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Article

Gold Nanoparticles Enhance Efficiency of In Vitro Gene Transcription-Translation System

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Abstract

Herein we report that the in vitro gene transcription-translation efficiency can be dramatically enhanced by gold nanoparticles of 5nm in diameter. The addition of less than or equal to 1.2 nM of gold nanoparticles of 5 nm in diameter into rapid-translation-system (RTS) reagents increased the transcription-translation efficiency up to 30% and shortened the reaction time to 4 h, with the same or higher translation yields. Gold nanoparticles did not decrease the yields' bioactivity. The results show that gold nanoparticles of 5 nm may act as a bio-catalyst in the RTS reaction. This innovation has great potential in applications such as large-scale protein fabrication, gene transcription-translation regulation, and studies of structure and function of toxic protein.

Keywords: Gold Nanoparticle, Rapid Translation System, Efficiency

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1. Introduction

The in vitro replications of some important in vivo biological processes have significant potential for fundamental studies and applications development in biology and medicine. For example, the polymerase chain reaction (PCR) [1] and the rapid translation system (RTS) [2] were developed to realize specific targets such as obtaining a lot of gene fragments or peptides within several hours. However, it is very difficult for in vitro replication courses to achieve the same efficiency and specificity as in vivo courses, because in vivo biochemistry reactions involve the synergistic action of a lot of biomolecules, like the operation of a molecular machine [3-6]. Moreover, because of current technical difficulties, it is impossible for in vitro mimicking biochemical reaction courses to be reproduced as in vivo biochemical reactions. For example, RTS uses a coupled transcription-translation reaction for in vitro protein synthesis [7-10], in which the transcription and translation occur simultaneously, while the T7-RNA-polymerase transcribes the template gene and the ribosomes from the E. coli lysate start to translate the 5'-end of the nascent mRNA. So far, this cell-free synthesis system provides some advantages and has great potential both as an analytical tool and a method for the efficient production of recombinant proteins. Although RTS has been applied successfully to in-vitro protein synthesis, key factors such as efficiency and production bioactivity still need improvement. Currently, there are some methods to improve RTS efficiency and production bioactivity: for example, decreasing the reaction temperature to 20 °C. adding a second mixture of amino acids into the reaction, extending the reaction time, and using mutation T7 RNA polymerase to replace the real T7 RNA polymerase [11-15]. However, these methods still cannot improve the efficiency and yield bioactivity of RTS to the levels that are required in modern biomedicine.

Nanoparticle-based nanotechnology provides a new functional platform for medical chemistry and biomedical engineering, because nanoparticles have unique physical, chemical, optical, and mechanical properties [16-19]. Specifically, the use of nanoparticles as bio-catalysts has been very successful [20]. For example, silver nanoparticles can be used to catalyze

the chemiluminescence of luminal [20]. Also, nanoparticles such as carbon nanotubes [21] and gold nanoparticles [22] have been used as supports for enzymes. Nanoparticles also enhance the stability of adsorbed proteins relative to micro- or macro-scale supports, thereby helping to preserve or enhance enzyme bioactivity in nanocomposites [19, 22-25]. Furthermore, recent studies show that nanoparticles can function as replacement for some proteins or enzymes. For example, nanoparticles such as single-walled carbon nanotubes [25] and gold nanoparticles(GNPs) [26-28] can improve the efficiency or specificity of PCR, which highly suggest that nanoparticles are capable of acting as a biocatalyst to enhance the bio-activity of Taq DNA polymerase and as a single-strand binding protein (SSB) [29] to improve strict base-pairing in the course of PCR. Since numerous enzyme-catalyzed biochemical reactions are involved in the course of RTS [30, 31], it is very possible that gold nanoparticles could improve both the bioactivities of enzymes such as aminoacyl-tRNA synthetases, RNA polymerase, ribozymes, etc, and the efficiency of coupling mRNA to ribosome and also amino acids to tRNA, thereby speeding up the course of transcription and translation. In other respects, gold nanoparticles could possibly act as a single-strand binding protein (SSB) in RTS, similar to their role in the course of PCR [25, 28], ensuring correct molecule-pairing such as that between amino acids and tRNA and between mRNA and ribosomes. Therefore, it is likely that gold nanoparticles may markedly enhance the efficiency of RTS. However, the coupled in-vitro transcription-translation system is a complicated process, in which transcription and translation are processed simultaneously and all the necessary components for transcription and translation (as well as the synthesized products) are mixed together [2-7], all of which involves the synergistic action of lot of biomolecules. So far, there have been no reports on the possible effects of the addition of gold nanoparticles to this complicated system.

