reduced by any of several strong reducing agents with varied structures and net charges.[9] They suggested that the spectroscopic and chemical properties of the blue chromophore in N_2OR III might be consistent with its formulation as a [Cu(I)–SR•] site "stabilized" by additional sulfur ligation.[9] These suggestions concerning the nature of the blue N_2O reductase center are not necessarily inconsistent, since [Cu(II)–SR^-] and [Cu(I)–SR•] may simply be resonance forms describing a highly covalent paramagnetic site. Such considerations have been raised in discussions of the Cu_A site in cytochrome oxidase.[15] Certainly the number, intensities, and energies of the transitions observed in the visible and near-infrared CD spectra of N_2OR III are consistent with formulating the blue chromophore as a near-tetrahedral [Cu(II)–SR^-] site, most probably with pronounced delocalization. On the basis of our data, we cannot unequivocally distinguish between the limiting possibilities, since too little is known about the electronic properties of copper(I)–thiyl radicals.

N_2O reductase contains nine tryptophans per subunit; five of these are in the carboxyl-terminal half of the protein, which contains the Cu_A-type site. Two tryptophan residues are located within two or three residues of a conserved cysteine (cys-618), which is part of the presumed Cu_A site. The tryptophan fluorescence quenching (Figure 8) in N_2OR I and N_2OR V, relative to the apo protein, is pronounced and seems to correlate with the Cu_A content rather than the total copper content. This suggests that the Cu_A-type sites may form a "fluorescence-energy sink" when occupied. Such a result would not be unexpected given the paramagnetism and the number of low-energy electronic states associated with these sites. It remains to be seen if the fluorescence is sensitive to the redox state of the Cu_A-type sites or other copper sites in N_2O reductase. The initial results do suggest that fluorescence spectroscopy will be useful in studies of N_2O reductase and that additional studies are warranted.

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Stoichiometry of Electron Uptake and the Effect of Anions and pH on the Molybdenum and Heme Reduction Potentials of Sulfite Oxidase

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Microcoulometric titrations of chicken liver sulfite oxidase under varied conditions at 25 °C have shown that the enzyme requires three electrons for complete subunit reduction. Reduction potentials (vs NHE) for the molybdenum site at pH 9.00 in the absence of coordinating anions (Mo(VI/V) = 0.057 V, Mo(V/IV) = −0.233 V) are considerably more negative than at pH 6.00 in 0.10 M KCl (Mo(VI/V) = 0.131 V, Mo(V/IV) = −0.086 V), consistent with the formation of chloride complexes of the Mo(V) and Mo(IV) states. Phosphate ($H_2PO_4^-$) competes effectively with chloride for the Mo(V) site of the enzyme at lower concentrations (0.020 M phosphate vs 0.10 M KCl, pH 7.00) and complexes the Mo(VI) state, as seen in the significant negative shifts of the reduction potentials (Mo(VI/V) = 0.038 V, Mo(V/IV) = −0.239 V). Dissociation constants for proton and phosphate complexes of the Mo(VI) state and the chloride complex of the Mo(IV) state have been estimated. The heme reduction potentials decrease with increase in pH and are relatively unaffected by anions. The results are consistent with the results of recent EPR and EXAFS studies of the enzyme regarding active-site structure.

Introduction

Sulfite oxidase (sulfite: ferricytochrome *c* oxidoreductase EC 8.1.2.1) is a dimeric enzyme that contains one molybdopterin center and one cytochrome b_5 type heme in each monomeric subunit.[2,3] The enzyme catalyzes the physiologically important oxidation of sulfite to sulfate, with the oxidation of substrate occurring at the molybdenum center.[4-6] The postulated catalytic cycle for the enzyme requires a two-electron change in the oxidation state of the molybdenum site (Mo(VI)/(Mo(IV)) and a one-electron change in the oxidation state of the heme site (Fe(III)/Fe(II)).[4,7] Data for the electron uptake of sulfite oxidase have not been previously reported. Electron uptake results for both nitrate reductase (*Chlorella vulgaris*) and milk xanthine oxidase, obtained by microcoulometry, show that their molybdenum centers are reduced by two electrons.[8,9]

