Inorg. Chem. **2004**, *43*, 1175–1183

Effect of Equatorial Ligands of Dirhodium(II,II) Complexes on the Efficiency and Mechanism of Transcription Inhibition *in Vitro*

Helen T. Chifotides,[†] Patty K.-L. Fu,[‡,§] Kim R. Dunbar,*[,†] and Claudia Turro*[,‡]

Departments of Chemistry, The Ohio State University, Columbus, Ohio 43210, and Texas A&M University, College Station, Texas 77843

Received April 24, 2003

The nature of the equatorial ligands spanning the dirhodium core was shown to affect the ability and mechanism of various lantern-type complexes to inhibit transcription in vitro. The inhibition of transcription by $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ appears to proceed predominantly via binding of the complexes to T7-RNA polymerase (T7-RNAP) and is dependent on the concentration of enzyme and Mg^{2+} ions in solution. The concentrations of the aforementioned complexes required to inhibit 50% of the transcription, C_{inh}^{50}, are similar to that measured for activated cisplatin, whereas a significantly higher concentration of $Rh_2(\mu\text{-}HNCOCH_3)_4$ is required to effect similar inhibition; the inhibition induced by $Rh_2(\mu\text{-}HNCOCH_3)_4$ does not involve binding to T7-RNAP. The spectral changes observed for each complex upon addition of enzyme are consistent with $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ binding to the enzyme and may involve partial displacement of the equatorial (*eq*) groups by the Lewis basic sites of T7-RNAP. In contrast, addition of enzyme to solutions of $Rh_2(\mu\text{-}HNCOCH_3)_4$ does not result in significant spectral changes, a finding consistent with lack of enzyme dependence in the transcription inhibition. These differences in reactivity and transcription inhibition mechanism among complexes with different bridging ligands are explained by variations of the Lewis acidity of the axial (*ax*) sites in the series of complexes $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $Rh_2(\mu\text{-}HNCOCH_3)_4$. The Lewis acidity of the *ax* sites is expected to affect the initial interaction of the complexes with the biomolecules, followed by their rearrangement to *eq* positions if the bridging ligands are labile.

Introduction

Disruption of transcription and related processes is recognized as a principal mode of action of potent antitumor and antiviral agents currently in use or undergoing clinical trials,[1−4] including actinomycin D,[5−7] anthracycline antibiotics,[8,9] daunorubicin,[10,11] and cisplatin,[12,13] among others.[14−23] Most of these drugs inhibit the transcription process through their interaction with the template DNA, binding to the promoter sequence or to other sequences in the template, and DNA modification or damage.[5−13] In some cases, however, the suppression of transcription arises from drug binding to the active site of RNA polymerase or by blocking

* To whom correspondence should be addressed. E-mail: turro@chemistry.ohio-state.edu (C.T.); dunbar@mail.chem.tamu.edu (K.R.D.).
† Texas A&M University.
‡ The Ohio State University.
§ Present address: Food and Drug Administration, 5100 Paint Branch, College Park, MD 20740.

(1) Darnell, J. E., Jr. *Nat. Rev. Cancer* **2002**, *2*, 740−749.
(2) Arlt, A.; Schaefer, H. *Int. J. Clin. Pharm. Ther.* **2002**, *40*, 336−347.
(3) Kim, R.; Tanabe, K.; Emi, M.; Uchida, Y.; Inoue, H.; Toge, T. *Anti-Cancer Drugs* **2003**, *14*, 3−11.
(4) Karamouzis, M. V.; Gorgoulis, V. G.; Papavassiliou, A. G. *Clin. Cancer Res.* **2002**, *8*, 949−961.
(5) Imamichi, T.; Murphy, M. A.; Adelsberger, J. W.; Yang, J.; Watkins, C. M.; Berg, S. C.; B., Michael W.; L., Richard A.; Guo, J.; Levin, J. G.; Lane, H. C. *J. Virol.* **2003**, *77*, 1011−1020.
(6) Chang, T.-C.; Tsai, L.-C.; Hung, M.-W.; Chu, L.; Chu, J.-T.; Chen, Y.-C. *Biochem. Pharmacol.* **1997**, *53*, 969−977.
(7) Phillips, D. R.; Crothers, D. M. *Biochemistry* **1986**, *25*, 7355−7362.
(8) Bakker, M.; van der Graaf, W. T. A.; Groen, H. J. M.; Smit, E. F.; De Vries, E. G. E. *Curr. Pharm. Des.* **1995**, *1*, 133−144.
(9) Jiang, X. R.; Macey, M. G.; Kelsey, S. M.; Collins, P. W.; Gutteridge, C. N.; Miki, T.; Adachi, K.; Yamabe, S.; Newland, A. C. *J. Chemother.* **1993**, *5*, 334−343.
(10) Marin, S.; Mansilla, S.; Garcia-Reyero, N.; Rojas, M.; Portugal, J.; Pina, B. *Biochem. J.* **2002**, *368*, 131−136.
(11) Portugal, J.; Martin, B.; Vaquero, A.; Ferrer, N.; Villamarin, S.; Priebe, W. *Curr. Med. Chem.* **2001**, *8*, 1−8.
(12) Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467−2498.
(13) O′ Dwyer, P. J.; Stevenson, J. P.; Johnson, S. W. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Wiley-VCH: Weinheim, Germany, 1999; pp 31−69.
(14) (a) Chu, W.; Shinomya, M.; Kamitori, K. Y.; Kamitori, S.; Carlson, R.. G.; Weaver, R. F.; Takusagawa, F. *J. Am. Chem. Soc.* **1994**, *116*, 7971−7982. (b) Takusagawa, F.; Carlson, R. G.; Weaver, R. F. *Bioorg. Med. Chem.* **2001**, *9*, 719−725.

10.1021/ic034438m CCC: $27.50
Published on Web 01/10/2004

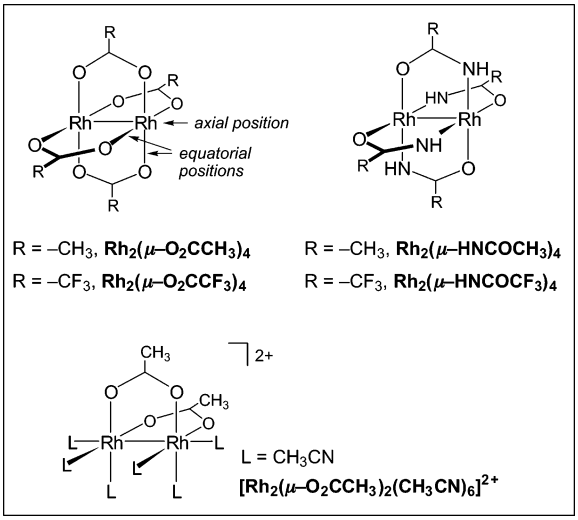

Figure 1. Schematic diagram of the dirhodium complexes.

the DNA/RNA channel[24,25] or targeting transcription factors.[26] Although many of these drugs are effective, a current concern is drug-induced resistance and the efficacy of each drug against certain cancers but not others, which demands the search for new compounds as potential future drugs.[27−32]

Dirhodium(II) tetracarboxylate complexes, $Rh_2(\mu\text{-}O_2CR)_4$ (R = Me, Et, Pr, Bu; Figure 1), exhibit carcinostatic activity against Ehrlich ascites and leukemia L1210 tumors.[33] More recently, these and related compounds have also been shown to be potent antibacterial and antitumor agents.[34] The fluorinated complexes $Rh_2(\mu\text{-}O_2CCF_3)_4$ and $Rh_2(\mu\text{-}HNCOCF_3)_4$, whose structures are shown in Figure 1, were found to significantly increase the survival rate of mice bearing Ehrlich ascites;[35] moreover, the LD50 value of $Rh_2(\mu\text{-}HNCOCF_3)_4$ both *in vitro* (U937 and K562 human leukemia cells and Ehrlich ascitic tumors) and *in vivo* was reported to be of the same order of magnitude as that of cisplatin.[36] Although the mechanism of action of the dirhodium complexes and their mode of binding to double-stranded DNA (*ds*-DNA) remain unknown, it has been established that the complexes inhibit DNA replication in vitro.[37] In addition, we recently reported that it requires a lower concentration of $Rh_2(\mu\text{-}O_2CCH_3)_4$ and $[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2]^{2+}$ (phen = 1,10-phenanthroline) than cisplatin to inhibit 50% of the transcription by T7-RNA polymerase under similar experimental conditions in vitro.[38] It appears, however, that the dirhodium(II,II) complexes achieve inhibition through binding to the enzyme,[38] whereas cisplatin is well known to covalently bind to ds-DNA by forming primarily 1,2-d(GpG) intrastrand cross-links, which result in the hijacking of various proteins to the platinated sites, thus disrupting their normal function and the transcription process.[39−41] In particular, it is thought that the binding of the HMG-domain proteins to the major cisplatin 1,2-

