Preparation of Cationic Gold Nanoparticles for Gene Delivery

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Abstract: Cationic gold nanoparticles modified with 2-aminoethanethiol, 8-amino-1-octanethiol, and 11-amino-1-undecanethiol were prepared by $NaBH_4$ reduction of $HAuCl_4$ in the presence of thiols in water or a water/ethanol mixture solvent. The formation of gold nanoparticles depended on the kind of solvent, and the concentration of $NaBH_4$, $HAuCl_4$, and thiols. A low concentration of $HAuCl_4$ and a high concentration of $NaBH_4$ facilitated the formation of gold nanoparticles. A high concentration of $HAuCl_4$ solution resulted in aggregation and a low concentration of $NaBH_4$ did not induce the formation of gold nanoparticles. The size of the gold nanoparticles increased with an increase of the concentration of the $HAuCl_4$ solution, while it decreased with an increase of the concentration of the $NaBH_4$ solution. When thiols were present, the $HAuCl_4$/thiol ratio must be in the range of 1:1 to 1:2 for the formation of gold nanoparticles in both the water and water/ethanol mixture solvents. Modification with thiol molecules changed surface charge of the gold nanoparticles from negative to positive, and stabilized the gold nanoparticles. The cationic gold nanoparticles promoted gene transfection. A high ratio of gold nanoparticles to DNA increased transfection efficiency. However, the length of the alkyl groups had no evident effect.

INTRODUCTION

Gene transfection has been developed as a powerful technique for elucidating gene function, and for treating and controlling diseases [1]. Viral and nonviral methods can be used for gene transfection. Viral methods have high transfection efficiency. However, the viral methods have the problems of immunogenicity and induction of oncogenic responses. The nonviral methods include physical and chemical methods [2, 3]. Physical methods such as electroporation, particle bombardment, and fine-needle naked DNA injection have been developed to facilitate gene transfection. Chemical methods by using cationic carriers such as cationic lipids, liposomes, polyethylenimine, polyamidoamine, polypropylenimine, poly-L-lysine, polyallylamine, and cationic dextran can form complexes with DNA and exhibit transfection activity. The nonviral methods have the advantages of safety, ease of preparation, reproducibility, and stability. However, the main drawbacks of nonviral-mediated gene transfection are related to low transfection efficiency. Development of new nonviral methods to facilitate high transfection efficiency is strongly desirable.

In recent years, gold and inorganic nanoparticles have been used in alternative approaches to gene transfection [4-12]. Cationic gold and silica nanoparticles have been used for efficient transfection of 293T and COS-1 cells. Prow *et al.* used gold nanoparticles, semiconductor nanocrystals, and magnetic nanoparticles that were modified with biotin-labeled transcriptionally active PCR products for gene transfection [7]. Sandhu *et al.* reported gene transfection using gold nanoparticles modified with *N,N,N*-trimethyl(11-mercaptoundecyl) ammonium chloride and alkylthiol with several chain lengths [10]. Niidome *et al.* reported preparation of gold nanoparticles coated with 2-aminoethanethiol and their application in gene transfection [12]. However, the effect of the alkyl group has not been elucidated. In this study, cationic gold nanoparticles modified with various thiols were prepared and used for gene transfection. The effects of length of the alkyl group in thiols, and the Au/DNA ratio were investigated.

EXPERIMENTAL

Preparation of Gold Nano-Particles in Aqueous Solution

Each of five aqueous solutions of $NaBH_4$ (50 µL) was dropwisely added to five aqueous solutions of $HAuCl_4$ (5 mL) in tubes with shaking in the dark. The 25 reaction solutions were kept shaking for another three hours at room temperature in the dark. The concentration of the $HAuCl_4$ aqueous solution was 0.24, 1.20, 2.40, 12.20, and 24.00 mM while the concentration of the $NaBH_4$ aqueous solution was 2.64, 5.28, 26.4, 264, and 2640 µM.

Preparation of 2-Aminoethanethiol-Modified Gold Nanoparticles in Aqueous Solution

The preparation scheme of gold nanoparticles in the presence of thiols is shown in Fig. (**1**). 2-Aminoethanethiol was first added to an aqueous solution of $HAuCl_4$ in a tube. Then, 50 µL of $NaBH_4$ aqueous solution was dropwisely added with shaking in the dark. The mixture solution was kept shaking for another three hours at room temperature in the dark. The concentrations of the aqueous solutions of $HAuCl_4$ and 2-aminoethanethiol were 0.24~2.4 mM and 0.18~7.2 mM, respectively. The concentration of the $NaBH_4$ aqueous solution was 2.64, 5.28, and 26.4 µM.