The heme reduction potentials for the chicken liver and beef liver enzymes have been previously measured at room temperature by spectroelectrochemical titration at pH 7.00 in the presence of chloride and phosphate ions.[10,11]

The molybdenum reduction potentials for the beef liver enzyme have been previously determined by potentiometric titration using low-temperature EPR spectroscopy at pH 7.00 and 9.00 in the

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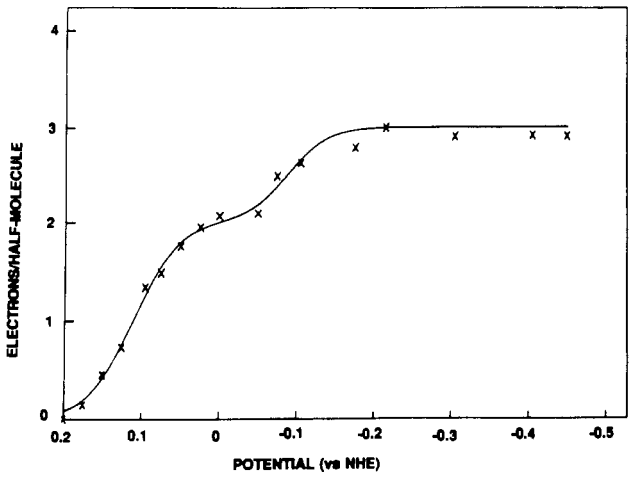

Figure 1. Microcoulometric titration of chicken liver sulfite oxidase. E (V vs NHE) is plotted vs n (e^-/heme). The titration was performed in 0.020 M universal buffer, containing 0.10 M KCl, at pH 6.0 in the presence of dye mediators, as described in the Experimental Section. Each experimental point represents the electron uptake following the addition of approximately 3.00×10^{-9} mol of enzyme subunits to the titration vessel (~3.0-mL volume). The solid curve represents the nonlinear least-squares "best fit" to the data points using the potentials given in Table I.

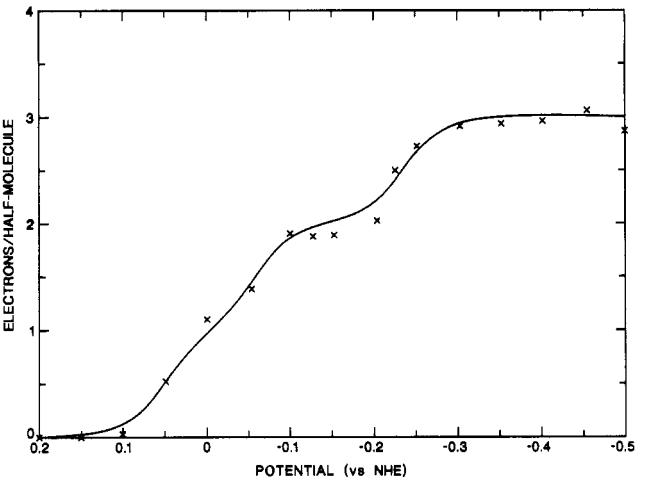

Figure 2. Microcoulometric titration of chicken liver sulfite oxidase. E (V vs NHE) is plotted vs n (e^-/heme). The titration was performed in 0.020 M universal buffer, containing 0.10 M sodium *p*-toluenesulfonate, at pH 9.00 in the presence of dye mediators, as described in the Experimental Section. Each experimental point represents the electron uptake following the addition of approximately 3.00×10^{-9} mol of enzyme subunits to the titration vessel (~3.0-mL volume). The solid curve represents the nonlinear least-squares "best fit" to the data points using the potentials given in Table I.

presence of chloride ion,[11] and the room-temperature molybdenum potentials for the chicken liver enzyme, obtained by potentiometric titration and room-temperature EPR spectroscopy at pH 7.00, have recently been reported.[10]

