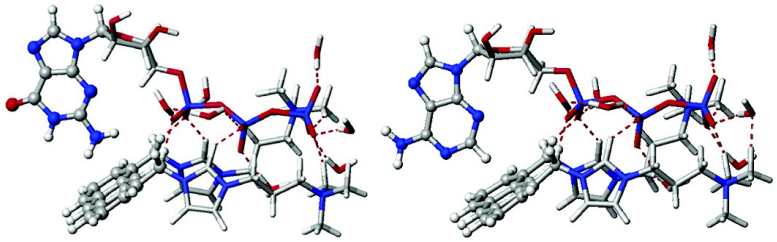
Communication

Fluorescent GTP-Sensing in Aqueous Solution of Physiological pH

Ji Young Kwon, N. Jiten Singh, Ha Na Kim, Sook Kyung Kim, Kwang S. Kim, and Juyoung Yoon

J. Am. Chem. Soc., **2004**, 126 (29), 8892-8893• DOI: 10.1021/ja0492440 • Publication Date (Web): 30 June 2004



J|A|C|S
COMMUNICATIONS

Published on Web 06/30/2004

Fluorescent GTP-Sensing in Aqueous Solution of Physiological pH

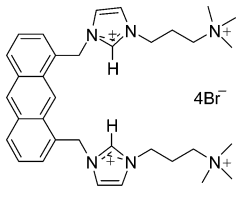
Ji Young Kwon,† N. Jiten Singh,‡ Ha Na Kim,† Sook Kyung Kim,† Kwang S. Kim,*,‡ and Juyoung Yoon*,†

Department of Chemistry and Division of Nano Sciences, Ewha Womans University, 11-1 Daehyon-Dong, Sodaemun-Ku, Seoul 120-750, Korea, and National Creative Research Initiative Center for Superfunctional Materials, Department of Chemistry, Division of Molecular and Life Sciences, Pohang University of Science and Technology, San 31, Hyojadong, Namgu, Pohang 790-784, Korea

Received February 11, 2004; E-mail: jyoon@ewha.ac.kr; kim@postech.ac.kr

With the aid of supramolecular chemistry, recognition and sensing of anionic anaylates has recently emerged as a key research field.[1] In this regard, fluorescence is an important detection method due to its simplicity and high detection limit.[2] Even though considerable efforts have been devoted to developing fluorescent chemosensors for various anions in the past decades,[2e,3] there have been relatively few reports on adenosine 5′-triphosphate (ATP)-selective receptors that show the fluorescent changes[4] or color changes.[5] ATP is known to be the universal energy currency in all of the biological systems and has been a significant target for the design of molecular receptors. Even though guanosine 5′-triphosphate (GTP) also plays an important role in biological systems, to our knowledge, no fluorescent chemosensor that selectively communicates with GTP in 100% aqueous solution has been reported thus far. Recently, Anslyn and co-workers[4a] have reported the intelligent combinatorial library-based sensors to differentiate between the structurally similar compounds ATP and GTP with the help of principal component analysis (PCA). However, their quantitative binding studies have not yet been made available. Herein, we report for the first time a new water-soluble imidazolium anthracene derivative, which not only differentiates the structurally similar compounds GTP and ATP but also acts as a potential fluorescent chemosensor for GTP in 100% aqueous solution (pH = 7.4, 10 mM HEPES). Our new fluorescent chemosensor senses GTP by a chelation-enhanced fluorescence quenching (CHEQ) effect, whereas it displays a chelation-enhanced fluorescence (CHEF) effect for ATP, ADP, and AMP.

In contrast to the typical hydrogen-bonding type for the anion binding such as amide, pyrrole, urea, etc., the benzene-based tripodal imidazolium receptors utilize strong $(C-H)^{+}-X^{-}$ hydrogen bonding for halide anion recognition.[6] In this regard, host **1** was



1

synthesized by the reaction of 1,8-bis-(imidazolylmethyl)anthracene[6a] with (3-bromopropyl)-trimethylammonium bromide in acetonitrile followed by recrystallization (acetonitrile/methanol = 1:1, v/v) in 70% yield (see Supporting Information).

