Archives of Biochemistry and Biophysics
Vol. 381, No. 1, September 1, pp. 25–30, 2000
doi:10.1006/abbi.2000.1951, available online at http://www.idealibrary.com on IDEAL®

Decavanadate Inhibits Catalysis by Ribonuclease A[1]

June M. Messmore*,[2] and Ronald T. Raines*,†,[3]
*Department of Biochemistry and †Department of Chemistry, University of Wisconsin–Madison, Madison, Wisconsin 53706

Received February 18, 2000, and in revised form May 12, 2000

Pentavalent organo-vanadates have been used extensively to mimic the transition state of phosphoryl group transfer reactions. Here, decavanadate ($V_{10}O_{28}^{6-}$) is shown to be an inhibitor of catalysis by bovine pancreatic ribonuclease A (RNase A). Isothermal titration calorimetry shows that the K_d for the RNase A · decavanadate complex is 1.4 μM. This value is consistent with kinetic measurements of the inhibition of enzymatic catalysis. The interaction between RNase A and decavanadate has a coulombic component, as the affinity for decavanadate is diminished by NaCl and binding is weaker to variant enzymes in which one (K41A RNase A) or three (K7A/R10A/K66A RNase A) of the cationic residues near the active site have been replaced with alanine. Decavanadate is thus the first oxometalate to be identified as an inhibitor of catalysis by a ribonuclease. Surprisingly, decavanadate binds to RNase A with an affinity similar to that of the pentavalent organo-vanadate, uridine 2′,3′-cyclic vanadate.

Key Words: **active site; coulombic interaction; oxometalate; vanadate; vanadium.**

Vanadium is one of the important transition elements in biology (1, 2). In aqueous solutions, vanadium (V) occurs in a number of oxometalate forms collectively termed vanadates. In neutral dilute solutions, the predominant vanadium oxometalate is monomeric vanadate, $H_2VO_4^-$. In concentrated solutions, the speciation of vanadium oxometalates is complex (3, 4). Monomeric vanadate predominates only at concentrations below 1 mM or at pH values above 8. The species that occur at higher concentrations or under neutral to acidic conditions are multimers that arise through the sharing of oxo ligands.

Vanadates are versatile. In addition to its capacity for self-association, monomeric vanadate possesses the ability to form complexes with a variety of biochemical ligands. Alcohols, carboxylic acids, amines, and phosphates can all participate (5). A single vanadium can be coordinated to as many as eight other atoms, but six coordinating atoms is most typical. A variety of geometries have been observed, including tetrahedral, octahedral, and trigonal bipyramidal. Multimeric oxovanadates can form larger complexes with additional ligands. When free thiols act as ligands, they can participate in redox chemistry, resulting in the reduction of vanadium (V) to vanadium (IV). The thermodynamic and kinetic stabilities of vanadate coordination complexes fall within a wide spectrum.

Bovine pancreatic ribonuclease A (RNase A; EC 3.1.27.5) is a much studied enzyme that has served as the prototype in the development of new ribonuclease inhibitors (6–8). Indeed, the first vanadium-containing enzyme inhibitor, uridine 2′,3′-cyclic vanadate, was an inhibitor of RNase A (9). Subsequently, monomeric and polymeric oxovanadates were found to inhibit other enzymes that catalyze the transfer of phosphoryl groups (10, 11). Yet, monomeric vanadate does not to bind to RNase A (12), and small amounts of decavanadate have been reported to have no effect on catalysis by RNase A (9). Hence, with the exception of the original nucleoside complex, no vanadate has been reported to inhibit catalysis by RNase A.

Polymeric oxovanadates exist as polyanions at physiological pH. Likewise, many of the known inhibitors of RNase A are polyanions, such as heparin, polyvinylsulfate, aurintricarboxylic acid, and polymers of acidic amino acids (13, 14). Given the precedent for polyanionic inhibitors and the historical link between vanadate and RNase A, we suspected that polymeric oxovanadates could inhibit catalysis by RNase A. Here, we report that RNase A is indeed

[1] This study was supported by NIH Grant GM44783.
[2] Permanent address: Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226.
[3] To whom correspondence should be addressed. Fax: (608) 262-8588. E-mail: raines@biochem.wisc.edu.