It is, therefore, the first report on these effects in this letter by our group. We found that the gold nanoparticles with a 5 nm-diameter can markedly improve both the efficiency and the bioactivity of yield of the in-vitro transcription-translation system. This new finding has great potential in applications such as large-scale protein fabrication, gene transcription-translation regulation, and studies on structure and function of toxic protein.

2. Experiments

2.1 PCR primers and PCR cycles

The fragment A1, corresponding to amino acids 1006 to 1118 of BRCAA1 protein [32], was selected. This region includes a new breast cancer antigen epitope SSKKQKRSHK. Two primers with introduction of a COOH-terminal His-tag were designed and chemically synthesized by Gibico Inc.,Germany. Their sequences were as follows: primer1: 5'-CTT TAA GAA GGA GAT ATA CCA TGA GAG TGA AAG ATG CTC AG-3'; primer2: 5'-TGA TGA TGA GAA CCC CCC CCA CTC CAT TTG TAA ACT TTG G-3'. The PCR product was 339 bp in length. PCR reaction 1 was used to obtain specific fragment A1: 10× PCR buffer 3 uL, 2.5 mm deoxynucleotide triphosphate 3 uL, primer 1 and primer 2 each 1 uL, brcaa1 vector template 2 uL, 25 mm $MgCl_2$ 3 uL, and Taq enzyme 1uL Sterilized water was added until the total volume was up to 30 uL. PCR condition: predenature at 94 °C for 4 minutes, then 94 °C for 1minute, 55 °C for 1minute, 72 °C for 1minute, 30 cycles, finally extend at 72 °C for 7 minutes. The PCR product was purified with a PCR purification kit.

2.2 RTS reaction with or without gold nanoparticles

PCR reaction 2 and a rapid-translation system (RTS) *E. coli* Linear Template Generation Set and His-tag kit (Roche Diagnostics Inc., Germany) were used to obtain specific fragment A2 of 720 bp in length with regulatory elements and His-tag. Fragment A2 was purified with a PCR purification kit. An RTS 100 E. coli kit (Roche Diagnostics Inc., Germany) was used to translate the purified fragment A2 into corresponding peptides in the ProteoMaster (Roche Diagnostic Inc., Germany), under the following reaction condition: 50 uL total volume, 30°C for 6 h. Different amounts of gold nanoparticles with a diameter of 5 nm (Sigma Inc, USA, 75.1 nmol L^{-1}) were added to the RTS reagent, the total volume of each tuber still was kept at 50 uL, and the reaction condition did not change.

2.3 Yield analysis of RTS

The protein products were analyzed by 10% SDS-polyacrylamide gel and thenstained by Coomassie brilliant blue and quantified by ultraviolet spectrophotometer. The peptide bio-activity was confirmed by Western blotting and by using a rabbit anti-human new antigen epitope IG G antibody (Abcam Company, UK), as discussed in references [32]. Enzymatic assays of aminoacyl-tRNA synthetase with or without GNPs were performed according to manual(aminoacyl-tRNA synthetase, A3646-10KU from ESwerichia coli, crude,Sigma).