As shown in Figure 1, compound **1** (6 μM) displayed a CHEF effect with ATP, even though **1** also displayed relatively small

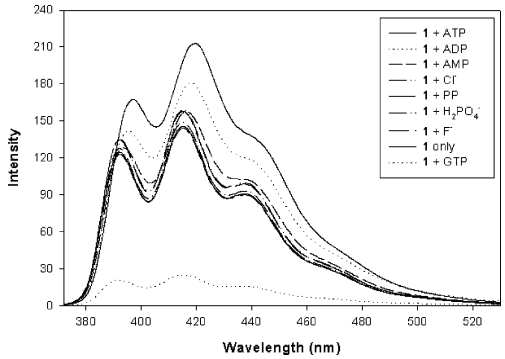


Figure 1. Fluorescent emission changes of **1** (6 μM) upon addition of tetrabutylammonium salts of HSO_4^-, Cl^-, F^-, $H_2PO_4^-$, and pyrophosphate and sodium salts of AMP, ADP, ATP, and GTP (300 equiv) at pH 7.4 (50mM HEPES) (excitation at 367 nm).

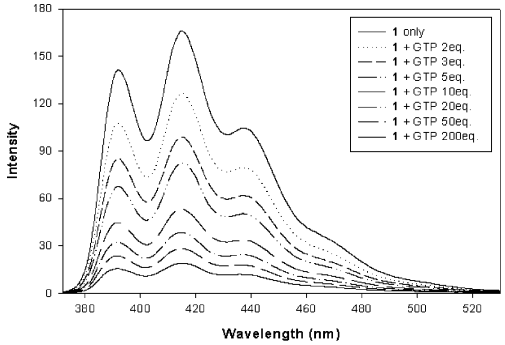


Figure 2. Fluorescent titrations of **1** (3 μM) upon addition of sodium salt of GTP at pH 7.4 (10 mM HEPES) (excitation at 367 nm).

CHEF effects for ADP and AMP. On the other hand, there was a significant CHEQ effect with slight red-shift for GTP. The guanine base in GTP acts as a fluorescence quencher, which results in the CHEQ effect on host **1**. There were almost no fluorescent changes even when 300 equiv of pyrophosphate, $H_2PO_4^-$, F^-, or Cl^- were added. All of the fluorescent experiments were done in 100% aqueous solution at pH 7.4 (10 or 50 mM HEPES). Figure 2 clearly shows the CHEQ effects with increasing GTP concentration. From the fluorescent titrations the association constants for GTP, ATP, ADP, and AMP are 87000, 15000, 610, and 120 M^{-1} (errors < 10%), respectively (Table 1).[7] In addition, the Job-plot analysis indicates the formation of 1:1 complexes.

Addition of **1** equiv of GTP (Figure 3, also see Supporting Information) or ATP (see Supporting Information) to a 2 mM solution of **1** at pD 7.4 in D_2O caused significant upfield shifts of the aromatic protons of the anthracene moiety of **1** in addition to the H-8 of GTP (0.45), H-2 and H-8 of ATP (0.41 and 0.56, respectively), and the anomeric protons. Similar chemical shifts of

† Ewha Womans University.
‡ Pohang University of Science and Technology.

10.1021/ja0492440 CCC: $27.50

Table 1. Experimental Free Energy Changes in Aqueous Solution for the **1**−Anion Complexes (kcal/mol)[a]

	GTP	ATP	ADP	AMP
$K_a(M^{-1})^a$	87000	15000	614	121
$-\Delta G_{expt}$	6.73	5.69	3.80	2.84

[a] Association constants K_a (M^{-1}) were measured using the fluorescence titrations. ΔG_{expt} is the change in Gibbs free energy in solution measured in 100% aqueous solution at pH 7.4 fluorescence titrations.

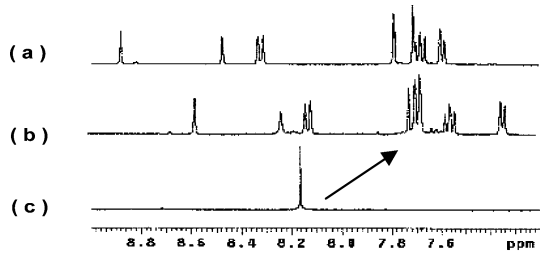


Figure 3. Partial NMR spectra for (a) host **1** (2 mM), (b) **1**+ GTP (1.1 equiv) and (c) GTP at pD 7.4.