0003-9861/00 $35.00

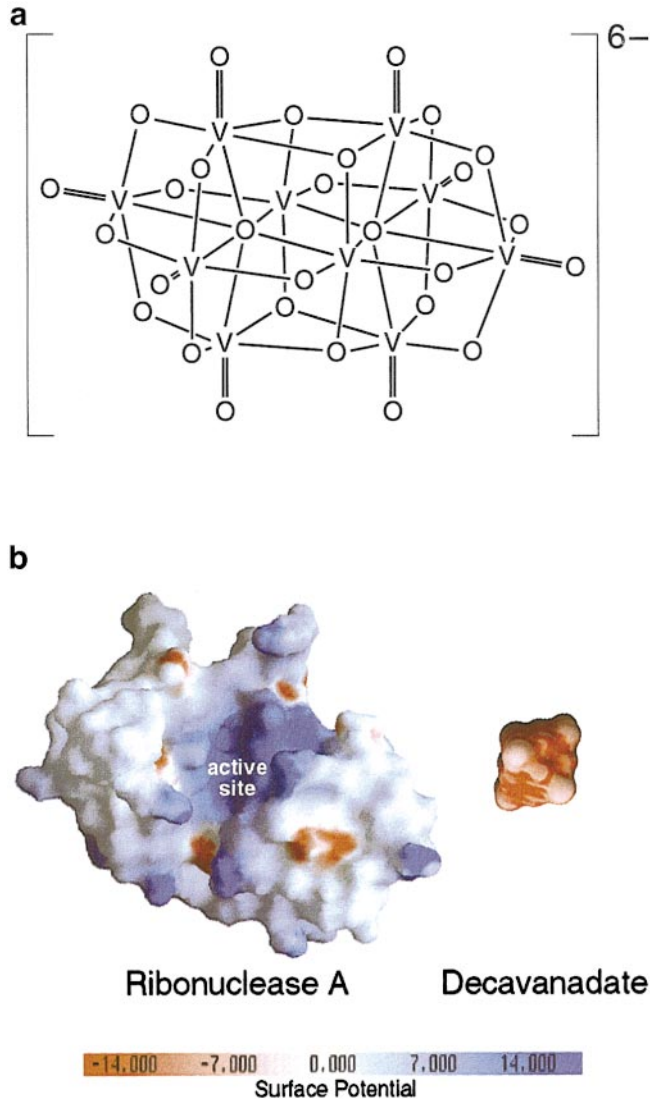

FIG. 1. (a) Structure of decavanadate, which is $V_{10}O_{28}^{6-}$ (4). (b) Electrostatic potential maps of the surface of wild-type ribonuclease A and decavanadate. Atomic coordinates for RNase A and decavanadate are from pdb:6RSA and the Cambridge data base, respectively. Maps were created with the program GRASP (42). Each of the 18 bridging surface oxygens of decavanadate was assigned a charge of $-\frac{1}{3}$, as the primary species present at pH 6.0 is $V_{10}O_{28}^{6-}$ (23). Histidine residues were assigned a charge of $\frac{1}{2}$. Otherwise, full charges and default parameters within GRASP were used.

strongly inhibited by a polymeric oxovanadate—decavanadate (Fig. 1a).

MATERIALS AND METHODS

Enzyme design. RNase A has 14 arginines and lysines among its 124 residues. Its p*I* is 9.3 (15). The active site of RNase A is especially cationic (Fig. 1b). That character comes from two histidine residues and Lys41. In addition to its cationic active site, RNase A has cationic subsites for binding to multiple phosphoryl groups in a polymeric RNA substrate (16, 17). For example, Lys66 binds to the closest phosphoryl group on the 5′ side of the scissile bond, and Lys7 and Arg10 bind to the closest phosphoryl group on the 3′ side of the scissile bond. To probe the characteristics of the binding of RNase A to decavanadate, we created enzyme variants in which either Lys41 or Lys7, Arg10, and Lys66 were replaced with an alanine residue.