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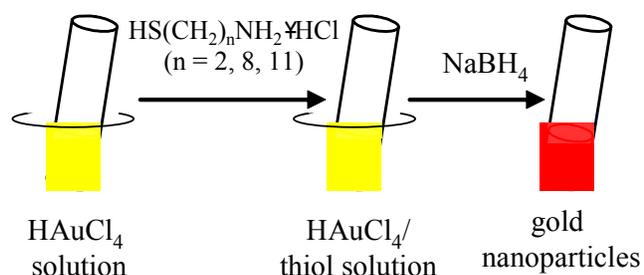
$$HS(CH_2)_nNH_2 \cdot HCl$$
$$(n = 2, 8, 11)$$

NaBH$_4$

HAuCl$_4$ solution

HAuCl$_4$/ thiol solution

gold nanoparticles

Fig. (1). Preparation scheme of thiol-modified gold nanoparticles.

Preparation of Gold Nanoparticles in Mixture Solvent

50 μL of NaBH$_4$ solution in water/ethanol（v/v ratio of 1:1）was dropwisely added to 5 mL of HAuCl$_4$ solution in water/ethanol (1:1) with shaking in the dark. The reaction solution was kept shaking for another three hours at room temperature in the dark. The concentration of the HAuCl$_4$ aqueous solution was 0.24, 2.40, and 24.0 mM. The concentration of the NaBH$_4$ aqueous solution was 5.28, 26.4, 264, and 2640 μM.

Preparation of 2-Aminoethanethiol-Modified Gold Nanoparticles in Mixture Solvent

50 μL of NaBH$_4$ solution in water/ethanol (1:1) was dropwisely added to a mixture solution of HAuCl$_4$ and 2-aminoethanethiol in water/ethanol (1:1, 5 mL) with shaking in the dark. The reaction solution was kept shaking for another three hours at room temperature in the dark. The concentrations of the aqueous solutions of HAuCl$_4$ and 2-aminoethanethiol were 0.24~2.4 and 0.24~4.8 mM, respectively. The concentration of the NaBH$_4$ aqueous solution was 5.28 and 26.4 μM.

Preparation of 8-Amino-1-Octanethiol-Modified Gold Nano-Particles in Mixture Solvent

50 μL of NaBH$_4$ solution in water/ethanol (1:1) was dropwisely added to a mixture solution of HAuCl$_4$ and 8-amino-1-octanethiol in water/ethanol (1:1, 5 mL) with shaking in the dark. The reaction solution was kept shaking for another three hours at room temperature in the dark. The concentrations of the aqueous solutions of HAuCl$_4$, and 8-amino-1-octanethiol were 0.24~2.4 mM and 0.18~7.2 mM, respectively. The concentration of the NaBH$_4$ aqueous solution was 5.28, 26.4, and 264 μM.

Preparation of 11-Amino-1-Undecanethiol-Modified Gold Nano-Particles in Mixture Solvent

The 11-amino-1-undecanethiol-modified gold nanoparticles were prepared in the same way as the 8-amino-1-octanethiol-modified gold nanoparticles. The concentrations of the aqueous solutions of HAuCl$_4$, 11-amino-1-undecanethiol, and NaBH$_4$ were 2.40 mM, 3.65 mM, and 5.28 μM, respectively.

Purification, Size, and Zeta Potential Measurements of Gold Nanoparticles

After 3 hours of shaking, the reaction solutions were diluted 8-fold with MilliQ water and centrifuged at 1000-12000 rpm. The grain size distributions before and after centrifugation were compared to determine the optimal centrifugation speed. The centrifugation speed at which the main grain size peaks did not change was used as the optimal centrifugation speed. The prepared naked and thiol-modified gold nanoparticles were purified and collected at the optimized centrifugation speed. The size of the nanoparticles was analyzed by use of a dynamic light scattering spectrophotometer (DLS 6000, Otsuka Electronics Co., Ltd, Osaka, Japan). Zeta-potential measurements were performed on a zeta potential analyzer (Delsa 440SX, Beckman Coulter, Inc., Fullerton, CA).

RESULTS

The reaction of NaBH$_4$ and HAuCl$_4$ was conducted by changing the concentrations of the two aqueous solutions as shown in Table **1**. The gold nanoparticles could be prepared with a solution with a low concentration of HAuCl$_4$ but not with a solution with a high concentration of HAuCl$_4$. At high concentrations of HAuCl$_4$, the solution turned red immediately after the addition of the NaBH$_4$ solution. The suspension solution was unstable and gold particles aggregated within one hour. The formation of gold nanoparticles required a solution with a high concentration of NaBH$_4$. Gold nanoparticles could not be obtained with a solution with a low concentration of NaBH$_4$. The mean diameter of the naked gold nanoparticles was between 11.4 and 80.1 nm. The solution with a high concentration of HAuCl$_4$ resulted in large-sized nanoparticles. Although the gold nanoparticles formed in solutions at 26.4 and 264 μM of NaBH$_4$ were almost the same size, the highest concentration of 2640 μM resulted in formation of small-sized gold nanoparticles.