2.4 Statistical Analysis

Each experiment was repeated three times in duplicate. The results were presented as mean ± SD. Statistical differences were evaluated evaluated using the t-test and considered significance at $P < 0.05$.

3 Results and Discussion

3.1 Characterization of gold nanoparticles and gold nanopartilces-yields composites

The gold nanoparticles were characterized by high-resolution transmission electron microscopy (HR-TEM) and ultraviolet spectrophotometer (UV). As shown in Fig. 1, the gold nanoparticles are 5 nm in diameter and have a UV absorption peak at 522 nm. The gold nanoparticles wrapped by the yield made by the rapid translation

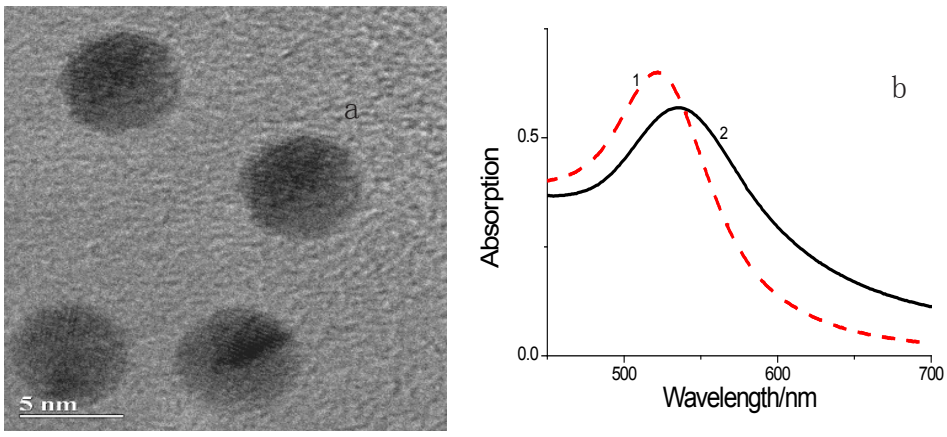

Fig. 1 Characterization of gold nanoparticles of 5nm in diameter by HR-TEM and UV-spectroscopy. a. HR-TEM image of gold nanoparticles; b. UV-spectra of gold nanoparticles before RTS reaction (1) and after RTS reaction (2).

system (RTS) have a UV absorption peak at 536 nm; this peak represents a 14 nm right-shift, which strongly suggests that there is a strong interaction between gold nanoparticles and synthesized yield.

3.2 Effects of gold nanoparticles on efficiency of RTS

The first round gene fragment was obtained by common PCR using brcaa1 vector as the template. The fragment A1 then was used as the template for the second round-PCR, which produced the fragment A2 with the necessary translational elements. Finally, the purified fragment A2 was used as the template for RTS. As shown in Fig. 2A, the fragment A1 was 339 bp in length, and the fragment A2 was 720 bp in length.

Next, 0.24 nm, 0.48 nm, 0.72 nm, 0.96 nm, 1.2 nm, 2.4 nm, 4.8 nm, 7.2 nm, 9.6 nm, and 12 nm of 5 nm-diameter gold nanoparticles were added into different reaction tubes with the RTS reagents, and then the standard procedure of RTS was used to transcribe and translate the fragment A2 into short peptides. As shown in Fig. 2B, in the concentration range of less than or equal to 1.2 nm, as the amount of gold nanoparticles in the RTS reagent gradually increased, the yield also gradually increased. The efficiency of synthesized yields was enhanced by 30% compared to the manual method, which shows that gold nanoparticles can improve the efficiency of in-vitro transcription–translation system. However, as shown in Fig. 2C in the concentration range of 1 uL to 10 uL, as the amount of gold nanoparticles in the RTS reagent gradually increased, the yield peptides gradually decreased, showing that superfluous gold nanoparticles can inhibit the synthesis of peptide.