EPR studies of the Mo(V) state of sulfite oxidase have provided important information concerning the effects of pH and coordinating anions on the molybdenum site of the enzyme. Distinctly different Mo(V) EPR signals are obtained, depending on pH and chloride and phosphate concentrations.[12,13] Low pH and high chloride concentrations result in the low-pH form (also called the chloride form), while high pH and low chloride concentrations give the high-pH form. Addition of sufficient phosphate results in the formation of a molybdenum(V) phosphate signal, which may be converted to the high-pH form by raising the pH or to the low-pH form by addition of sufficient chloride.[12,13] Estimates of the equilibrium constants for the different Mo(V) complexes have been made by Bray et al.[12]

Recent EXAFS studies of sulfite oxidase in all three oxidation states have provided evidence for the binding of chloride to the Mo(V) and Mo(IV) states and have demonstrated the presence of two oxo ligands in the Mo(VI) state and one oxo ligand in the Mo(V) and Mo(IV) states, as well as two or three thiolate sulfur ligands in all states.[14] Recent EPR work has provided evidence for the binding of two phosphate ligands in the Mo(V) state.[15]

Microcoulometry gives information concerning the number of electrons the redox sites of the enzyme accept as a function of potential, but it does not identify individual electron acceptors.[8,9] It provides room-temperature values for reduction potentials, avoiding possible low-temperature effects of potentiometric–EPR methods.[9] It requires, however, relatively high concentrations (0.05–0.10 M) of electrolyte.

The electron uptake of chicken liver sulfite oxidase and the effects of pH and chloride and phosphate ions on its reduction potentials, as well as estimates of molybdenum(IV) chloride and molybdenum(VI) phosphate dissociation constants not previously determined are reported here. The results provide electrochemical evidence that supports the active-site anion complexes proposed in previous EPR[12,15] and EXAFS studies.[14]

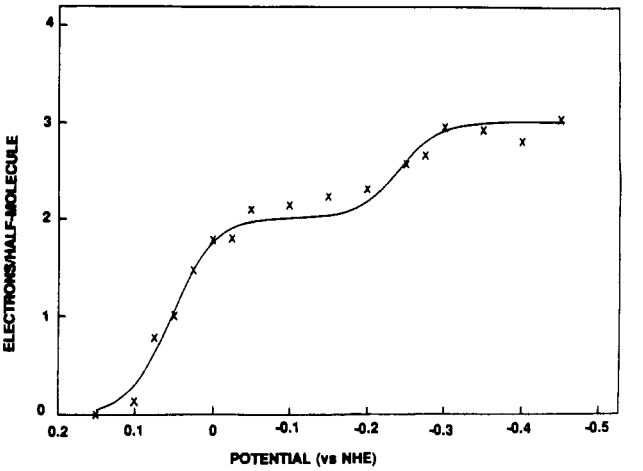

Figure 3. Microcoulometric titration of chicken liver sulfite oxidase. E (V vs NHE) is plotted vs n (e^-/heme). The titration was performed in 0.020 M potassium phosphate buffer, containing 0.10 M KCl, at pH 7.00 in the presence of dye mediators, as described in the Experimental Section. Each experimental point represents the electron uptake following the addition of approximately 3.00×10^{-9} mol of enzyme subunits to the titration vessel (~3.0-mL volume). The solid curve represents the nonlinear least-squares "best fit" to the data.

Results

Figure 1 shows the results of the microcoulometric titration of sulfite oxidase under conditions (pH = 6.00, 0.020 M universal buffer, 0.10 M KCl) that should result in 100% of the low-pH (chloride) form of the Mo(V) EPR signal.[12] Three electrons/monomer were required for complete reduction of the enzyme. The results indicate two electrons were taken up in the 0.200 to 0.000 V region, followed by a one-electron uptake in the −0.050 to −0.150 V region. The best least-squares fit to the data, assuming three one-electron reductions, gave 0.131, 0.090, and −0.086 V as the midpoint potentials.

Figure 2 shows the results under conditions (pH = 9.00, 0.020 M universal buffer, 0.10 M sodium *p*-toluenesulfonate) that should give 100% of the high-pH form of the Mo(V) signal.[12] Three electrons/monomer were required for complete reduction of the enzyme. One electron was taken up in each of the potential regions 0.100 to 0.000, 0.000 to −0.100, and −0.200 to −0.300 V. The best least-squares computer fit of the data gave 0.051, −0.057, and −0.233 V as the midpoint potentials.

