Volume 31
Number 17
August 19, 1992

Inorganic Chemistry

Communications

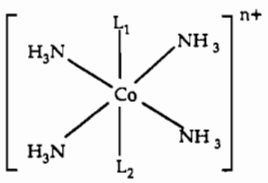
Inert Cobalt Complexes as Mechanistic Probes of the Biochemistry of Magnesium Cofactors. Application to Topoisomerase I

Soodan Kim and J. A. Cowan*

Evans Laboratory of Chemistry, The Ohio State University, 120 West 18th Avenue, Columbus, Ohio 43210

Received February 19, 1992

Topoisomerases are important enzymes in DNA replication. They promote the relaxation of supercoiled DNA (Figure 1) by creating a transient break in the polynucleotide backbone that allows the passage of the intact strand through the gap.[1,2] Eukaryotic and prokaryotic type I topoisomerases are activated by divalent magnesium. Understanding the catalytic mechanism has proven to be a difficult experimental problem, and the general features of the reaction pathway, especially the role of the essential metal cofactor, are poorly understood.[3] Previous studies on ribonuclease H have shown that the inert complex $Co(NH_3)_6^{3+}$ is an effective mimic of $Mg(H_2O)_6^{2+}$.[4] We have found that topoisomerase I is activated by $Co(NH_3)_6^{3+}$, and so the essential metal cofactor must act as an outer-sphere complex. This may serve either a *structural* role (stabilizing the enzyme–substrate complex by formation of bridging hydrogen bonds and reducing electrostatic interactions between the negatively-charged polynucleotide chain and residues at the active site) or a *catalytic* role (by promotion of nucleophilic or base catalysis by an outer-sphere mechanism).[4] Herein we describe a strategy to obtain further insight into the functional role of the metal cofactor by employing a series of *inert* cobalt–ammine derivatives:



$L_1 = NH_3$, $L_2 = H_2O, HO^-, Cl^-, CO_3^{2-}, NO_2^-, C_2O_4^{2-}$

$L_1 = NO_2^-$ $L_2 = NO_2^-$

$[Co(en)_3]^{3+}$, *trans*-$[Co(en)_2(NO_2^-)_2]^+$

whereby rational design of the inner coordination sphere may be used to probe the chemistry of the outer coordination sphere. Previously Cleland and Mildvan have used inert cobalt(III) and chromium(III) nucleotide complexes to test the stereochemistry and regiochemistry of enzymatic reactions employing ATP chelates as substrates.[5] Our approach is quite distinct inasmuch as cobalt(III) complexes, in particular $Co(NH_3)_6^{3+}$, are used as probes of the outer-sphere chemistry of Mg^{2+}(aq) in enzymes that do not necessarily have a requirement for ATP; i.e., the focus is on the intrinsic chemistry of the metal cofactor.

The cobalt derivatives used in this work were synthesized and purified by literature procedures.[6] Activation of topoisomerase was determined from the fraction of relaxed (and partially relaxed) plasmid DNA by agarose gel electrophoresis (Figure 1). Table I summarizes important observations resulting from the use of a variety of inert complexes to promote activity of topoisomerase I. First, there is no direct correspondence between the level of activity and the net charge on the complex, and so electrostatic stabilization of the enzyme–substrate complex may play a minimal role. Chemical and steric interactions of the inner-sphere ligands with functionality in the enzyme–substrate pocket appear to dominate. Second, cobalt–ammine complexes appear to activate topoisomerase more readily than Mg^{2+}(aq), irrespective of net charge. This is unlikely to result from nonsaturating binding by magnesium at the active site since the enzyme is optimally activated at the magnesium concentration used,[2] while similar reaction profiles were obtained over a range of $[M^{n+}]$ (3–10 mM). The increased number of hydrogen-bond donors on NH_3 relative to H_2O is most likely responsible. The enhanced reactivity of the carbonato complex may arise from the ionizable ligand (pK_a 7.1, 0 °C), which can promote ionization of a catalytic residue. Nonionizable ligands (Cl^- and NO_2^-) are better probes of