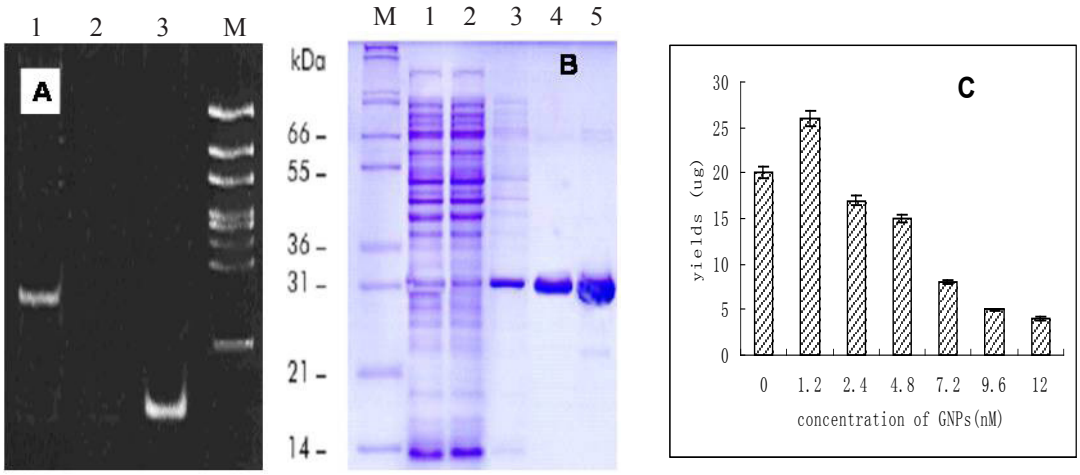

Fig. 2 Electrophoresis analysis of PCR products and RTS yields

A: Image of PCR products by 1% agarose gel electrophoresis. M is λDNA Hind III Marker; No.1 lane is fragment A2; No. 2 lane: negative control; No.3 lane: fragment A1.

B: Image of RTS yield by 15% SDS-PAGE electrophoresis. M is protein marker; No.1 lane is RTS yield without GNPs; No.2 lane is yield with 0.24 nM GNPs; No.3 lane is yield with 0.48nM GNPs; No.4 lane is yield with 0.96 nM GNPs; No.5 lane is yield with 1.2 nM GNPs.

C: RTS yields respectively with 0 nm, 1.2 nm, 2.4 nm, 4.8 nm, 7.2 nm, 9.6 nm and 12 nm GNPs in the RTS reactions.

To further confirm that gold nanoparticles can affect the efficiency of RTS, we prepared 6 tubes of RTS reagents, added 1uLlof gold nanoparticles to each tube, and then put the tubes into a Roche ProteinMaster instrument (Roche Diagnostics Inc.,Germany) for peptide synthesis under different times such as 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h. As shown in Fig. 3A, from 1h to 4h the peptide yields gradually increased, at 4 h the maximum yield of peptides was obtained, and at 5 h and 6 h the peptide yields leveled off. It should be noted that the control group needed 6 hours to reach maximum yield. The results show that gold nanoparticles with a diameter of 5 nm can shorten the time of peptide synthesis to 4 h.

