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Gold nanoparticles functionalised by Gd-complex of DTPA-bis(amide) conjugate of glutathione as an MRI contrast agent

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ABSTRACT

The work is directed toward the synthesis of gold nanoparticles (Au NPs) coated with paramagnetic Gd-complex of DTPA-bis(amide) conjugate of glutathione (GdL) for use as a highly efficient MRI contrast agent. Well-dispersed spherical Au NPs coated with gadolinium complexes, abbreviated as Au@GdL, have been obtained; the mean size of Au@GdL is 5–7 nm, and the numbers of GdL are 1.36×10^4 per Au NP. Au@GdL exhibits high longitudinal ($r1$) and transverse ($r2$) relaxivities of 1.87×10^5 and 3.02×10^5 mM^{-1} s^{-1}, respectively.

Magnetic resonance imaging (MRI) is a leading candidate for diagnostic medical imaging because it has an infinite penetration depth and higher spatial resolution (micrometer rather than several millimeters) with non-invasive in vivo visualization.[1,2] In recent times, a great deal of effort has been made to explore the usage of nanoparticles in magnetic resonance imaging to improve the sensitivity of MRI.[3–6] Although nanoparticle-based approaches have been focused mainly on transverse relaxation ($T2$) effects of superparamagnetic nanoparticles, another type of nanoplatform coated with paramagnetic complexes can be employed as strong longitudinal relaxation ($T1$) MRI contrast agents (CAs), due to their ability to carry large payloads of active paramagnetic centers. These systems may be used as target-specific CAs even at extremely low concentrations. It is known that the nanoparticles with size below 100 nm can escape the uptake of the reticuloendothelial system in the liver, lung and spleen, in vivo, which results in a strong MRI signal in the tissue.[7]

For ultra-sensitive $T1$ CAs, much interest has recently been devoted to the design and the synthesis of gold nanoparticles coated with paramagnetic Gd-complexes for use as $T1$ MRI CAs.[8] Indeed, it has been demonstrated that with Au@Gd(DTDTPA), pronounced enhancement in contrast can be achieved. We have also been involved for some time in the design and the synthesis of some new DTPA-bis(amides) and their Gd-complexes for use in MRI.[9,10] In one case, we have observed that Gd-complexes of DTPA-bis(amide) conjugates of tranexamic esters exhibit much higher $r1$ relaxivity than Omniscan®; the highest $r1$ reaches up to 2.6 times as high as that for Omniscan®.[11]

These observations have encouraged us to pursue further MRI CAs with even higher $r1$ relaxivity by anchoring the similar above-mentioned Gd-complex on the gold nanoparticles. We now wish to report the synthesis and relaxivity properties of Gd-complex of DTPA-bis(amide) conjugate of glutathione coated on gold nanoparticles. Glutathione is a well-known peptide which finds many biological applications.

Scheme 1 shows the preparative method leading to the formation of the Gd-coated Au NPs. The synthesis initially involves the preparation of DTPA-bis(amide) conjugate of glutathione (L) from the reaction of DTPA-bis(anhydride) with two equivalents of glutathione in DMF at 80 °C for 24 h. The subsequent reaction of L with Gd_2O_3 in water under reflux led to the formation of the corresponding gadolinium(III) complex (GdL) as a white solid. The gold nanoparticles (Au NPs) were synthesized in situ by reducing $HAuCl_4$ with a five molar excess of $NaBH_4$ in the presence of GdL in water.

The resulting Au NPs coated by gadolinium complex (Au@GdL) were isolated as a black solid by centrifugation. Each Gd-complex undergoes oligomerization to form disulfide bonds as shown in the scheme. Complex formation as $[Gd(L)(H_2O)]\cdot xH_2O$ can be

