

Enhanced Optical Sensing of Biologically Important Anions with Functionalized Gold Nanoparticles

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Gold nanoparticles surface-modified with amide ligands optically sense biologically important anions with the high sensitivity: the detection limit is increased about three orders of magnitude higher than that originally expected from the anion binding ability of neutral amide ligands.

Key words: nanoparticle, optical sensing, anion, molecular recognition

INTRODUCTION

Gold and silver nanoparticles have been of increasing interest in applications to biological and chemical nanosensors. In recent years, optical¹⁻³ and electrochemical^{4,5} nanoprobe have been developed by modifying the surface of metal nanoparticles with various functional molecules. Gold nanoprobe capable of optically sensing biological macromolecules such as DNA have been extensively studied.¹ However, there are few nanoparticle-type optical probes for biologically important ions and molecules. As gold nanoparticles have the intense plasmon band in the visible region,⁶ they must have considerable potential for optical detection of ions and molecules as well as macromolecules. We reported that the amide-functionalized gold nanoparticle **1b** optically senses anions with significantly high sensitivity.⁷ In this study, we have synthesized a new nanoparticle **1a** surface-modified with amide ligands containing a short alkyl chain. The optical sensing ability of **1a** has been explored in comparison with that of **1b**.

EXPERIMENTAL

UV-vis titration: A 2×10^{-8} M solution of **1** in CH_2Cl_2 was prepared, and a 2-ml portion was transferred to a 1-cm UV cell. A 1.5×10^{-4} M solution of anions in CH_2Cl_2 was made using the stock solution of **1**, and small aliquots were added to the solution of **1**. The absorbance

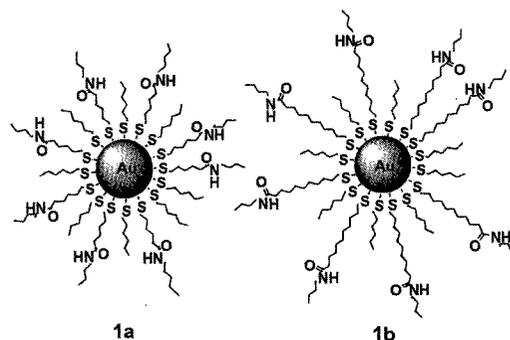


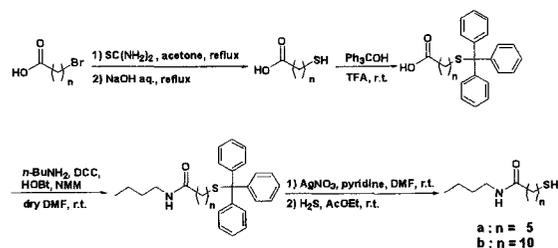
Figure 1.

changes of **1** at 520 nm were monitored as a function of anion concentration.

RESULTS AND DISCUSSION

The thiol-ligand syntheses are shown in Scheme 1. The surface-functionalized gold nanoparticle **1a** was prepared through a similar synthetic route of **1b**.⁷ The exchange reactions of hexanethiolate-protected gold nanoparticles⁸ with $n\text{-BuNHCO}(\text{CH}_2)_n\text{SH}$ in CH_2Cl_2 for 24 h were carried out for the 1:1 mole ratio of $n\text{-BuNHCO}(\text{CH}_2)_n\text{SH}$ to $\text{CH}_3(\text{CH}_2)_5\text{S-Au}$. After purification via sequential precipitation, the gold nanoparticle **1a** was characterized by elemental analysis, powder X-ray diffraction (XRD), and UV-vis spectroscopies. The mean diameters of the gold core determined by XRD were 4.2 ± 0.5 nm for **1a**. Assuming a truncated octahedral shape⁹ predicts that the core of **1a** contain 2556 Au atoms. Given the values for elemental analysis of **1a** (C: 6.70%; H: 0.74%; N: 0.63%), there are 69 $\text{C}_6\text{H}_{13}\text{S-Au}$ and 260 $\text{BuNHCO}(\text{CH}_2)_n\text{S-Au}$ ligands. The CH_2Cl_2 solution of **1a** shows wine-red color being characteristic of the plasmon band at 520 nm ($\epsilon = 4.74 \times 10^6 \text{ M}^{-1}\text{cm}^{-1}$).