Materials. Wild-type RNase A and its K41A and K7A/R10A/K66A variants were prepared as described elsewhere (18–20). The concentration of RNase A solutions was determined by measuring the absorbance at 277.5 nm and using $\epsilon = 0.72$ mL mg^{-1} cm^{-1} (21).

Sodium metavanadate, $NaVO_3$, was obtained from Aldrich Chemical (Milwaukee, WI). Poly(cytidylic acid) [poly(C)][4] was from Midland Certified Reagents (Austin, TX). Uridine 2′,3′-cyclic phosphate (U>p) was from Sigma Chemical (St. Louis, MO). 2-(*N*-Morpholino)ethanesulfonic acid (Mes), imidazole, and sodium succinate were from Sigma Chemical (St. Louis, MO). Tris–HCl was from Fisher (Chicago, IL).

Stock solutions of decavanadate. Monomeric vanadate is known to oligomerize to dimers, tetramers, pentamers, and decamers. Vanadate dimers, tetramers, and pentamers interconvert on the time scale of milliseconds to seconds (22). Stock solutions of decavanadate were prepared in a manner that maximized the proportion of vanadate that was present as the decamer. These solutions contained $NaVO_3$ (>40 mM) and NaCl (0.6 M). Aqueous HCl was used to adjust the pH to between 3 and 5. Because pH values were slow to stabilize, the pH was checked 24 h later to ensure that equilibrium had been established. Under these conditions, all but trace amounts of the vanadium was in the form of decavanadate (23), which is slow to disassemble upon dilution (24). Stock solutions appeared bright yellow to orange in color, which is diagnostic of the presence of decavanadate.

Isothermal titration calorimetry. Thermodynamic parameters for the binding of decavanadate binding to RNase A were determined by isothermal titration calorimetry (ITC). ITC was performed with a Micro Calorimetry System calorimeter from MicroCal (Northhampton, MA). ITC experiments were performed at 25°C. Binding experiments are generally performed by injecting ligands into dilute protein solutions (25). The inverse approach of injecting protein into ligand was adopted here to avoid the background signals generated by the dilution of a concentrated solution of vanadate multimers.

A concentrated solution of RNase A (~7 mg/mL) was dialyzed against 30 mM sodium succinate buffer (pH 6.0) overnight prior to an ITC experiment. Within 30 min of the beginning of the experiment, a 20 mM decavanate solution was diluted with 30 mM succinate buffer (pH 6.0) to 20 μM decavanadate. Both the decavanadate and protein solutions were degassed by vacuum.

The reference cell was filled with water. The degassed protein solution was placed in the 250-μL injection syringe, while the degassed, dilute decavanadate solution was transferred to the sample cell. A small aliquot of protein solution was retained for protein quantitation. Each 10-μL injection of protein occurred over 12.6 s, and injections were made at intervals of 240 s. Controls were performed using buffer alone in the syringe in place of the protein solution or buffer alone in the cell in place of the ligand solution. The heat of dilution of protein into buffer was observed to be negligible. The small exothermic signal resulting from injecting buffer into the dilute decavanadate solution was used to correct the experimental data before using the program ORIGIN (MicroCal Software; Northhampton, MA) to determine the binding parameters n (the equivalents of decavanadate bound per protein molecule) and K_d (the equilibrium dissociation constant of the RNase A · decavanadate complex).

[4] Abbreviations used: poly(C), poly(cytidylic acid); Mes, 2-(*N*-morpholino)ethanesulfonic acid; ITC, isothermal titration calorimetry; U>p, uridine 2′,3′-cyclic phosphate; RI, ribonuclease inhibitor protein.

