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Potential Oxidative Stress of Gold Nanoparticles by Induced-NO Releasing in Serum

Hong Ying Jia,[†] Yang Liu,*[,†] Xue Ji Zhang,*[,‡] Lu Han,[†] Li Bo Du,[†] Qiu Tian,[†] and Yuan Chao Xu[†]

State Key Laboratory for Structural Chemistry of Unstable and Stable Species, Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China, and Department of Chemistry, University of South Florida, 4202 East Fowler Avenue, CHE-205A, Tampa, Florida 33620-5250

Received October 11, 2008; E-mail: yliu@iccas.ac.cn; xzhang@cas.usf.edu

Among the known organic and inorganic nanoparticles, gold nanoparticles have attracted considerable attention for their potential applications in biology and medicine.[1] As early as 1618, Francisci Antonii, a philosopher and medical doctor, published the first book on the medical use of colloidal gold.[2] However, the gold nanoparticles may possibly transport across the skin of the body and into the cell due to their small size (most range in size below 50 nm). Consequently, there have been a few reports on the potential toxicity concerning gold nanoparticles, such as cytotoxicity,[3] limited biocompatibility, or oxidative stress.[4] Even so, little attention has been focused on the interaction between gold nanoparticles and some special endogenous proteins or polypeptide in tissues or body fluids. Herein, we have focused on assessing whether and how xenobiotic gold nanoparticles initiate the releasing of nitric oxide (NO) upon the interaction with endogenous RSNO in blood serum, and additionaly, how the potential oxidative stress in the process has been discussed, accordingly.

Gold nanoparticles were prepared by the reduction of aqueous chloroaurate with sodium citrate (Supporting Information S1) and characterized using transmission electron microscopy (TEM). The TEM image of the gold nanoparticles clearly shows a spherical morphology with a nearly uniform particle size of 13 nm and presents good monodispersion in solution (Figure 1A).

Production of NO induced by the gold nanoparticles was monitored on the Apollo 4000 system equipped with a NO microsensor. In the assay, we added 80 μL fresh serum into various concentrations of gold nanoparticles (10, 20, 40, 80 μM gold atoms). It was interesting to observe that the signal of nitric oxide (NO) was detectable immediately after the addition of gold nanoparticles to the blood serum, as illustrated in the inner picture of Figure 1B. In contrast, the signal amplitude remained relatively constant while pure water was supplied. Further insight into the effect of concentration of gold nanoparticles on NO-release was obtained by comparing the signal intensities of the released NO, as illustrated in the main picture of Figure 1B. It shows that the signal level of NO-release increases with increasing the concentration of gold nanoparticles, which implies that there is a dose-dependent increase in reactive nitrogen species (RNS) level upon addition of gold nanoparticle to serum.

Gold nanoparticles have been shown to catalyze NO generation whenever they come into contact with fresh blood serum. It is well documented that NO has a relative short lifetime in blood because of its reactivity with various blood components. In constrast, a more abundant and stable form of NO in blood is *S*-nitroso adducts with thiol group (RSNOs), such as *S*-nitrosoalbumin (AlbSNO), *S*-nitrosocysteine (CysNO), and *S*-nitrosoglutathione (GSNO).[5] These compounds may function as NO carrying systems, prolonging the half-life and spatial impact of NO.[6] One well-known reaction of RSNO decomposition to yield NO is catalyzed by metal ions, such as Cu^{2+}, Cu^{+}, or Fe^{2+}.[7] On the other hand, recent reports[8] have shown that, at the nanoscale level, gold becomes a highly efficient catalyst. Therefore, we may reasonably presume that the NO-release results from the catalysis of gold nanoparticles to RSNOs.

To prove the hypothesis, we chose GSNO as a model compound instead of the whole RSNO in blood serum. Referring to the range of the concentration of GSNO (from 120 to 180 nM) detected in the plasma,[9] a final concentration of 156 nM of GSNO was added to different concentrations of gold nanoparticles (10, 20, 50, 182, 455, 910 μM, respectively). Similar to the observation in the main picture of Figure 1B, the amounts of NO released, as presented in Figure 2A, were directly proportional to the concentrations of gold nanoparticles applied in the solution. In contrast, we cannot observe any signal pulse when the concentration of gold nanoparticles is equal to or below 10 μM. To examine the signal response to the addition of GSNO, the various concentrations of GSNO were consecutively dripped into a solution of gold nanoparticles. The main picture of Figure 2B demonstrates to us a stairs−like graph which indicates that the NO signal rises from the addition of GSNO. A fine correlation with $R = 0.9989$ in the inner picture of Figure 2B further indicates the linear response to the concentration of GSNO. As a result, our finding also implies that the gold nanoparticles may induce NO-release via catalyzing GSNO decomposition in physical conditions. In this manner, GSNO and other nitrosothiols can be quantitively determined and analyzed in solution or even in blood serum.

Furthermore, we found that the signal magnitudes of NO-release from serum (Figure 1B) are approximately 10 times higher than that from GSNO solution (Figure 2A), assuming the concentration of GSNO is 156 nM on average in blood serum. It means that, besides GSNO, gold nanoparticles can catalyze the decomposition of other endogenous NO-thiol adducts, such as AlbSNO or CysNO.

To elucidate the mechanism of NO-release and to identify whether the gold nanoparticle acts as an eletron transfer mediator or other—the formation of thiolate occurs in the reaction—the constituents of the final product were analyzed via X-ray photoelectron spectroscopy (XPS).

