J|A|C|S
COMMUNICATIONS

Published on Web 08/22/2002

An Artificial Guanine that Binds Cytidine through the Cooperative Interaction of Metal Coordination and Hydrogen Bonding

Fabrizio Mancin and Jik Chin*

Department of Chemistry, University of Toronto, 80 St. George St., Toronto, Ontario, Canada M5S 3H6

Received May 15, 2002

Over the past decade, there has been much interest in developing receptors for DNA bases. Some of these receptors are metal based[1] (e.g., (cyclen)Zn, Chart 1),[2] while others are purely organic.[3] Nucleobase receptors may be useful for sensing nucleotides involved in signal transduction (e.g., cAMP and cGMP) or for detecting the levels of therapeutic agents (e.g., Lamivudine or AZT).[4] Furthermore, nucleoside analogues that bind tightly and selectively to DNA bases may be useful for making antigene or antisense agents that suppress gene expression at the transcriptional or translational level.[5] Nucleoside analogues may also be used to develop novel base pairs[6,7] (e.g., (dipic)Cu(pyr), Chart 1).[8] Many of the receptors reported to date bind to the nucleobases in nonaqueous solvents through hydrogen bonding.[3] A major challenge is in developing receptors that bind tightly to the nucleobases in polar solvents such as water or DMSO. Our interest in understanding the cooperative interaction between metal coordination and hydrogen bonding[9,10] led to the development of a Cd(II) complex (**L**Cd, Chart 1) that binds tightly and selectively to cytidine in DMSO.

The cadmium complex (**L**Cd(Cl)) was prepared by adding 1 equiv each of $Cd(Cl)_2$ and NaOH to a methanolic solution of the ligand[11] (**L**H). The formation of the complex between cytidine and the receptor was followed by ^{1}H NMR in d_6-DMSO. Titration of cytidine with the metal complex resulted in a downfield shift (Figure 1) of the signals due to the amino (H_a and H_b, Chart 1) and aromatic hydrogens (H_5 and H_6), while the signals due to the sugar hydrogens show only minor changes. The dramatic downfield shift of one of the amino hydrogens (H_a) is indicative of hydrogen bonding, and the downfield shift of the aromatic hydrogens is expected from coordination of cytidine to the metal complex. The equilibrium constant for binding of cytidine to the receptor was determined by plotting the ^{1}H NMR signals of cytidine as a function of the receptor concentration and fitting the data according to the equation for 1:1 complexation (Figure 2).[12] On the basis of these results, we suggest that cytidine coordinates to the metal complex to give **L**Cd**C** as shown in Chart 1.

In designing the cytidine receptor, we tried to mimic the Watson−Crick interaction between guanine and cytosine (**G**−**C**, Chart 1). The receptor−cytidine interaction (**L**Cd**C**) resembles the guanine−cytosine interaction except that one of the Watson−Crick hydrogen bonds is replaced with a coordinative bond. Molecular mechanics computation[13] shows that the size of the metal ion is important for the hydrogen bonds to form in **L**Cd**C**. For example, if the cadmium ion (covalent radius 1.48 Å) in **L**Cd**C** is replaced with a smaller metal ion such as zinc (1.25 Å), the ligand (**L**) and the substrate (**C**) are brought about one-half of an angstrom too close for the hydrogen bonds to form. Molecular mechanics computation shows that **L**Cd**C** is essentially planar in structure and ideally positioned for formation of the hydrogen bonds. In contrast, the structure of **L**Zn**C** is twisted due to steric interactions (Figure 3). We do not observe any binding of cytidine to the Zn(II) complex (**L**Zn) under the same conditions used for formation of **L**Cd**C** (Table 1), even though the two metal ions are similar in many respects.

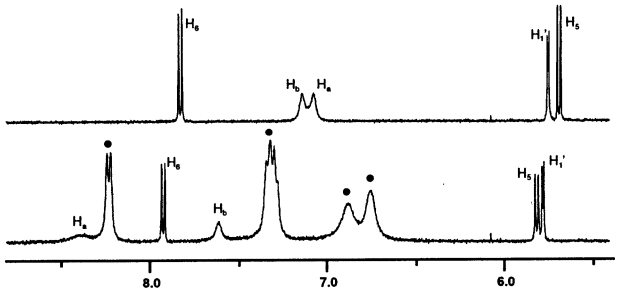
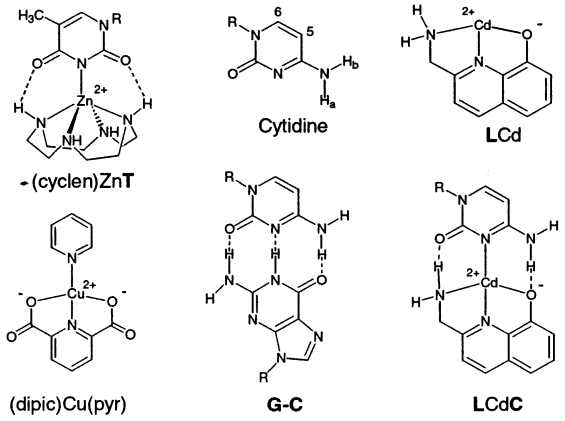


Figure 1. ^{1}H NMR spectra of (a) cytidine (3.0 mM), (b) cytidine (3.0 mM) and **L**Cd (15 mM, 60% of the ternary complex formed). The symbol (●) indicates the signals relative to **L**Cd.