IC_{50} values for inhibition of catalysis. RNase A catalyzes the cleavage of poly(C) and the hydrolysis of U>p. We used both substrates and wild-type RNase A, K41A RNase A, and K7A/R10A/K66A RNase A in assays designed to detect inhibition by decavanadate and to examine its characteristics. Assays were performed at 25°C in 0.80 mL of buffer (10 mM Tris–HCl, pH 7.0, or 0.10 M Mes–NaOH, pH 6.0) containing NaCl (0–0.29 M) substrate (50 μM poly(C) or 1.1 mM U>p), decavanadate (0–1.0 mM), and enzyme. The concentrations of the two substrates are less than the values of K_m. The initial rates of poly(C) cleavage and U>p hydrolysis were monitored by changes in the absorbance at 286 and 250 nm, respectively. The concentration of decavanadate that reduced the initial velocity to 50% (±10%) was taken to be the IC_{50} value.

Steady-state kinetic analyses. Steady-state assays of poly(C) cleavage were used to characterize further the inhibition of wild-type RNase A by decavanadate. Poly(C) was purified by precipitation from aqueous ethanol (70% v/v) prior to use. Small aliquots of poly(C) stock solutions were quantitated for total cytidyl concentration by their absorbance at 268 nm using $\epsilon = 6200\ M^{-1}\ cm^{-1}$ (26). Assays of poly(C) cleavage were performed at 25°C in 10 mM Tris–HCl buffer (pH 7.0) containing NaCl (0.10 M). Decavanadate was added from the concentrated stock immediately before enzyme addition. Cleavage of poly(C) was monitored by changes in absorbance at 250 nm using $\Delta\epsilon = 2380\ M^{-1}\ cm^{-1}$ (27). Steady-state kinetic parameters were determined by fitting initial velocity data to the Michaelis–Menten equation with the program DELTAGRAPH (Deltapoint Software, San Francisco, CA). The subsequent hyperbolic fit of $(K_m/V)_{apparent}$ was also made with the program DELTAGRAPH.

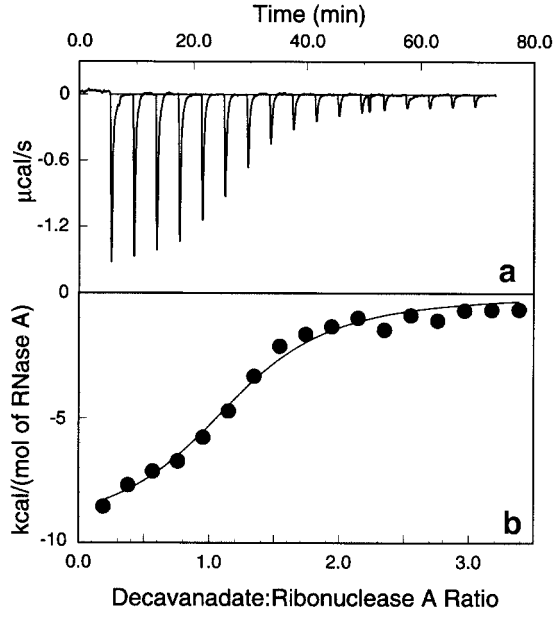

FIG. 2. Thermograms for the binding of decavanadate to wild-type ribonuclease A. (a) Heat released upon injection of RNase A into the decavanadate solution. (b) Heat released as a function of the molar ratio of RNase A to decavanadate. Binding was measured by isothermal titration calorimetry at 25°C in 30 mM sodium succinate buffer (pH 6.0). The value of K_d derived from these data is 1.4 ± 0.3 μM.

RESULTS

Calorimetry. Isothermal titration calorimetry was used to evaluate the binding of decavanadate to RNase A in the absence of substrate. When protein was injected into a solution of vanadate, an exothermic reaction was observed, as shown in Fig. 2a. Least-squares estimates of binding parameters give a value of 1.2 ± 0.1 for n. This value is consistent with binding to decavanadate. If we assume that the stoichiometry of binding is 1:1, then the K_d derived from calorimetric data is 1.4 ± 0.3 μM. The fit to these values of n and K_d is shown in Fig. 2b.