Table 1.   **Formation and Size of Gold Nanoparticles Prepared in Water with Various Concentrations of HAuCl$_4$ and NaBH$_4$**

HAuCl$_4$ (mM) NaBH$_4$ (μM)	0.24	1.20	2.40	12.2	24.0
2.64	-	-	-	×	×
5.28	-	-	-	×	×
26.4	42.9±3.2	49.4±2.1	76.6±5.2	×	×
264	43.4±2.5	42.2±1.1	80.1±6.5	×	×
2640	11.4±1.7	39.3±0.3	54.3±2.2	×	×

(-): no formation of nanoparticles; (×): aggregation.

Reduction of HAuCl$_4$ by NaBH$_4$ was also conducted under the presence of 2-aminoethanethiol. The concentrations of aqueous solutions of HAuCl$_4$ and 2-aminoethanethiol were controlled in the range of 0.24~2.4 mM and 0.18~7.2 mM, respectively. The concentration of the aqueous solutions of NaBH$_4$ was 2.64, 5.28, and 26.4 μM. With the presence of 2-aminoethanethiol, the concentration of the NaBH$_4$ solution did not affect the formation of gold nanoparticles. Gold nanoparticles could be prepared with all three concentrations of NaBH$_4$. The NaBH$_4$ concentrations of 2.64 and 5.28 μM, at which the gold nanoparticles did not form in the

absence of 2-aminoethanethiol, also facilitated the formation of gold nanoparticles when 2-aminoethanethiol was present. The effects of the concentrations of $HAuCl_4$ and 2-aminoethanethiol solutions are summarized in Table **2**. The concentration ratio of $HAuCl_4$/2-aminoethanethiol must be in the range from 1:1 to 1:2 to facilitate the formation of gold nanoparticles. A high percentage of $NaBH_4$ resulted in aggregation and a low percentage did not induce the formation of nanoparticles. With the presence of 2-aminoethanethiol, the gold nanoparticles formed more readily than with the absence of 2-aminoethanethiol.

Table 2. **Formation of 2-Aminoethanethiol-Modified Gold Nanoparticles Prepared with Various Concentrations of $HAuCl_4$ and 2-Aminoethanethiol in Water. The Concentration of $NaBH_4$ was 5.28 µM**

HauCl₄ (mM) Thiol (mM)	0.24	0.36	0.48	0.96	1.2	1.8	2.4
0.18	×	×	×	×	×	×	×
0.24	○	×	×	×	×	×	×
0.36	○	○	×	×	×	×	×
0.72	-	○	○	×	×	×	×
1.2	-	-	-	○	○	×	×
1.8	-	-	-	○	○	○	×
2.4	-	-	-	-	○	○	○
3.6	-	-	-	-	-	○	○
4.8	-	-	-	-	-	-	○
7.2	-	-	-	-	-	-	-

○: formation of nanoparticles; (-): no formation of nanoparticles; (×): aggregation.

A mixture solution of water and methanol (1:1) was used to prepare gold nanoparticles to investigate the effect of solvent on the formation of gold nanoparticles. Naked gold nanoparticles were prepared under the same conditions as those used in water except the solvent was changed from water to the mixture solvent. Similar results were obtained with both the mixture and water solvents except for the $NaBH_4$ solution with a concentration of 2640 µM (Table **3**). At this concentration, the gold nanoparticles were larger than those formed in the $NaBH_4$ solution with a concentration of 264 µM, which was opposite of the results obtained in water. The 2-aminoethanethiol-modified gold nanoparticles could not be prepared using the water/ethanol mixture solvent because the particles aggregated and precipitated after reaction.

