rapid initial deflection at 426 nm (Soret maximum for alkaline-reduced low-spin CCP), which is followed by an *increase* in absorbance with $t_{1/2} < 1.5$ ms, indicating the presence of a transient species with a higher extinction at this wavelength than the equilibrium low-spin species. This transient then decays with $t_{1/2} < 40$ ms to the equilibrium low-spin species. It can be concluded, however, that this transient consists of a nonequilibrium low-spin heme since the resonance Raman data mentioned earlier demonstrate that ligand recombination is complete within <1 μs. In addition, the multiphasic decay of the 434-nm transient (isosbestic between equilibrium ferrous high- and low-spin CCP) indicates multiple conformational interactions of the protein in response to the rebinding of the σ ligand. These processes are distinct from the ligand recombination itself and may represent more global protein dynamics conformationally linked to the heme active site.

In summary, we have observed that the photodissociation of an endogenous σ ligand from ferrous CCP in the high-pH form induces ligand recombination dynamics that are unique from those of other protein systems. The multiphasic behavior and long time scale involved indicate that these processes are intimately associated with global protein dynamics. This study demonstrates that the conformational flexibility of CCP may be directly linked to the heme active site and hence peroxidase activity.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health [GM33330 (M.R.O.) and GM22432 (S.I.C.)].

A New Approach toward the Inhibition of Ribonucleases: A Water-Stable Ribonucleoside–Technetium Chelate

Y.-C. Jack Chen and Kim D. Janda*

The Scripps Research Institute
Departments of Molecular Biology and Chemistry
10666 North Torrey Pines Road
La Jolla, California 92037

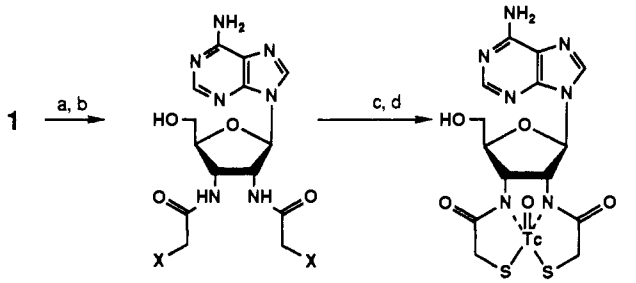
Received September 26, 1991

The ribonuclease-mediated hydrolysis of RNA is believed to proceed via a cyclic 2′,3′-monophosphate transition state in which the attacking 2′-hydroxyl and the departing 5′-nucleoside occupy the axial positions of a pentacoordinate trigonal-bipyramidal phosphorane.[1] Since phosphorus does not form a stable pentacoordinate complex, other elements, namely, vanadium,[2a-d] have been used to model the postulated transition state for RNA hydrolysis.[2e] Biophysical studies, including X-ray crystallography, have been performed on the complex formed between vanadate, a ribonucleoside, and RNase A.[3] All the evidence points toward the formation of a trigonal-bipyramidal vanadate ribonucleoside complex bound in the active site of the enzyme. Unfortunately, the complexes exist only when bound to RNAse A and disproportionate in aqueous solution into a complex mixture.[2,4] Recently, a cyclic vanadium(V) chloro alkoxide has been synthesized and the crystal structure of the compound determined.[4] It is unlikely that this complex is stable in aqueous media. Thus, no water-soluble metal-based pentacoordinate analogues that are potent inhibitors of ribonucleases exist. In this communication, we report the synthesis of the first pentacoordinate metallochelate of a ribonucleoside **4** and its potency as an inhibitor of ribonuclease U2.

The inhibitor **4** is a derivative of a class of technetium-99 complexes that are used as imaging agents in nuclear medicine.[5,6] These metallocycles are stable in aqueous solution and exist as pentacoordinate species free in solution.[5-7] Diffractable crystals of one ethylenediamine complex have been obtained and its X-ray structure, independent of protein, solved showing that the parent complex adopts a square-pyramidal geometry.[7] Molecular modeling suggested that if the ethylenediamine is incorporated as part of a ribonucleoside, the resulting constrained complex adopts a geometry that approaches a trigonal bipyramid.[8] The technetium chelate was synthesized from 2′,3′-diamino-2′,3′-dideoxyadenosine, **1**,[9] in three steps (Scheme I), and purified by reverse-phase chromatography on a Sep-Pak C-18 cartridge (Millipore). Analysis of the complex by HPLC (RP C-18) showed the product to be a 2.4:1 mixture of diastereomers.[10]

Addition of the diastereomeric mixture **4** to assays of ribonuclease U2 with diribonucleotide substrates (ApA or ApU) yielded a concentration-dependent inhibition of U2 activity.[11]

Scheme I.[a] Synthesis of Ribonuclease Inhibitor **4**



[a] Key: (a) 2 equiv of $(ClCH_2CO)_2O$, CH_3CN, DMF, 0 °C → 25 °C, 12 h; (b) $C_6H_5COS^-Na^+$, EtOH, 78 °C, 3 h, 20% from **1**; (c) EtOH, 5 N NaOH, 78 °C, 5 min; (d) 0.95 equiv of Tc(VII), $Na_2S_2O_4$, 82 °C, 30 min, 30% from **3**.