(15) Kester, H. A.; Blanchetot, C.; Den Hertog, J.; Van der Saag, P. T.; Van der Burg, B. *J. Biol. Chem.* **1999**, *274*, 27439−27447.
(16) Taatjes, D. J.; Gaudiano, G.; Resing, K.; Koch, T. *J. Med. Chem.* **1997**, *40*, 1276−1286.
(17) (a) Cullinane, C.; Mazur, S. J.; Essigmann, J. M.; Phillips, D. R.; Bohr, V. A. *Biochemistry* **1999**, *38*, 6204−6212. (b) Trimmer, E. E.; Essigmann, J. M. *Essays Biochem.* **1999**, *34*, 191−211.
(18) Aune, G. J.; Furuta, T.; Pommier, Y. *Anti-Cancer Drugs* **2002**, *13*, 545−555.
(19) Gojo, I.; Zhang, B.; Fenton, R. G. *Clin. Cancer Res.* **2002**, *8*, 3527−3538.
(20) Gillet, R.; Bobichon, H.; Trentesaux, C. *Biol. Cell* **2002**, *94*, 267−273.
(21) Jaspers, N. G. J.; Raams, A.; Kelner, M. J.; Ng, J. M. Y.; Yamashita, Y. M.; Takeda, S.; McMorris, T. C.; Hoeijmakers, J. H. J. *DNA Repair* **2002**, *1*, 1027−1038.
(22) Gajate, C.; An, F.; Mollinedo, F. *J. Biol. Chem.* **2002**, *277*, 41580−41589.
(23) Rokhlin, O. W.; Glover, R. A.; Taghiyev, A. F.; Guseva, N. V.; Seftor, R. E. B.; Shyshynova, I.; Gudkov, A. V.; Cohen, M. B. *J. Biol. Chem.* **2002**, *277*, 33213−33219.
(24) Campbell, E. A.; Korzheva, N.; Mustaev, A.; Murakami, K.; Nair, S.; Goldfarb, A.; Darst, S. A. *Cell* **2001**, *104*, 901−912.
(25) Luo, G.; Lewis, R. A. *Biochem. Pharmacol.* **1992**, *44*, 2251−2258.
(26) Chao, S.-H.; Price, D. H. *J. Biol. Chem.* **2001**, *276*, 31793−31799.
(27) Goldie, J. H. *Cancer Metast. Rev.* **2001**, *20*, 63−68.
(28) Tolomeo, M.; Simoni, D. *Curr. Med. Chem.: Anti-Cancer Agents* **2002**, *2*, 387−401.
(29) Broxterman, H. J.; Georgopapadakou, N. *Drug Resist. Updates* **2001**, *4*, 197−209.
(30) Schoendorf, T.; Neumann, R.; Benz, C.; Becker, M.; Riffelmann, M.; Goehring, U.-J.; Sartorius, J.; von Koenig, C.-H. W.; Breidenbach, M.; Valter, M. M.; Hoopmann, M.; Di Nicolantonio, F.; Kurbacher, C. M. *Recent Res. Cancer Res.* **2003**, *161*, 111−116.
(31) Kanzaki, A.; Takebayashi, Y.; Ren, X.-Q.; Miyashita, H.; Mori, S.; Akiyama, S.-I.; Pommier, Y. *Mol. Cancer Ther.* **2002**, *1*, 1327−1334.
(32) Papadopoulos, K. P.; Sherman, W. H. *Am. J. Cancer* **2002**, *1*, 323−340.
(33) (a) Erck, A.; Rainen, L.; Whileyman, J.; Chang, I.; Kimball, A. P.; Bear, J. L. *Proc. Soc. Exp. Biol. Med.* **1974**, *145*, 1278−1282. (b) Bear, J. L.; Gray, H. B.; Rainen, L.; Chang, I. M.; Howard, R.; Serio, G.; Kimball, A. P. *Cancer Chemother. Rep., Part 1* **1975**, *59*, 611−620. (c) Howard, R. A.; Spring, T. G.; Bear, J. L. *Cancer Res.* **1976**, *36*, 4402. (d) Erck, A.; Sherwood, E.; Bear, J. L.; Kimball, A. P. *Cancer Res.* **1976**, *36*, 2204−2209. (e) Howard, R. A.; Sherwood, E.; Erck, A.; Kimball, A. P.; Bear, J. L. *J. Med. Chem.* **1977**, *20*, 943−946. (f) Bear, J. L.; Howard, R. A.; Dennis, A. M. *Curr. Chemother.* **1978**, 1321−1323. (g) Howard, R. A.; Kimball, A. P. Bear, J. L. *Cancer Res.* **1979**, *39*, 2568−2573. (h) Rao, P. N.; Smith, M. L.; Pathak, S.; Howard, R. A.; Bear, J. L. *J. Natl. Cancer Inst.* **1980**, *64*, 905−911. (i) Bear, J. L. In *Precious Metals 1985: Proceedings of the Ninth International Precious Metals Conference*; Zysk, E. E., Bonucci, J. A., Eds.; Int. Precious Metals: Allentown, PA, 1986; pp 337−344.
(34) (a) Bien, M.; Pruchnik, F. P.; Seniuk, A.; Lachowicz, T. M.; Jakimowicz, P. *J. Inorg. Biochem.* **1999**, *73*, 49−55. (b) Pruchnik, F. P.; Starosta, R.; Ciunik, Z.; Opolski, A.; Wietrzyk, J.; Wojdat, E.; Dus, D. *Can. J. Chem.* **2001**, *79*, 868−877. (c) Pruchnik, F. P.; Jakimowicz, P.; Ciunik, Z.; Zakrzewska-Czerwinska, J.; Opolski, A.; Wietrzyk, J.; Wojdat, E. *Inorg. Chim. Acta* **2002**, *334*, 59. (d) Pruchnik, F. P.; Banbula, M.; Ciunik, Z.; Chojnacki, H.; Latocha, M.; Skop, B.; Wilczok, T.; Opolski, A.; Wietrzyk, J.; Nasulewicz, A. *Eur. J. Inog. Chem.* **2002**, 3214−3221. (e) Pruchnik, F. P.; Banbula, M.; Ciunik, Z.; Chojnacki, H.; Latocha, M.; Skop, B.; Wilczok, T. *Appl. Organomet. Chem.* **2002**, *16*, 587−592.
(35) (a) Esposito, B. P.; Faljoni-Alário, A.; Silva de Menezes J. F.; Felinto de Brito, H.; Najjar, R. *J. Inorg. Biochem.* **1999**, *75*, 55−61. (b) Esposito, B. P.; Zyngier, S. B.; Najjar, R.; Paes R. P.; Ueda, S. M. Y.; Barros, J. C. A. *Met. Based Drugs* **1999**, *1*, 17−18. (c) Esposito, B. P.; de Oliviera, E.; Zygnier, S. B.; Najjar, R. *J. Braz. Chem. Soc.* **2000**, *11*, 447−452. (d) Esposito, B. P.; Najjar, R. *Coord. Chem. Rev.* **2002**, *232*, 137−149.
(36) (a) Reibscheid, E. M.; Zyngier, S.; Maria, D. A.; Mistrone, R. J.; Sinisterra, R. D.; Couto, L. G.; Najjar, R. *Braz. J. Med. Biol. Res.* **1994**, *27*, 91−94. (b) Esposito, B. P.; Zyngier, S. B.; Ribeiro de Souza A. and Najjar, R. *Met. Based Drugs*, **1997**, *4*, 333−338.
(37) Dale, L. D.; Dyson, T. M.; Tocher, D. A.; Tocher, J. H.; Edwards, D. I. *Anti-Cancer Drug Des.* **1989**, *4*, 295−302.
(38) Sorasaenee, K.; Fu, P. K.-L.; Angeles-Boza, A. M.; Dunbar, K. R.; Turro, C. *Inorg. Chem.* **2003**, *42*, 1267−1271.
(39) (a) Yarnell, A. T.; Oh, S.; Reinberg, D.; Lippard, S. J. *J. Biol. Chem.* **2001**, *276*, 25736−41. (b) Cohen, S. M.; Jamieson, E. R.; Lippard, S. J. *Biochemistry* **2000**, *39*, 8259−8265. (c) Zamble, D. B.; Lippard, S. J. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Wiley-VCH: Weinheim, Germany, 1999; pp 73−110.
(40) (a) Wozniak, K.; Blasiak, J. *Acta Biochim. Pol.* **2002**, *49*, 583−596. (b) Wozniak, K.; Walter, Z. *Cell Biol. Int.* **2002**, *26*, 495−503.

intrastrand cross-links enhances cisplatin cytotoxicity by blocking the repair of these lesions.[42] The properties of the various dirhodium(II,II) complexes reported to date, including their binding to nucleobases,[43−45] dinucleotides,[46] and DNA dodecamer single strands,[47] make them valuable candidates as potential antitumor drugs. Herein, we have undertaken the evaluation of the ability of various dirhodium(II,II) complexes to inhibit transcription *in vitro*.

