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Simultaneous N7,O6-Binding of Guanine to Two Zinc Centers and Its Possible Biological Significance

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The reaction of $ZnCl_2$ with 9-ethylguanine (9-EtGH) produced a novel dinuclear Zn(II) complex, $[Zn_2Cl_4(H_2O)(\mu$-9-EtGH-*N7*,*O6*)(9-EtGH-*N7*), **1**. The X-ray structure analysis (monoclinic, $P2_1$ (No. 4), $a = 11.0636(6)$ Å, $b = 6.6546(4)$ Å, $c = 15.9630(9)$ Å, $\beta = 101.069(1)°$, $V = 1153.4(1)$ Å^3, $Z = 2$) revealed that one of the tetrahedrally coordinated Zn(II) atoms binds to the N7 site of 9-EtGH and to the exocyclic O6 atom of another 9-EtGH molecule. The remaining Zn(II) atom binds to the N7 site of the second 9-EtGH moiety.

As an essential biological element, zinc is present in several proteins, including those interacting with DNA.[1] The structural and catalytic role of zinc in DNA-binding proteins is now quite well established.[2] However, there are also several biological processes in which direct interactions between zinc ions and nucleic acids are encountered. Binding of Zn(II) ions to the 5 S RNA gene sequence of *Xenopus borealis* produces strong bending of the DNA.[3] A significant enhancement of kinking of circular DNA has been attributed to direct Zn(II) binding.[4] Interaction of Zn(II) with the AGCT sequences induces conformational changes in negatively supercoiled DNA.[5] Zinc is also important for the stabilization of intramolecular purine·purine−pyrimidine DNA triplexes (*H-DNA) formed in the $d(GA{\cdot}CT)_n$ sequences.[6]

It has been suggested that all these events are caused by direct covalent binding of the Zn(II) ions to the guanine bases, specifically to their N7 sites. Binding of this kind has been characterized in numerous model compounds.[7] Additionally, the importance of the binding has been demonstrated by several NMR investigations of Zn(II)−oligonucleotide complexes[8] as well as by an X-ray crystallographic report on the interaction between Zn(II) ions and $tRNA^{Phe}$.[9]

Several earlier spectroscopic studies[10,11] suggested that Zn(II) might also interact with the exocyclic O6 site of guanine. Furthermore, an X-ray analysis of the dimeric Mo, Ru, and Rh complexes with 9-ethylguanine indicated that, in principle, metal binding to O6 is attainable.[12] In this Communication, we present data on a Zn(II) complex with 9-ethylguanine in which the metal ion binds simultaneously to N7 of one guanine and O6 of another guanine moiety. The crystalline dimeric compound, $[Zn_2Cl_4(H_2O)(\mu$-9-EtGH-*N7*,*O6*)(9-EtGH-*N7*) (**1**) (9-EtGH = 9-ethylguanine), was obtained in the reaction between 9-EtGH and $ZnCl_2$ (molar ratio 1:5) in methanol at 65 °C[13] (Scheme 1). The X-ray structure of the compound[14] is presented in Figure 1. The zinc center adopts a distorted tetrahedral geometry by binding to the N7 site of a 9-ethylguanine ligand, the O6 site of another 9-ethylguanine moiety and two chlorine atoms. The second 9-ethylguanine base is bound through its N7 atom to another zinc atom.

The geometry around this metal center is also tetrahedral with the remaining ligands being a water molecule and two chlorine atoms. The Zn−N7 distances are close to those previously found in Zn(II) complexes with guanosine mono-

