J|A|C|S
COMMUNICATIONS

Published on Web 09/23/2004

Isothermal Titration Calorimetry Studies on the Binding of DNA Bases and PNA Base Monomers to Gold Nanoparticles

A. Gourishankar,[†] Sourabh Shukla,[‡] Krishna N. Ganesh,*[,†] and Murali Sastry*[,‡]

Organic Chemistry (Synthesis) and Materials Chemistry Divisions, National Chemical Laboratory, Pune 411 008, India

Received June 1, 2004; E-mail: sastry@ems.ncl.res.in; kng@ems.ncl.res.in

DNA-based nanotechnology has generated interest in a number of applications due to the specificity, programmability, and reproducibility of DNA interaction with nanoparticles. Gold nanoparticles (AuNPs) modified with DNA find use in diverse fields such as DNA chips,[1] DNA sensors,[2] drug/DNA delivery,[3] imaging,[4] and biodiagnostics[5] and in structured nanoparticle assemblies for electronics.[6] Similar applications are envisaged for the DNA analogue, peptide nucleic acids (PNAs), wherein the sugar−phosphate backbone is replaced by a polyamide backbone (structures for the PNA nucleobases are shown in Supporting Information, S1).[7] The remarkable utility and versatility of such systems is attributed to the very nature of AuNP−DNA interaction, which in turn is determined by the differential affinity of the nucleobases (adenine, guanine, thymine, and cytosine), nucleosides, and oligonucleotide sequences to the gold nanoparticles.[8] Previous studies on understanding the nature of interaction of nucleobases with nanogold have made use of various spectroscopic techniques such as surface-enhanced Raman spectroscopy (SERS),[9] Fourier transform infrared (FTIR),[10] and reflection absorption infrared (RAIR) spectroscopies,[11] which are clearly indirect methods for estimation of the strength of the abovementioned interactions.

Isothermal titration calorimetry (ITC) is a sensitive technique that measures the heat of reaction of two aqueous solutions when one is titrated against the other and has emerged as a powerful tool for studying the thermodynamic as well as kinetic aspects of complexation in biological systems. ITC has been used with much success in studies related to protein−protein interactions,[12] protein−DNA interactions,[13] protein−lipid interactions,[14] etc. We show here that ITC may be used to directly observe the energetics of interaction of the DNA bases as well as the corresponding PNA base monomers with AuNPs. Our results suggest that the binding affinity of nucleobases toward AuNPs is different from those obtained in earlier studies.[10,11] We believe that the differential binding strengths of the four nucleobases can be exploited to develop alternative strategies of designing oligonucleotides for gold nanoparticle complexation without the need to thiolate oligonucleotides.

Chloroauric acid ($HAuCl_4$), sodium borohydride, adenine (A), guanine (G), cytosine (C), and thymine (T) were obtained from Aldrich Chemicals and used as received. Aqueous gold nanoparticles were synthesized by borohydride reduction of chloroauric acid (10^{-4} M of aqueous solution of $HAuCl_4$) resulting in a ruby red solution with gold nanoparticles of dimensions 65 ± 7 Å.[15] The pH of the solution was adjusted to physiological pH using dilute hydrochloric acid, and the solution was dialyzed for 24 h. All four amino ethyl glycyl (*aeg*)-PNA monomers were synthesized in the laboratory as described earlier.[7a] The capping of the gold nanoparticles with the PNA base monomers and nucleobases was achieved by mixing 1.47 mL of 10^{-4} M AuNP with 300 μL of 0.25 mM PNA monomers and 2.5 mM DNA bases.

[†] Organic Chemistry (Synthesis) Division.
[‡] Materials Chemistry Division.

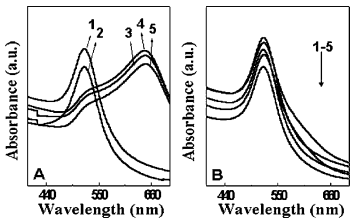

Figure 1. (A) UV−vis absorption spectra recorded from AuNPS modified with PNA base monomers. Curve 1 corresponds to as-prepared AuNPs. Curves 2−5 correspond to AuNP−PNA−T, AuNP−PNA−G, AuNP−PNA−C, and AuNP−PNA−A solutions, respectively. (B) UV−vis absorption spectra of gold nanoparticles modified with DNA bases. Curves 1−5 correspond to the as-prepared AuNPs, AuNP−T, AuNP−A, AuNP−G, and AuNP−C solutions, respectively. The spectra have been displaced vertically for clarity. The UV−vis spectra were recorded on a JASCO dual-beam spectrophotometer (V-570) operated at a resolution of 2 nm.