IC_{50} values. Decavanadate inhibits catalysis of both poly(C) cleavage and U>p hydrolysis by RNase A, as shown in Table I. The extent of inhibition is relatively independent of substrate, buffer type, and pH (6.0 or 7.0). Nonetheless, the ability of decavanadate to inhibit catalysis does vary with salt concentration and with the type of enzyme.

Kinetic analyses. Steady-state kinetic analysis of inhibition indicates that the vanadate inhibitor competes against poly(C) for binding to RNase A (Fig. 3a). When $(K_m/V_{max})_{apparent}$ is plotted versus vanadate concentration, the linear relationship expected for simple competitive inhibition is not observed. The relationship can, however, be fitted to a hyperbola (Fig. 3b). The implications of this relationship are discussed below.

DISCUSSION

Vanadate forms a multitude of complexes and has versatile redox chemistry (1, 2). Accordingly, multiple mechanisms for the loss of enzymatic activity in the presence of decavanadate are possible. Full activity of inhibited RNase A can be recovered by dialyzing the enzyme against 1 M NaCl and then against dilute buffer (data not shown). This observation rules out any irreversible reaction with the enzyme. Still, decavanadate could interact with the substrate in such a way as to make the substrate unavailable to the enzyme. This possibility is difficult to reconcile with the inhibition being observed with both cyclic mononucleotide and linear polymeric substrates (Table I). Moreover, the calorimetric data indicate that decavanadate binds tightly to protein in the absence of substrate (Fig. 2). A substrate–decavanadate interaction is therefore not necessary to explain the loss of enzymatic activity. Moreover, the loss of enzyme activity is observed at decavanadate concentrations far below those of the substrate.

If decavanadate does not alter the protein irreversibly and does not sequester the substrate, then it must be a reversible inhibitor. Because buffer functional groups can potentially participate in vanadate complexes, buffer concentrations were kept low (10–30 mM). Changing the buffer species did not significantly alter the extent of inhibition (Table I), and we therefore

TABLE I

Effect of Salt Concentration and Active-Site Charge on IC_{50} Values for Decavanadate Inhibition of Catalysis by Ribonuclease A[a]

Ribonuclease A	[NaCl] (M)	Buffer	pH	Substrate	IC_{50} (μM)
Wild type	0.00	10 mM Tris–HCl	7.0	U>p	0.27
Wild type	0.10	10 mM Tris–HCl	7.0	U>p	1.0
Wild type	0.10	10 mM Tris–HCl	7.0	Poly(C)	1.2
Wild type	0.29	10 mM Tris–HCl	7.0	U>p	5.0
Wild type	0.10	0.10 M MES–NaOH	6.0	Poly(C)	4.0
K41A	0.10	0.10 M MES–NaOH	6.0	Poly(C)	15
K7A/R10A/K66A	0.10	0.10 M MES–NaOH	6.0	Poly(C)	>60

[a] Assays were performed at 25°C with [substrate] $< K_m$.

conclude that inhibition does not require coordination to a buffer functional group.

Decavanadate is known to inhibit some kinases and phosphorylases, as well as the glycolytic enzyme aldolase (10, 11). Reported K_i and IC_{50} values range from 62 μM for hexokinase to 45 nM for phosphofructokinase (28, 29). The IC_{50} values observed for RNase A are within this range (Table I).

The ability of decavanadate to inhibit an enzyme cannot be predicted from macroscopic properties alone. The enzymes inhibited by decavanadate are all preorganized to bind phosphoryl groups. For example, RNase A has four known subsites that interact with the phosphoryl groups of an RNA substrate (16, 17). Nonetheless, decavanadate inhibition is by no means a general property of all such enzymes. Creatine kinase, pyruvate kinase, galactokinase, and inorganic pyrophosphatase are all enzymes that bind to phosphoryl groups at multiple sites but are not affected by decavanadate (29). Likewise, inhibition by decavanadate is not simply a consequence of the enzyme being cationic. High isoelectric point (pI) does not correlate with susceptibility to decavanadate inhibition. The pI of pyruvate kinase (cow muscle) is 8.9 (30); it is not inhibited by decavanadate. The pI of RNase A is 9.3 (15) and that of phosphofructokinase (pig liver) is only 5.0 (31); yet both are inhibited by decavanadate.