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(1) Coleman, J. E. *Annu. Rev. Biochem.* 1992, *61*, 897.
(2) Lipscomb, W. N.; Strater, N. *Chem. Rev.* **1996**, *96*, 2375.
(3) Nickol, J.; Rau, D. C. *J. Mol. Biol.* **1992**, *228*, 1115.
(4) Han, W.; Lindsay, S. M.; Dlakic, M.; Harrington, R. E. *Nature* **1997**, *386*, 563.
(5) Kang, S.; Wells, R. D. *J. Biol. Chem.* **1994**, *269*, 9528.
(6) Casasnovas, J. M.; Huertas, D.; Ortiz-Lombardia, M.; Kypr, J.; Azorin, F. *J. Mol. Biol.* **1993**, *233*, 671.
(7) *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1996; Vol. 32.
(8) (a) Froystein, N. A.; Davis, J. T.; Reid, B. R.; Sletten, E. *Acta Chem. Scand.* **1993**, *47*, 649. (b) Jia, X.; Zon, G.; Marzilli, L. G. *Inorg. Chem.* **1991**, *30*, 228.
(9) Rubin, J. R.; Wang, J.; Sundaralingam, M. *Biochim. Biophys. Acta* **1983**, *756*, 111.
(10) Duguid, J.; Bloomfield, V. A.; Benevides, J.; Thomas, G. J. *Biophys. J.* **1993**, *65*, 1916.
(11) Marzilli, L. G. *Advances in Inorganic Biochemistry Vol. 3. Metal Ions in Genetic Information Transfer*; Eichhorn, G. L., Marzilli, L. G., Eds.; Elsevier/North-Holland: New York, 1981; p 47.
(12) (a) Catalan, K. V.; Hess, J. S.; Maloney, M. M.; Mindiola, D. J.; Ward, D. L.; Dunbar, K. R. *Inorg. Chem.* **1999**, *38*, 3904. (b) Crawford, C. A.; Day, E. F.; Saharan, V. P.; Folting, K.; Huffman, J. C.; Dunbar, K. R.; Christou, G. *Chem. Commun.* **1996**, 1113.
(13) Synthesis of $[Zn_2Cl_4(H_2O)(\mu$-9-EtGH-*N7*,*O6*)(9-EtGH-*N7*)] (**1**): To a suspension of finely divided 9-EtGH (33 mg, 0.18 mmol) in methanol (5 mL) was added a solution of $ZnCl_2$ (125 mg, 0.92 mmol) in 10 mL of methanol with stirring. The reaction mixture was stirred for 18 h at 65 °C. The resulting clear solution was filtered and then 5 mL of ethanol added. The filtrate was allowed to concentrate upon slow evaporation at 20 °C (ca. two weeks) to yield colorless crystals (18.7 mg; 32%). Anal. Calcd for $C_{14}H_{20}N_{10}O_3Cl_4Zn_2$ (Found): C, 25.90 (26.01); N, 21.58 (21.60); H, 3.11 (3.09)%. IR (KBr, cm^{-1}): 1712 vs, 1633 vs, 1594 vs, 1489 s, 1349 s, 628 m. ^{1}H NMR (300 MHz, D_2O, TSP): $\delta = 7.74$ (s, 1H), 3.99 (q, 2H, CH_2), 1.35 (t, 3H, CH_3).

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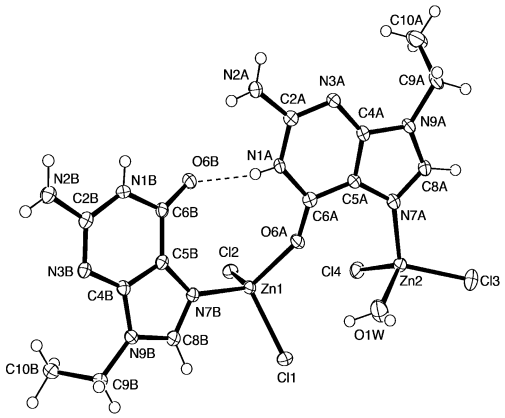
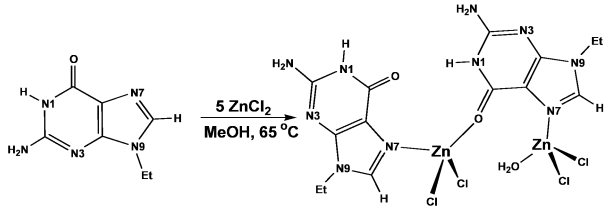


Figure 1. ORTEP drawing of **1** with 50% thermal ellipsoids. Selected bond distances (Å): Zn1−Cl1, 2.2670(5); Zn1−Cl2, 2.2474(6); Zn1−O6A, 1.986(1); Zn1−N7B, 2.013(2); Zn2−Cl3, 2.2179(6); Zn2−Cl4, 2.2390(6); Zn2−O1W, 2.007(2); Zn2−N7A, 1.995(2). Selected bond angles (deg): Cl1−Zn1−Cl2, 112.91(2); O6A-Zn1−N7B, 103.89(5); Zn1−O6A−C6A, 150.3(1); Cl3−Zn2−Cl4, 114.59(2); O1W−Zn2−N7A, 102.29(9).

Scheme 1



1

phosphate (GMP),[15] and the Zn1−O6A separation of 1.986(1) Å is quite similar to that observed in the trinuclear zinc complex with 1-methylcytosine.[16] Interestingly, the distance between the metal centers (4.609 Å) is in the range found in multi-zinc enzymes, where two or three zinc atoms coexist as catalytic and cocatalytic sites.[17] The complex is additionally stabilized by a strong intramolecular hydrogen bond N1A−H···O6B (N1A···O6B, 2.641 Å), bridging the two 9-ethylguanine ligands.