Figure 1A,B shows the UV−vis absorption spectra recorded from AuNPs modified with DNA bases and PNA base monomers, respectively. In the case of PNA base monomer interaction with AuNPs (Figure 1A), binding is indicated by the appearance of a longitudinal plasmon band at ca. 670 nm, indicative of aggregation of the nanoparticles. In the case of PNA−thymine monomer interaction with AuNPs, the 670 nm band does not arise, suggesting weak interaction with the particles. A similar trend is seen in the reaction of nucleobases with gold nanoparticles (Figure 1B). While we do not observe a longitudinal absorption band (suggesting that the PNA base monomers interact more strongly with AuNPs than the corresponding nucleobases), the transverse plasmon band at ca. 520 nm broadens upon AuNP complexation with G, C, and A. These results show that both the thymine nucleobase and thymine−PNA monomer interact much more weakly with AuNPs than the other nucleobases/PNA base monomers. UV−vis spectroscopy is, however, unable to differentiate between the strength of interaction of the remaining three nucleobases/PNA monomers with AuNPs.

Although the TEM images (Supporting Information, S2) are unable to highlight the differential binding affinities among the four nucleobases and the PNA monomers, they support the higher binding affinities of the latter over the former. The ITC responses recorded during titration of the different nucleobases and PNA base monomers with the AuNPs after correction for dilution effects (Supporting Information, S3) are shown in Figure 2. The main curves in Figure 2 show the raw data obtained during each injection, while the insets correspond to the integrated calorimetric response plotted against the total volume of titrate added. The following observations may be made: in all experiments, the interaction is exothermic (A−H); however, the magnitude of the response is a strong function of the nucleobase−AuNP complex and thus enables a direct evaluation of the strength of the interaction. The three nucleobases C, A, and G (insets, Figure 2A−C, respectively) and the corresponding PNA monomers (insets, Figure 2E−G, respectively) show a classical binding behavior with a sigmoidal response.

10.1021/ja046785g CCC: $27.50

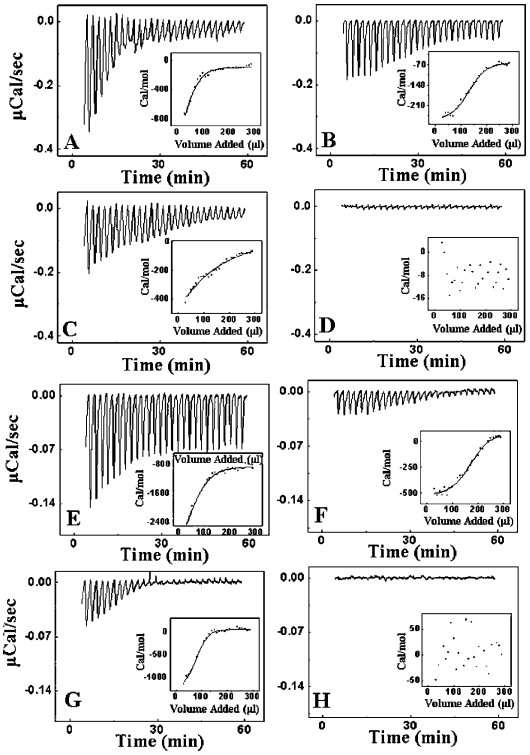

Figure 2. ITC data recorded for AuNP−DNA base interactions and AuNP−PNA monomer interaction. Curves A−D correspond to AuNP interactions with C, A, G, and T, respectively. Curves E−H correspond to AuNP interaction with PNA−C, PNA−A, PNA−G, and PNA−T, respectively. ITC experiments were performed in a Micro-Cal VP-ITC instrument at 4 °C, wherein 300 μL of 2.5 mM aqueous solution of DNA bases and 0.25 mM aqueous solution of PNA monomers at pH 7 were injected in equal steps of 10 μL into 1.47 mL of dialyzed borohydride-reduced gold hydrosol.

On the other hand, the titration of T (Figure 2D) and the PNA analogue (Figure 2H) did not elicit a strong calorimetric response, with a fair degree of scatter existing in the data. The exothermicity of the interaction of the PNA base monomers during titration with AuNPs (Figure 2E−G) is much higher than for the corresponding DNA bases. The sigmoidal nature of the binding curves may be explained on the basis of increasing coverage of the gold nanoparticles by the nucleobases/PNA base analogues, which lowers the available surface area for complexation during succeeding titrations. The exothermic tail in each case arises from dilution effects of the DNA/PNA bases during titration (Supporting Information, S3) on completion of nanoparticle surface coverage by the DNA/PNA bases. From the number of injections required for saturation of the calorimetric response, an equilibrium coverage of 0.2 mM/0.025 mM, 0.35 mM/0.028 mM, and 0.39 mM/0.039 mM was determined for C, G, and A and the corresponding PNA base analogues, respectively. These results show that C, G, and A and their PNA base analogues bind strongly with gold nanoparticles, while T binds much more weakly.

