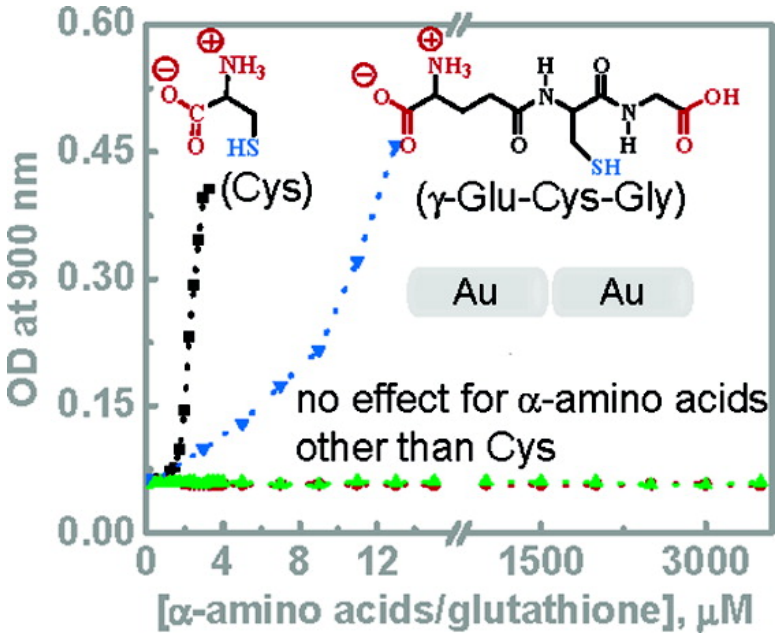
Communication

Selective Detection of Cysteine and Glutathione Using Gold Nanorods

P. K. Sudeep, S. T. Shibu Joseph, and K. George Thomas

J. Am. Chem. Soc., **2005**, 127 (18), 6516-6517• DOI: 10.1021/ja051145e • Publication Date (Web): 13 April 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 31 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 04/13/2005

Selective Detection of Cysteine and Glutathione Using Gold Nanorods

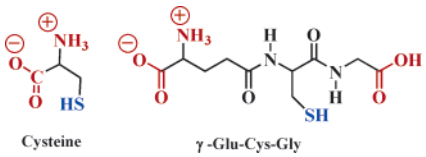
P. K. Sudeep, S. T. Shibu Joseph, and K. George Thomas*

Photosciences and Photonics, Regional Research Laboratory (CSIR), Trivandrum 695 019, India

Received February 22, 2005; E-mail: georgetk@md3.vsnl.net.in

Although significant advancement has been made on the stoichiometric recognition of metal cations through optical signal transduction, sensors for the selective detection of organic molecules are rather few.[1] One of the major difficulties associated with the selective detection of α-amino acids and peptides using chemosensors is their structural similarity, incorporating both carboxylic and amino groups.[1d] Among various peptides, glutathione (γ-Glu-Cys-Gly) has gained much attention in recent years due to its vital biological functions: (i) keeping the cysteine thiol group in proteins in the reduced state and (ii) protecting the cells from oxidative stress by trapping free radicals that damage DNA and RNA.[2] It has been suggested that there is a direct correlation between aging and reduced glutathione concentrations in intracellular fluids. However, its selective detection using chemosensors is difficult due to the presence of several functional groups (Chart 1). Herein we report a novel strategy for the selective detection of micromolar concentrations of cysteine and glutathione by exploiting the interplasmon coupling in Au nanorods.

Chart 1



Gold nanorods (average aspect ratio of 2.3) were prepared by adopting a photochemical method.[3a] Gold nanorods possess two plasmon absorption bands (trace a, Figure 1A): a shorter wavelength ($\epsilon_{515nm} = 0.34 \times 10^{10}$ M^{-1} cm^{-1}) originating from the transverse absorption and one at a longer wavelength ($\epsilon_{650nm} = 0.58 \times 10^{10}$ M^{-1} cm^{-1}), from the longitudinal absorption.[3b,c] The possibility of using Au nanorod based assays for the detection of target molecules through their regiospecific modification was earlier proposed by invoking a simple quasistatic treatment for the nanorod-nanorod plasmon resonance spectra at different distances and orientations.[4]

