The intense and broad band e cannot be assigned in great detail. It certainly contains ionizations from the U–O σ bonds and probably overlaps the O–C σ ionizations as well.

The change from He I to He II radiation produces notable changes in relative PES band intensities, as shown in Figure 4. The most striking effect is the remarkable increase in intensity of band b. This intensity increase is in accord with the calculations since the MOs that contribute to band b have the largest metal 5f contributions (Table III). Hence, if the Gelius model for PE cross sections is adopted,[15] the observed intensity change reproduces the well-known increases of the U 5f and 6d cross sections relative to those of O and C under He II excitation.[11,16] Interestingly, the relative intensities of the remaining bands of the He II spectrum are in better agreement than the He I spectrum with the statistical values based on proposed occupancies. This effect is frequently observed on passing from He I to He II spectra and could be due to a less pronounced effect of the second-order terms in the plane wave approximation for molecular photoelectron cross sections under the more energetic He II radiation.[17] It is also of interest to note that the He II intensities suggest that the increase in the U 5f cross section is slightly greater than that of the U 6d. In our previous study of Cp$_4$U,[14] it appeared that this order was reversed, i.e. U 6d > U 5f. We suggest that this underscores the need for additional experimental and theoretical studies of heavy-element cross sections.

Conclusions

We believe that the study of the electronic structure of U(OCH$_3$)$_6$ presented here is significant for several reasons. It demonstrates the utility of the DV-Xα molecular orbital method for describing the bonding energetics of metal–organic actinide molecules. It also underscores our previous claim that the correct assignment of PE spectra for f-element complexes almost by necessity requires a suitable calculational description of the bonding in them.[14]

The electronic structure of U(OCH$_3$)$_6$ is, not surprisingly, more complex than that of truly octahedral molecules such as UX$_6$ (X = F, Cl). It is surprising, however, that the U(VI) center is capable of producing such a large (ca. 3–4 eV) splitting in the oxygen π orbitals. It is precisely these largely unexpected metal–ligand interactions in such systems that encourages us that the electronic structure of f-element complexes will continue to show significant variances from those of transition-metal analogues and that investigations such as the present one are essential if these variances are to be completely understood.

Acknowledgment. This research was supported by the NSF (Grants CHE8306255 to T.J.M. and DMR8214966 to D.E.E.) and by the NATO research grants program (Grant 068/84 to I.F. and T.J.M.). B.E.B. gratefully acknowledges the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support.

Registry No. U(OCH$_3$)$_6$, 69644-82-2.

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Contribution from the Department of Chemistry,
Amherst College, Amherst, Massachusetts 01002

Thermodynamics of Azide and Thiocyanate Binding to Bovine Copper–Zinc Superoxide Dismutase

David M. Dooley* and Michele A. McGuirl

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Thermodynamic parameters (K, $\Delta H°$, $\Delta S°$) have been measured for N$_3^-$ and SCN$^-$ binding to bovine copper–zinc superoxide dismutase. Comparative data have also been obtained for the anation reactions of Cu(II)–diethylenetriamine (dien). The thermodynamics of N$_3^-$ and SCN$^-$ binding to superoxide dismutase are very different. Moreover, the enzyme + SCN$^-$ reaction cannot be described by a single equilibrium constant and is ionic strength dependent. However, it is clear that $\Delta S°$ is far more important in the enzyme–anion reactions as compared to Cu(dien)$^{2+}$ ligand substitution, which is dominated by $\Delta H°$.

Anion binding to bovine copper–zinc superoxide dismutase (Cu,Zn-SOD) has been extensively investigated, in part because the coordination of small anions (e.g. N$_3^-$, CN$^-$, SCN$^-$) may model the interaction between the enzyme and superoxide.[1] Azide and cyanide are potent inhibitors of Cu,Zn-SOD and are known to coordinate to Cu(II). Conflicting results have been reported with regard to thiocyanate binding;[2,3] Bertini and co-workers have presented evidence for imidazole (from a nonbridging histidine) displacement by anions, including SCN$^-$,[2] but this has been disputed.[3] In contrast to N$_3^-$ and CN$^-$, SCN$^-$ binding does not perturb the solvent water ^{1}H NMR T_1.[2] SCN$^-$ has been shown to displace the bridging imidazolate in the 4-Cu derivative of Cu,Zn-SOD.[4] Thus, it seems likely that SCN$^-$ binds differently than N$_3^-$ and CN$^-$. In addition, the fact that phosphate has recently been shown to inhibit Cu,Zn-SOD and to influence its reactivity toward potential Cu(II) ligands[5] complicates the interpretation of previous work carried out in phosphate buffers. Although some equilibrium constants for anion binding to bovine Cu,Zn-SOD have been reported[1–6], $\Delta H°$ and $\Delta S°$ have not been determined. Such thermodynamic data are essential to under-