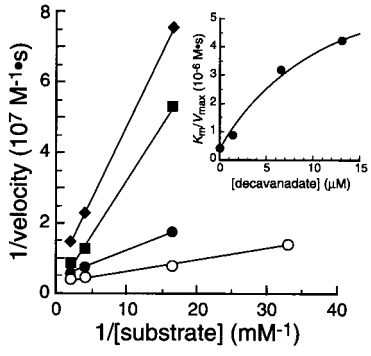

FIG. 3. Effect of decavanadate on catalysis of poly(cytidylic acid) cleavage by wild-type ribonuclease A. Lineweaver–Burk plots are shown for four concentrations of decavanadate: 13 μM (◆), 6.5 μM (■), 1.3 μM (●), and 0.0 μM (○). Experiments were performed in 10 mM Tris–HCl buffer (pH 7.0) at 25°C. Inset: Slope replot of the kinetic data fitted to Eq. [1] with K_i = 0.41 μM and K_I = 11.5 μM.

Inhibition by decavanadate does not tend to follow a simple competitive model. For example, decavanadate inhibits hexokinase noncompetitively with respect to both ATP and glucose (29). Decavanadate inhibits phosphofructokinase in a complex allosteric manner. Inhibition is antagonized by positive allosteric effectors and is synergistic with ATP, suggesting that decavanadate may interact with both the substrate and effector binding sites (28). For RNase A, the data likewise suggest a model with more subtlety than a simple competition with poly(C) for the active site. Inhibition is less severe at higher levels of the substrate (Fig. 3a), but the slope replot (Fig. 3b) fits a hyperbola better than a straight line. One model capable of explaining this pattern arises from an inhibitor that can bind to an enzyme in a fashion that does not preclude substrate binding and turnover, yet alters K_m. In this model

$$(K_m/V_{max}) = (K_m/V_{max})_{[I]=0} \times \left(\frac{1 + [I]/K_i}{1 + [I]/K_I}\right), \qquad [1]$$

where K_i = [E][I]/[EI] and K_I = [ES][I]/[EIS] (32). Figure 3b shows the least-squares fit to this equation where K_i = 0.41 μM and K_I = 11.5 μM. Such hyperbolic competitive inhibition is not unexpected for a large polyanionic ligand inasmuch as its binding weakly to an enzymic subsite (rather than the active site) is likely to affect K_m without precluding catalysis.

To characterize the basis for the interaction of RNase A with decavanadate, we collected kinetic

data in solutions of different pH and salt concentrations, as well as with less cationic RNase A variants (Table I). Although the pK_a of $HV_{10}O_{28}^{5-}$ is 6.14 (23), the IC_{50} values vary little between pH 6.0 and pH 7.0. Apparently, $HV_{10}O_{28}^{5-}$ and $V_{10}O_{28}^{6-}$ have similar affinities for RNase A. Yet, the IC_{50} values are higher in solutions of high salt concentration and with the less cationic variants. RNase A has a positive electrostatic potential around its active site. The dimensions of the active-site cleft can accommodate decavanadate (Fig. 1b). This positive potential is shielded by salt and reduced in the K41A and K7A/R10A/K66A variants (33). Thus, our data indicate that coulombic forces contribute favorably to the RNase A–decavanadate interaction. This finding is consistent with the decrease in k_{cat}/K_m for the cleavage of RNA (another polyanion) at high salt concentration (34).