Approaches for the detection of α-amino acids and peptides include the use of chemosensors[5] and spherical nanoparticles.[6] Utilization of Au nanorods as probes for the detection of cysteine/glutathione have several advantages compared to spherical Au nanoparticles.[6,7] Analyte-induced aggregation of spherical Au nanoparticle results in a decrease in the plasmon absorption at around 520 nm and the formation of a long wavelength band.[8] However, various functional groups such as amines, thiols, and carboxylic acids present in amino acids and other organic molecules also influence their plasmon absorption. In contrast, the presence of monofunctional molecules containing such functional groups do not influence the plasmon absorption of Au nanorods (vide infra), allowing excellent selectivity for cysteine/glutathione. Furthermore,

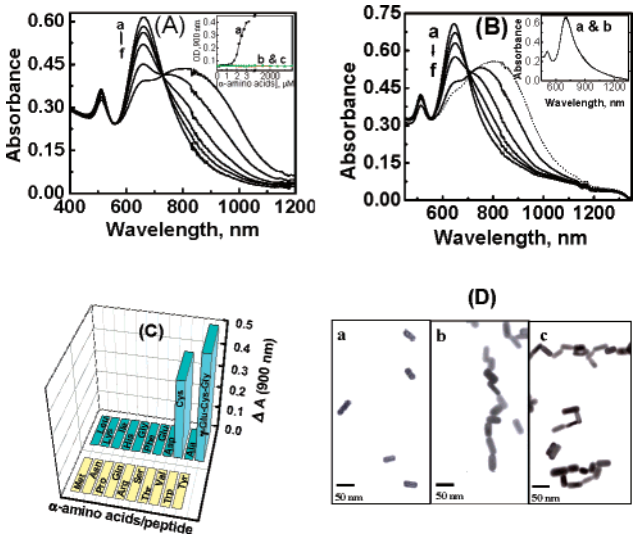


Figure 1. (A, B) Absorption spectral changes of Au nanorods (0.12 nM) in acetonitrile/water (4:1) on addition[9] of (A) cysteine at (a) 0 (b) 1.75 (c) 2.0 (d) 2.25 (e) 2.5 and (f) 3 μM or (B) glutathione at (a) 0, (b) 7, (c) 9, (d) 11, (e) 13, and (f) 14 μM. (C) 3D plot showing the selectivity of cysteine (3 μM), glutathione (12 μM), and other α-amino acids (10 μM). (D) TEM images of Au nanorods in the absence (a) and presence (b and c) of cysteine under identical conditions. Figure 1A (inset): changes in optical density at different concentrations of (a) cysteine, (b) tyrosine, and (c) leucine. Figure 1B (inset): effect of addition of 1-hexylmercaptan at (a) 0 and (b) 10 μM.

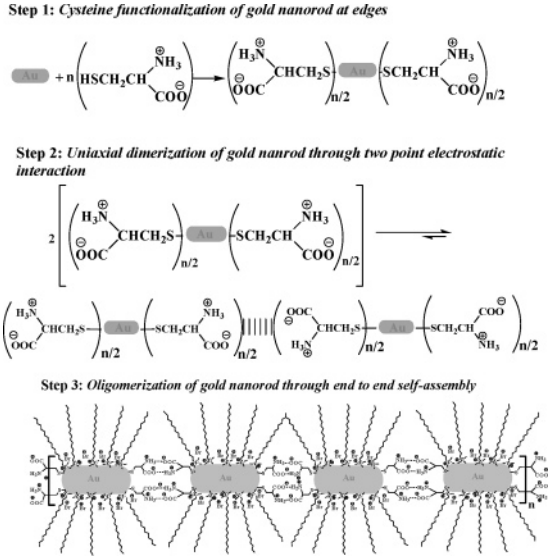
the anisotropic features of Au nanorods allow their orientation in lateral or axial (end-to-end) fashion, resulting in interplasmon coupling.[4]

The effect of various α-amino acids/glutathione on the absorption spectra of Au nanorods (0.12 nM) was investigated in a mixture (4:1) of acetonitrile and water.[9] In the case of both cysteine and glutathione, a dramatic decrease in the intensity of the longitudinal surface plasmon absorption band, with a concomitant formation of a new band at 850 nm, was observed (Figure 1A,B). The clear isosbestic point centered at 730 nm suggests the existence of two types of gold nanorods in the solution. Interestingly, the intensity of the transverse band is more or less unaffected in this concentration range. In the case of α-amino acids other than cysteine, no noticeable spectral changes were observed even in the millimolar concentration range (see Figure 1), indicating that the thiol functionality in cysteine/glutathione is essential for spectral changes. Similar spectral changes were also observed in the case of cystine.