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standing the ligand-substitution chemistry and, by inference, the substrate binding. Here we report the thermodynamics of azide and thiocyanate binding to bovine Cu,Zn-SOD. Further insight into the reactivity of the protein metal site is provided by comparative thermodynamic data for the ligand-substitution reactions of the diethylenetriamine (dien) copper(II) complex, which has been proposed as a reasonable model for the Cu(II) site in SOD.[7]

Experimental Section

Bovine Cu,Zn-SOD was purchased from DDI (Mountain View, CA) and used as received. Protein concentrations were measured by using the extinction coefficients (per mole of SOD): $\epsilon_{258} = 13\,200$ M^{-1} cm^{-1}; $\epsilon_{680} = 300$ M^{-1} cm^{-1}.[8] Azide and thiocyanate solutions were freshly prepared prior to each experiment. Experiments were routinely carried out in 5.0 mM PIPES buffer (pH 7.0) containing 0.1 M NaCl. Titrations were performed in a thermostated cell holder equipped with 1.0-cm cells by using a Cary 219 spectrophotometer, interfaced to an Apple IIe computer. Base line subtraction and data manipulations were done digitally. Small volumes of concentrated anion solutions were added in order to minimize dilution effects, but the data were corrected when necessary. Absorption changes as a function of temperature were obtained as difference spectra: SOD + N_3^-/SCN$^-$ vs. SOD + Cl$^-$. Generally, the sample and reference cells were maintained at the same temperature; if not, the data were corrected by using the temperature dependence of the SOD vs. SOD spectrum, measured independently. Samples were allowed to equilibrate at least $^1/_2$ h following each change in temperature. Temperatures were measured by inserting a calibrated thermocouple into the cell assembly next to the cuvette. Corrections for small temperature-dependent base line changes were made as required. Equilibrium constants were determined by a standard method.[9] $\Delta H°$ and $\Delta S°$ were obtained as described by Gorman and Darnall[10] or by using the extinction coefficients obtained from titration experiments to calculate K at each temperature. The validity of these methods for Cu,Zn-SOD was checked by performing titrations with azide at 30 and 15 °C; $\Delta\epsilon$ for the SOD–azide complex was temperature independent and $\Delta H°$ calculated from these experiments agreed with the value obtained from the temperature dependence at fixed [N_3^-], within the estimated experimental uncertainty. Extinction coefficients are quoted per mole of Cu, except where noted.