The bacteriophage T7-RNA polymerase (T7-RNAP) system was chosen for this study because its transcription reaction is one of the simplest in nature.[48,49] The polymerases in other organisms, such as bacteria, archaea, and eukaryotes, are multisubunit and transcription often requires transcription factors working in conjunction with the polymerase.[48−50] The single-subunit T7-RNAP enzyme is capable of initiation, elongation, and termination, and its mechanism and kinetics have been investigated extensively and are well understood.[51−53] Despite these differences, the similarity of the active site of T7-RNAP to that of all other polymerases renders it an ideal model system.[53−56]

The studies of the mechanism of transcription by T7-RNAP and the structures of various key intermediates provide a detailed picture of the enzyme's action.[51−53] A

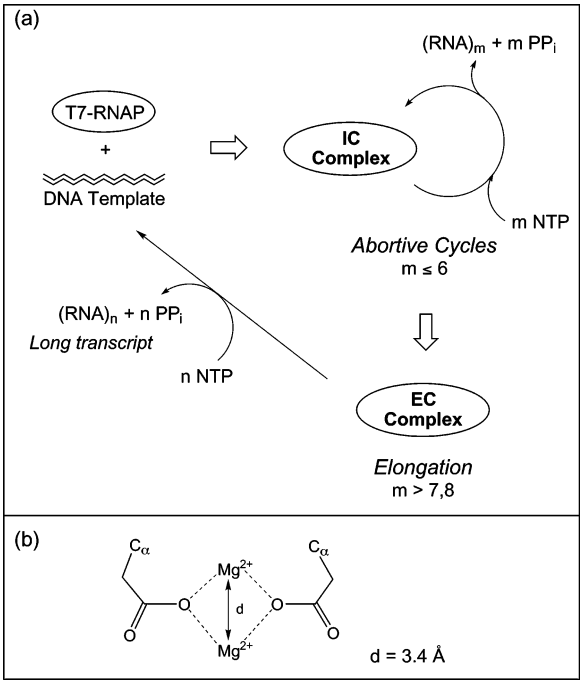

Figure 2. Schematic representation of (a) the transcription process and (b) the active site of T7-RNAP showing binding of Mg^{2+} ions.

schematic representation of the overall T7-RNAP transcription process, which starts with binding of the enzyme to the promoter sequence of the template DNA, is shown in Figure 2a.[51−53,57] It has been shown that, following this initial binding, the initiation complex (IC) undergoes a number of abortive cycles that result in the production of short mRNA transcripts that are 2 to ∼6 nucleotides in length.[51−53,57,58] When the transcript reaches a length of ∼7−8 nucleotides, a transition takes place to the elongation complex (EC), which ultimately produces a long mRNA transcript.[51−53,57,58] The crystal structure of the T7-RNAP enzyme (99 kDa) has been reported,[59] as well as that of the adduct with lysozyme,[60] the complex with the ds-DNA promoter,[61] the IC,[62] and EC complexes.[63,64] The structure of the IC complex has been shown to be similar to that of the resting enzyme, whereas significant structural reorganization occurs during the transition to the EC complex.[65]

The active site of T7-RNAP is composed of two Mg^{2+} ions bound to two aspartate residues, Asp537 and Asp812, with a binding constant of 5.0×10^2 M^{-1}, for each ion

(41) Fuertes, M. A.; Castilla, J.; Alonso, C.; Perez, J. M. *Curr. Med. Chem.* **2003**, *10*, 257−266.
(42) (a) Zamble, D. B.; Mikata, Y.; Eng, C. H.; Sandman, K. E.; Lippard, S. J. *J. Inorg. Biochem.* **2002**, *91*, 451−462. (b) Trimmer, E. E.; Zamble, D. B.; Lippard, S. J.; Essigmann, J. M. *Biochemistry* **1998**, *37*, 352−362. (c) McA'Nulty, M. M.; Whitehead, J. P.; Lippard, S. J. *Biochemistry* **1996**, *35*, 6089−6099. (d) Huang, J.-C.; Zamble, D. B.; Reardon, J. T.; Lippard, S. J.; Sancar, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10394−10398.
(43) (a) Dunbar, K. R.; Matonic, J. H.; Saharan, V. P.; Crawford, C. A.; Christou, G. *J. Am. Chem. Soc.* **1994**, *116*, 2201−2202. (c) Day, E. F.; Crawford, C. A.; Folting, K.; Dunbar, K. R.; Christou, G. *J. Am. Chem. Soc.* **1994**, *116*, 9339−9340. (d) Crawford, C. A.; Day, E. F.; Saharan, V. P.; Folting, K.; Huffman, J. C.; Dunbar, K. R.; Christou, G. *Chem. Commun.* **1996**, 1113−1114. (e) Catalan, K. V.; Mindiola, D. J.; Ward, D. L.; Dunbar, K. R. *Inorg. Chem.* **1997**, *36*, 2458−2460. (f) Catalan, K. V.; Hess, J. S.; Maloney, M. M.; Mindiola, D. J.; Ward, D. L.; Dunbar, K. R. *Inorg. Chem.* **1999**, *38*, 3904−3913.
(44) Rubin, J. R.; Haromy, T. P.; Sundaralingam, M. *Acta Crystallogr.* **1991**, *C47*, 1712−1714.
(45) Aoki, K.; Salam, M. A. *Inorg. Chim. Acta* **2001**, *316*, 50−58.
(46) (a) Chifotides, H. T.; Koshlap, K. M.; Pérez, L. M.; Dunbar K. R. *J. Am. Chem. Soc.* **2003**, *125*, 10703−10713. (b) Chifotides, H. T.; Koshlap, K. M.; Pérez, L. M.; Dunbar K. R. *J. Am. Chem. Soc.* **2003**, *125*, 10714−10724. (c) Chifotides, H. T.; Dunbar, K. R. Unpublished results.
(47) (a) Asara, J. M.; Hess, J. S.; Lozada, E.; Dunbar, K. R.; Allison, J. *J. Am. Chem. Soc.* **2000**, *122*, 8−13. (b) Chifotides, H. T.; Koomen, J. M.; Kang, M.; Dunbar K. R.; Tichy, S.; Russell, D. Manuscript in preparation.
(48) Cheetham, G. M. T.; Steitz, T. A. *Curr. Opin. Struct. Biol.* **2000**, *10*, 117−123.
(49) McAllister, W. T. *Cell. Mol. Biol. Res.* **1993**, *39*, 385−391.
(50) McAllister, W. T. *EMBO J.* **1997**, *11*, 15−25.
(51) (a) Cheetham, G. M. T.; Jeruzalmi, D.; Steitz, T. A. *Cold Spring Harbor Symp. Quant. Biol.* **1998**, *63*, 263−267. (b) Cheetham, G. M. T.; Steitz, T. A. *Curr. Opin. Struct. Biol.* **2000**, *10*, 117−123.
(52) (a) McAllister, W. T. *Nucleic Acids Mol. Biol.* **1997**, *11*, 15−25. (b) Doublié, S.; Tabor, S.; Long, A. M.; Richardson, C. C.; Ellengerger, T. *Nature* **1998**, *391*, 251−258. (c) Sousa, R. *Nucleic Acids Mol. Biol.* **1997**, *11*, 1−14.
(53) (a) Arnold, S.; Siemann, M.; Scharnweber, K.; Werner, M.; Baumann, S.; Reuss, M. *Biotechol. Bioeng.* **2001**, *72*, 548−561. (b) Ujvári, A.; Martin, C. T. *J. Mol. Biol.* **1997**, *273*, 775−781.
(54) Steitz, Thomas A. *Harvey Lect.* **1999**, *93*, 75−93.
(55) Sousa, R. *Trends Biochem. Sci.* **1996**, *21*, 186−191.
(56) Tunitskaya, V. L.; Kochetkov, S. N. *Biochemistry* **2002**, *67*, 1124−1135.
(57) Kochetkov, S. N.; Rusakova, E. E.; Tunitskaya, V. L. *FEBS Lett.* **1998**, *440*, 264−267.
(58) Jia, Y.; Patel, S. S. *Biochemistry* **1997**, *36*, 4223−4232.
(59) Sousa, R.; Chung, Y. J.; Rose, J. P.; Wang, B. C. *Nature* **1993**, *364*, 593−599.
(60) Jeruzalmi, D.; Steitz, T. A. *EMBO J.* **1998**, *17*, 4101−4113.
(61) Cheetham, G. M. T.; Jeruzalmi, D.; Steitz, T. A. *Nature* **1999**, *399*, 80−83.
(62) Cheetham, G. M. T.; Steitz, T. A. *Science* **1999**, *286*, 2305−2309.
(63) (a) Tahirov, T. H.; Temiakov, D.; Anikin, M.; Patlan, V.; McAllister, W. T; Vassylyev, D. G.; Yokoyama, S. *Nature* **2002**, *420*, 43−50. (b) Temiakov, D.; Tahirov, T. H.; Anikin, M.; McAllister, W. T.; Vassylyev, D. G.; Yokoyama, S. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2003**, *59*, 185−187.
(64) Yin, Y. W.; Steitz, T. A. *Science* **2002**, *298*, 1387−1395.
(65) Ma, K.; Temiakov, D.; Jiang, M.; Anikin, M.; McAllister, W. T. *J. Biol. Chem.* **2002**, *277*, 43206−43215.