The appearance of a new red shifted band at 850 nm in the presence of cysteine/glutathione results from the coupling of the plasmon absorption of Au nanorods assisted through self-assembly.[4,10] In the case of gold nanorods there are two modes of plasmon oscillation (lateral and axial); each of these mode can, in principle, couple with plasmon bands of the neighboring rods.[4] For explaining self-assembly of Au nanorods, various ionic forms of cysteine may be considered that are highly dependent on the pH

10.1021/ja051145e CCC: $30.25

Scheme 1. Mechanism of Self-Assembly of Au Nanorods



of the solution (Supporting Information).[11] The isoelectric point of cysteine is reported as 5.02 and the pH of the Au nanorod solution in CH_3CN-H_2O mixture is ~5.6. This indicates the existence of the zwitterionic form of cysteine in solution, which can assist an end to end self-assembly through a two-point electrostatic interaction. TEM studies showed that the Au rods before the addition of cysteine are randomly distributed, whereas cysteine-bound rods are preferentially self-assembled in an end to end fashion (Figure 1D).

It is interesting to note that no spectral changes are observed at lower concentrations of cysteine (inset of Figure 1A) and glutathione. In the first step, the thiol group of cysteine/glutathione molecules is selectively functionalized onto the edges of Au nanorods, leaving the zwitterionic groups for further interaction (Scheme 1). It has been recently shown that the edges of gold nanorods are dominated by {111} facets and the lateral sides are dominated by {100} and {110} planes.[12,13] In the second step, the appended zwitteronic groups at the ends of Au nanorods assist the coupling through a two-point electrostatic interaction in a cooperative fashion. The spectral changes in Figure 1A,B featuring a clear isosbestic point support the coupling between two rods. In the presence of excess cysteine, a gradual shift in the absorption band to the NIR region is observed, indicating the stepwise assembly of nanorods. Coexistence of assembled nanorods of different lengths in solution probably results in the deviation from the isosbestic point.

To further verify the role of two-point electrostatic interaction, we have investigated the effect of (i) varying the pH of the medium and (ii) addition of 1-alkylmercaptan. Cysteine molecules are present in other ionic forms at higher pH.[11] Primary amines (methylamine solution and octadecylamine) were used for deprotonating amino acids, and the absorption spectral changes of Au nanorods were found to be negligible on addition of cysteine (Supporting Information), indicating that the linear organization does not occur. Also, we have observed that the addition of 1-hexylmercaptan (inset of Figure 1B) does not influence the plasmon absorption of Au nanorods. The {111} planes in Au nanorods occupy only a relatively smaller area compared to the total surface area, and thiols preferentially bind to this plane.[12,13] Hence plasmon bands remain unaffected at lower concentrations of 1-alkylmercaptans. Both of these results further confirm the role of zwitterionic group in self-assembly.

One of the significant features of the present system is its ability to detect cysteine/glutathione in the presence of various other α-amino acids. The spectra of Au nanorods remain unaffected on addition of millimolar concentrations of various α-amino acids (depending upon the solubility) other than cysteine. Interestingly, we could selectively detect micromolar concentrations of cysteine/glutathione from a pool of α-amino acids, and this makes Au nanorods versatile for sensing biologically important molecular systems bearing thiol and zwitterionic groups.

Acknowledgment. The authors thank the Council of Scientific and Industrial Research (CMM 220239) and the Department of Science and Technology (SP/S5/NM-75/2002), Government of India for financial support and Dr. Murali Sastry (NCL, Pune) and Dr. Annie John (Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum) for TEM measurements. This is contribution No. RRLT-PPD-193 from the Regional Research Laboratory, Trivandrum, India.

Supporting Information Available: Details on the synthesis, purification and characterization of Au nanorods, its interaction with various α-amino acids and primary amines. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963. (b) deSilva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (c) Tong, A.; Yamauchi, A.; Hayashita, T.; Zhang, Z.; Smith, B. D.; Terame, N. *Anal. Chem.* **2001**, *73*, 1530. (d) Ait-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296.

(2) Mathews, C. K., van Holde, K. E., Ahern, K. G. *Biochemistry*; Addison-Wesley Publishing Company: San Francisco, 2000.

(3) (a) Kim, F.; Song, J. H.; Yang, P. D. *J. Am. Chem. Soc.* **2002**, *124*, 14316. (b) El-Sayed, M. A. *Acc. Chem. Res.* **2001**, *34*, 257. (c) See Supporting Information.