The 8-amino-1-octanethiol- and 11-amino-1-undecanethiol-modified gold nanoparticles were prepared in the water/ethanol mixture solvent because 8-amino-1-octanethiol and 11-amino-1-undecanethiol could be dissolved in the mixture solvent. The preparation condition was determined by preparing the 8-amino-1-octanethiol-modified gold nanoparticles in the mixture solvent of water and ethanol (1:1). At first, the $NaBH_4$ concentration was fixed at 5.28 µM. The results, shown in Table **4**, are similar to those obtained with the 2-aminoethanethiol-modified gold nanoparticles prepared in water. The concentration ratio of $HAuCl_4$/8-amino-1-octanethiol must be in the range from 1:1 to 1:2 to induce the formation of gold nanoparticles. By fixing the concentration of $HAuCl_4$, the effect of the concentration of $NaBH_4$ on the formation of gold nanoparticles was also investigated. The results are shown in Table **5**. Gold nanoparticles aggregated at low concentrations of 8-amino-1-octanethiol and did not form at high concentrations of 8-amino-1-octanethiol. High concentrations of $NaBH_4$ resulted in large-size gold nanoparticles. Under the condition of the mixture solvent with the presence of 8-amino-1-octanethiol, the concentrations of $HAuCl_4$, $NaBH_4$, and 8-amino-1-octanethiol affected the formation of the gold nanoparticles. The 11-amino-1-undecanethiol-modified gold nanoparticles were prepared under the same condition as that of the 8-amino-1-octanethiol-modified gold nanoparticles.

Table 3. **Formation and Size of Gold Nanoparticles Prepared with Various Concentrations of $HAuCl_4$ and $NaBH_4$ in Water/Ethanol Mixture Solvent**

HAuCl₄ (mM) NaBH₄ (µM)	0.24	2.40	24.0
5.28	-	-	×
26.4	40.6±1.8	51.7±3.1	×
264	28.1±1.7	46.7±2.9	×
2640	31.7±1.1	67.7±5.2	×

(-): no formation of nanoparticles; (×): aggregation.

Table 4. **Formation of 8-Amino-1-Octanethiol-Modified Gold Nanoparticles Prepared with Various Concentrations of $HAuCl_4$ and 8-Amino-1-Octanethiol in Water/Ethanol Mixture Solvent. The Concentration of $NaBH_4$ was 5.28 µM**

HAuCl₄ (mM) Thiol (mM)	0.24	0.36	0.48	0.96	1.2	1.8	2.4
0.18	×	×	×	×	×	×	×
0.24	×	×	×	×	×	×	×
0.36	○	○	×	×	×	×	×
0.72	-	○	○	×	×	×	×
1.2	-	-	-	○	○	×	×
1.8	-	-	-	○	○	○	×
2.4	-	-	-	-	○	○	○
3.6	-	-	-	-	-	-	○
4.8	-	-	-	-	-	-	-
7.2	-	-	-	-	-	-	-

○: formation of nanoparticles; (-): no formation of nanoparticles; (×): aggregation.

Table 5. **Formation and Size of 8-Amino-1-Octanethiol-Modified Gold Nanoparticles Prepared with Various Concentrations of 8-Amino-1-Octanethiol and NaBH$_4$ in Water/Ethanol Mixture Solvent. The Concentration of HAuCl$_4$ was 2.40 mM**

Thiol (mM) / NaBH$_4$ (µM)	1.2	2.4	3.6	4.8	7.2
5.28	×	44.7±6.1	39.5±4.2	39.4±2.6	-
26.4	×	34.1±7.5	39.9±3.3	-	-
264	×	66.3±3.1	112.8±16.9	-	-

(-): no formation of nanoparticles; (×): aggregation.

Finally, the gold nanoparticles used for gene transfection were prepared under the conditions shown in Table **6**. Naked, 8-amino-1-octanethiol-modified, and 11-amino-1-undecanethiol-modified gold nanoparticles were prepared in a water/ethanol mixture solvent, while the 2-aminoethanethiol-modified gold nanoparticles were prepared in water. The naked gold nanoparticles were negatively charged, while the modified gold nanoparticles were positively charged. The cationic thiols bound to the gold nanoparticles and changed their electrostatic properties.

The 2-aminoethanethiol-, 8-amino-1-octanethiol-, and 11-amino-1-undecanethiol-modified gold nanoparticles were used for transfection of the plasmid DNA coding EGFP. The Au/DNA (w/w) ratios used for transfection were 1:1, 5:1, 10:1, 15:1, and 20:1. Observation with fluorescence microscopy demonstrated that all the thiol-modified gold nanoparticles had some effect on gene transfection (Fig. **2**) and the effect increased with an increase in the Au/DNA (w/w) ratio. No evident effect of the length of the alkyl groups was detected.