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Scheme I

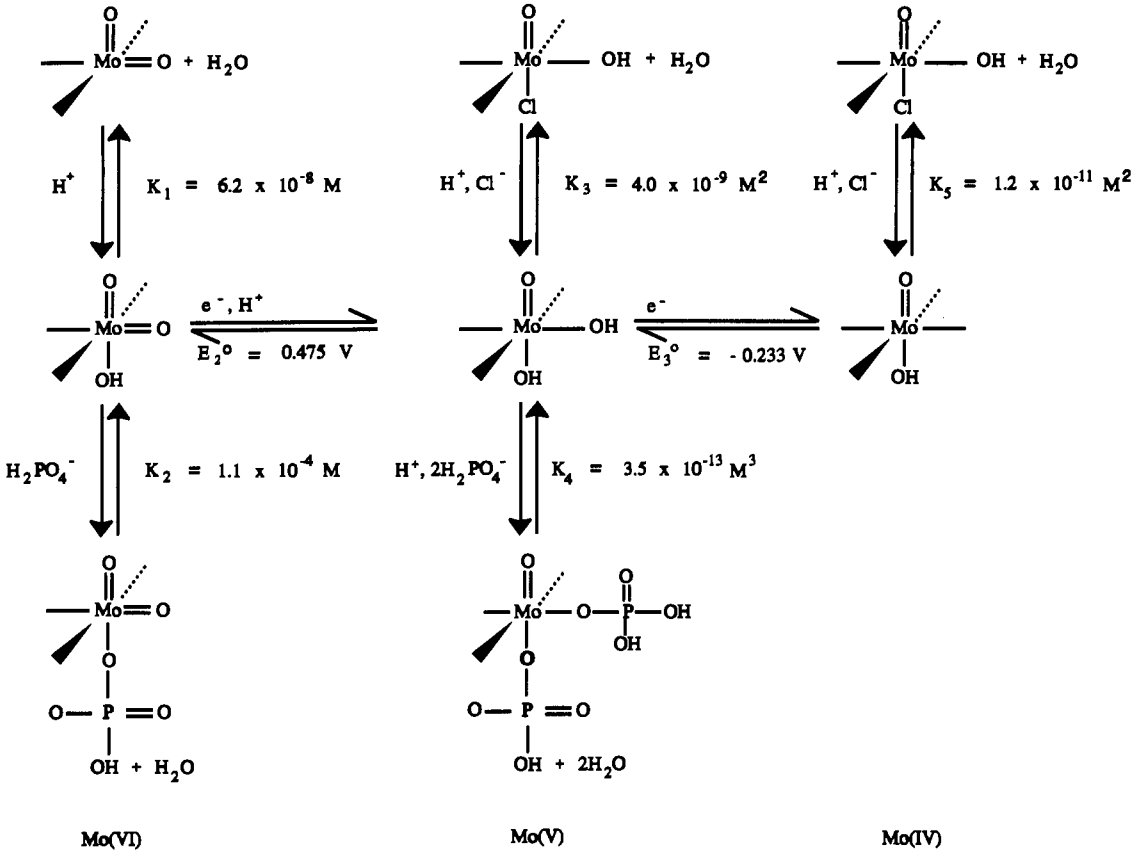

Figure 3 shows the results under conditions (pH = 7.00, 0.020 M universal buffer, 0.10 M KCl, 0.020 M phosphate) in which the low-pH, high-pH and phosphate Mo(V) signals should all be present.[12] Again, three electrons/monomer were required for complete reduction of the enzyme. Two electrons were taken up in the 0.150 to −0.050 V region, and a third was taken up in the −0.200 to −0.300 V region. A computer fit of the data gave midpoint potentials of 0.068, 0.038, and −0.239 V. These results are found in Table I.