Chart 1



The results reported in Table 1 highlight the effectiveness of the Cd(II) complex for binding cytidine. The combination of metal coordination and hydrogen bonding leads to a substantial increase in the complexation between the receptor (**L**Cd) and cytidine. This receptor binds cytidine about 30 times more tightly than does guanosine ($K = 3.7$ M^{-1} at 37 °C).[14] It is about 20 times more effective than ligand free $CdCl_2$ ($K = 7.2$ M^{-1}) or $ZnCl_2$ ($K = 3.0$ M^{-1})[12] despite the fact that the ligand (**L**) reduces the Lewis acidity of the cadmium ion. Selectivity of the receptor (**L**Cd) is another remarkable feature. Adenosine and guanosine have potential metal binding sites (N7 on guanosine and N1 and N7 on adenine). However, only cytidine may coordinate and form two hydrogen bonds with the receptor.

* To whom correspondence should be addressed. E-mail: jchin@chem.utoronto.ca.

10.1021/ja026922e CCC: $22.00

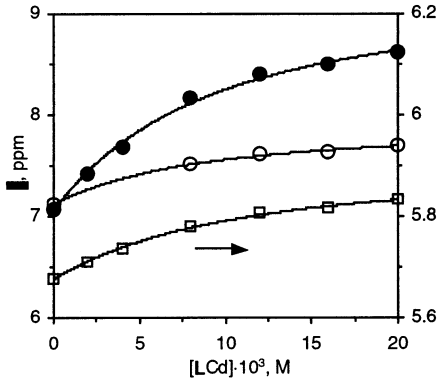


Figure 2. NMR titration of cytidine with **L**Cd, chemical shift changes relative to the cytidine protons H_a (●), H_b (○), and H_5 (□) as a function of the **L**Cd concentration (lines: best fit). Conditions: [cytidine] = 2.0 mM, d_6-DMSO, 25 °C.

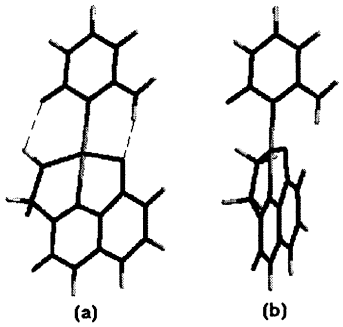


Figure 3. Computed structures of (a) **L**Cd**C** (hydrogen bond indicated with dashed lines) and (b) **L**Zn**C**.

Table 1. Association Constants for the Complexes between the Nucleosides and Receptors at 25 °C

receptor	nucleoside	K, M^{-1}
LZn	**C**	<5
LCd	**C**	117
LCd	**A**	<2
LCd	**G**	<2
LCd	**T**	<2

The energy of a hydrogen bond between uncharged groups is about 1.0−1.4 kcal mol^{-1}, whereas that involving a charged group is slightly higher (1.5−2.8 kcal mol^{-1}).[15] The energy of a hydrogen bond between a positively charged donor and negatively charged acceptor is about 4 kcal mol^{-1}.[16] In general, it is difficult to obtain significant increases in binding of molecules with a single hydrogen bond in water.[17] However, we have recently shown that the equilibrium constant for binding of hydroxide to a metal complex in water can be increased over 100-fold by exchanging a single weak hydrogen bond that does not have charge complimentarity (1 kcal mol^{-1}) with a strong one that does (4 kcal mol^{-1}).[10] The amino nitrogen of cytosine is partially positive, while the carbonyl oxygen of cytosine is partially negative. The hydrogen bond acceptor in **L**Cd is negative, whereas the donor is partially positive. This charge complimentarity between the hydrogen bond donor and acceptor groups in **L**Cd and **C** may be the reason for the high selectivity and sensitivity of the receptor (Table 1).