Decavanadate binds to RNase A with an affinity on par with two other small molecules that are useful prophylactics in laboratory manipulations of RNA. Aurintricarboxylic acid has been reported to have an IC_{50} value near 1 μM (14), and uridine 2′,3′-cyclic vanadate has a K_i of 0.45 μM, though this species is a minor component of a complex and dynamic mixture (12). Also comparable is the IC_{50} near 2 μM observed for tyrosine–glutamic acid copolymers of $M_r \sim 3500$ (35). None of these inhibitors has nearly the affinity ($K_d < 10^{-13}$ M) of the 50-kDa ribonuclease inhibitor protein (RI) (36, 37), which is also often used experimentally to suppress ribonucleolytic activity in mammalian tissues and extracts. But in comparison to RI, decavanadate is both inexpensive and inert. Moreover, decavanadate is not prone to oxidation, as is RI (38). Finally, decavanadate binds to RNase A much more tightly than do other inorganic anions, such as phosphate [K_i = 14 mM at pH 7.0 (39)] and pyrophosphate [K_i = 1.3 mM at pH 7.0 (39)].

Prospectus. The successful manipulation of RNA often depends on suppression of all ribonucleolytic activity (40, 41). Consequently, any new strategies to inhibit ribonucleases may be useful in many biochemical protocols. We find that low concentrations of decavanadate can have a significant impact on the catalytic activity of RNase A. This finding intimates that other polyoxometalates may also be capable of inhibiting catalysis by ribonucleases.

ACKNOWLEDGMENTS

We are grateful to Dr. B. M. Fisher for providing K7A/R10A/K66A RNase A and to K. J. Woycechowsky and C. Park for comments on the manuscript. Calorimetry data were collected at the University of Wisconsin–Madison Biophysical Instrumentation Facility, which is supported by the University of Wisconsin–Madison and by Grant BIR-9512577 (NSF).

REFERENCES

1. Srivastava, A. K., and Chiasson, J.-L. (Eds.) (1995) Vanadium Compounds: Biochemical and Therapeutic Applications (Developments in Molecular and Cellular Biochemistry, Vol. 16), Kluwer Academic, Dordrecht.
2. Tracey, A. S., and Crans, D. C. (Eds.) (1998) Vanadium Compounds: Chemistry, Biochemistry, and Therapeutic Applications (ACS Symposium Series, Vol. 711), Am. Chem. Soc., Washington, DC.
3. Petterson, L. (1994) *in* Polyoxometalates: From Platonic Solids to Anti-retroviral Activity (Pope, M. T., and Müller, A., Eds.), pp. 27–40, Kluwer Academic, Dordrecht.
4. Rehder, D. (1995) *in* Metal Ions in Biological Systems: Vanadium and Its Role in Life (Sigel, H., and Sigel, A., Eds.), Vol. 31, pp. 147–209, Dekker, New York.
5. Crans, D. C. (1995) *in* Metal Ions in Biological Systems: Vanadium and Its Role in Life (Sigel, H., and Sigel, A., Eds.), pp. 147–209, Dekker, New York.
6. Stowell, J. K., Widlanski, T. S., Kutateladze, T. G., and Raines, R. T. (1995) *J. Org. Chem.* **60,** 6930–6936.
7. D'Alessio, G., and Riordan, J. F. (Eds.) (1997) Ribonucleases: Structures and Functions, Academic Press, New York.
8. Raines, R. T. (1998) *Chem. Rev.* **98,** 1045–1065.
9. Lindquist, R. N., Lynn, J. L., Jr., and Lienhard, G. E. (1973) *J. Am. Chem. Soc.* **95,** 8762–8768.
10. Crans, D. C. (1994) *Comments Inorg. Chem.* **16,** 35–76.
11. Stankiewicz, P. J., Tracey, A. S., and Crans, D. C. (1995) *in* Metal Ions in Biological Systems: Vanadium and Its Role in Life (Sigel, H., and Sigel, A., Eds.), pp. 287–324, Dekker, New York.
12. Leon-Lai, C. H., Gresser, M. J., and Tracey, A. S. (1996) *Can. J. Chem.* **74,** 38–48.
13. Richards, F. M., and Wyckoff, H. W. (1971) *in* The Enzymes (Boyer, P. D., Ed.), Vol. IV, pp. 647–806, Academic Press, New York.
14. Hallick, R. B., Chelm, B. K., Gray, P. W., and Orozco, E. M. J. (1977) *Nucleic Acids Res.* **4,** 3055–3064.
15. Ui, N. (1971) *Biochim. Biophys. Acta* **229,** 567–581.
16. Fisher, B. M., Grilley, J. E., and Raines, R. T. (1998) *J. Biol. Chem.* **273,** 34,134–34,138.
17. Nogués, M. V., Moussaoui, M., Boix, E., Vilanova, M., Ribó, M., and Cuchillo, C. M. (1998) *Cell. Mol. Life Sci.* **54,** 766–774.
18. delCardayré, S. B., Ribó, M., Yokel, E. M., Quirk, D. J., Rutter, W. J., and Raines, R. T. (1995) *Protein Eng.* **8,** 261–273.
19. Fisher, B. M., Ha, J.-H., and Raines, R. T. (1998) *Biochemistry* **37,** 12,121–12,132.
20. Messmore, J. M. (1999) Ph.D. thesis, University of Wisconsin—Madison.
21. Sela, M., Anfinsen, C. B., and Harrington, W. F. (1957) *Biochim. Biophys. Acta* **26,** 502–512.
22. Crans, D. C., and Schelble, S. M. (1990) *Biochemistry* **29,** 6698–6706.
23. Petterson, L., Hedman, B., Andersson, I., and Ingri, N. (1983) *Chem. Scripta* **22,** 254–264.
24. Goddard, J. B., and Gonas, A. M. (1973) *Inorg. Chem.* **12,** 574–579.
25. Cooper, A., and Johnson, C. M. (1994) *in* Methods in Molecular Biology: Microscopy, Optical Spectroscopy, and Macroscopic Techniques (Jones, C., Mulloy, B., and Thomas, A. H., Eds.), pp. 137–150, Humana Press, Totowa, NJ.
26. Yakovlev, G. I., Moiseyev, G. P., Bezborodova, S. I., Both, V., and Sevcik, J. (1992) *Eur. J. Biochem.* **204,** 187–190.