Assignment of the reduction potentials to the Mo(VI/V), Mo(V/IV), and Fe(III/II) couples was made by comparison with previously reported values obtained by spectroelectrochemical and EPR methods that identify the species present at a given potential. Barber and Kay reported the Fe(III/II) potential of the chicken liver enzyme at pH 7.00 in 0.050 M MPOS buffer and unspecified chloride concentration to be 0.077 V, with a pH dependency of 0.027 V/pH unit from pH 5.00 to 7.00 and no pH dependence above pH 7.00.[10] Previously, a value of 0.084 V for this couple at pH 7.00 in 0.100 M phosphate had been obtained,[1] indicating little effect of buffer composition. Our results (Table I) at pH 6.00 in 0.10 M KCl give a value of 0.063 V when calculated for pH 7.00. This value and our value for this couple at pH 7.00 in 0.10 M KCl and 0.070 M phosphate (0.068 V) are clearly within experimental error (±0.015 V) of the previous values. The value we obtained at pH 9.00 in 0.10 M sodium *p*-toluenesulfonate, 0.051 V, slightly but significantly lower than the average of the other values (0.073 ± 0.009 V), was also assigned to this couple, since it is the highest of the three values obtained under these conditions. The small difference (0.022 V) may indicate a small pH dependency, or it may result from minor effects of buffer composition or ionic strength.

Barber and Kay have reported room-temperature values of 0.110 V for the Mo(VI/V) and −0.060 V for the Mo(V/IV) couples for the chicken liver enzyme.[10] Their reported Mo(V) EPR signal is that of the chloride species, indicating that appreciable, but unspecified, amounts of chloride were present.[10] Values for the beef liver enzyme of 0.117 and −0.110 V at pH 7.00 and 0.038 and −0.163 V at pH 9.00 in 0.050 M Tris–HCl buffer for these couples have been obtained at low temperature.[11] On the basis of these results, the Mo(VI/V) and Mo(V/IV) couples are assigned the values of 0.131 and −0.086, −0.059 and −0.233, and 0.038 and −0.239 V at pH 6.00 in 0.10 M KCl, pH 9.00 in 0.10 M sodium *p*-toluenesulfonate, and pH 7.00 in 0.10 M KCl plus 0.020 M phosphate, respectively. Clearly, both pH and anions have a strong effect on the molybdenum reduction potentials of the enzyme, consistent with the EPR[12,15] and EXAFS[14] results.

Table I. Reduction Potentials (V vs NHE) for Sulfite Oxidase

E_1[Fe(III/II)]	E_2[Mo(VI/V)]	E_3[Mo(V/IV)]	note
0.090	0.131	−0.086	*a*, *i*
0.051	−0.057	−0.233	*b*, *i*
0.068	0.038	−0.239 (exp)	*c*, *i*
		−0.158 (calc)	
0.077	0.110 (exp)	−0.060 (exp)	*d*, *i*
	0.070 (calc)	−0.090 (calc)	*e*, *i*
0.084			*f*, *i*
	0.117	−0.110	*g*, *j*
	0.038	−0.163	*h*, *j*

[a] This work, pH 6.00, 0.020 M universal buffer, 0.10 M KCl. [b] This work, pH 9.00, 0.020 M universal buffer, 0.10 M sodium *p*-toluenesulfonate. [c] This work, pH 7.00, 0.020 M universal buffer, 0.10 M KCl, 0.020 M phosphate. [d] Reference 10, pH 7.00, 0.050 M MOPS buffer, unspecified KCl concentration. [e] This work, pH = 7.00, 0.10 M KCl. [f] Reference 11, pH 7.00, 0.100 M phosphate. [g] Reference 11, pH 7.00, 0.005 M Tris–HCl. [h] Reference 11, pH 9.00, 0.005 M Tris–HCl. [i] Chicken liver enzyme. [j] Beef liver enzyme.