These ligands are not exactly coplanar as the angle between them is 26.6°. It should also be emphasized that both the N7 and O6 sites of guanine are exposed toward the major groove of the DNA duplex. The present structure indicates that these two sites could be simultaneously involved in binding Zn(II) ions, without invoking significant steric clashes.

The binding mode presented here may have some significance for several biological interactions involving Zn(II) ions. For instance, these ions have been found to promote the temperature-dependent unwinding and rewinding of DNA.[18] It has been postulated that the function of Zn(II) ions in those processes is to hold the separate strands together, possibly by the formation of guanine N7−zinc−guanine N7 cross-links.[19] The present structure suggests also that the guanine N7−zinc−guanine O6 cross-linking could be quite feasible, enhancing the ability of zinc to control the renaturation process.

DNA quadruplexes containing guanine quartets are believed to play an important role in several regions of the genome, including telomeres.[20] Several divalent transition cations dissociate the quadruplex structures.[21] It has been suggested[22] that Zn(II) ions prevent the quadruplex formation by binding to the guanine sites N7 and O6 that are required for quadruplex assembly. Our model compound provides the first structural evidence that such a simultaneous binding may indeed happen.

Finally, the ability of Zn(II) ions to bind to the O6 site of guanine could potentially be utilized in the design of the inhibitors of *O6*-alkylguanine-DNA alkyltransferase (Atase), a DNA repair protein responsible for removing alkyl groups from the guanine residues.[23] High concentrations of Atase in tumor cells increase the resistance of these cells to the *O6*-alkylating agents used in chemotherapy. Several organic compounds have been designed and synthesized as inhibitors of Atase.[24] The design of a Zn(II)-based inhibitor seems feasible, taking into account the high affinity of zinc for the cysteine residues involved in the inactivation processes.

Supporting Information Available: Crystallographic data of **1** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Crystal data for **1**: monoclinic space group $P2_1$ (No. 4); a = 11.0636(6) Å, b = 6.6545(4) Å, c = 15.9630(9) Å, β = 101.069(1)°; V = 1153.4(1) Å^3; Z = 2; d_{calcd} = 1.869 Mg/m^3; Mo Kα radiation, λ = 0.71073 Å; T = −120 °C; $2\theta_{max}$ = 65°; 16002 reflections were collected on a Bruker SMART APEX CCD diffractometer, of which 8202 with $I > 2\sigma(I)$ were used in the structure determination and refinement (R_{int} = 0.0259). The intensities were corrected for absorption using the Bruker SADABS program[25] with the transmission factors ranging 0.63−0.80. The structure was solved by direct methods of the Bruker SHELXTL program.[26] Full-matrix least-squares refinement on F^2 yielded the final R1 of 0.0270 and wR2 of 0.0471. All hydrogen atoms were located in difference Fourier maps and refined with isotropic displacement parameters. The final difference map was essentially featureless with the highest peak of 0.93 e/Å^3.

(15) Miller, S. K.; VanDerveer, D. G.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1048.
(16) Fusch, E. C.; Lippert, B. *J. Am. Chem. Soc.* **1994**, *116*, 7204.
(17) Vallee, B. L.; Falchuk, K. H. *Physiol. Rev.* **1993**, *73*, 79.
(18) Jia, X.; Marzilli, L. G. *Biopolymers* **1991**, *31*, 23.
(19) Shin, Y. A.; Eichhorn, G. L. *Biochemistry* **1968**, *7*, 1026.
(20) Blackburn, E. H. *Cell* **1994**, *77*, 621.
(21) Hardin, C. C.; Watson, T.; Corregan, M.; Bailey, C. *Biochemistry* **1992**, *31*, 833.
(22) Hardin, C. C.; Perry, A. G.; White, K. *Biopolymers* **2001**, *56*, 147.
(23) Pegg, A. E. *Cancer Res.* **1990**, *50*, 6119.
(24) McElhinney, R. S.; Donnelly, D. J.; McCormick, J. E.; Kelly, J.; Watson, A. J.; Rafferty, J. A.; Elder, R. H.; Middleton, M. R.; Willington, M. A.; McMurry, T. B. H.; Margison, G. P. *J. Med. Chem.* **1998**, *41*, 5265.
(25) Sheldrick G. M. *SADABS: Program for Empirical Absorption of Area Detector Data*; University of Goetingen: Goetingen, Germany, 1996.
(26) Sheldrick, G. M. *SHELXTL Version 5.1 Reference Manual*; BRUKER AXS, Inc.: Madison, WI, 1997.