Results and Discussion

When N_3^- or SCN$^-$ is added to solutions of Cu,Zn-SOD in 5.0 mM PIPES and 0.1 M NaCl (pH 7.0), new absorption bands are evident at 375 nm ($\Delta\epsilon = 1300$ M^{-1} cm^{-1}) and 355 nm ($100 \leq \Delta\epsilon \leq 300$ M^{-1} cm^{-1}), respectively. Comparable data for the Cu(dien)$^{2+}$–X$^-$ complexes are as follows: X$^-$ = N_3^-, $\lambda = 345$ nm, $\Delta\epsilon = 2200$ M^{-1} cm^{-1}; X$^-$ = SCN$^-$, $\lambda = 314$ nm, $\Delta\epsilon = 900$ M^{-1} cm^{-1}. The energy and intensity of the bands are most consistent with a ligand-to-metal charge-transfer (LMCT) assignment, N_3^-/SCN$^-$ → Cu(II). The half-filled orbital in a tetragonal Cu(II) complex is $d_{x^2-y^2}$, which is located approximately in the equatorial plane. Consequently, the relatively low intensity of the SOD–SCN$^-$ LMCT transition may reflect poorer overlap with the Cu(II) $d_{x^2-y^2}$ orbital[11] and therefore a somewhat different structure than Cu(dien)$^{2+}$–SCN$^-$. Equilibrium constants for SOD–anion complex formation, from the titration data in Figures 1 and 2, are $K(N_3^-) = 88 \pm 2$ M^{-1} and K(SCN$^-$) = 22 ± 3 M^{-1}. Our value for $K(N_3^-)$ is in excellent agreement with the value obtained by Mota de Freitas and Valentine[5] at constant ionic strength ($I = 0.15$) in a noncoordinating buffer. The data in Figure 2 unambiguously establish that SCN$^-$ coordinates to Cu(II) in bovine Cu,Zn-SOD under our conditions, and they are reasonably consistent with earlier results obtained in unbuffered solutions by NMR relaxation measurements ($K = 40 \pm 10$ M^{-1}).[2b] Clearly the affinity of Cu,Zn-SOD for thiocyanate decreases at high [SCN$^-$]; hence SCN$^-$ binding cannot be adequately described by a single equilibrium constant. Attempts to fit the SCN$^-$ titration data to two equilibrium constants were also unsuccessful. As expected, a Scatchard plot of the N_3^- titration is linear with $(1/k) = 90$ M^{-1} and $n = 2$.[12] SCN$^-$ binding gives a curved Scatchard plot, consistent with $n_1 = 2$ and $(1/k_1) = 20$ M^{-1}, but no reliable value of $(1/k_2)$ can be estimated. At 15 °C, in 5.0 mM PIPES containing 0.3 M NaCl, the affinity of SOD for thiocyanate decreases significantly. Much smaller, but significant deviations from linearity are observed in the ΔA vs. $\Delta A/L$ plot; analysis yields 3.0 M^{-1} $< K <$ 6.0 M^{-1} with the entire curve described by K(SCN$^-$) = 4.0 M^{-1}, $\Delta\epsilon \simeq 200$ M^{-1} cm^{-1} (correlation coefficient for a linear fit = 0.98). Therefore, the nonlinearity in the thiocyanate data (Figure 2, inset) can be attributed (at least) in part to ionic strength effects on the ligand-substitution reaction. Ionic strength variations do not significantly perturb the Cu,Zn-SOD absorption spectrum in the 300–400-nm range. However, the SOD–Cu(II)–NCS$^-$ structure itself may be slightly ionic strength dependent since $\Delta\epsilon$ differs at low and high ionic strength. Refinement of the 2-Å Cu,Zn-SOD structure indicates that two potential anion-binding sites exist near the Cu(II),[13] which may

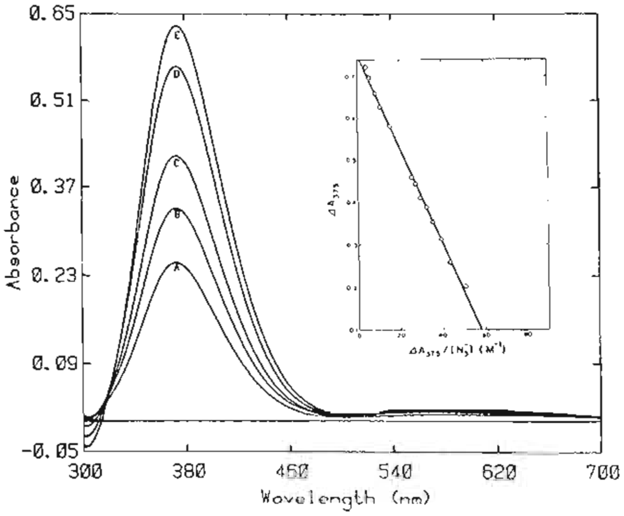

Figure 1. Titration of 0.30 mM copper–zinc superoxide dismutase with azide at 14.3 °C in 5.0 mM PIPES and 0.1 M NaCl (pH 7.0). [N_3^-] = 5.8 mM (A), 9.5 mM (B), 16.5 mM (C), 52.3 mM (D), and 198 mM (E). Inset: slope of plot equals $-K^{-1}$.