E° values were calculated from the results at pH 9.00, 0.10 M sodium *p*-toluenesulfonate, and 0.020 M universal buffer, in which no chloride or phosphate complexes are present[12] (see Scheme I):

$$E_2 = E_2^\circ + \frac{RT \ln [H^+]}{nF} \qquad E_2 = 0.131 \text{ V}$$

$$E_2^\circ = 0.475 \text{ V}$$

$$E_3 = E_3^\circ = -0.233 \text{ V}$$

Table II. Dissociation Constants for Sulfite Oxidase[d]

$K_1 = 6.2 \times 10^{-8}$ M (calc)[a]	$K_4 = 3.5 \times 10^{-13}$ M^3[c]
$K_2 = 1.1 \times 10^{-4}$ M (calc)[a]	$K_5 = 1.2 \times 10^{-11}$ M^2 (calc)[a]
$K_3 = 4.0 \times 10^{-9}$ M^2[b]	

[a] This work; see Results. [b] Average of room temperature values.[12] [c] Calculated from values reported for phosphate complexes at 120 K.[12] [d] The estimated errors in K_i are ±20%.

K_1 was estimated from the results at pH 6.00, 0.100 M KCl, and 0.020 M universal buffer by using a value of K_3 obtained from the room-temperature results of Bray et al.:[12]

$$E_2 = E_2^\circ - \frac{RT}{nF}\ln\left[\frac{K_1 + [H^+]}{K_1}\right] - \frac{RT}{nF}\ln\left[\frac{K_3}{K_3 + [H^+][Cl^-]}\right] + \frac{RT\ln[H^+]}{nF}$$

$$E_2 = 0.131 \text{ V} \qquad K_3 = 4.0 \times 10^{-9} \text{ M}^2$$

$$K_1 = 6.2 \times 10^{-8} \text{ M}$$

K_2 was estimated from the results at pH 7.00, 0.10 M KCl, 0.020 M phosphate, and 0.020 M universal buffer by using a value for K_4 estimated from the low-temperature (120 K) results of Bray et al.:[12]

$$E_2 = E_2^\circ - \frac{RT}{nF}\ln\left[\frac{K_1K_2 + K_1[H_2PO_4^-] + K_2[H^+]}{K_1K_2}\right] - \frac{RT}{nF}\ln\left[\frac{K_3K_4}{K_3K_4 + K_3[H^+][H_2PO_4^-]^2 + K_4[H^+][Cl^-]}\right] + \frac{RT\ln[H^+]}{nF}$$

$$E_2 = 0.038 \text{ V} \qquad K_4 = 3.5 \times 10^{-13} \text{ M}^3$$

$$K_2 = 1.1 \times 10^{-4} \text{ M}$$

K_5 was estimated from the results at pH 6.00, 0.10 M KCl, and 0.020 M universal buffer:

$$E_3 = E_3^\circ - \frac{RT}{nF}\ln\left[\frac{K_3 + [H^+][Cl^-]}{K_3}\right] - \frac{RT}{nF}\ln\left[\frac{K_5}{K_5 + [H^+][Cl^-]}\right]$$

$$E_3 = -0.086 \text{ V}$$

$$K_5 = 1.2 \times 10^{-11} \text{ M}^2$$

No evidence was found from the electrochemical data for the presence of a phosphate complex of the Mo(IV) state.

Values for the constants are found in Table II.

Discussion

Several schemes have been proposed to explain the EPR and EXAFS results for sulfite oxidase.[12,14] That of Bray et al.,[12] modified by the EXAFS results of George et al.,[14] has been used to interpret our results and to estimate dissociation constants for complexes of the Mo(VI) and Mo(IV) states (Scheme I).[16]

(16) Scheme I is based upon substitution of ligands trans to an oxo group in pseudooctahedral six-coordinate oxo- and dioxomolybdenum complexes. The exact coordination number and detailed coordination geometry of the molybdenum center in sulfite oxidase are still unknown, but model chemistry has shown that ligands trans to a terminal oxo group are generally more labile than the corresponding cis ligands. However, five-coordinate dioxomolybdenum(VI) complexes[19] with trigonal-bypyramidal geometry and six-coordinate dioxomolybdenum(VI) complexes with a replaceable ligand cis to the oxo groups[20] are also known, and both types of compounds give oxo-transfer reactions to yield monooxomolybdenum(IV) compounds. No charges have been specified for the various species in Scheme I because the charges on the remaining coligands are unknown.