The anion sensing properties of **1a** was investigated by UV-vis titrations with H_2PO_4^- , HSO_4^- , AcO^- , NO_3^- , Cl^- , Br^- , and I^- . The addition of anions (as their Bu_4N^+ salts) to a solution of **1a** in CH_2Cl_2 at 20°C caused the aggregation of **1a**



Scheme 1.

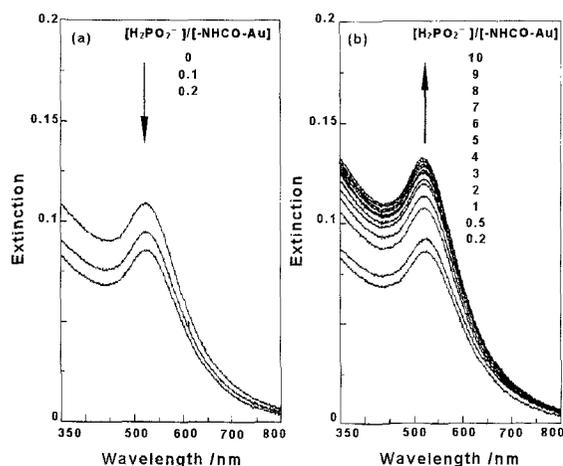


Figure 2. Change in the UV-visible spectrum of Au nanoparticle **1** upon addition of H_2PO_4^- in CH_2Cl_2 at 293K: $[\mathbf{1a}] = 2.28 \times 10^{-8}$ M, $[\text{H}_2\text{PO}_4^-] = 0.5.86 \times 10^{-5}$ M.

leading to dramatic changes in the plasmon band. When 0 – 0.2 equiv of anion/ $\text{BuNHCO}(\text{CH}_2)_5\text{S-Au}$ was added to the solution of **1a** ($[\mathbf{1a}] = 2.28 \times 10^{-8}$ M), the plasmon band decreased by 7–25% in intensity with a slight shift in wavelength (Figure 2a). The decrease in extinction is due to the screening of nanoparticles embedded deeply within the aggregate interior resulted in the reduction of the plasmon band.¹⁰ Increasing anion fraction would lead to the disaggregation of the suprananoparticles composed of **1a** and anions because of Coulombic repulsions between the negatively charged nanoparticles by the anion binding. In fact, further addition of anions to the solution of **1a** caused an increase in the intensity (Figure 2b). In particular, the addition of excess amount of H_2PO_4^- increased the extinction intensity of **1a** solution more than that of original solution. This might reflect that a part of the nanoparticles **1a** already aggregated before adding the anions. As shown in Figure 3, the nanoparticle **1a** showed much less spectral changes than **1b** did. The amide groups of **1a** are placed near the surface of hexanethiolate monolayer. Upon the binding of anions, it is difficult for them to form the ideal complex leading to the aggregations of **1a** because of steric hindrance between the terminal butyl group and the hexanethiolate monolayer. The anion-induced aggregates of the nanoparticle **1a** would be smaller than those of **1b**. Lazarides and Schatz showed using coupled dipole approximations that a dramatic red shift occurs in extinction wavelength when the interparticle distances in the aggregates decrease to less than the average particle radius.¹¹ Neither of the nanoparticles **1a** and **1b** showed the significant red shift of the plasmon band. Nanoparticles **1a** and **1b** within the anion-induced aggregates might not be close enough to cause the color change. The UV-vis titration curves for anions in CH_2Cl_2 shows the absorption spectra were restored to the

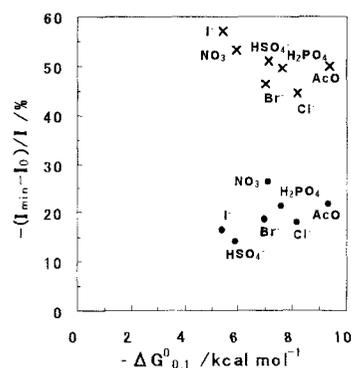
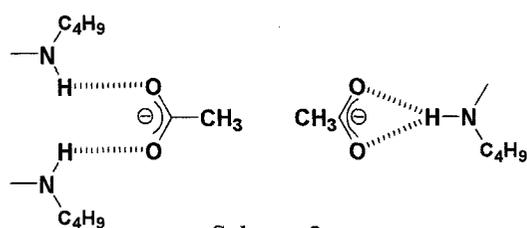