binding to the two ligands in an equivalent manner.[66] It should be pointed out that these two carboxylate groups in the active site are conserved in all polymerases, and their mutation results in strongly impaired activity.[67−71] It has been shown that the two aspartate residues in T7-RNAP simultaneously bind to both ions providing the two Mg^{2+} ion catalytic site (Figure 2b).[72] In addition, it has been shown that, in T7-RNAP, the terminal carboxylate group is positioned near the active site and its esterification results in a decrease in the Mg^{2+}-dependent catalytic activity.[62,73]

Given that dirhodium tetracarboxylate complexes are known to be powerful inhibitors of transcription,[33] this study explores the effect of the structure variation of the bridging ligands on the quantity of mRNA produced. The molecular structures of the complexes investigated, $Rh_2(\mu\text{-}O_2CR)_4$ (R = CH_3, CF_3), $Rh_2(\mu\text{-}HNCOR)_4$ (R = CH_3, CF_3), and *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$, are shown in Figure 1. In the experiments reported herein only the long mRNA transcripts are detected (Figure 2a), and the differences in the amount of mRNA produced as a function of various experimental conditions are attributed to variations in the mechanism of inhibition. The present results are compared to those obtained in the presence of cisplatin, and possible binding motifs of the complexes to the enzyme are discussed.

Experimental Section

Materials. The starting material $Rh_2(\mu\text{-}O_2CCH_3)_4$ was a gift from Johnson-Matthey and was used without further purification. Acetamide, trifluoroacetamide, trifluoroacetic acid, trifluoroacetic anhydride, ciplatin, $AgNO_3$, and anhydrous chlorobenzene were purchased from Aldrich. Agarose, ethidium bromide, EDTA, Tris/HCl, $MgCl_2$, and RNA loading solution were purchased from Sigma and used as received. The pGEM linear DNA control template (3995 bp) was purchased from Promega, whereas the T7-RNA polymerase (50 units/μL) and 5× RNA transcription buffer were purchased from Life-Technologies (Rockville, MD). $Rh_2(\mu\text{-}O_2CCF_3)_4$,[74−76] $Rh_2(\mu\text{-}HNCOCF_3)_4$,[77] $Rh_2(\mu\text{-}HNCOCH_3)_4$,[78,79] and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6](BF_4)_2$[80] were prepared according to literature procedures and characterized by standard methods. Activated cisplatin, *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$, was prepared by reacting an aqueous solution of cisplatin with 1.97 equiv of $AgNO_3$ for 1 day at room temperature in the dark. The reaction mixture was centrifuged for 10 min (Jouan A12-14 microcentrifuge) to remove AgCl. The aqueous layer was separated from the precipitate, and the solution was centrifuged for another 10 min; this process was repeated twice.

Methods. The transcription assay has been previously reported.[38,81] In the *in vitro* transcription experiment, the pGEM linear DNA template (120 μM bases) was used with T7-RNAP, resulting in two transcripts of length 1065 and 2346 bases, and each trial was conducted 3 times. The transcription reaction was conducted for 30 min at 37 °C (40 mM Tris/HCl, pH = 8.0) in nuclease-free water in the presence of 1.25 units of T7-RNAP, 8 mM $MgCl_2$, 25 mM NaCl, and 1.0 mM of each ATP, CTP, GTP, and UTP. The inhibition of mRNA production by the dirhodium complexes was detected in vitro by the measurement of the mRNA produced upon addition of increasing amounts of metal complex to the assay. The concentration of each complex at which 50% of the RNA is transcribed, C_{inh}^{50}, was calculated by interpolation of the integrated areas of the imaged mRNA signal of each lane of the gel conducted with various concentrations of a given complex. Modifications of these methods were utilized in the various control assays, including those designed to determine the role of binding of the complexes to T7-RNAP. The effect of the Mg^{2+} ion concentration, using 7.5 and 15 mM $MgCl_2$, on the transcription reaction in the presence of the various dirhodium adducts was monitored by comparing the mRNA produced both in the presence and absence of the metal complexes at each Mg^{2+} ion concentration.

All metal stock solutions were dissolved in pure H_2O except for that of $Rh_2(\mu\text{-}HNCOCF_3)_4$, which was prepared in 7:3 H_2O/MeOH (v/v) due to the limited solubility of the compound in H_2O. Control experiments indicate that the amount of methanol introduced into each transcription assay from the stock solution does not interfere with transcription.

The extinction coefficient of bacteriophage T7-RNA polymerase at 280 nm (ϵ_{280} = 138 000 M^{-1} cm^{-1}) was calculated from the amino acid sequence by a method reported in the literature[82] and was estimated to be ∼153 μM in the stock solutions used for the spectroscopic titrations.

Instrumentation. Absorption measurements were performed on a Shimadzu UV 1601PC spectrophotometer. The ethidium bromide stained agarose gels (1%) were imaged on an AlphaImager 2000 transilluminator (Alpha Innotech Corp.).

Results and Discussion

Transcription Inhibition. The inhibition of transcription by each metal complex was determined by recording the imaged mRNA produced during the transcription reaction as a function of complex concentration, while keeping the concentrations of all other components constant. A typical imaged gel is shown in Figure 3a for $Rh_2(\mu\text{-}HNCOCF_3)_4$, where the produced mRNA decreases relative to the control