(1) Wang, J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665–697. Osheroff, N. *Pharmacol. Ther.* **1989**, *41*, 223–241.
(2) Der Garabedian, P. A.; Mirambeau, G.; Vermeersch, J. J. *Biochemistry* **1991**, *30*, 9940–9947.
(3) Champoux, J. J. *J. Mol. Biol.* **1978**, *118*, 441–446. Been, M. D.; Champoux, J. J. *J. Mol. Biol.* **1984**, *180*, 515–531. Bonven, B. J.; Gocke, E.; Westergaard, O. *Cell* **1985**, *41*, 541–551. Pulleyblank, D. E.; Ellison, M. J. *Biochemistry* **1982**, *21*, 1155–1161. Caserta, M.; Amadei, A.; Camilloni, G.; Di Mauro, E. *Biochemistry* **1990**, *29*, 8152–8157.
(4) Jou, R.; Cowan, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 6685–6686.
(5) Cleland, W. W.; Mildvan, A. S. *Adv. Inorg. Biochem.* **1979**, *1*, 163. Cornelius, R. D.; Cleland, W. W. *Biochemistry* **1975**, *17*, 3279. Danenberg, K. D.; Cleland, W. W. *Biochemistry* **1975**, *14*, 28.
(6) Gould, E. S.; Taube, H. *J. Am. Chem. Soc.* **1964**, *86*, 1318. Basolo, F.; Murmann, R. K. *Inorg. Synth.* **1946**, *2*, 171. Holtzclaw, H. F.; Sheetz, D. P.; McCarty, B. D. *Inorg. Synth.* **1946**, *4*, 176. Schlessinger, G. G. *Inorg. Synth.* **1967**, *9*, 160. Olson, G. L. *J. Chem. Educ.* **1969**, *46*, 508. Saffin, P.; Taube, H. *J. Am. Chem. Soc.* **1960**, *82*, 13. Buckingham, D. A.; Marty, W.; Sargeson, A. M. *Inorg. Chem.* **1974**, *13*, 2165. Palmer, W. G. *Experimental Inorganic Chemistry*; Cambridge University Press: Cambridge, U.K., 1954; p 537.
(7) Each set of experiments was carried out on two distinct plasmid systems: φX174 RF (Bethesda Research Laboratories) and pAXHP [a synthetic gene (unpublished results) is cloned into the *Eco*RI/*Hind*III site of a commercially available expression vector pAX4A$^+$ (United States Biochemical)].
(8) Wilkins, R. G. *The Study of Kinetics and Mechanism of Reactions of Transition Metal Complexes*; Allyn and Bacon: Boston, MA, 1974; p 318.
(9) Basolo, F.; Pearson, R. G. *Mechanisms of Inorganic Reactions*; John Wiley: New York, 1967.

0020-1669/92/1331-3495$03.00/0

Table I. Relative Trends for Activation of Topoisomerase I by Inert Cobalt Complexes[a,b]

1	$[Co(NH_3)_5(CO_3)]^+ > [Co(NH_3)_5(NO_2)]^{2+} \gg$ *trans*-$[Co(NH_3)_4(NO_2)_2]^+$
2	$[Co(NH_3)_6]^{3+} > [Co(en)_3]^{3+} \gg$ *trans*-$[Co(en)_2(NO_2)_2]^+$
3	$[Co(NH_3)_5Cl]^{2+} > [Co(NH_3)_5(NO_2)]^{2+} > [Co(NH_3)_5(OH)]^{2+} > Mg^{2+}(aq)$
4	$[Co(NH_3)_6]^{3+} \sim [Co(NH_3)_5(H_2O)]^{3+} \sim [Co(NH_3)_5(C_2O_4)]^+ > Mg^{2+}(aq)$