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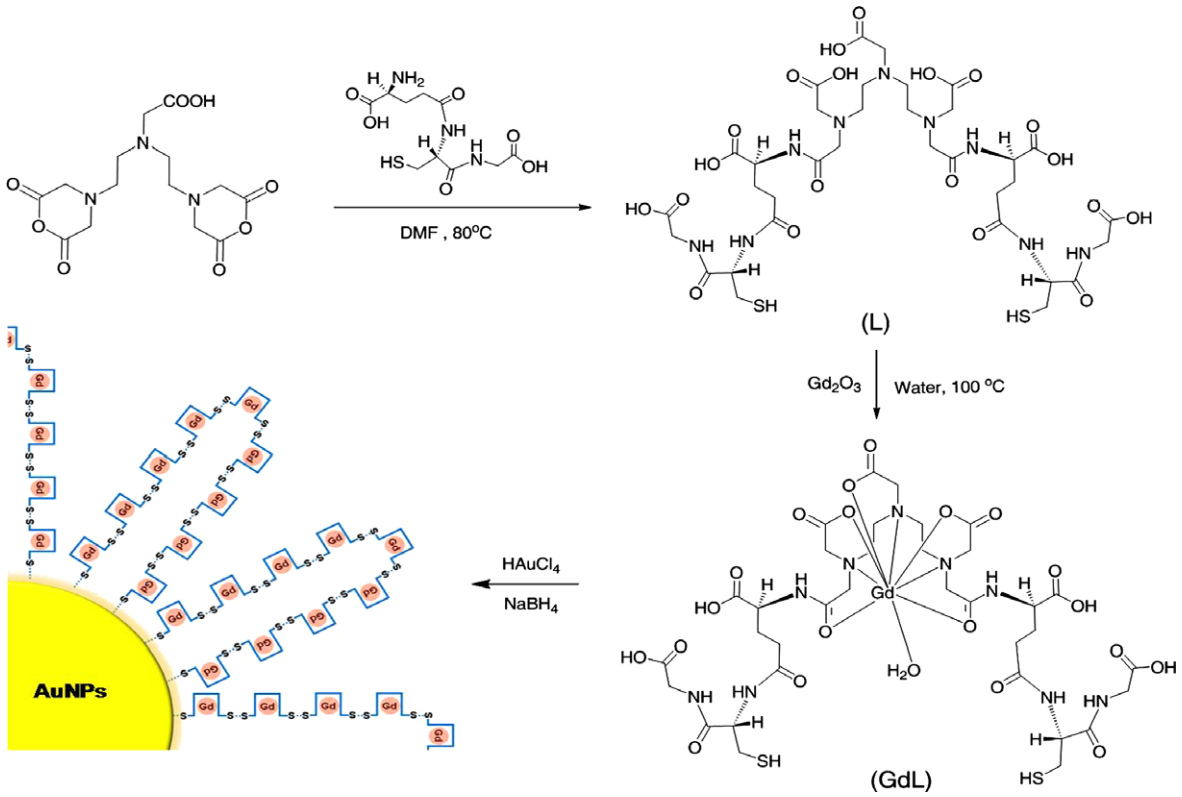

Scheme 1. Synthetic scheme leading to the formation of Au@GdL.

confirmed by analytical and various spectroscopic techniques (Supporting Information). For instance, the ESI-MS spectrum exhibits the molecular peak at 1127 Da corresponding to $[M]^+ - H_2O$. Figure 1 shows the powder X-ray diffraction for Au@GdL. The peaks at 38.2°, 44.4°, 64.5°, 77.5° and 81.7° correspond to the planes of (111), (200), (220), (311) and (222), respectively, the pattern of which is typical of Au NPs.[12]

The TEM image in Figure 2 shows well-dispersed spherical particles of Au@GdL with the mean size of 5–7 nm. DLS analysis also confirms this observation. The visible absorption spectrum shows a band at ca. 540 nm corresponding to the excitation of surface Plasmon vibrations. This observation is consistent with that made with mono-dispersed gold nanoparticles of the same size.[13]

The binding of GdL to the Au NP surface can be confirmed by FT-IR. Figure 3 shows the S–H stretching band at 2550 cm^{-1} in GdL

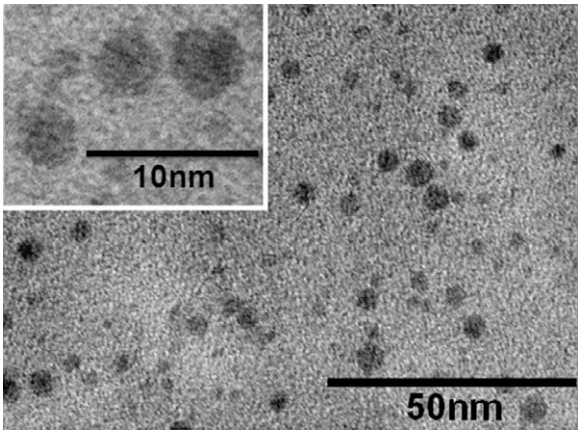

Figure 2. TEM image of Au@GdL.