(66) Woody, A.-Y. M.; Eaton, S. S.; Osumi-Davis, P. A.; Woody, R. W. *Biochemistry* **1996**, *35*, 144−152.
(67) Larder, B. A.; Purifoy, D. J.; Powell, K. L.; Darby, G. *Nature* **1987**, *327*, 716−717.
(68) (a) Osumi-Davis, P. A.; Aguilera, M. C.; Woody, R. W.; Woody, A. Y. *J. Mol. Biol.* **1992**, *226*, 37−45. (b) Osumi-Davis, P. A.; Sreerama, N.; Volkin, D. B.; Middaugh, C. R.; Woody, R. W.; Woody, A. Y. *J. Mol. Biol.* **1994**, *237*, 5−19. (c) Woody, A.-Y. M.; Eaton, S. S.; Osumi-Davis, P. A.; Woody, R. W. *Biochemistry* **1996**, *35*, 144−152.
(69) (a) Bonner, G.; Patra, D.; Lafer, E. M.; Sousa, R. *EMBO J.* **1992**, *11*, 3767−3775. (b) Bonner, G.; Lafer, E. M.; Sousa, R. *J. Biol. Chem.* **1994**, *269*, 25120−25128.
(70) Date, T.; Yamamoto, S.; Tanihara, K.; Nishimoto, Y.; Matsukage, A. *Biochemistry* **1991**, *30*, 5286−5292.
(71) Polesky, A. H.; Dahlberg, M. E.; Benkovic, S. J.; Gindley, N. D. F.; Joyce, C. M. *J. Biol. Chem.* **1992**, *267*, 8417−8428.
(72) Brautigam, C. A.; Steitz, T. A. *Curr. Opin. Struct. Biol.* **1998**, *8*, 54−63.
(73) Lykke-Andersen, J.; Christiansen, J. *Nucleic Acids Res.* **1998**, *26*, 5630−5635.
(74) Rempel, G. A.; Legzdins, P.; Smith, H.; Wilkinson, G. *Inorg. Synth.* **1972**, *13*, 90.
(75) Cotton, F. A.; Felthouse, T. R. *Inorg. Chem.* **1980**, *19*, 2347−2351.
(76) Telser, J.; Drago, R. S. *Inorg. Chem.* **1984**, *23*, 1798−1803.
(77) Dennis, A. M.; Korp, J. D.; Bernal, I.; Howard, R. A.; Bear, J. L. *Inorg. Chem.* **1983**, *22*, 1522−1529.
(78) Ahsan, M. Q.; Bernal, I.; Bear, J. L. *Inorg. Chem.* **1986**, *25*, 260−265.
(79) Doyle, M. P.; Bagheri, V.; Wandless, T. J.; Harn, N. K.; Brinker, D. A.; Eagle, C. T.; Loh, K.-L. *J. Am. Chem. Soc.* **1990**, *112*, 1906−1912.
(80) Pimblett, G.; Garner, C. D.; Clegg, W. *J. Chem. Soc., Dalton Trans.* **1986**, 1257−1263.
(81) (a) Fu, P. K.-L.; Turro, C. *Chem. Commun.* **2001**, 279−280. (b) Fu, P. K.-L.; Bradley, P. M.; Turro, C. *Inorg. Chem.* **2003**, *42*, 878−884.
(82) Gill, S. C.; von Hippel, P. H. *Anal. Biochem.* **1989**, *182*, 319−326.

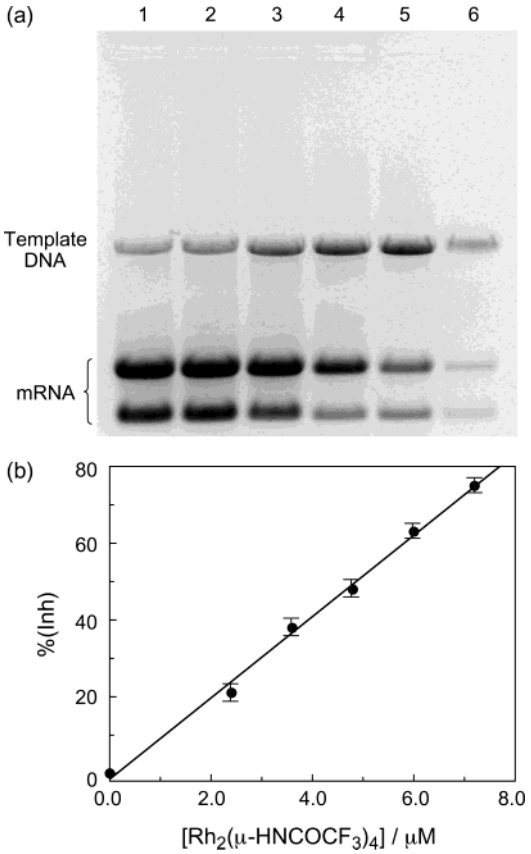

Figure 3. (a) Ethidium bromide stained agarose gel (1%) of transcribed mRNA in the presence of various concentrations of $Rh_2(\mu\text{-}HNCOCF_3)_4$. Key (lane no., [$Rh_2$(II,II)] ($\mu$M)): 1, 0.0; 2, 2.4; 3, 3.6; 4, 4.8; 5, 0.60; 6, 7.2. Both the DNA template and mRNA are imaged on the gel (labeled). (b) Plot of percent transcription inhibition, % Inh, as a function of $Rh_2(\mu\text{-}HNCOCF_3)_4$ concentration. The linear fit was used to calculate C_{inh}^{50} value and to estimate the binding constant (see text).

lane (no metal complex, lane 1) as the complex concentration is increased (lanes 2−6). The concentration of each complex required to inhibit 50% of the transcription, C_{inh}^{50}, was determined from interpolation of plots of percent inhibition as a function of increasing complex concentration. A typical plot is shown in Figure 3b for $Rh_2(\mu\text{-}HNCOCF_3)_4$, and the C_{inh}^{50} values for all the complexes are listed in Table 1. The measured C_{inh}^{50} value for activated cisplatin, *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$, is 4.1 μM under similar experimental conditions (Table 1). It is evident from Table 1 that the C_{inh}^{50} values for $Rh_2(\mu\text{-}O_2CCF_3)_4$ and $Rh_2(\mu\text{-}HNCOCF_3)_4$ are comparable to that of activated cisplatin, whereas that of $Rh_2(\mu\text{-}HNCOCH_3)_4$ is larger by a factor of 3. In contrast, the C_{inh}^{50} of $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ is lower than that of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ by a factor of 2 under similar experimental conditions.

Various control experiments were conducted to rule out the participation of other species in the inhibition. To ensure that the dirhodium complexes do not cause degradation of the transcribed mRNA or interfere with the ethidium bromide imaging, each complex was added (at [Rh_2(II,II)] $\sim C_{inh}^{50}$) following the transcription reactions conducted in the absence of inhibitors. In all cases, the presence of the added complexes did not result in a decrease of the mRNA produced relative to the control lanes, to which metal complexes were not added. Owing to the known preference of adenine and its derivatives to bind axially to dirhodium(II,II) complexes,[33a,44,83−87] transcription inhibition experiments were conducted in the presence of additional ATP. Assuming a binding constant for dirhodium complexes with ATP of $\sim 10^3$ M^{-1} (1:1 axial adduct),[83] an increase in ATP from 1 to 2 mM would be expected to result in ~30% increase in transcription inhibition when keeping all other conditions constant. No difference in the amount of mRNA produced was observed upon this 2-fold increase in ATP concentration with [Rh_2(II,II)] $\sim C_{inh}^{50}$, an indication that a Rh_2(II,II)−ATP adduct is not the species that inhibits transcription. Furthermore, the presence of 5−10 μM of free ligand (CF_3COOH, CF_3CONH_2, CH_3CONH_2, CH_3CN), during the transcription assay, does not result in changes in the amount of mRNA produced, a fact inconsistent with a mechanism that involves the dissociated ligand as the species inducing the inhibition. Furthermore, it was recently shown that the C_{inh}^{50} of Rh(III) is quite high (1.25 mM);[81b] thus, it is clear that the mechanism of inhibition by the dinuclear complexes differs significantly from that of the free rhodium ion.

Enzyme Concentration Dependence. The possible role of the complexes binding to the enzyme as their mechanism of transcription inhibition was investigated by comparison of the relative amount of mRNA produced upon 3-fold increase in the concentration of T7-RNAP in the presence of each complex, while keeping the concentration of all other components constant. The ratio of the relative mRNA produced in the presence of 3× and 1× enzyme, $R_{Enz}(3\times/1\times)$, for each complex was calculated from eq 1,

$$R_{Enz}(3\times/1\times) = P(3\times)/P(1\times) \quad (1)$$

where $P(3\times)$ and $P(1\times)$ represent the percent mRNA produced in the presence of $\sim C_{inh}^{50}$ metal complex relative to that in the absence of inhibitor under the same experimental conditions, using 7.5 and 2.5 units of enzyme, respectively. The $R_{Enz}(3\times/1\times)$ values calculated for all the complexes, as well as the percent mRNA transcribed, are summarized in Table 1. A value of $R_{Enz}(3\times/1\times) \sim 1.0$ for a given complex is indicative of a mechanism of transcription inhibition that does not proceed via binding of the complex to the enzyme with a binding constant $K_{Rh}^{Enz} > 10^4$ M^{-1} under these experimental conditions (assuming a 1:1 complex between the Rh_2(II,II) complex and the enzyme and 100% inactive Rh_2-bound T7-RNAP). Conversely, if enzyme binding to the dirhodium complex (with $K_{Rh}^{Enz} > 10^4$ M^{-1}; see Supporting Information) is necessary for inhibition to take place, additional enzyme is expected to result in

(83) Rainen, L.; Howard, R. A.; Kimball, A. P.; Bear, J. L. *Inorg. Chem.* **1975**, *14*, 2752−2754.
(84) Pneumatikakis, G.; Hadjiliadis, N. *J. Chem. Soc., Dalton Trans.* **1979**, 596−599.
(85) Aoki, K.; Yamazaki, H. *J. Chem. Soc., Chem. Commun.* **1980**, 186−188.
(86) (a) Farrell, N. *J. Chem. Soc., Chem. Commun.* **1980**, 1014−1016. (b) Farrell, N. *J. Inorg. Biochem.* **1981**, *14*, 261−265.
(87) Rubin, J. R.; Sundaralingam, M. *J. Biomol. Struct. Dyn.* **1984**, *2*, 525−530.