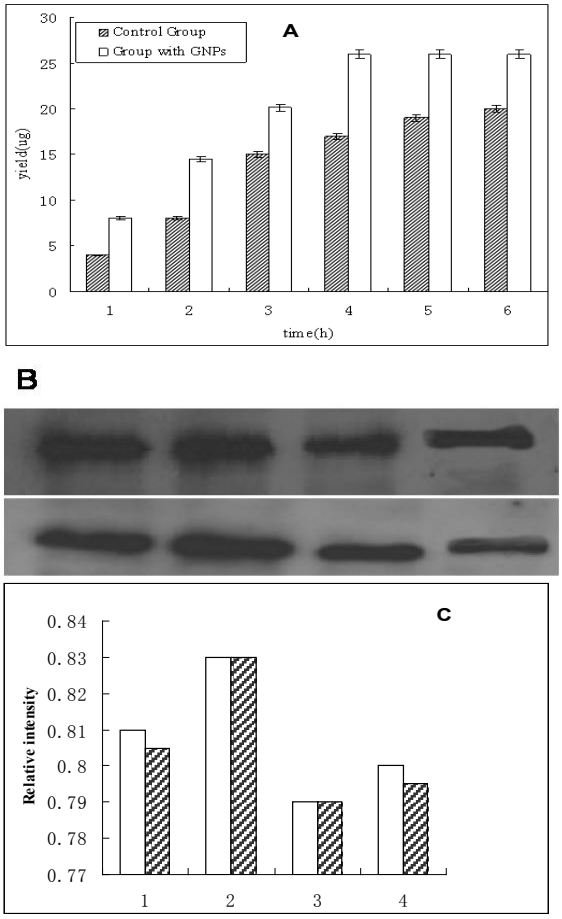

Fig. 3 (A) The amounts of yields at different times. Control group: RTS reaction without GNPs; Test group: RTS reaction with 1.2 nm GNPs.
(B) Bioactivity analysis of RTS yields by Western Blotting. Blank: No. 1 to No.4: 40 ng, 50 ng, 20 ng, 30 ng of RTS yields with 0.24 nm, 0.48 nm, 2.4 nm, 3.6 nm GNPs, respectively; Black diagonal: No.1 to No.4: 40 ng 50 ng, 20 ng, 30 ng RTS yields without GNPs, respectively.
(C) Relative scanning intensity of hybridization signals in Fig. 3B.

To further investigate the function of gold nanoparticles with a diameter of 5 nm in the course of peptide synthesis, we used the Western blot analysis to compare the bioactivity of peptide yields from RTS reagents with and without 1.2 nM gold nanoparticles. As shown in Fig. 3B and C, the intensity of yield from RTS reagents with gold nanoparticles is stronger than that from RTS reagents without gold nanoparticles, however, there is no statistical difference between the bioactivities of the peptide yields from the two RTS reagents ($p > 0.05$). These results show that gold nanoparticles with a diameter of 5 nm do not decrease the bioactivity of synthesized peptides, and that they even may improve the bioactivity of yields.

4. Potential mechanism

In short, the gold nanoparticles with a diameter of 5 nm can improve the efficiency of in vitro gene transcription-translation system. In order to investigate the mechanism underlying this improvement, we hypothesized that the gold nanoparticles may improve the bioactivity of T7 RNA polymerase, aminoacyl-tRNA synthetase and ribosome. In order to confirm this point, we compared with enzymatic bioactivity of aminoacyl-tRNA synthetase with or without GNPs. As shown in Fig. 4, GNPs can improve the efficiency of tRNA-14C Arginine composite synthesis, which suggest that GNPs can act as a bio-catalyst [19, 22-24] to improve the bioactivity of those enzymes such as aminoacyl-tRNA synthetase and ribosome enzyme, which finally results in the enhancement of in vitro gene transcription–translation efficiency.

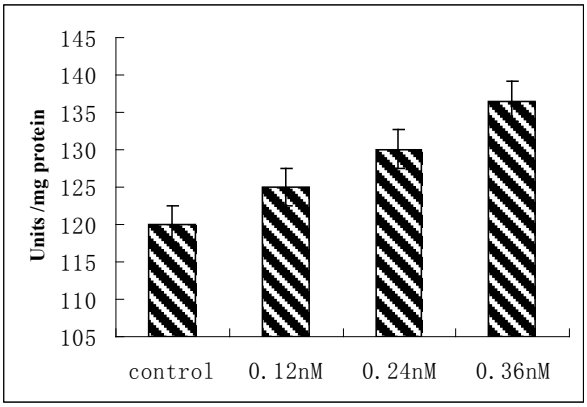

Fig. 4 Units /mg protein (aminoacyl-tRNA synthetase) with different concentration of GNPs