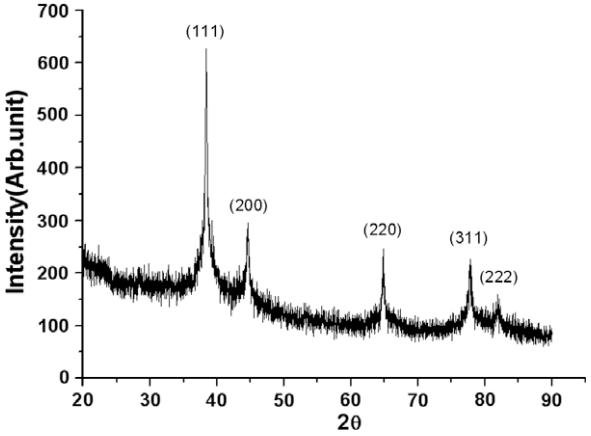

Figure 1. The powder X-ray diffraction pattern of Au@GdL.

(curve a) is absent in Au@GdL (curve b).[14] Other conspicuous changes found in the IR spectrum of Au@GdL are that the bands in the range of 1500–2000 cm^{-1} are reduced in intensity. A probable implication is that the carboxylic groups in L may also be involved in the binding to the gold surface.[15]

Inductively coupled plasma mass spectrometry (ICP-MS) reveals the total numbers of GdL per Au NP to be about 1.36×10^4. This value is derived based on the size of Au@GdL NPs as 7 nm, and compares well with theoretical calculation known in the literature.[16]

Table 1 shows relaxation times ($T1$, $T2$) and relaxivities ($r1$, $r2$) of Omniscan®, GdL and Au@GdL at the 1 mM concentration at 293 K and 1.5 T. GdL exhibits $r1$ and $r2$ relaxivities approximately three times higher than those of Omniscan®. These differences can be clearly observed by their $T1$ and $r1$ maps at varying concentrations shown in Figure 4. For instance, the biggest difference in

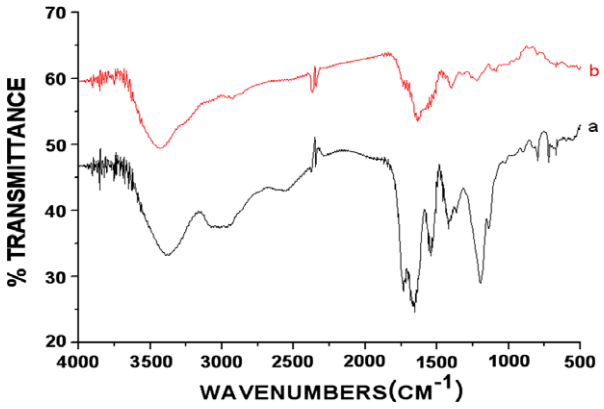

Figure 3. The FT-IR spectra of GdL (a) and Au@GdL (b).

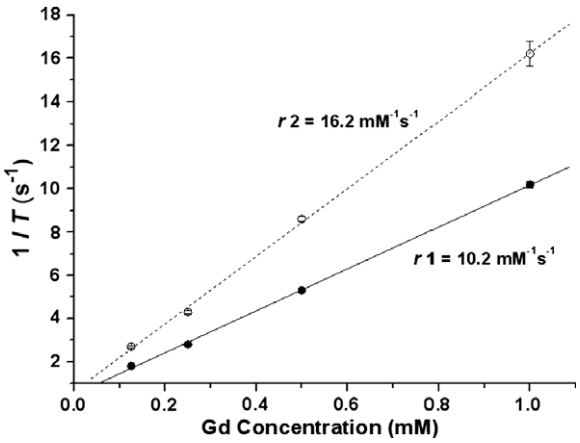

Figure 5. The relaxivity of Au@GdL as a function of [Gd].

Table 1
Experimental relaxation times and relaxivities of Ominiscan®, GdL and Au@GdL complex at the 1 mM concentration and 293 K

	*T*1	*r*1	*T*2	*r*2
Omniscan®	306.73 ± 0.15	3.30 ± 0.02	267.29 ± 4.53	3.70 ± 0.06
GdL	95.13 ± 1.66	10.50 ± 0.18	86.41 ± 2.02	11.60 ± 0.26
Au@GdL		1.87×10^5		3.02×10^5

the *R*1 maps between Au@GdL and Omniscan® is observed at the 1 mM concentration. Figure 5 shows the plots of *r*1 and *r*2 relaxivites of Au@GdL as a function of nanoparticle concentration [Gd]. Our system exhibits exceptionally high relaxivities ($r1 = 1.87 \times 10^5$ and $r2 = 3.02 \times 10^5$ mM^{-1} s^{-1}) per mM concentration of Au NPs. These values can be compared well with those of silica-based multilayered nanoparticles,[4] and compared even better with those of an analogous system.[17,18] Such high relaxivities with our system may be rationalized in terms of not only the intrinsic nature of monomeric GdL (cf. Table 1) but also a great number of GdL loading per Au NP. The MTT assay (Supporting Information) shows that our Au@GdL system is non-toxic at the clinical concentrations, further demonstrating a potential application as an imaging agent.