Table 1. Metal Complex Concentrations Required To Inhibit 50% of the Transcription, C_{inh}^{50}, Relative Effect of [T7-RNAP] and [Mg^{2+}] on mRNA Production, and pK_a Values of the Bridging Ligands

metal complex	C_{inh}^{50}/μM	% mRNA 1× Enz[a]	% mRNA 3× Enz[b]	$R_{Enz}(3\times/1\times)$[c]	$R_{Mg^{2+}}(2\times/1\times)$[d]	pK_a
$Rh_2(\mu\text{-}O_2CCF_3)_4$	5.3	57	80	1.4	0.5	0.52[e]
$Rh_2(\mu\text{-}HNCOCF_3)_4$	4.8	60	88	1.5	0.5	10.36[f]
$[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$	2.6	46	93	2.0	0.7	4.75[e]
$Rh_2(\mu\text{-}HNCOCH_3)_4$	12	65	62	1.0	1.0	15.1[e]
cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$	4.1	70	71	1.0	1.0	

[a] 2.5 units of T7-RNAP (1×). [b] 7.5 units of T7-RNAP (3×). [c] Ratio of mRNA produced per unit time in the presence of 3× to that with 1× T7-RNAP, each relative to the reaction under the same conditions in the absence of metal complex (see text). Average of 3 trials (6% error). [d] Ratio of mRNA produced per unit time in the presence of 15 mM to that with 7.5 mM Mg^{2+} ions, each relative to the reaction under the same conditions in the absence of metal complex. Average of 3 trials (6% error). [e] From ref 94. [f] From ref 95.

decreased inhibition or higher amount of mRNA produced, thus resulting in $R_{Enz}(3\times/1\times) > 1.0$. In such cases, a higher relative amount of unbound T7-RNAP will be available to produce mRNA when the enzyme concentration is increased, while the inhibitor concentration is held constant (at $\sim C_{inh}^{50}$). Activated cisplatin, which is known to inhibit the transcription process via binding to the template DNA and not the enzyme,[12,13] does not induce an increase in the amount of transcribed mRNA upon 3-fold increase in T7-RNAP per unit reaction time, thus resulting in $R_{Enz}(3\times/1\times) = 1.0$ (Table 1). Similar results are also observed for $Rh_2(\mu\text{-}HNCOCH_3)_4$ (Table 1), consistent with a mechanism of transcription inhibition that does not involve binding of this complex to the enzyme. For $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$, however, a relative increase in the mRNA produced is observed per unit reaction time upon 3-fold increase in T7-RNAP ($R_{Enz}(3\times/1\times) > 1.0$), a finding indicative of inhibition that involves binding of the dirhodium complexes to T7-RNAP (Table 1). Assuming a 1:1 complex between the rhodium complexes and T7-RNAP, which results in 100% inhibition of the bound enzyme, plots of % Inh vs $[Rh_2]_0$ will have a slope of $\sim 100(K_{Rh}^{Enz})$ and an intercept of 0. Such a plot and the linear fit of the data is shown for $Rh_2(\mu\text{-}HNCOCF_3)_4$ in Figure 3b. This treatment of the inhibition data results in $K_{Rh}^{Enz} \sim 1 \times 10^5\ M^{-1}$ for $Rh_2(\mu\text{-}O_2CCF_3)_4$ and $Rh_2(\mu\text{-}HNCOCF_3)_4$ and $K_{Rh}^{Enz} \sim 2 \times 10^5\ M^{-1}$ for $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$. Assuming competitive binding for the dirhodium complexes with ATP (1:1 complex, $K_{Rh,ATP} \sim 10^3\ M^{-1}$),[83] the slopes of the plots of % Inh vs $[Rh_2]$ result in $K_{Rh}^{Enz} \sim 2 \times 10^5\ M^{-1}$ for $Rh_2(\mu\text{-}O_2CCF_3)_4$ and $Rh_2(\mu\text{-}HNCOCF_3)_4$ and $K_{Rh}^{Enz} \sim 4 \times 10^5\ M^{-1}$ for $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ (Supporting Information).

Similar dependence of transcription inhibition on the enzyme concentration per unit reaction time was recently reported for $Rh_2(\mu\text{-}O_2CCH_3)_4$ and $[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2]^{2+}$.[38] The greatest effect on enzyme concentration is observed for $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$. This finding could be related to stronger binding of the complex to T7-RNAP due to electrostatic interactions that are not possible for neutral complexes or could be related to greater lability of the monodentate equatorial (*eq*) acetonitrile ligands relative to *eq* bidentate bridging ligands in the other complexes.[47b] Although multiple simultaneous mechanisms of inhibition are possible, for the transcription inhibition experiments that exhibit dependence on the enzyme concentration, the dominant mechanism of inhibition must involve enzyme binding to the complex.

Additional evidence that the binding of $Rh_2(\mu\text{-}HNCOCF_3)_4$, $Rh_2(\mu\text{-}O_2CCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ to the enzyme plays an important role in transcription inhibition is apparent from experiments in which the complexes were incubated with T7-RNAP for 30 min at 37 °C prior to the addition of the nucleotides and the DNA template, which initiated the transcription process. For example, preincubation of 2.4 μM $Rh_2(\mu\text{-}HNCOCF_3)_4$ with T7-RNAP results in 32(4)% enhanced transcription inhibition, whereas similar preincubation of T7-RNAP with $Rh_2(\mu\text{-}HNCOCH_3)_4$ or activated cisplatin results in no change in the transcribed mRNA (compared to experiments with no preincubation of T7-RNAP with the metal complexes).

Possible Inhibition Mechanisms. For $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$, all of which inhibit transcription via binding to T7-RNAP, various possible mechanistic schemes can be envisioned. There are possibilities that include interference with the function and binding of the enzyme to the template DNA. In one scenario, inhibition may proceed via binding of the complexes to the template DNA or to both the enzyme and the DNA, which may lower the affinity of the latter to the former. Alternatively, the association of the rhodium complexes to T7-RNAP may interfere with the binding between the enzyme and the template.

A 2-fold increase in template DNA concentration does not affect the inhibition by these complexes; however, it has been shown to decrease the inhibition by the DNA-binding drug cisplatin.[81] For example, the percent mRNA produced remains constant in the presence of 2.4 μM $Rh_2(\mu\text{-}HNCOCF_3)_4$ upon 2-fold increase in template DNA concentration. These results are not unexpected, since it has been shown that neutral dirhodium complexes bind duplex DNA with binding constants of $\sim 10^3\ M^{-1}$.[38] Under the present experimental conditions, the concentration of free DNA template remains largely unaffected by the presence of ~ 5 μM Rh_2(II,II) complex (Supporting Information). Therefore, binding of the complexes to template DNA is an unlikely mechanism of inhibition. The binding of the complexes to the enzyme, such that it interferes with binding of T7-RNAP to the DNA promoter sequence, can also be ruled out. Owing to the strong binding of the enzyme to the promoter, with a binding constant $\sim 10^8\ M^{-1}$,[53] a complex concentration of 4 μM ($\sim C_{inh}^{50}$) with $K_{Rh}^{Enz} \sim 10^5\ M^{-1}$ does not result in a significant decrease in the calculated enzyme-bound template

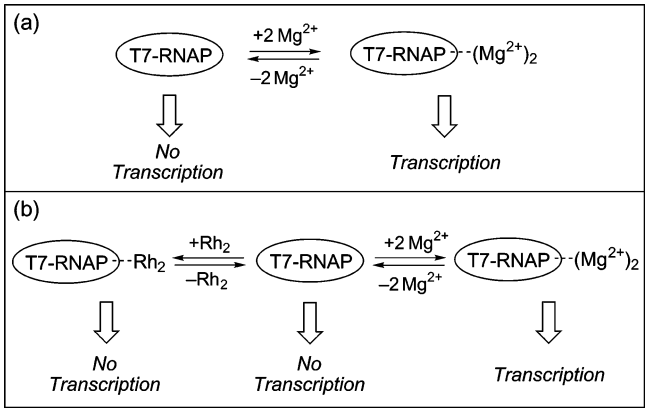

Figure 4. Schematic representation of the equilibrium of Mg^{2+} ions binding to R7-RNAP resulting in transcription in (a) the absence and (b) the presence of competitive binding by a dirhodium complex.