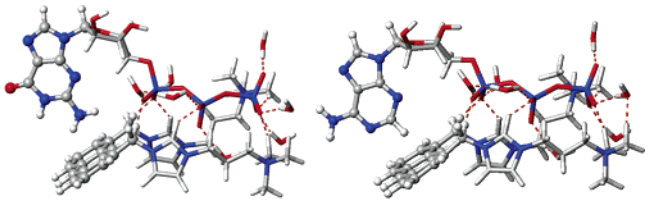


Figure 4. Computed geometries of **1**−GTP and **1**−ATP complexes. While stick represents the results at the medium level of theory (PM3), ball-and-stick represents those at high level (MP2/6-31G*). The dotted lines represent the H-bond distances less than 2.0 Å.

hydrogen in positions 2 and 7, 3 and 6, and 4 and 5 of anthracene and different chemical shifts for hydrogen at positions 9 and 10 suggest additional interactions of the nucleic bases with the middle ring of anthracene moiety of **1** in the T-shape.

We note that host **1** shows a selective binding with GTP over ATP, ADP, AMP, pyrophosphate, $H_2PO_4^-$, F^-, and Cl^-. The selectivity for GTP (K_a = 87000 M^{-1}) is about 6 times that for ATP, and over 100 times those for ADP, AMP, pyrophosphate, $H_2PO_4^-$, F^-, and Cl^-. Figure 4 shows theoretically optimized structures of host **1** complexed with GTP/ATP.[8] The vertical distance from the H atoms in NH_2 (GTP) and from the H atom at the C-2 position (ATP) of nucleic bases to the plane of the anthracene ring for **1**−GTP and **1**−ATP are ∼2.4 Å, indicating π−H interactions. The selectivity toward GTP over ATP by **1** is likely due to the difference in π−H interaction strength between **1**−GTP and **1**−ATP complexes. The calculated binding energy between anthracene and guanine in water solution is 1.0 kcal/mol larger than that between anthracene and adenine, which may explain the difference in binding energy between **1**−GTP and **1**−ATP. Given that the π−H interaction tends to increase with the increased dipole strength of the counter-molecule solvating the anthracene, the calculated results are consistent with the fact that the dipole moments of guanine and adenine perpendicular to the anthracene ring are 6.37 and 1.45 D, respectively. Furthermore, given that the dipole moment of the solvent water is ∼3 D, it would explain the fluorescence quenching/enhancing effect by GDP/ATP, since molecules solvating the anthracene with larger/smaller dipole moments than the solvent water tend to enhance/reduce the vertical emission transition more and thereby to reduce/enhance the fluorescent transition more.

In conclusion, we have shown that a new water-soluble and fluorescent imidazolium receptor **1** effectively and selectively recognizes the biologically important GTP over other structurally similar anions in aqueous solution of physiological pH 7.4.

Acknowledgment. This work was supported by Korean Science and Engineering Foundation (R02-2003-000-10022-0), the Creative Research Initiative of the Korean Ministry of Science and Technology, and BK21.

Supporting Information Available: Experimental details of the synthesis of the compound; the fluorescent titration spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For recent reviews for anion receptors, see; (a) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486. (b) Snowden, T. S.; Anslyn, E. V. *Chem. Biol.* **1999**, *3*, 740. (c) Antonisse, M. M. G.; Reinhoudt, D. N. *Chem. Commun.* **1998**, 143. (d) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609. (e) Rudkevich, D. M.; Brzozka, Z.; Palys, M.; Visser, H. C.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 467. (e) Clifford, T.; Mason, S.; Llinares, J. M.; Bowman-James, K. *J. Am. Chem. Soc.* **2000**, *122*, 1814. (f) Mahoney, J. M.; Beatty, A. M.; Smith, B. D. *J. Am. Chem. Soc.* **2001**, *123*, 5847.