Table I. Substrate and Inhibition Constants of Ribonuclease U2[a]

substrate	K_m (mM)	V_{max} (pmol/min)	K_i (μM)
ApA	3.1	47.4	134
ApU	18.7	95.8	220

[a] Assays were performed as described.[11]

(1) (a) Saenger, W.; Suck, D.; Eckstein, F. *Eur. J. Biochem.* **1974**, *46*, 559. (b) Usher, D. A.; Erenrich, E. S.; Eckstein, F. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 115.

(2) (a) Lindquist, R. N.; Lynn, J. L., Jr.; Lienhard, G. E. *J. Am. Chem. Soc.* **1973**, *95*, 8762. (b) Berger, S. L.; Birkenmeier, C. S. *Biochemistry* **1979**, *18*, 5143. (c) Puskas, R. S.; Manley, N. R.; Wallace, D. M.; Berger, S. L. *Biochemistry* **1982**, *21*, 4602. (d) Tracey, A. S.; Gresser, M. J.; Liu, S. *J. Am. Chem. Soc.* **1988**, *110*, 5869. (e) Recently, a hydrolytically stable pentacoordinate phosphorus complex has been reported. The structure, however, does not resemble a ribonucleoside analogue. Moriarty, R. M.; Hiratake, J.; Liu, K. *J. Am. Chem. Soc.* **1990**, *112*, 8575.

(3) (a) Wlodawer, A.; Miller, M.; Sjölin, L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3628. (b) Borah, B.; Chen, C.-w.; Egan, W.; Miller, M.; Wlodawer, A.; Cohen, J. S. *Biochemistry* **1985**, *24*, 2058. (c) Alber, T.; Gilbert, W. A.; Ponzi, D. R.; Petsko, G. A. *Ciba Found. Symp.* **1983**, *93*, 4. (d) Brünger, A. T.; Brooks, C. L., III; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 8458. (e) Wlodawer, A.; Sjölin, L. *Biochemistry* **1983**, *22*, 2720.

(4) Crans, D. C.; Felty, R. A.; Miller, M. M. *J. Am. Chem. Soc.* **1991**, *113*, 265.

(5) Lever, S. Z.; Burns, H. D.; Kervitsky, T. M.; Goldfarb, H. W.; Woo, D. V.; Wong, D. F.; Epps, L. A.; Kramer, A. V.; Wagner, H. N., Jr. *J. Nucl. Med.* **1985**, *26*, 1287.

(6) (a) Costello, C. E.; Brodack, J. W.; Jones, A. G.; Davison, A.; Johnson, D. L.; Kasina, S.; Fritzberg, A. R. *J. Nucl. Med.* **1983**, *24*, 353. (b) Fritzberg, A. R.; Kuni, C. C.; Klingensmith, W. C., III; Stevens, J.; Whitney, W. P. *J. Nucl. Med.* **1982**, *23*, 592. (c) Kasina, S.; Fritzberg, A. R.; Johnson, D. L.; Eshima, D. *J. Med. Chem.* **1986**, *29*, 1933. (d) Efange, S. M. N.; Kung, H. F.; Billings, J.; Guo, Y.-Z.; Blau, M. *J. Nucl. Med.* **1987**, *28*, 1012. (e) Kung, H. F.; Molnar, M.; Billings, J.; Wicks, R.; Blau, M. *J. Nucl. Med.* **1984**, *25*, 326. (f) Kung, H. F.; Yu, C. C.; Billings, J.; Molnar, M.; Blau, M. *J. Med. Chem.* **1985**, *28*, 1280.

(7) Kung, H. F.; Guo, Y.-Z.; Yu, C. C.; Billings, J.; Subramanyam, V.; Calabrese, J. C. *J. Med. Chem.* **1989**, *32*, 433.

(8) Chem-3D, Cambridge Scientific Computing, Inc.

(9) (a) Chen, Y.-C. J.; Hansske, F.; Janda, K. D.; Robins, M. J. *J. Org. Chem.* **1991**, *56*, 3410. (b) Robins, M. J. In *Nucleosides, Nucleotides, and their Biological Applications*; Barascut, J.-L., Imbach, J.-L., Eds; INSERM: Paris, 1979; pp 13-35.