(Supporting Information). However, this concentration of Rh_2(II,II) complex results in ~50% transcription inhibition (Table 1).

Reduction in the catalytic activity of T7-RNAP through binding of the rhodium complexes to the enzyme could result in partial or total inhibition of a given Rh_2-bound protein. It is apparent from Figure 3b and numerous other similar inhibition experiments with the complexes that the mRNA production decreases linearly with complex concentration. Typically, for each complex, a given concentration is reached at which no mRNA is observed (100% inhibition). A scenario where the enzyme activity is reduced (less than 100%) through binding of the complexes would be expected to result in a plateau in the plot of % mRNA vs [Rh_2] at a value lower than 100% inhibition since even when all the rhodium complex is bound, some mRNA should still be produced. Furthermore, the linearity of the plots is indicative of a single predominant mechanism of inhibition operative in this complex concentration range under these experimental conditions. The inactivation of T7-RNAP upon binding of these dirhodium complexes may take place through competition of Rh_2(II,II) with Mg^{2+} ions for the Lewis basic residues in the enzyme's active site, which can be probed through the dependence of the transcription inhibition on the magnesium ion concentration.

Magnesium Ion Dependence. Binding to the enzyme appears to be an important mechanism for inhibition of transcription for the complexes $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$. The binding of these complexes to the magnesium-dependent active site (Figure 2b) cannot be ascertained from the enzyme concentration dependence, since binding to other areas involved in important functions, such as residues that make specific contacts with the template DNA or that block the mRNA exit channel are also possible. As shown schematically in Figure 4a, an increase in the concentration of Mg^{2+} ions should result in a higher amount of activated enzyme (with two bound Mg^{2+} ions) and, thus, enhanced production of transcribed mRNA. If there is competitive binding of the dirhodium complexes with one or both Mg^{2+} ions required for transcription (Figure 4b), in the presence of ~$C_{inh}{}^{50}$ of a given complex, it is expected that a 2-fold increase in the concentration of Mg^{2+} ions would result in less transcribed mRNA, relative to that with the same increase in Mg^{2+} ion concentration in the absence of metal complex (Figure 4b). The values of $R_{Mg^{2+}}(2\times/1\times)$ listed in Table 1 were calculated from the ratio of the percent mRNA produced in the presence of ~$C_{inh}{}^{50}$ of a given complex with 7.5 mM (1×) and 15 mM (2×) $MgCl_2$. The changes in mRNA produced, with a 2-fold increase in Mg^{2+} ion concentration, in the presence of 4.1 μM activated cisplatin and 12 μM $Rh_2(\mu\text{-}HNCOCH_3)_4$, are similar to that observed in the absence of metal complex (Table 1), thus resulting in $R_{Mg^{2+}}(2\times/1\times) = 1.0$. These results are consistent with lack of enzyme concentration dependence on the transcription inhibition for both complexes discussed above. In contrast, for the complexes that exhibit enzyme-dependent transcription inhibition, namely $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$, there is a significantly lower increase in mRNA produced, relative to that in the absence of metal complex at [Rh_2(II,II)] ~ $C_{inh}{}^{50}$ (Table 1), with $R_{Mg^{2+}}(2\times/1\times)$ between 0.5 and 0.7.

These results are indicative of competitive binding between these dirhodium complexes and the Mg^{2+} ions, which are known to bind to the active site of the enzyme and are vital to the transcription process (Figure 2b).[67] A possible interpretation of these findings is that the dirhodium complexes bind more tightly than the Mg^{2+} ions to the two aspartate residues at the active site (Figure 2b), whose binding constant has been reported to be $K_{Mg^{2+}} = 5 \times 10^2$ M^{-1}.[66] If this is indeed the case, then Mg^{2+} ions would not be able to displace enzyme-bound Rh_2(II,II); therefore, any enzyme deactivated by binding of dirhodium complexes would not be reactivated through the presence of excess Mg^{2+} ions in solution. Assuming competitive binding between the Rh_2 complexes and Mg^{2+} ions at the active site of Mg^{2+}-free T7-RNAP and assuming that only the enzyme with two bound Mg^{2+} ions is active and other forms are 100% inactive, a value of $R_{Mg^{2+}}(2\times/1\times) = 0.86$ can be calculated (Supporting Information). Although this ratio is larger than those measured, which range from 0.5 to 0.7 (Table 1), the calculation clearly shows that under the present experimental conditions this 2-fold increase in Mg^{2+} ion concentration is expected to result in a value of $R_{Mg^{2+}}(2\times/1\times) < 1.0$ if competitive binding is taking place.

Binding of Complexes to T7-RNAP. Figure 5a shows that, upon addition of 7.5 μM and 10.5 μM T7-RNAP to a solution containing 75 μM $Rh_2(\mu\text{-}HNCOCH_3)_4$ (40 mM Tris/HCl, pH = 8), the observed changes in the electronic absorption spectrum are minimal. These results are consistent with the lack of enzyme dependence of the transcription inhibition by $Rh_2(\mu\text{-}HNCOCH_3)_4$, indicating that there is little interaction between the complex and T7-RNAP. In contrast, addition of 7.5 and 10.5 μM T7-RNAP to 75 μM solutions of $Rh_2(HNCOCF_3)_4$, $Rh_2(\mu\text{-}O_2CCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)(CH_3CN)_6]^{2+}$ (40 mM Tris/HCl, pH = 8), all of which exhibit enzyme concentration dependent inhibition of transcription, results in marked increase in the absorption in the 350−370 nm region. The spectral changes for $Rh_2(\mu\text{-}HNCOCF_3)_4$ upon addition of enzyme are depicted in Figure 5b. A similar increase in the absorption maximum at

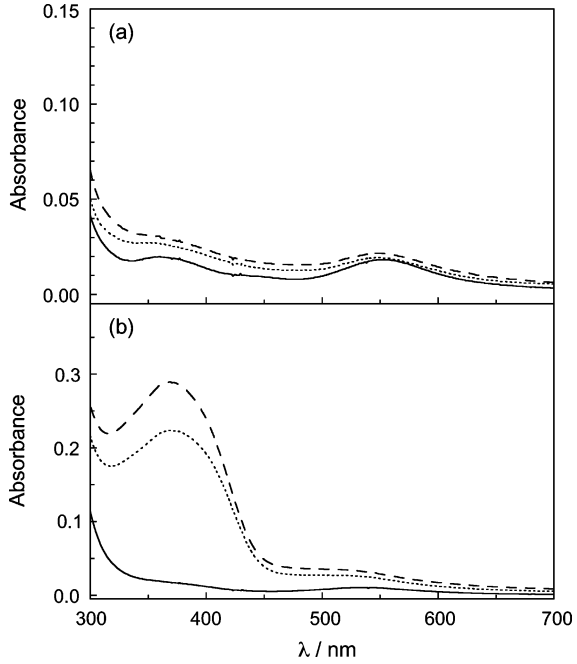

Figure 5. Electronic absorption spectra in 40 mM Tris/HCl, pH 8 buffer of 75 μM (a) $Rh_2(\mu\text{-}HNCOCF_3)_4$ and (b) $Rh_2(\mu\text{-}HNCOCF_3)_4$ (—) and upon addition of 7.5 μM (---) and 10.5 μM (— — —) T7-RNAP.