(4) Gluodenis, M.; Foss, C. A. *J. Phys. Chem. B* **2002**, *106*, 9484.

(5) (a) Rusin, O.; St Luce, N. N.; Agbaria, R. A.; Escobedo, J. O.; Jiang, S.; Warner, I. M.; Dawan, F. B.; Lian, K.; Strongin, R. M. *J. Am. Chem. Soc.* **2004**, *126*, 438. (b) Ros-Lis, J. V.; Garcia, B.; Jimenez, D.; Martinez-Manez, R.; Sancenon, F.; Soto, J.; Gonzalvo, F.; Valldecabres, M. C. *J. Am. Chem. Soc.* **2004**, *126*, 4064.

(6) (a) Verma, A.; Nakade, H.; Simard, J. M.; Rotello, V. M. *J. Am. Chem. Soc.* **2004**, *126*, 10806. (b) Zhong, Z. Y.; Patskovskyy, S.; Bouvrette, P.; Luong, J. H. T.; Gedanken, A. *J. Phys. Chem. B* **2004**, *108*, 4046. (c) Naka, K.; Itoh, H.; Tampo, Y.; Chujo, Y. *Langmuir* **2003**, *19*, 5546. (d) Zhang, F. X.; Han, L.; Israel, L. B.; Daras, J. G.; Maye, M. M.; Ly, N. K.; Zhong, C. J. *Analyst* **2002**, *127*, 462. (e) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078.

(7) For general reviews and articles, see: (a) Daniel, M.-C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293. (b) Shenhar, R.; Rotello, V. M. *Acc. Chem. Res.* **2003**, *36*, 549. (c) Thomas, K. G.; Kamat, P. V. *Acc. Chem. Res.* **2003**, *36*, 888. (d) Rao, C. N. R.; Kulkarni, G. U.; Thomas, P. J.; Edwards, P. P. *Chem. Eur. J.* **2002**, *8*, 29 (e) Sastry, M.; Rao, M.; Ganesh, K. N. *Acc. Chem. Res.* **2002**, *35*, 847. (f) Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128. (g) Shipway, A. N.; Katz, E.; Willner, I. *ChemPhysChem* **2000**, *1*, 18. (h) Imahori, H.; Fukuzumi, S. *Adv. Mater.* **2001**, *13*, 1197. (i) Jackson, A. M.; Myerson, J. W.; Stellacci, F. *Nat. Mater.* **2004**, *3*, 330.

(8) (a) Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. *J. Phys. Chem. B* **2003**, *107*, 668. (b) Hao, E.; Schatz, G. C.; Hupp, J. T. *J. Fluoresc.* **2004**, *14*, 331. (c) Hallock, A. J.; Redmond, P. L.; Brus, L. E. *Proc. Natl. Acad. Sci.* **2005**, *102*, 1280.

(9) After stabilization: cysteine (4 min) and glutathione (60 min).

(10) Thomas, K. G.; Barazzouk, S.; Ipe, B. I.; Joseph, S. T. S.; Kamat, P. V. *J. Phys. Chem. B* **2004**, *108*, 13066.

(11) Yakobke, H. D.; Jeschkert, H. *Amino Acids, Peptides and Proteins: An Introduction*; Akademie Verlag: Berlin, 1977.

(12) (a) Caswell, K. K.; Wilson, J. N.; Bunz, U. H. F.; Murphy, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 13914. (b) Herna'ndez, R. M.; Richter, L.; Semancik, S.; Stranick, S.; Mallouk, T. E. *Chem. Mater.* **2004,** *16*, 3431

(13) (a) Tian, M.; Wang, J.; Kurtz, J.; Mallouk, T. E.; Chan, M. H. W. *Nano Lett.* **2003**, *3*, 919. (b) Perez-Juste, J.; Liz-Marzan, L. M.; Carnie, S.; Chan, D. Y. C.; Mulvaney, P. *Adv. Funct. Mater.* **2004**, *14*, 571. (c) Johnson, C. J.; Dujardin, E.; Davis, S. A.; Murphy, C. J.; Mann, S. *J. Mater. Chem.* **2002**, *12*, 1765. (d) Wang, Z. L.; Mohamed, M. B.; Link, S.; El-Sayed, M. A. *Surf. Sci.* **1999**, *440*, L809.

JA051145E