As illustrated in the XPS plot (Supporting Information, Figure S1A), the peak S $2p_{3/2}$ with a binding energy of 161.9 eV corresponds to the thiolate bound to the surface of the nanoparticles.[10] The S 2p spectra gave only weak signals due to the small scattering cross-section of the S atoms and the low amount of S atoms sited on the surface of gold nanoparticles. Another evidence for the formation of the thiolate comes from a spectrum of the Au $4f_{7/2}$ and Au $4f_{5/2}$ bands, as shown in Figure S1B, which occur at 84.1 and 87.8 eV, respectively. On thiolate formation one would

[†] Institute of Chemistry, Chinese Academy of Sciences.
[‡] University of South Florida.

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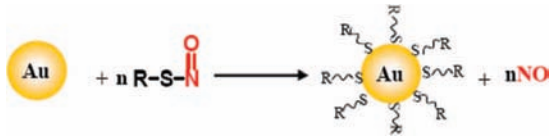
Scheme 1. The Mechanism for the Production of NO in Serum Containing Gold Nanoparticles



expect a significant proportion of the outer gold atoms to be oxidized from Au^0 to Au^1. The oxidation results in the Au $4f_{7/2}$ peak position shifting from 83.8 to 84.1 eV. Comparatively, because the particle size prepared for our work is around 13 nm and somewhat larger than those reported in the literature,[11] the higher number of gold atoms are located in gold cores of the nanoparticles. Thus, the less Au^1 state there exists, and consequently, the shift is not so large as those in the reports.

Further evidence for the thiolate formation initiated by the gold nanoparticles was shown in Figure S2. In the Figure, the signal magnitudes of NO-release were comparatively measured in GSNO solution when using normal citrate-protected gold nanoparticles or the GSH-protected gold nanoparticles, respectively. The so-called GSH-protected gold nanoparticles meant that the thiolate was pregenerated via GSH treatment and the surface of the particles was preoccupied. As expected, the rate of NO-release in the GSH-protected nanoparticles became evidently slower than that in the gold nanaparticles without GSH protection. The residual NO signals from the GSH-protected nanoparticles, even with most outer Au atoms being occupied, may result from the ligand exchange reaction on the surface of the nanoparticles.[12] The NO production catalyzed by the gold nanoparticles can be graphically demonstrated in Scheme 1.

NO is known to react rapidly with superoxide and then to produce a harmful peroxynitrite ($ONOO^-$) species.[13] Peroxynitrite interacts with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated damages.[14] Meanwhile, these reactions trigger cellular responses ranging from subtle modulations of cell signaling to overwhelming oxidative injury, committing cells to necrosis or apoptosis.[15] In vivo, peroxynitrite generation represents a crucial pathogenic mechanism in some conditions. Hence, gold nanoparticles probably exert, directly and/or indirectly, toxic effects on living body by a dose-dependent increase in RNS levels after the treatment of the nanoparticles.

In conclusion, the gold nanoparticles can catalyze NO production from endogenous RSNOs in blood serum. The process is ascribed to the formation of the Au-thiolate on the surface of gold

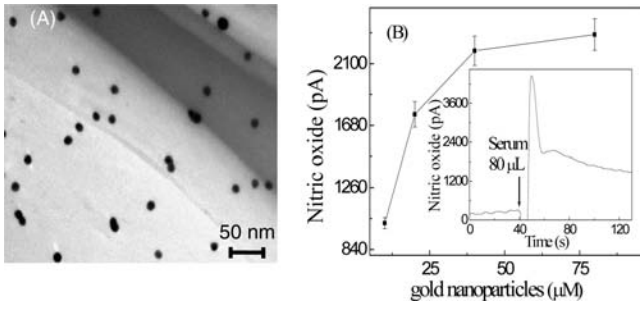


Figure 1. (A) TEM micrograph of gold nanoparticles; (B) the amount of induced-NO release in serum by gold nanoparticles solution (10, 20, 40, 80 μM). The inset is induced-NO release by 20 μM gold nanoparticles.

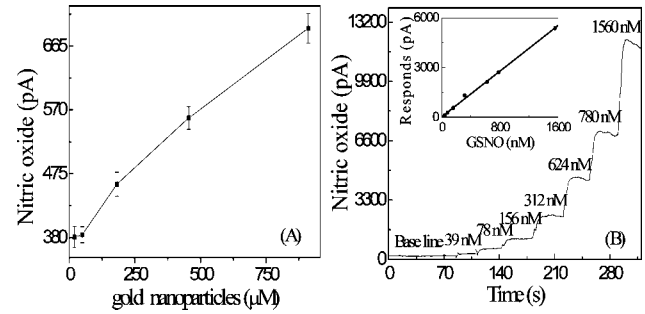


Figure 2. (A) The amount of NO release from final concentration of 156 nM GSNO addition to the different concentrations of gold nanoparticles solution (20, 50, 182, 455, 910 μM); (B) the amount of NO release due to different concentrations of GSNO addition to the gold nanoparticles solution. The inset is the current of NO versus concentration of GSNO.

nanoparticles. Furthermore, whenever the gold nanoparticles are used as a probe in the living body, a drug, or a component in drug excipient, it must be with considerable caution to avoid the oxidative stress, accordingly.

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Supporting Information Available: Experimental details and supporting results. This material is available free of charge via the Internet at http://pubs.acs.org.

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