$\lambda < 400$ nm was also recorded for 75 μM $Rh_2(\mu\text{-}O_2CCH_3)_4$ upon addition of 7.5 and 10.5 μM T7-RNAP (40 mM Tris/HCl, pH = 8).[38]

The spectral changes observed for $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}O_2CCH_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(O_2CCH_3)_2(CH_3CN)_6]^{2+}$ in this study are markedly different from those previously reported for the binding of $Rh_2(\mu\text{-}O_2CCH_3)_4$ and $[Rh_2(\mu\text{-}O_2CCH_3)_2(bpy)_2(OH_2)_2]^{2+}$ (bpy = 2,2′-bipyridine) to human serum albumin (HSA).[88] For the latter compounds, the blue shift of the lowest energy band ($MM\pi^* \rightarrow MM\sigma^*$) is attributed to axial (*ax*) binding of the dirhodium complexes to the histidine residues in the protein,[88] since cysteine binding is known to result in a dramatic increase in the absorption in the visible region ($\lambda < 550$ nm) for $Rh_2(O_2CCH_3)_4$.[88a] The spectra for solutions of $Rh_2(\mu\text{-}O_2CCH_3)_4$ treated with HSA, however, reveal an increase in the absorption at $\lambda < 400$ nm, a spectral feature that was not observed in the UV titration with histidine.[88a] This increase in intensity is not discussed by the authors.[88a]

At the present time, the species that absorbs strongly in the 350−370 nm region, formed in the presence of the dirhodium complexes upon addition of T7-RNAP, remains unknown. Some possibilities, however, have been ruled out. Addition of excess cysteine to 75 μM $Rh_2(HNCOCF_3)_4$ (40 mM Tris/HCl, pH = 8), results in the appearance of a new peak at $\lambda_{max} = 412$ nm which is not present in the spectra of the compound in the presence of T7-RNAP (Figure 5b). Furthermore, simple *ax* binding of histidine residues or other Lewis basic sites in the protein is unlikely, since such interactions do not result in marked spectral changes in the 350−370 nm region.[88]

A possible explanation for these new spectral features is the partial displacement of the *eq* ligands of the dirhodium compounds by electron-donating residues of the enzyme, which would result in large structural and electronic changes to the dinuclear core. The pK_a values of the free protonated ligands listed in Table 1 are found to correlate with the lability of the bridging groups.[47b] It is evident from the pK_a values that CH_3CONH^- is the least labile (strongest base) of all the ligands utilized in this study, which is in accord with the lack of reactivity of $Rh_2(\mu\text{-}HNCOCH_3)_4$ with T7-RNAP (Figure 5a). Moreover, the presence of the acetamide bridging groups on the dirhodium core renders the *ax* positions of $Rh_2(\mu\text{-}HNCOCH_3)_4$ much weaker Lewis acids with respect to *ax* bonding,[78] which may also account for its higher $C_{inh}{}^{50}$ value compared to the other dirhodium adducts (Table 1). Conversely, the electron-withdrawing effect of the trifluoro (CF_3) group renders the complexes $Rh_2(\mu\text{-}HNCOCF_3)_4$ and $Rh_2(\mu\text{-}O_2CCF_3)_4$ less electron-rich and therefore more susceptible to *ax* binding (an effect in agreement with the lower $C_{inh}{}^{50}$ values for these compounds). These observations are consistent with initial *ax* interactions of the enzyme with these dirhodium adducts followed by rearrangement to *eq* sites, if the bridging group is labile.[47b,89−92] The higher $C_{inh}{}^{50}$ value for $Rh_2(\mu\text{-}HNCOCF_3)_4$ as compared to $Rh_2(\mu\text{-}O_2CCF_3)_4$ may be due to the potential hydrogen bonding of the NH functional group on the trifluoroacetamidato ligand to the side groups of the *ax* ligand; such favorable hydrogen bonds have been documented for dirhodium adducts with nucleobases.[44,45,93]

Conclusions

The nature of the bridging ligands spanning the dirhodium(II,II) core was shown to affect the ability and mechanism of the various complexes to inhibit transcription *in vitro*. The results of this study indicate that inhibition of transcription by $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ proceeds predominantly via binding of the complexes to T7-RNAP and is dependent on the concentration of enzyme and Mg^{2+} ions in solution. Since it is known that two aspartate residues are bound to the Mg^{2+} ions in the active site of the enzyme during transcription, Mg^{2+} ion concentration dependence of the inhibition by these complexes suggests that competitive binding of the dirhodium complexes with the T7-RNAP active site is occurring. Another important finding is that the concentrations of these

(88) (a) Trynda, L.; Pruchnik, F. *J. Inorg. Biochem.* **1995**, *58*, 69−77. (b) Trynda-Lemiersz, L.; Pruchnik, F. P. *J. Inorg. Biochem.* **1997**, *60*, 187−192.

(89) Crawford, C. C.; Matonic, J. H.; Streib, W. E.; Huffman, J. C.; Dunbar, K. R.; Christou, G. *Inorg. Chem.* **1993**, *32*, 3125−3133.

(90) (a) Perlepes, S. P.; Huffman, J. C.; Matonic, J. H.; Dunbar, K. R.; Christou G. *J. Am. Chem. Soc.* **1991**, *113*, 2770−2771. (b) Crawford, C. A.; Matonic, J. H.; Huffman, J. C.; Kirsten, F.; Dunbar, K. R.; Christou G. *Inorg. Chem.* **1997**, *36*, 2361−2371.

(91) Cotton, F. A.; Hillard, E. A.; Liu, C. Y.; Murillo, C. A.; Wang, W.; Wang, X. *Inorg. Chim. Acta* **2002**, *337*, 233−246.

(92) Rotondo, E.; Mann, B. E.; Piraino, P.; Tresoldi, G. *Inorg. Chem.* **1989**, *28*, 8, 3070−3073.

(93) Aoki, K.; Hoshino, M.; Okada, T.; Yamazaki, H.; Sekizawa, H. *J. Chem. Soc., Chem. Commun.* **1986**, 314−316.

(94) *CRC Handbook of Chemistry and Physics*, 81st ed.; Lide, D. R., Ed.; CRC Press: New York, 2000−2001; pp 8-46−8-47

(95) Kashik, T. V.; Rassolova, G. V.; Ponomareva, S. M.; Medvedeva, E. N.; Yushmanova, T. I. Lopyrer, V. A. *Izv. Akad. Nauk SSSR, Ser. Chim.* **1982**, *10*, 2230−2234.

dirhodium complexes required to inhibit 50% of the transcription, C_{inh}^{50}, are comparable to that measured for activated cisplatin, whereas a significantly higher concentration of $Rh_2(\mu\text{-}HNCOCH_3)_4$ is required to effect similar inhibition. Our results show that the dominant mechanism of transcription inhibition by $Rh_2(\mu\text{-}HNCOCH_3)_4$ does not involve binding to T7-RNAP. Spectral changes measured for each complex after the addition of enzyme are consistent with strong binding of $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ to the enzyme, which may involve partial displacement of the *eq* groups by Lewis basic sites of the enzyme. In contrast, addition of enzyme to solutions of $Rh_2(\mu\text{-}HNCOCH_3)_4$ does not result in significant spectral changes, consistent with lack of enzyme dependence in the transcription inhibition. These intriguing differences in the reactivity and the mechanism of transcription inhibition between dirhodium complexes with different bridging groups may be explained by invoking the variation in the Lewis acidity of the *ax* site of the series of complexes $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $Rh_2(\mu\text{-}HNCOCH_3)_4$. The Lewis acidity of the *ax* position is expected to affect the initial interaction of the complexes with the biomolecules, followed by their rearrangement to *eq* sites if the bridging ligands are labile. Since the transcription inhibition by $Rh_2(\mu\text{-}O_2CCF_3)_4$, $Rh_2(\mu\text{-}HNCOCF_3)_4$, and $[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ is dependent on the concentration of the enzyme and Mg^{2+} ions, an interaction between these complexes or their partially solvated forms and the aspartate residues in the active site of the enzyme is certainly a possibility. Studies aimed at gaining further understanding of these binding interactions are currently in progress.

Acknowledgment. K.R.D. thanks Johnson-Matthey for a generous gift of $Rh_2(\mu\text{-}O_2CCH_3)_4$. C.T. thanks the National Institutes of Health (Grant RO1 GM64040-01) for their generous support, as well as The Arnold and Mabel Beckman Foundation for a Young Investigator Award. K.R.D. thanks the State of Texas for an ARP grant (010366-0277-1999) and the Welch Foundation (Grant A1449) for financial support.

Note Added after ASAP: This paper was posted ASAP on January 10, 2004, with errors in footnotes 46a and 46b. The version posted on January 20, 2004, contains the correct footnotes.

Supporting Information Available: Kinetic equations, equilibria, and assumptions used in calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

IC034438M