It is interesting to compare our cytidine receptor with the thymidine receptor reported by Kimura et al. ((cyclen)Zn**T**, Chart 1).[2] Both receptors bind to the target nucleobase through cooperative interaction of metal coordination and hydrogen bonding. While the zinc ion in the thymidine receptor is smaller than the cadmium ion in the cytidine receptor, the bent structure of the strained cyclen complex allows hydrogen bonding to take place. In contrast to the globular structure of the receptor−thymidine complex, the receptor−cytidine complex (**L**Cd**C**) should be planar (Figure 3), resembling the natural base pair.

A number of interesting novel base pairs have been reported[6] including those involving metal ions.[7] Planar metallo base pairs ((dipic)Cu(pyr), Chart 1)[8] can benefit from stacking interactions within the duplex DNA. The planar receptor−cytidine structure (**L**Cd**C**) could make the receptor an ideal artificial nucleobase for antisense applications.[18]

To our knowledge, **L**Cd represents the first cytidine selective receptor that can operate in polar solvents. This result opens the way to sensing cytosine based compounds such as lamivudine and to the synthesis of antisense molecules with enhanced binding affinity. The general strategy presented here for developing the artificial guanine should also be applicable for developing artificial cytosine, adenine, and thymine.

Acknowledgment. We thank the Natural Sciences and Engineering Council of Canada for financial support of this work. F.M. thanks financial support from MURST under the framework of the "Supramolecular Devices" project.

References

(1) (a) Marzilli, L. G.; Kistenmacher, T. J. *Acc. Chem. Res.* **1977**, *10*, 146−152. (b) Fabbrizzi, L.; Licchelli, M.; Mancin, F.; Pizzeghello, M.; Rabaioli, G.; Taglietti, A.; Tecilla, P.; Tonellato, U. *Chem.-Eur. J.* **2002**, *8*, 94−101.
(2) Shionoya, M.; Ikeda, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 3848−3859.
(3) (a) Baudoin, O.; Gonnet, F.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Tabet, J.-C.; Lehn, J.-M. *Chem.-Eur. J.* **1999**, *5*, 2762−2771. (b) Wilds, C. J.; Maier, M. A.; Tereshko, V.; Manoharan, M.; Egli, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 115−117.
(4) (a) Whelen, A. C.; Persing, D. H. *Annu. Rev. Microbiol.* **1996**, *50*, 349−373. (b) Sidransky, D. *Science* **1997**, *278*, 1054−1059.
(5) (a) Monia, B. P.; Johnston, J. F.; Geiger, T.; Muller, M.; Fabbro, D. *Nat. Med. (N. Y.)* **1996**, *2*, 668−675. (b) Matteucci, M. D.; Wagner, R. W. *Nature* **1996**, *384*, 20−22.
(6) (a) Piccirilli, J. A.; Krauch, T.; Moroney, S. E.; Benner, S. A. *Nature* **1990**, *343*, 33−37. (b) Guckian, K. M.; Krugh, T. R.; Kool, E. T. *Nat. Struct. Biol.* **1998**, *5*, 954−959.
(7) (a) Tanaka, K.; Shionoya, M. *J. Org. Chem.* **1999**, *64*, 5002−5003. (b) Atwell, S.; Weizman, H.; Tor, Y. *J. Am. Chem. Soc.* **2001**, *123*, 3375−3376.
(8) Meggers, E.; Spraggon, G.; Schultz, P. G. *J. Am. Chem. Soc.* **2001**, *123*, 12364−12367.
(9) Wall, M.; Linkletter, B.; Williams, D.; Lebuis, A.-M.; Hynes, R. C.; Chin, J. *J. Am. Chem. Soc.* **1999**, *121*, 4710−4711.
(10) Chin, J.; Chung, S.; Kim, D. *J. Am. Chem. Soc.* **2002**, *124*, 10948−10949.
(11) Stevenson, R. L.; Freiser, H. *Anal. Chem.* **1967**, *39*, 1354−1358.
(12) Marzilli, L. G.; De Castro, B.; Caradonna, J. P.; Stewart, R. C.; Van Vuuren, C. P. *J. Am. Chem. Soc.* **1980**, *102*, 916−924.
(13) Molecular mechanics computation was performed using Quantum Cache version 3.2 from Oxford Molecular Ltd. Square planar geometry was assumed for clarity of view for both the Cd(II) and the Zn(II) complexes.
(14) Newmark, R. A.; Cantor, C. R. *J. Am. Chem. Soc.* **1968**, *90*, 5010−5017.
(15) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin, 1991.
(16) Fersht, A. R.; Shi, J. P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M.; Winter, G. *Nature* **1985**, *314*, 235−238.
(17) Perrin, C. L.; Nielson, J. B. *Annu. Rev. Phys. Chem.* **1997**, *48*, 511−544.
(18) Cd(II) may need to be replaced with less toxic metal ions. The equilibrium constant for binding of Cd(II) to **L** is about 10^{11} M^{-1}.

JA026922E