DISCUSSION

Gold nanoparticles were prepared by NaBH$_4$ reduction of HAuCl$_4$ in water or water/ethanol mixture solvents under the absence or presence of thiols. All the conditions affected the formation and size of the gold nanoparticles. A low concentration of HAuCl$_4$ and a high concentration of NaBH$_4$ promoted the formation of gold nanoparticles. A solution with a high concentration of HAuCl$_4$ resulted in aggregation and a low concentration of NaBH$_4$ did not facilitate the formation of gold nanoparticles. The size of the gold nanoparticles increased with an increase in the concentration of HAuCl$_4$, while it decreased with an increase in the concentration of NaBH$_4$. It is thought that the high concentration of NaBH$_4$ might result in quick reduction of HAuCl$_4$, which results in the formation of small-sized gold nanoparticles. The NaBH$_4$ reduction of water might compete with the reduction of HAuCl$_4$. The low concentration of NaBH$_4$ solution might react with water other than HAuCl$_4$, which could explain the failure to form gold nanoparticles in a solution with a low concentration of NaBH$_4$.
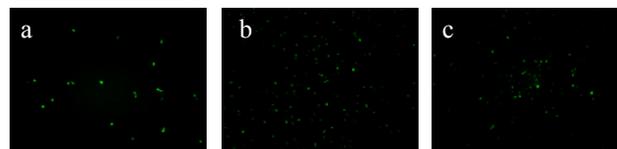
Fig. (2). Fluorescence micrographs of COS-7 cells transfected with EGFP plasmid by 2-aminoethanethiol- (**a**), 8-amino-1-octanethiol- (**b**) and 11-amino-1-undecanethiol-modified (**c**) gold nanoparticles at Au/DNA (w/w): 20 : 1.

With the absence of thiols, the water and water/ethanol mixture solvents showed similar effects except for the solution with the high concentration of NaBH$_4$. At a high concentration of NaBH$_4$, the two solvents had opposite effects.

When thiols were present, the concentration ratio of HAuCl$_4$/thiol was more important in the formation of gold nanoparticles than the concentrations and the kind of solvent. The HAuCl$_4$/thiol ratio must be in the range from 1:1 to 1:2 for forming gold nanoparticles in both water and water/ethanol mixture solvents. A high ratio of NaBH$_4$ resulted in aggregation and a low ratio inhibited the formation of nanoparticles. The gold nanoparticles could form more readily in the presence of thiol than in the absence of thiol. This might be due to the protective effect of the aggregation of the gold nanoparticles from the thiol molecules. However, no 2-aminoethanethiol-modified gold nanoparticles formed in the water/ethanol mixture solvent because the particles aggregated and precipitated after reaction. The reason for this is not clear. The thiol molecules assembled on the surfaces of the gold nanoparticles changed the surface properties of the gold nanoparticles from a negative charge to a positive

Table 6. **Preparation of Naked and Thiol-Modified Gold Nanoparticles and their Properties**

Measurement Date / Thiol	Solvent	Thiol (mM)	HAuCl$_4$ (mM)	NaBH4 (µM)	Size (µm)	Zeta Potential (mV) pH 4.0	pH 7.0
naked	water/ethanol	0	0.24	26.4	40.6±1.8	-10.0±1.2	-9.6±3.1
2-aminoethanethiol	water	3.65	2.40	5.30	31.6±5.6	14.3±4.7	0.1±0.2
8-amino-1-octanethiol	Water/ethanol	3.65	2.40	5.30	35.9±1.8	6.6±4.1	0.1±0.1
11-amino-1-undecanethiol	Water/ethanol	3.65	2.40	5.30	34.3±3.8	5.7±2.8	0.3±0.6

charge. The assembled thiol molecules could also protect the aggregation of the gold nanoparticles and stabilize the system.

The cationic gold nanoparticles could compact the DNA plasmid and facilitated transfection of the EGFP plasmid. A high ratio of gold nanoparticles to DNA increased transfection efficiency but the length of the alkyl groups had no evident effects. It has been reported that the size of the nanoparticles and their ratios to DNA have been shown to affect transfection efficiency. Niidome *et al.* reported that a high Au/DNA ratio could increase transfection efficiency [12]. Prow *et al.* reported that the larger the nanoparticles, the better the transfection efficiency [7]. Sandhu *et al.* reported that maximum transfection was achieved at an Au/plasmid mole ratio of 2200:1 [10].

CONCLUSIONS

Gold nanoparticles modified with 2-aminoethanethiol, 8-amino-1-octanethiol, and 11-amino-1-undecanethiol were prepared by $NaBH_4$ reduction of $HAuCl_4$ in water or water/ethanol mixture solvents under the presence of respective thiols. Modification of the thiol molecules changed the electrostatic properties of the gold nanoparticles from a negative charge to a positive charge. The cationic gold nanoparticles interacted with negatively charged plasmid DNA and their complex could be used for gene transfection. A high ratio of gold nanoparticles to DNA increased transfection efficiency.

ACKNOWLEDGEMENT

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