27. delCardayré, S. B., and Raines, R. T. (1994) *Biochemistry* **33,** 6032–6037.
28. Choate, G., and Mansour, T. E. (1979) *J. Biol. Chem.* **254,** 11,457–11,462.
29. Boyd, D. B., Kustin, K., and Niwa, M. (1985) *Biochim. Biophys. Acta* **827,** 472–475.
30. Cardenas, J. M., Dyson, R. D., and Strandholm, J. J. (1973) *J. Biol. Chem.* **248,** 6931–6937.
31. Massey, T. H., and Deal, W. C., Jr. (1973) *J. Biol. Chem.* **248,** 56–62.
32. Roberts, D. V. (1977) Enzyme Kinetics (Elmore, D. T., Leadbetter, A. J., and Schofield, K., Eds.), Cambridge Univ. Press, London.
33. Fisher, B. M., Schultz, L. W., and Raines, R. T. (1998) *Biochemistry* **37,** 17,386–17,401.
34. Park, C., and Raines, R. T. (2000) *FEBS Lett.* **468,** 199–202.
35. Sela, M. (1962) *J. Biol. Chem.* **237,** 418–421.
36. Lee, F. S., Shapiro, R., and Vallee, B. L. (1989) *Biochemistry* **28,** 225–230.
37. Vicentini, A. M., Kieffer, B., Mathies, R., Meycheck, Hemmings, B. A., Stone, S. R., and Hofsteenge, J. (1990) *Biochemistry* **29,** 8827–8834.
38. Kim, B.-M., Schultz, L. W., and Raines, R. T. (1999) *Protein Sci.* **8,** 430–434.
39. Anderson, D. G., Hammes, G. C., and Walz, F. G., Jr. (1968) *Biochemistry* **7,** 1637–1645.
40. Poulson, R. (1977) *in* The Ribonucleic Acids (Stewart, P. R., and Letham, D. S., Eds.), 2nd ed., pp. 333–337, Springer-Verlag, New York.
41. Berger, S. L., and Birkenmeier, C. S. (1979) *Biochemistry* **18,** 5143–5149.
42. Nicholls, A., and Honig, B. (1991) *J. Comp. Chem.* **12,** 435–445.