(10) Compound **4** exhibited satisfactory spectral and analytical analyses.

0002-7863/92/1514-1488$03.00/0

Lineweaver-Burk analysis of initial velocities suggest that the inhibition by **4** is competitive with respect to either substrate with K_i values of 134 μM and 220 μM for ApA and ApU, respectively (Table I).[12] The inclusion of uridine (4 mM) in the assay for ApU hydrolysis resulted in no detectable loss of activity, indicating that the observed inhibition is not due to product inhibition. A similar experiment with adenosine (4 mM) for ApA hydrolysis resulted in an inhibition of activity similar to that for the case of addition of 60 μM **4**. In a separate experiment, ribonuclease U2 was incubated with a radiolabeled RNA substrate (5'-AAGUAAGAGACAAGAUACAU-3'), and the products were separated on a denaturing polyacrylamide gel and visualized by autoradiography. Inclusion of adenosine to 2 mM or free technetium-99 (54 mM) in the limit digests resulted in no observable loss of activity. Addition of 600 μM **4** to the limit digests, however, resulted in a 95% loss of ribonuclease activity (data not shown). These findings strongly suggest that inhibition is caused by the complex and is not due to inhibition by either free adenosine or adventitious binding of technetium-99 to the enzyme.

Lindquist et al.[2a] have calculated the K_i for cyclic UMP hydrolysis by RNase A by uridine-vanadate complexes to be 8-12 μM. The strength of binding is attributed to the resemblance of the complexes to the transition state for the hydrolysis reaction. A solved X-ray crystal structure of a ribonuclease-uridine-vanadate complex indicates that the uridine-vanadate ester assumes a distorted trigonal-bipyramidal geometry in the active site of RNase A with an angle of 162° between the apical substituents.[4] Thus, the uridine-vanadate ester most likely resembles a complex formed along the reaction path for the hydrolysis reaction. While the parent technetium complexes are known to exist as square-pyramidal species, our constrained technetium-ribonucleoside complex may assume a geometry intermediate between a square-pyramidal and a trigonal-bipyramidal structure. The K_i values we have determined are only 13-28-fold weaker than the K_i calculated for the uridine-vanadate complex, which corresponds in a loss of binding energy of 1.6-2.1 kcal/mol. This suggests that the technetium complex may also resemble a complex formed along the reaction path of phosphodiester hydrolysis, albeit further away from the idealized transition state.[13] A crystallographic analysis of **4** should shed light on this hypothesis.[14]

We have synthesized a technetium-based hapten that appears to be a reasonable approximation of the putative transition state as judged by its potent, competitive inhibition of ribonuclease U2 activity. Our experimentally determined K_i values are only an order of magnitude less than the calculated K_i for the uridine-vanadate complexes, which are generally regarded as excellent mimics of the transition state for phosphodiester hydrolysis. This, coupled with the fact that the technetium-complex is stable in aqueous solution, makes them ideal candidates for use in immunization protocols for the purpose of generating catalytic antibodies capable of phosphodiester hydrolysis. These complexes, and others like them, would have potential as general inhibitors of ribonucleases in preparative RNA isolation or as inhibitors of other enzymes that catalyze phosphoryl-transfer reactions such as 3',5'-cAMP phosphodiesterase. We are currently exploring such opportunities.

Acknowledgment. This investigation has been aided by a grant from The Scripps Research Institute. Y.-C.J.C. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

(11) The assay mixture contained 10 units of sequencing-grade ribonuclease U2 (Pharmacia-LKB), 16.5 mM sodium citrate (pH 3.5), 0.85 mM EDTA, 3.5 M urea, and the desired concentration of substrate and inhibitor in a final volume of 50 μL. Assays were performed at 52 °C ± 1 °C; 5-μL aliquots were removed and products separated on an Alltech Adsorbosphere HS C-18 reverse-phase column using a mixed isocratic-gradient elution system. The mobile phase contained 4% acetonitrile/96% 50 mM NH_4OAc, pH 4.5, at a flow rate of 1 mL/min with a gradient to 50% acetonitrile from 10 to 20 min. Products were monitored at 260 nm and quantitated by comparison to standard curves derived by injection of authentic compounds. Initial velocities for cAMP, adenosine (or uridine), and ApA (or ApU) were used for the kinetic plots. All velocities were observed to be linear within this range; no product inhibition was observed under the assay conditions. The range of substrate concentrations used to determine K_m and V_{max} values was 63.8 μM to 3.88 mM for ApA (Sigma) and 382 μM to 18.3 mM for ApU (Sigma). K_i values were determined from Dixon plots using inhibitor concentrations ranging from 40 to 300 μM. ApA, adenylyl(3'-5')adenosine; ApU, adenylyl(3'-5')uridine.

