GTP-nanoceria-ABTS decreased a lot and was almost as light as that in the absence of NTPs. In the CT-NPs case, the decrease in color intensities happened in GTP-nanoceria-ABTS and ATP-nanoceria-ABTS systems. The decrease in color intensities reflects the corresponding decrease in reaction activities, which is caused by the loss of NTPs. As

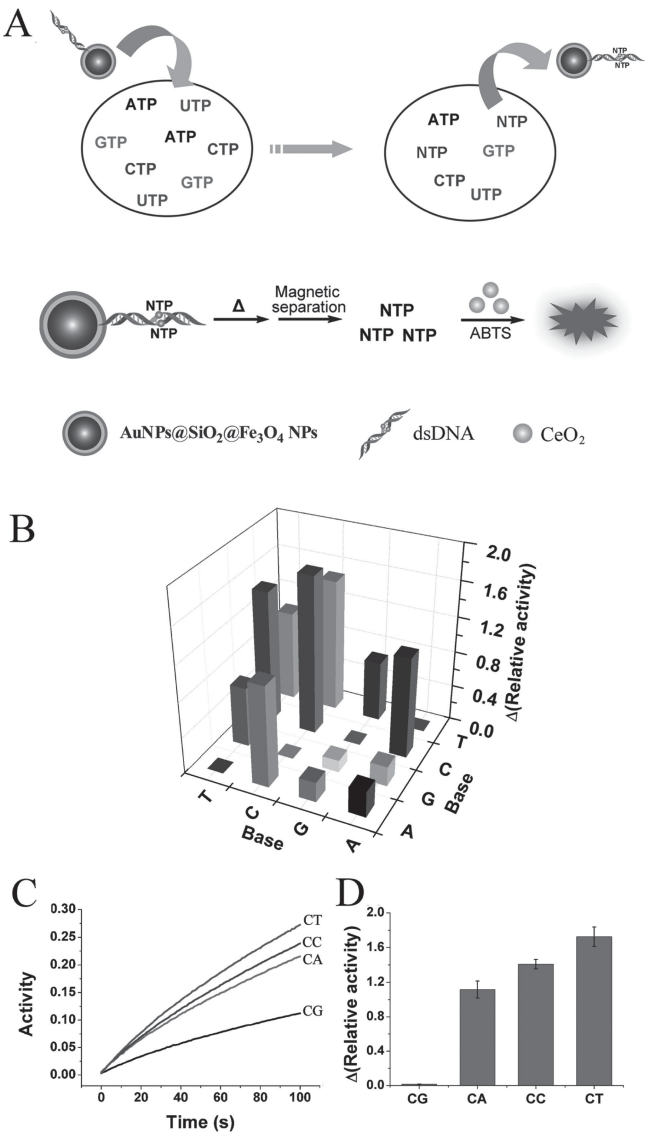

Figure 5. A) Scheme for one-pot SNP typing assay. B) The standard values of Δ(relative activity) for all types of SNP. Δ(relative activity) = $(A\text{-}A_0)/A_0$, where A_0 denotes the activity of nanoceria in the absence of NTPs and A denotes the activity of nanoceria in the presence of corresponding NTPs for different SNP types. C) The oxidase activities of nanoceria for CG, CA, CC, and CT DNA after treatment as in (A). D) The Δ(relative activity) values of (C).

describe above, the NTPs could be complementary to the corresponding mismatched moiety and be separated out along with the dsDNA-NPs. Taken together, the results demonstrate that, besides spectroscopic methods, the magnetically assisted reusable probe could also easily screened out the types of SNP within a 96-well screening plate.

2.3. One-Pot Method for SNP Typing

Finally, a one-pot method was proposed to increase the speed of SNP typing (**Figure 5**A). Briefly, instead of incubation with the four NTPs individually as in the two methods described above, the dsDNA-NPs were added to the solution of NTP mixtures. It was suggested that the mismatched sites could capture the corresponding NTPs via base-pairing.[9f] Considering that internal complementarity among the NTPs may take place, the concentrations of NTPs were in large excess of dsDNA. After incubation, the dsDNA-NPs were separated by a magnet and re-dispersed in pH 4.0 Na_2HPO_4 buffer with a final concentration of 8 μM. The solution was heated to make the complementary NTPs detach from the dsDNA-NPs (Figure 5A). Then, the dsDNA-NPs were separated by a magnet. The composition of the rest solution was dependent on the types of mismatched bases of dsDNA. In the case of completely complementary dsDNA, there were no NTPs left in the solution. For the X–X mismatch case (the same kind of base), the rest solution was supposed to contain one species of NTPs with a concentration of about 16 μM. For the X–Y mismatch case (different kinds of bases), two species of NTPs would be left with concentrations of about 8 μM for each. Due to the different compositions of the resulting solution, the improved efficiencies of nanoceria were expected to be different from each other and the SNP types could be detemined by the related activities.

Nanoceria oxidase activity-improvement assays were then carried out for the rest solutions. The standard activities for all types of SNP were obtained from the corresponding NTPs mixtures. The values of $(A\text{-}A_0)/A_0$ are shown in Figure 5B (the raw data is given in Table S4), where A_0 refers to the activity of nanoceria in the absence of NTPs and A is the activity of nanoceria in the presence of corresponding NTPs for different SNP types. To demonstrate the utility of the method, the CG, CA, CC, and CT were supposed to be dsDNA samples with unknown SNP types. After treatment by the one-pot method (Figure 5A), the nanoceria oxidase activity-improvement assays were then carried out for the rest solutions and the results are shown in Figure 5C. The values of $(A\text{-}A_0)/A_0$ were also calculated (Figure 5D). According to the standard plot (Figure 5B), CG was completely complementary to the dsDNA, where CA, CC, and CT were theorised to be C–A, C–C, and C–T mismatched dsDNA, respectively, which agrees well with the experiment. The results demonstrate the applicability of the one-pot method.