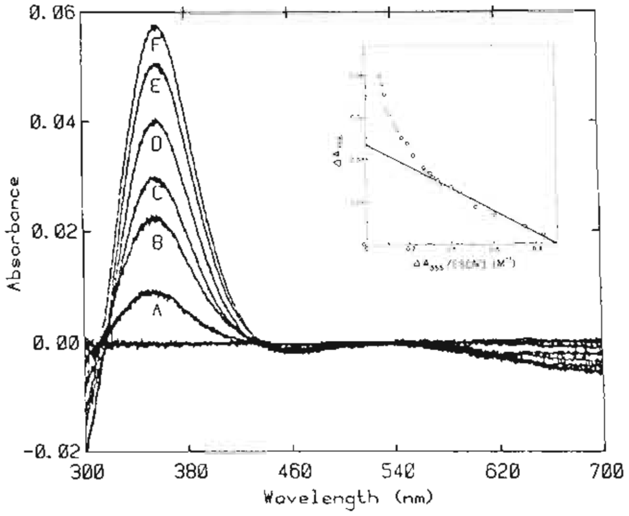

Figure 2. Titration of 0.26 mM copper–zinc superoxide dismutase with thiocyanate at 15.3 °C in 5.0 mM PIPES and 0.1 M NaCl (pH 7.0). [SCN$^-$] = 16.4 mM (A), 53 mM (B), 100 mM (C), 212 mM (D), 430 mM (E), and 744 mM (F). Inset: slope at low [SCN$^-$] is taken to be $-K^{-1}$, $\Delta\epsilon \simeq 100$ M^{-1} cm^{-1}.

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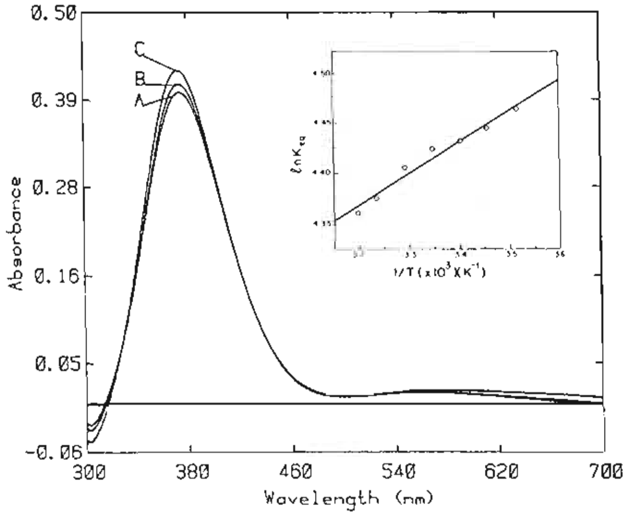

Figure 3. Temperature dependence of the LMCT transition characteristic of the superoxide dismutase-N_3^- complex: [SOD] = 0.36 mM; [N_3^-] = 10.0 mM. Temperatures ranged from 39.75 (A) to 11.40 °C (C). Inset: van't Hoff plot.

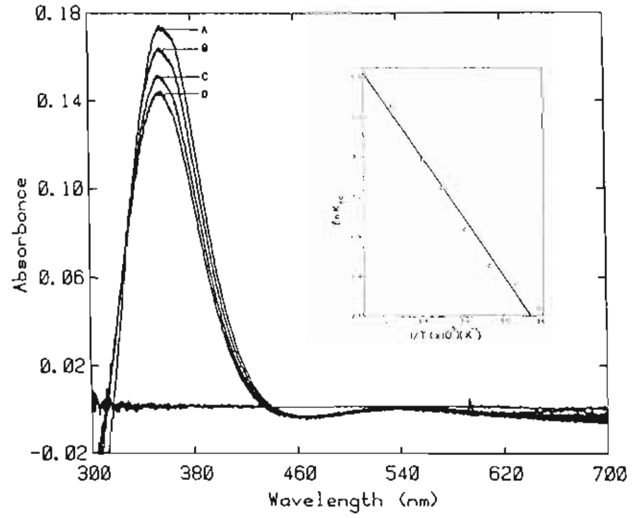

Figure 4. Temperature dependence of the LMCT transition characteristic of the superoxide dismutase-SCN^- complex: [SOD] = 1.35 mM; [SCN^-] = 98.0 mM. Temperatures ranged from 33.8 (A) to 5.6 °C (D). Inset: van't Hoff plot.