(12) Since these values represent a mixture of the diastereomers of **4**, the K_i values for the isomer recognized by the enzyme could be at least one-half of the reported values.

(13) One can also argue that the presence of the methylene moieties in **4** contribute to the hydrophobicity of the molecule and would disfavor binding of the complex in the hydrophilic pocket of the active site. If this is indeed the case, then the geometry of the complex would in fact more closely approximate a trigonal-bipyramidal species.

(14) It is interesting to note that the K_i value for free vanadium(IV), 60-65 μM, is not significantly different from the K_i value for the uridine-vanadate complex (8-12 μM). In our compound, both the ribonucleoside and the technetium are necessary for inhibition.

Reactions of Bis(indenyl)dimethyltitanium with Phenylsilane: Synthesis of a Novel μ-Indenyl μ-Dihydrido Mixed-Valence Ti(III)/Ti(II) Compound

François Gauvin,[†] James Britten,[†] Edmond Samuel,*[,‡] and John F. Harrod*[,†]

Department of Chemistry, McGill University
Montreal, Canada H3A 2K6
Laboratoire de Chimie Organométallique de l'ENSCP
URA 403 CNRS
11 rue Pierre et Marie Curie, 75005 Paris, France
Received June 18, 1991

Bis(cyclopentadienyl) complexes of titanium and zirconium have a remarkable ability to catalyze the dehydrocoupling of organosilanes.[1-4] Dimethyltitanocene (DMT) has been studied in some detail, and a number of novel silyltitanium(III) complexes have been prepared, either by direct reaction of silanes with DMT[1c] or by addition of ligands to such reactions.[5]

The chemistry of indenyl complexes is of interest because of their potential application as stereoselective catalysts.[6-8] It was found that bis(indenyl)dimethyltitanium complexes function relatively poorly as catalysts for dehydrocoupling, compared to their zirconium analogues[8] or to DMT. Initially, the reaction of bis(indenyl)dimethyltitanium (**1**) with phenylsilane is very similar to that observed with DMT.[1c] Following mixing of a catalytic amount of **1** with phenylsilane, there is an induction period of a few minutes, during which there is no observable reaction. The end of the induction period is signaled by a sudden change in color from orange to blue/green, accompanied by a vigorous gas evolution and the complete disappearance of NMR signals due to **1**. In contrast to the case of DMT, no new NMR signals due to bimetallic Ti(III) complexes are observed. In addition, the initial activity of the catalyst rapidly declines to a very low level. During the period of declining activity, dark colored crystalline material deposits on the wall of the reaction vessel and the initially broad NMR signals of organic and organosilicon products sharpen. One

[†] McGill University.
[‡] ENSCP.

(1) (a) Aitken, C. A.; Barry, J.-P.; Gauvin, F.; Harrod, J. F.; Malek, A.; Rousseau, D. *Organometallics* **1989**, *8*, 1732. (b) Harrod, J. F. *Inorganic and Organometallic Polymers*; Zeldin, M., Wynne, K., Allcock, H. R., Eds.; ACS Symposium Series 360; American Chemical Society: Washington, DC, 1988; Chapter 7. (c) Aitken, C. A.; Harrod, J. F.; Samuel, E. *J. Am. Chem. Soc.* **1986**, *108*, 4059.

(2) (a) Tilley, T. D. *Comments Inorg. Chem.* **1990**, *10*, 37. (b) Woo, H.-S.; Tilley, T. D. *J. Am. Chem. Soc.* **1989**, *111*, 8043. (c) Woo, H.-S.; Tilley, T. D. *J. Am. Chem. Soc.* **1989**, *111*, 3757.

(3) (a) Corey, J. Y.; Chang, L. S.; Corey, E. G. *Organometallics* **1987**, *6*, 1596. (b) Chang, L. S.; Corey, J. Y. *Organometallics* **1989**, *8*, 1885. (c) Corey, J. Y.; Zhu, X.-H.; Bedard, T. C. Organosilicon Symposium, 23rd, Midland, MI, April 20-21, 1990; Abstract D1.

(4) Campbell, W. H.; Hilty, T. K.; Yurga, L. *Organometallics* **1989**, *8*, 2615.

(5) Samuel, E.; Mu, Y.; Harrod, J. F.; Dromzee, Y.; Jeannin, Y. *J. Am. Chem. Soc.* **1990**, *112*, 3435.

(6) Ewen, J. A. *J. Am. Chem. Soc.* **1984**, *106*, 6355.

(7) Kaminsky, W.; Kulper, K.; Brintzinger, H.; Wild, F. R. W. P. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 507.

(8) Gauvin, F.; Harrod, J. F. *Can. J. Chem.* **1990**, *68*, 1638.

0002-7863/92/1514-1489$03.00/0