[a] Experimental conditions and a typical data set are detailed in the caption to Figure 1. Reactions with $[Co(NH_3)_5H_2O]^{3+}$ were obtained at pH 5.5 to obviate ligand ionization ($pK_a = 6.4$). Control experiments show no relaxation of supercoiled plasmid in the absence of enzyme for any of these complexes. Reactions were repeated at least three times with different batches of enzyme, and on two distinct plasmid systems.[7] The progress of each reaction was monitored at a variety of time intervals. [b] en = ethylenediamine.

electrostatic and H-bonding interactions.[10] Third, steric hindrance from the en ligands on $[Co(en)_3]^{3+}$ led to loss of activity relative to $[Co(NH_3)_6]^{3+}$, in contrast with previous observations for ribonuclease H.[4]

From these results we conclude that the metal cofactor acts as an outer-sphere complex and does not serve as a Lewis acid by direct coordination to the phosphate backbone, nor does it activate an inner-sphere nucleophile. Previously it has been demonstrated that formation of an intermediate tyrosine phosphate ester is a critical step on the reaction pathway.[11] It is likely that the metal cofactor promotes ionization of tyrosine (by lowering the pK_a) to form the nucleophilic tyrosinate anion, and also serves to stabilize this anion and the enzyme–polynucleotide complex via electrostatic interactions and bridging hydrogen bonds from Mg-bound H_2O. The metal ion may help to position the substrate for nucleophilic attack by tyrosine but is not active as a Lewis acid toward either the phosphate backbone or bound water.

Figure 1. The relaxation of supercoiled plasmid (pAXHP) by topoisomerase I was monitored by agarose gel electrophoresis (S = supercoiled, R = relaxed).[7] Each lane represents activation by a different metal cofactor: (1) $Mg^{2+}(aq)$, (2) $[Co(NH_3)_6]^{3+}$, (3) $[Co(NH_3)_5(CO_3)]^+$, (4) $[Co(NH_3)_5(NO_2)]^{2+}$, (5) $[Co(NH_3)_5Cl]^{2+}$, (6) $[Co(NH_3)_5(OH)]^{2+}$, (7) $[Co(NH_3)_5(C_2O_4)]^+$. A control lane (P) shows the unreacted plasmid. Reactions with complexes in the absence of enzyme gave no reaction. Each reaction mixture (10 μL) contained the following: 6 mM M^{n+}; 50 mM Tris (pH 8); 50 mM KCl; topoisomerase I (Bethesda Research Laboratory, 2 units); pAXHP (0.5 μg). After incubation at 0 °C for 30 min, the reaction was terminated with 1 μL of gel-loading buffer (5% bromophenol blue, 5% xylene cylanol EE, 5% orange C, 60% glycerol) and run on a 0.8% agorose gel in 1 × TAE buffer at 100 V for 45 min. As expected, the data support a processive rather than a distributive mechanism.[2]

In this paper, we have described a logical elaboration of the use of inert cobalt complexes to study outer-sphere activation of DNA-processing enzymes by essential metal cofactors. Systematic manipulation of the inner coordination sphere is straightforward for cobalt–ammine complexes. When coupled to rigorous analyses of thermodynamic and kinetic steady-state parameters (k_{cat}, K_m, and $K_{M^{n+}}$), this approach should provide greater mechanistic insight into the structural and catalytic details of the outer-sphere reaction pathways for these enzymes.

Acknowledgment. This work was supported by grants from the Elsa U. Pardee Foundation and the donors of the Petroleum Research Fund, administered by the American Chemical Society. J.A.C. is a Fellow of the Alfred P. Sloan Foundation.

(10) For *trans*-$[Co(NH_3)_4L_2]^+$, L = NO_2^- is better than Cl^- since the latter is appreciably labile.

(11) Eng, W.; Pandit, S. D.; Sternglanz, R. *J. Biol. Chem.* **1989**, *264*, 13373–13376. Lynn, R. M.; Bjornsti, M.; Caron, P. R.; Wang, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3559–3563.