Table I. Thermodynamic Parameters for Ligand Substitution Reactions[a]

reactions	K_{eq},[b] M^{-1}	$\Delta H°$, kJ/mol	$\Delta S°$, J/(mol K)	$\Delta G°$,[c] kJ/mol
SOD + N_3^-	88 ± 2	−4.0 ± 1.0	28 ± 2	−11.0 ± 1.0
SOD + SCN^-[d]	22 ± 3	11.7 ± 1.2	64 ± 4	−7.4 ± 2.4
$Cu(dien)^{2+}$ + N_3^-	75 ± 2	−10.6 ± 0.7	−2 ± 2	−10.0 ± 1.0
$Cu(dien)^{2+}$ + SCN^-	36 ± 2	−9.5 ± 1.0	−3 ± 3	−8.6 ± 1.0

[a] Error limits calculated from the standard errors of the slope and intercept. Where checked, the values were found to be reproducible within the quoted limits. [b] Determined via titrations at 288 K. [c] Calculated from $\Delta H°$ and $\Delta S°$ and from $-RT \ln K$ obtained from the temperature dependence. [d] Obtained under conditions corresponding to the linear region of the SCN^- titration curve (Figure 2), $I \cong 0.2$ M.

be a factor in the Cu,Zn-SOD reaction with thiocyanate. Bertini and co-workers have suggested that thiocyanate possibly binds to Cu(II) in more than one way.[2b]

$\Delta H°$ and $\Delta S°$ can be determined by measuring the temperature dependence of the LMCT bands for bovine Cu,Zn-SOD (Figures 3 and 4) and for $Cu(dien)^{2+}$ (Figures 5 and 6); the data are set out in Table I.[14] The rationale for comparing anion binding in Cu,Zn-SOD and $Cu(dien)^{2+}$ should be explicitly stated. First, the effective ligand fields are similar, as judged by the d-d transition envelopes (λ_{max}^{SOD} = 680 nm, λ_{max}^{dien} = 620 nm), so electronic effects on the ligand-substitution dynamics should be comparable. Second, $Cu(dien)^{2+}$ contains a single equatorial water molecule that is displaced by anions. Thus, if anion binding to SOD is governed only by the energetic and entropic effects associated with a simple ligand-substitution reaction, with water as the leaving group, the thermodynamics should be similar for SOD and $Cu(dien)^{2+}$ ligand substitution. This is a plausible mechanism for SOD-Cu(II) ligand-substitution reactions. Equatorial coordination for azide and cyanide is well established, and NMR relaxation experiments indicate that no rapidly exchanging coordinated water molecule is present in the azide and cyanide complexes.[15] In addition, large rearrangements of the nonlabile ligands are not required for equatorial anion coordination to $Cu(dien)^{2+}$ or Cu,Zn-SOD.[13] On the other hand, if the SOD

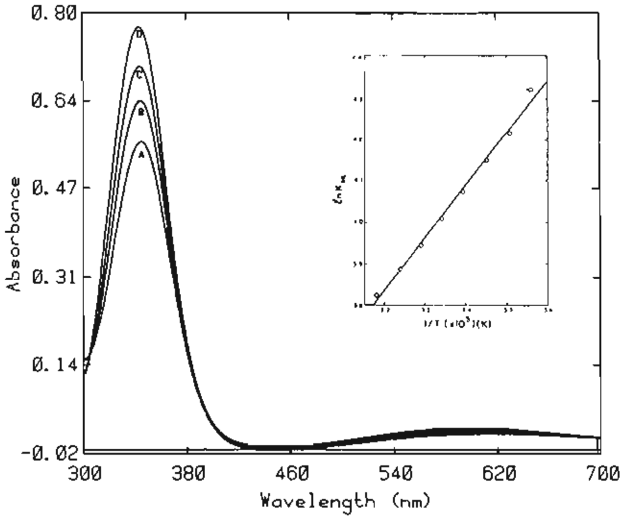

Figure 5. Temperature dependence of the LMCT transition characteristic of the $[Cu(dien)N_3]^+$ complex: [Cu^{2+}] = 1.0 mM; [N_3^-] = 7.94 mM. Temperatures ranged from 41.40 (A) to 7.85 °C (D). Inset: van't Hoff plot.