Figure 3. Plots of anion-induced maximum decreasing ratio $(I_{\min} - I_0)/I_0$ in extinction of **1a** (●) and **1b** (×) in CH_2Cl_2 vs. free energies $\Delta G^0_{0,1}$ of hydration in the gas phase for anions.

original state when excess of H_2PO_4^- , HSO_4^- , AcO^- , Cl^- , and NO_3^- was added to the solution. In contrast, the initial absorption intensity was not recovered even after addition of large excess of Br^- and I^- . There were also no spectral changes when these solutions were kept at 20°C for 24 h after addition of 10 equiv of anion/ $\text{BuNHCO}(\text{CH}_2)_5\text{S-Au}$ ($n = 5$ and 10). The aggregates of **1** and these anions were tightly held together and were hardly disaggregate by the component anions themselves.

The associate constants for anions with neutral amide ligands are generally not so high ($K_a < 100 \text{ M}^{-1}$) even in low dielectric solvents.¹² Optical probes containing such amide ligands would have a detectable response only in $>10^{-3}$ M concentration of anions if one assumed that optical probes have a detectable response in the concentration range from approximately $0.1 \times K_d$ to $10 \times K_d$.¹³ Therefore, it is noted that the nanoparticle **1a** is capable of optically sensing changes in anion concentration at a level of 10^{-9} M as well as **1b**. The detection limit is increased about three orders of magnitude higher than originally expected. This effectiveness cannot be understood only by the cooperative action of the amide ligands assembled on the particle surface. Interparticle van der Waals attractions may play a key role, which are quite strong in particular between metal nanoparticles because of the large polarizability of the gold cores.¹⁴

In order to elucidate the fundamental features of binding selectivity, optical sensing studies between **1** and anions were further performed in CH_2Cl_2 containing DMSO as the competitive solvent. At 1% DMSO/ CH_2Cl_2 , the maximum decreasing ratio $\Delta I_{\max} = (I_{\min} - I_0)/I_0$ in absorption intensity of **1** upon anion binding was in the following order: $\text{Cl}^- > \text{H}_2\text{PO}_4^- > \text{NO}_3^- > \text{AcO}^- > \text{HSO}_4^-$. As the amide ligands were randomly introduced on the surface of **1**, the binding selectivity should follow the ability of the guest anions to form hydrogen-bond to the amide ligands rather than their structural differences. The free energy of hydration $\Delta G^0_{0,1}$ (kcal/mol)¹⁵ in the gas phase for the anions were used as a



Scheme 2.

measure of hydrogen-bond acceptor strength of the guest anions although the pK_a values of acid (HX) in aqueous solution generally are used as a measure of the hydrogen bond acceptor strength of the corresponding anions (X^-).¹⁶ The nanoparticle **1b** showed the high binding selectivity for HSO_4^- and $H_2PO_4^-$ that work as the hydrogen bond donor as well as the hydrogen bond acceptor. While the plot of ΔI_{max} of **1a** against $\Delta G_{0,1}^\circ$ of the guest anions shows a good correlation between them except for AcO^- . The ΔI_{max} value for AcO^- was smaller than that expected from the relationship between the ΔI_{max} and the $\Delta G_{0,1}^\circ$. There are several binding geometries of the guest anions and the terminal amido groups. For the planer and bidentate AcO^- , some of them as shown in Scheme 2 are undesirable to induce the aggregates of **1a**.

CONCLUSION

We demonstrated that the amide-functionalized gold nanoparticles **1a** and **1b** optically recognize the anions. They have distinct advantages in sensitivity. The detection limit is increased about three orders of magnitude higher than that originally expected from the anion binding ability of neutral amide ligands. Although further improvements of the selectivity and the binding affinity are required for practical use, gold nanoparticles are particularly easy to build up their highly functionalized surface through ligand exchange reactions. The surface-functionalized gold nanoparticles are considerably potential as optical nanoprobe of biologically important ions and molecules.

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