The strengths of interaction of the nucleobases and PNA analogues with AuNPs may be evaluated on the basis of the exothermicity during the initial stages of interaction (i.e., when the AuNP surface is bare, Table 1). The strength of interaction of the nucleobases/PNA analogues decreases in the order C > G > A > T (Table 1). Amine groups have been shown to bind exceptionally strongly with aqueous AuNPs,[16] and we believe the weak interaction of T with the nanoparticles could be due to the absence of an exocyclic amino group in this nucleobase (Supporting Information, S1). While the weakest interaction of thymine and PNA-T is in agreement with previous results (G > A > C > T in ref 11),[9−11]

Table 1. AuNP−DNA/PNA Interaction Exothermicity in Cal/mol

	nucleobases	PNA monomers
cytosine	−732.19	−2444.92
guanine	−427.18	−1147.97
adenine	−249.29	−518.45
thymine		

we observe a different order in the strength of interaction of the other three bases/PNA base monomers using the more direct, calorimetric evaluation. In the study conceptually closest to ours, Mirkin and co-workers have used temperature-programmed desorption (TPD) to study the interaction of DNA bases and nucleosides with gold thin films.[11] We believe the differences in the strength of interactions of the DNA bases in our studies and that of the Mirkin group[11] could be attributed to differences in surface chemistry; in the case of solution-based AuNPs, the role of surface-bound $AuCl_4^-/AuCl_2^-$ ions[16] in modulating these interactions cannot be neglected. The differences could also be due the fact that DNA/PNA bases have been used in this study, while nucleosides were studied in earlier reports.[10,11] It is interesting to note that the PNA monomers bind much more strongly to the AuNPs than the DNA bases and that a concentration (0.25 mM) 10 times smaller than that of the DNA bases (2.5 mM) is enough to bring about the saturation of the gold nanoparticle surface. This may be attributed to a secondary binding interaction between AuNPs and the PNA base monomers and their more bulky size (Supporting Information, S1). However, this difference in binding does not alter the order of interaction of the PNA base monomers with AuNPs; they mirror the results obtained with the nucleobases.

Acknowledgment. This work was partially funded by a grant from the Department of Science and Technology (DST), Government of India, which is gratefully acknowledged.

Supporting Information Available: Structures of DNA bases and the corresponding PNA monomers (S1), TEM images (S2), and dilution control experiments (S3) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Moller, R.; Csaki, A.; Kohler, J. M.; Fritzche, W. *Nucleic Acid Res.* **2000**, *28*, e91(1−5).
(2) Liu, T.; Tang, J.; Jiang, L. *Biochem. Biophys. Res. Commun.* **2004**, *313*, 3−7.
(3) Woffendin, C.; Yang, Z.; Udaykumar; Xu, L.; Yang, N.; Sheehy, M. J.; Nabel, G. J. *Proc. Natl. Acad. Sci.* U.S.A. **1994**, *91*, 11581−11585.
(4) Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128−4158.
(5) Bielinska, A.; Eichman, J. D.; Lee, I.; Baker, J. R., Jr.; Balogh, L. *J. Nanopart. Res.* **2002**, *4*, 395−403.
(6) Nakao, H.; Shiigi, H.; Yamamoto, Y.; Tokonami, S.; Nagaoka, T.; Sugiyama, S.; Ohtani, T. *Nano Lett.* **2003**, *3*, 1391−1394.
(7) (a) Ganesh, K. N.; Nielsen, P. E. *Curr. Org. Chem.* **2000**, *4*, 931−943. (b) Chakrabarti, R.; Klibanov, A. M. *J. Am. Chem. Soc.* **2003**, *125*, 12531−12540.
(8) Storhoff, J. J.; Elghanian, R.; Mirkin, C. A.; Letsinger, R. L. *Langmuir* **2002**, *18*, 6666−6670.
(9) Jang, N. H. *Bull. Korean Chem. Soc.* **2002**, *23*, 1790−1800.
(10) Hiromi, K.; Petrovykh, D. Y.; Tarlov, M. J.; Whitman, L. J. *J. Am. Chem. Soc.* **2003**, 125, 9014−9015.
(11) Demers, L. M.; Ostblom, M.; Zhang, H.; Jang, N. H.; Liedberg, B.; Mirkin, C. A. *J. Am. Chem. Soc.* **2002**, *124*, 11248−11249.
(12) Thompson, G.; Owen, D.; Chalk, P. A.; Lowe, P. N. *Biochemistry* **1998**, *37*, 7885−7891.
(13) Kunne, A. G. E.; Sieber, M.; Meierhans, D.; Allemann, R. K. *Biochemistry* **1998**, *37*, 4217−4223.
(14) Wenk, M. R.; Seelig, J. *Biochemistry* **1998**, *37*, 3909−3916.
(15) Patil, V.; Malvankar, R. B.; Sastry, M. *Langmuir* **1999**, *15*, 8197−8206.
(16) Kumar, A.; Mandal, S.; Selvakannan, P. R.; Pasricha, R.; Mandale, A. B.; Sastry, M. *Langmuir* **2003**, *19*, 6277−6282.

JA046785G