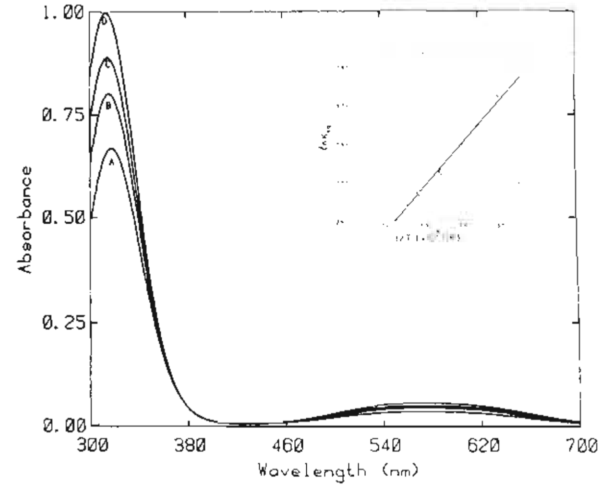

Figure 6. Temperature dependence of the LMCT transition characteristic of the $[Cu(dien)NCS]^+$ complex: [Cu^{2+}] = 3.0 mM; [SCN^-] = 13.8 mM. Temperatures ranged from 40.00 (A) to 9.25 °C (D). Inset: van't Hoff plot.

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(14) Thermodynamic parameters for the $Cu(dien)^{2+}$ reaction with azide have previously been measured in 0.1 M acetate buffer, pH 6.0, I = 0.25 M: K(25 °C) = 36 ± 1 M^{-1}, $\Delta H°$ = −9.2 ± 1.2 kJ mol^{-1}, $\Delta S°$ = 0 ± 4 J mol^{-1} deg^{-1}, in very good agreement with our values considering the difference in conditions. See: Holwerda, R. A.; Stevens, G.; Anderson, C.; Wynn, M. *Biochemistry* **1982**, *21*, 4403–4407.

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and $Cu(dien)^{2+}$ thermodynamics are very different, then the ligand-substitution reactions of the protein and $Cu(dien)^{2+}$ must be different, possibly reflecting the protein's direct influence on metal ion reactivity.

It is clear that the similarity of the equilibrium constants for anion binding to SOD and $Cu(dien)^{2+}$ is very misleading, since the relative contributions from $\Delta H°$ and $\Delta S°$ differ greatly. $\Delta H°$ and $\Delta S°$ for the SOD reaction with thiocyanate were measured under various conditions in addition to those cited in Table I. Assuming $\Delta\epsilon \simeq 200$ M^{-1} cm^{-1}, $\Delta H° = 16 \pm 2$ kJ mol^{-1} and $\Delta S° = 70 \pm 5$ J mol^{-1} K^{-1} in the standard buffer at relatively high thiocyanate concentrations, $[SCN^-] = 0.40$ M. In 5.0 mM PIPES, 0.3 M NaCl, and 0.1 M KSCN (pH 7.0), $\Delta H° = 6.0 \pm 1$ kJ mol^{-1} and $\Delta S° = 30 \pm 2$ J mol^{-1} K^{-1}. Independent of any assumptions about the magnitudes of K and $\Delta\epsilon$, the temperature dependence of the SOD LMCT transition establishes that $\Delta H°(SCN^-)$ is unambiguously positive. Absorbance changes displayed by the $SOD-SCN^-$ complex in the ligand-field region were nearly independent of temperature (Figure 4), which rules out the possibility that the $SOD-Cu(II)-NCS^-$ geometry is significantly temperature dependent over the range examined. Positive enthalpies and large positive entropies were consistently observed for SCN^- binding under all conditions employed. The thermodynamic data also suggest that the structures of the $Cu(dien)^{2+}$–anion and SOD–Cu(II)–anion complexes may not be the same (e.g. nonidentical leaving groups or final coordination numbers/geometries). Entropy effects are far more important in the SOD reactions and may reflect the influence of the protein structure on metal ion reactivity. Indeed, $\Delta S°$ can be readily interpreted by reference to the Cu,Zn-SOD crystal structure. The active-site channel, including the metal ion ligands and three buried water molecules, is very highly ordered.[13] Anion binding displaces at least some of the ordered water molecules and possibly disrupts the structure of the Cu(II) ligands and neighboring residues. Both effects should contribute to a positive $\Delta S°$. The more positive enthalpies for anion binding to SOD, compared to $Cu(dien)^{2+}$, may reflect histidine displacement[2c] or protein conformational changes. Further, the thermodynamics of N_3^- and SCN^- binding to bovine Cu,Zn-SOD are qualitatively different, in contrast to the $Cu(dien)^{2+}$ ligand-substitution reactions, where N_3^- and SCN^- coordination are thermodynamically very similar. Two possible sources for thermodynamic differences between azide and thiocyanate binding to the SOD are as follows: (1) N_3^- and SCN^- coordinate to Cu(II) in different ways; (2) protein structural perturbations (associated, for example, with the anions' relative sizes and the narrow width (<4 Å)[13] of the active-site channel) may be a function of the bound ions.[16] Experiments designed to test these possibilities (including experiments on modified SODs) are feasible. Thermodynamic measurements clearly will be quite helpful in understanding the active-site coordination chemistry of Cu,Zn-SOD. Moreover, our results indicate that thermodynamics measurements, combined with structural information, are a useful approach to identifying ways in which proteins modulate or control metal ion reactivity.