In summary, we have described the synthesis and characterization of Gold nanoparticles functionalized by Gd-complex of DTPA-bis(amide) conjugate of glutathione as an MRI contrast agent. The numbers of loading of GdL per gold nanoparticle are approximately 10.[4] Our system shows extremely high *r*1 and *r*2 relaxivities in the order 10^5 mM^{-1} s^{-1}. This is the first demonstration of possible application of Gd-coated Au NPs for clinical uses. Further work is underway to develop target-specific MRI CAs based on Au NPs.

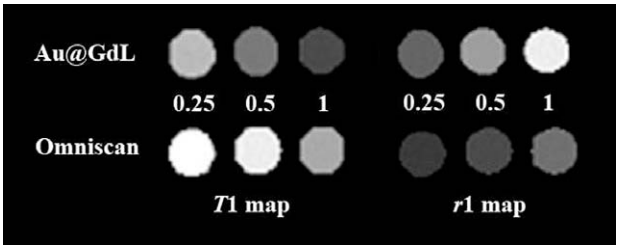

Figure 4. *T*1 and *r*1 maps of Au@GdL and Ominiscan® at different concentrations (in mM).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.017.

References and notes

1. Merbach, A. E.; Tóth, É. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; John Wiley: Chichester, UK, 2001.
2. Caravan, P. *Chem. Soc. Rev.* **2006**, *35*, 512.
3. Seo, W. S.; Lee, J. H.; Sun, X.; Suzuki, Y.; Mann, D.; Liu, Z.; Terashima, M.; Yang, P. C.; McConnel, M. V.; Nishimura, D. G.; Dai, H. *Nat. Mater.* **2006**, *5*, 971.
4. Kim, J. S.; Rieter, W. J.; Taylor, K. M. L.; An, H.; Lin, W.; Lin, W. *J. Am. Chem. Soc.* **2007**, *129*, 8962.
5. Xing, G.; Yuan, H.; He, R.; Gao, X.; Jing, L.; Zhao, F.; Chai, Z.; Zhao, Y. *J. Phys. Chem. B* **2008**, *112*, 6288.
6. Lanza, G. M.; Winter, P. M.; Caruthers, S. D.; Morawsk, A. M.; Schmieder, A. H.; Crowder, K. C.; Wickline, S. A. *J. Nucl. Cardiol.* **2004**, *11*, 733.
7. Moore, A.; Weissleder, R.; Bogdanov, A., Jr. *J. Magn. Reson. Imaging* **1997**, *7*, 1140.
8. Debouttiere, P.-J.; Roux, S.; Vocanson, F.; Billotey, C.; Beuf, O.; Favre-Reguillon, A.; Lin, Y.; Pellet-Rostaing, S.; Lamartine, R.; Perriat, P.; Tillement, O. *Adv. Funct. Mater.* **2006**, *16*, 2330.
9. Dutta, S.; Kim, S.-K.; Patel, D. B.; Kim, T.-J.; Chang, Y.-M. *Polyhedron* **2007**, *26*, 3799.
10. Dutta, S.; Kim, S.-K.; Lee, E. J.; Kim, T.-J.; Kang, D.-S.; Chang, Y.-M.; Kang, S. O.; Han, W.-S. *Bull. Korean Chem. Soc.* **2006**, *27*, 1038.
11. Dutta, S.; Park, J.-A.; Jung, J.-C.; Chang, Y.-M.; Kim, T.-J. *Dalton Trans.* **2008**, *16*, 2199.
12. Joint Committee on Powder Diffraction Standards (JCPDS) Card No. 04-0784, 2002.
13. Chechik, V.; Crooks, R. M. *Langmuir* **1999**, *15*, 6364.
14. Yonezawa, T.; Kunitake, T. *Colloids Surf. A* **1999**, *149*, 193.
15. Daniel, M.-C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293.
16. Lewis, D.; Day, T. M.; MacPherson, J. V.; Pikramenou, Z. *Chem. Commun.* **2006**, 1433.
17. Rieter, W. J.; Kim, J. S.; Taylor, K. M. L.; An, H.; Lin, W.; Tarrant, T.; Lin, W. *Angew. Chem. Int. Ed.* **2007**, *46*, 3680.
18. Alric, C.; Taleb, J.; Duc, G. L.; Mandon, C.; Billotey, C.; Meur-Herland, A. L.; Brochard, T.; Vocanson, F.; Janier, M.; Perriat, P.; Roux, S.; Tillement, O. *J. Am. Chem. Soc.* **2008**, *130*, 5908.