In addition, properties of GNPs may help to improve the efficiency of in vitro gene transcription–translation. First, since gold nanoparticles have a high surface/volume ratio, their addition to RTS reagents can significantly change the distribution of reaction components. All the necessary components for transcription and translation such as amino acids, DNA fragments, T7 RNA polymerases, ribosomes, and transcription factors, etc. can be attracted toward gold nanoparticles as the result of Van der Waals and electrostatic interactions [16, 24, 29, 30]. Therefore, the gold nanoparticles in the RTS solution may become small reaction centers. Second, at the beginning of gene transcription-translation in the course of RTS, as the temperature increases rapidly, the thermal entropy in the reagent and the surface energy of the gold nanoparticles also increase [33-35]. The result is

that all the components speed up the Brownian motion, which raises the probability of dynamic contact among gene fragment A2 and T7RNA polymerases, amino acids and tRNA, and mRNA and ribosomes around the gold nanoparticles. This contact, in turn, enhances the probability of right base-pairing and transcription between T7 RNA polymerase and gene fragment A2, and also between ribosome and mRNA fragments. During the course of peptide synthesis, the gold nanoparticles may act as single-strand binding proteins, similar to their role in the course of PCR [26, 28], which improves the chance that amino acids rightly bind with ribosome and mRNA templates, which, in turn decreases or even eliminates the probability of wrong base-pairing between amino acids and tRNAs and between ribosome and mRNA fragments, and enhance the synergistic action of all biomolecules in RTS reaction.

Regarding the effects of gold nanoparticles on the bioactivity of synthesized peptides, some reports show that gold nanoparticles can be used as supports for enzymes [17, 18], can enhance the stability of adsorbed enzyme molecules relative to micro- or macro-scale supports, and, finally, can preserve or enhance enzyme bioactivity in the nanocomposites; therefore, gold nanoparticles may act as a molecular "chaperone" [5, 6] in the course of RTS, stabilizing the structure of peptides, which means that gold nanoparticles do not decrease the bioactivity of synthesized peptides.

Together, all of the factors mentioned above have the possibility to improve the efficiency of RTS. However, this kind of dose-product effect requires an optimum concentration of gold nanoparticles; after passing a critical concentration point of 1.2 nm, gold nanoparticles decrease or inhibit in-vitro gene transcription-translation. The concrete mechanism underlying this inhibition still needs further clarification. Future work will focus on investigating the interaction between GNPs and these key enzymes such as T7 RNA polymerase, aminoacyl-tRNA synthetase and ribosome, and the potential mechanism of GNPs improving the synergetic function of a lot of biomolecules in the course of RTS.

Numerous reports have been mentioned above, but so far there have been no reported studies closely associated with the effects of nanoparticles on the course of gene transcription and translation. Our study, which is the first of its kind, shows that gold nanoparticles can markedly affect the course of gene transcription and translation like siRNA and microRNA.

5. Conclusion

In conclusion, our results directly demonstrate that gold nanoparticles of 5 nm in diameter can markedly improve the efficiency of a gene transcription-translation system, and that the smaller size and surface properties of the gold nanoparticles may be responsible for this improvement. Our results also indirectly suggest that gold nanoparticles may improve the bio-activity of T7 RNA polymerases, aminoacyl-tRNA synthetase and ribosome involved in the course of gene transcription-translation and, therefore, even may act as a biocatalyst. Based on our research, it is evident that gold nanoparticles have great potential for in-vitro applications such as large-scale protein fabrication, gene transcription-translation regulation, and studies of structure and function of toxic protein. It should be noted, however, that our results also indirectly demonstrate that gold nanoparticles of 5 nm in diameter that enter into human cells could interfere with gene transcription and protein synthesis, resulting in toxic effects on the human cells, which means that in-vivo applications could be limited.

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