(2) (a) *Fluorescent Chemosensors for Ion and Molecular Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993. (b) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302. (c) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1994**, 197. (d) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T. A.; Huxley, T. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (e) Martínez-Máñez, R.; Sancanón, F. *Chem. Rev.* **2003**, *103*, 4419.

(3) (a) Cho, E. J.; Moon, J. W.; Ko, S. W.; Lee, J. Y.; Kim, S. K.; Yoon, J.; Nam, K. C. *J. Am. Chem. Soc.* **2003**, *125*, 12376. (b) Ojida, A.; Inoue, M.; Mito-oka, Y.; Hamachi, I. *J. Am. Chem. Soc.* **2003**, *125*, 10184.

(4) (a) McCleskey, S. C.; Griffin, M. J.; Schneider, S. E.; McDevitt, J. T.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 1114. (b) Sancenón, F.; Benito, A.; Lloris, J. M.; Martínez-Máñez, R.; Pardo, T.; Soto, J. *Helv. Chim. Acta* **2002**, *85*, 1505. (c) Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542. (d) Kumar, C. V.; Asuncion, E. H. *J. Am. Chem. Soc.* **1993**, *115*, 8547. (e) Van Arman, S.; Czarnik, A. W. *Supramol. Chem.* **1993**, *1*, 99. (f) Schneider, H. J.; Blatter, T.; Palm, B.; Pfingstag, U.; Rudiger, V.; Theis, I. *J. Am. Chem. Soc.* **1992**, *114*, 7704. (g) Van Arman, S.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 5376. (h) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896. (i) Shi, Y.; Schneider, H. J. *J. Chem. Soc., Perkin Trans 2* **1999**, 1797.

(5) Sancenón, F.; Descalzo, A. B.; Martínez-Máñez, R.; Miranda, M. A.; Soto, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2640.

(6) (a) Yoon, J.; Kim, S. K.; Singh, N. J.; Lee, J. W.; Yang, Y. J.; Chellappan, K.; Kim, K. S. *J. Org. Chem.* **2004**, *69*, 581. (b) Kim, S. K.; Singh, N. J.; Kim, S. J.; Kim, H. G.; Kim, J. K.; Lee, J. W.; Kim, K. S.; Yoon, J. *Org. Lett.* **2003**, *5*, 2083. (c) Yun, S.; Ihm, H.; Kim, H. G.; Lee, C. W.; Indrajit, B.; Oh, K. S.; Gong, Y. J.; Lee, J. W.; Yoon, J.; Lee, H. C.; Kim, K. S. *J. Org. Chem.* **2003**, *68*, 2467. (d) Ihm, H.; Yun, S.; Kim, H. G.; Kim, J. K.; Kim, K. S. *Org. Lett.* **2002**, *4*, 2897. (e) Sato, K.; Arai S.; Yamagishi, T. *Tetrahedron Lett.* **1999**, *40*, 5219.

(7) (a) Conners, K. A. *Binding Constants*; Wiley: New York, 1987. (b) Association constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom.

(8) Geometry optimizations of **1**−ATP/−GTP complexes with seven water molecules near each oxygen atom of the triphosphate group of the ATP/GTP were carried out using ONIOM (MP2/6-31G* calculations for the anthracene moiety and nucleic base and PM3 calculations for the remaining part). The binding energy of **1**−GTP complex is calculated to be 10 kcal/mol more stable than that of **1**−ATP complex. This energy difference comprises the electrostatic term (∼8.5 kcal/mol mainly due to the interaction between the $(C-H)^+$ moieties of **1** and the phosphate groups of ATP/GTP) and the π−H interaction term (∼1.5 kcal/mol). The π−H binding energies of **1**−GTP and **1**−ATP in the gas phase are 3.5 and 2 kcal/mol, respectively. The difference (8.5 kcal/mol) in electrostatic interaction would be almost nullified in the presence of highly polar solvent (water), while the difference in π−H interaction is little affected by solvents. The MP2/6-31G* binding energy difference between the anthracene-guanine and anthracene-adenine in the gas phase and aqueous medium are 1.5 and 1.0 kcal/mol, respectively; the latter is close to the experimental energy difference (1.0 kcal/mol).

JA0492440