3. Conclusion

We reported that NTPs could be used as coenzymes to improve the oxidase-like activity of nanoceria and the improving efficiency was correlated with the type of NTP. The improvement effect was demonstrated to be as a result of the coupling of the oxidative reaction with the NTP hydrolysis reactions as the nanoceria had both oxidase-like and phosphatase-like activities. The difference of the improvement effect reflected different dephosphorylation catalytic activities of nanoceria to different NTPs. Furthermore, based on the NTP-promoted oxidase-like activity of nanoceria and the differences among the various NTPs, series effective colorimetric assays for typing of SNPs were developed. By virtue of their low cost, ease-of-use, high throughput, and high sensitivity, our developed colorimetric assays may open a new avenue for SNP typing. Considering the physiological functions and significances of NTPs as well as the

outstanding versatility of the nanoceria, the constructed system may also be used as a nanoreaction promoter, as our results indicate that NTPs can be not only the "energy currency" for the physiological processes, but also for the general chemical/enzyme reactions.

4. Experimental Section

Synthesis of Nanoceria: Nanoceria was synthesized according to the literature method.[7a] Briefly, a solution containing 1 M cerium (III) nitrate solution (2.17 g in 5.0 mL of water) was mixed with 1.0 M dextran. The above mixture was added by dropwise to 30.0 mL ammonium hydroxide solution (25%) while stirring was continued. After mixture, the mixture was continually stirred for another 24 h at room temperature. Then the suspension was centrifuged. After washing with distilled water three times, the precipitate was dispersed in 40 mL distilled water.

Nanoceria-Mediated Oxidation of ABTS in the Presence of NTPs: Nanoceria was suspended in 25 mM of Na_2HPO_4 buffer (pH 4.0) with a concentration of 10 μg/mL. Then NTPs of different concentrations were added followed by ABTS (1 mM). The reaction activities were detected by monitoring the absorption changes at 417 nm.

Nanoceria-Mediated NTP Hydrolysis and Free Inorganic Phosphate Determination: Briefly, CNPs (10 μg/mL for final concentration) were added to the NTPs solutions in 10 mM of Tris buffer (pH 4.0) and incubation for 1 h. The NTPs solutions in absence of CNPs were used as blanks. Then the amount of inorganic phosphate (Pi) released into solution during the reaction was measured using a malachite green-ammonium molybdate assay.[2] The sample's absorbance was read at 650 nm. A phosphate standard curve was generated using KH_2PO_4. The amount of the Pi generated by NTPs hydrolysis could then be calculated by the standard curve.

In-situ, Label-Free Assay for SNP Detection: For in-situ label-free SNP typing, the pre-annealing probe-target double strand DNA (20 μM) was added into the solution containing 8 μM of NTPs and incubated at 4 °C for 0.5 h (25 mM NaH_2PO_4, pH 4.0). Then, the nanoceria (10 μg/mL) and ABTS (1 mM) were added and the oxidase activities were measured.

SNP Typing by Multistep dsDNA-NPs Dethod: The SH-labeled prDNA (SH-prDNA) was conjugated to the AuNPs@SiO_2@Fe_3O_4 NPs through S–Au bond as described by Mirkin and co-workers.[17] From absorption changes of DNA concentrations before and after incubation, the density of SH-prDNA could be calculated. The conjugation was donated as prDNA-NPs. Before typing, the prDNA-NPs were annealed with the target DNA in phosphate buffered saline solutions (PBS, pH 7.4) and then separated by a magnet. After washing 3 times with PBS buffer to get rid of the unpaired DNA, the conjugation was incubated with NTPs (8 μM) for 2 h in pH 7.4 PBS, 4 °C. After magnetic separation, the residual NTPs were then diluted with pH 4.0 Na_2HPO_4 buffer (25 mM) to the same volume as before incubation. The nanoceria (10 μg/mL) and ABTS (1 mM) were then added to the residual NTPs solution and oxidase activity-improvement assays were tested.

SNP Typing by One-Pot dsDNA-NPs Method: Briefly, dsDNA-NPs (2 μM) were added into the solution of NTPs mixtures. The concentrations of NTPs were 400 μM for each. After incubation for 2 h, the dsDNA-NPs were separated by a magnet and re-dispersed in pH 4.0 Na_2HPO_4 buffer (25 mM) with a final concentration of 8 μM. Then the solution was heated to 35 °C to make the complementary NTPs detached from the dsDNA-NPs. The dsDNA-NPs were separated out by a magnet. The nanoceria oxidase activity-improvement assay was then carried out. The standard promoting activities for all types of SNP were obtained from the corresponding NTPs mixtures and donated as A. The activity of nanoceria in the absence of NTPs was also measured and donated as A_0. The standard chart was given with $(A-A_0)/A_0$ as the ordinate. To demonstrate the utility of the method, the CG, CA, CC, and CT DNA were supposed to be sample DNA with unknown SNP types, which was named as DNA 1, DNA 2, DNA 3 and DNA 4, respectively. After treatment by the one-pot method, the nanoceria oxidase activity-improvement assays were then carried out for the rest solutions. The values of $(A-A_0)/A_0$ were then calculated and the SNP types were determined by the standard chart.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work was supported by 973 Project (2011CB936004, 2012CB720602), and NSFC (21210002, 91213302).

Received: May 14, 2013
Published online: October 31, 2013

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