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Registry No. SOD, 9054-89-1; N_3^-, 14343-69-2; SCN^-, 302-04-5; $Cu(dien)^{2+}$, 45520-77-2.

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(16) A definitive explanation for the complex behavior associated with the SOD–thiocyanate reaction is not yet available. One reviewer suggested that SCN^- may bind to charged groups on the protein. This is certainly a reasonable proposal that is not excluded by our data, although one might expect similar behavior with azide, which was not observed. Until the interactions(s) between Cu,Zn-SOD and thiocyanate are better understood at the molecular level, the thermodynamic quantities for this reaction should be regarded as applicable only under conditions that closely correspond to those used here.

Contribution from the Department of Chemistry,
Anhui University, Hefei, Anhui, People's Republic of China

Oscillating Reactions Involving a Nickel Complex with a 13-Membered Tetraaza Macrocyclic Ligand in Acidic Bromate Medium

Xu Ji-de and Ni Shi-sheng*

Received December 10, 1984

New oscillating reactions with the participation of a macrocyclic nickel(II) complex ion $[Ni(ATH)]^{2+}$ as catalyst in acidic bromate medium are described. This complex ion contains the ligand 11,13-dimethyl-1,4,7,10-tetraazacyclotrideca-10,13-diene. The $[Ni(ATH)]^{2+}$ ion can undergo an irreversible oxidation reaction of $Ni(II) \rightarrow Ni(III)$. The reduction potential of the couple $[Ni(ATH)]^{3+}/[Ni(ATH)]^{2+}$ in a 9:1 ethanol–acetonitrile mixture is found to be 1.32 V. The concentration oscillations of this system are damped rapidly due to the consumption of the catalyst and are inhibited by I^- ion, if the I^- ion concentration exceeds 2×10^{-3} mol L^{-1}, but they are promoted somewhat, if the I^- concentration is below 1.2×10^{-3} mol L^{-1}. Acrylamide or acetonitrile cannot entirely inhibit the oscillation. In the absence of malonic acid or other organic substrate, the catalyst itself may serve as organic substrate to produce a few oscillations. In some respects, the $[Ni(ATH)]^{2+}$-catalyzed oscillating reactions behave similarly to the ferroin-catalyzed ones. The reaction trajectories of the $[Ni(ATH)]^{2+}$-catalyzed system are spiral, which may provide some useful information about the details of the chemical mechanism.

Introduction

Yatsimirskii et al.[1-5] have recently reported that several hexamethyl tetraaza macrocyclic complexes can act as catalysts in Belousov–Zhabotinskii[6,7] (B–Z) oscillating reactions. They also described new oscillating reactions, viz. the oxidation of Me_6-[14]-4,14-dieneN_4 complexes of copper and nickel by acidic bromate. However, the mechanisms of these oscillating reactions have not been discussed in detail. Until now, the oscillating reactions involving other polyaza macrocyclic complexes have not yet been reported.

In order to search for the possibilities that other polyaza macrocyclic complexes may participate in oscillating reactions,

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