The EXAFS results indicated no difference in the Mo(VI) site between high and low pH.[14] The value of E_2, calculated by using our values of E_2° and the molybdenum(V) chloride dissociation constant of Bray et al., at pH 6.00 in 0.10 M KCl, is significantly higher (0.073 V) than the observed value, suggesting, in fact, a pH-dependent structure for the Mo(VI) state. As pointed out by George et al., changes in the EXAFS data as a result of the loss of a weakly bound trans oxygen or nitrogen ligand, as postulated in Scheme I for the Mo(VI) state, might not be resolved.[14]

From the values E_2°, E_3°, and the appropriate dissociation constants, E_2 and E_3 at pH 7.00 in 0.10 M KCl may be calculated:

$$E_2 = E_2^\circ - \frac{RT}{nF}\ln\left[\frac{K_1 + [H^+]}{K_1}\right] - \frac{RT}{nF}\ln\left[\frac{K_3}{K_3 + [H^+][Cl^-]}\right] + \frac{RT\ln[H^+]}{nF}$$

$$E_3 = E_3^\circ - \frac{RT}{nF}\ln\left[\frac{K_3 + [H^+][Cl^-]}{K_3}\right] - \frac{RT}{nF}\ln\left[\frac{K_5}{K_5 + [H^+][Cl^-]}\right]$$

$$E_2 = 0.070 \text{ V}$$

$$E_3 = -0.090 \text{ V}$$

These values may be compared with those of Barber et al.,[10] obtained at pH 7.00 in 0.050 M MOPS buffer and unspecified chloride concentration, of 0.110 and −0.060 V. While the values for the Mo(V/IV) couple are just within experimental error, the Mo(VI/V) values differ by a small, but significant, amount. The reason for this difference is unknown but may be a result of different chloride ion concentration.

E_3 at pH 7.00 in 0.10 M KCl plus 0.020 M phosphate may also be calculated and compared with the experimental value, −0.239 V:

$$E_3 = E_3^\circ - \frac{RT}{nF}\ln\left[\frac{K_3K_4 + K_3[H_2PO_4^-]^2[H^+] + K_4[Cl^-][H^+]}{K_3K_4}\right] - \frac{RT}{nF}\ln\left[\frac{K_5}{K_5 + [Cl^-][H^+]}\right]$$

$$E_3 = -0.158 \text{ V}$$

This value is significantly lower (0.08 V) than the experimental value. The calculation uses a value for K_4 obtained from the results of Bray et al., at 120 K, which might be somewhat different at room temperature.[12] If it were, in fact, one pK unit higher, the calculated result (−0.216 V) would be in good agreement with the experimental value. In any case, considering the error in potentials (±0.015 V) and the difficulty in estimating dissociation constants from the results of Bray et al.,[12] the difference is not considered to be serious.

The results of the microcoulometric titrations indicate full reduction of the enzyme requires three electrons/subunit, corresponding to the Mo(VI/V), Mo(V/IV), and Fe(III/II) couples. As with previous results for nitrate reductase[8] and xanthine oxidase,[9] no evidence for reduction of the pterin moiety of the molybdenum cofactor, common to the three enzymes, was found.

Clearly, the reduction potentials of the molybdenum center are strongly dependent on pH and coordinating anions. Chloride binds to both Mo(V) and Mo(IV) states (Mo(IV) more strongly than Mo(V)), while phosphate binds to the Mo(VI) and Mo(V) states (Mo(VI) more strongly than Mo(V)) but not to the Mo(IV) state. These results support the EPR and EXAFS work of Bray et al.[12] and George et al.[14,15] and provide additional equilibrium constants for proton and phosphate binding to the Mo(VI) state and chloride binding to the Mo(IV) states of the enzyme.

Experimental Section

Sulfite oxidase was isolated from chicken liver by using the method of Kipke et al.[17] The purified enzyme used for the experiments had heme to protein ratios (A_{413}:A_{280}) of 0.60 and above. Analysis of representative enzyme preparations gave Mo:heme ratios of 1.03:1:00. Sulfite oxidase activity was assayed as previously described[18] by measuring the reduction of cytochrome c (0.20 M Tris-HCl, pH 8.5, with 2×10^{-4} M EDTA, 2×10^{-4} M cytochrome c, and 0.040 M sodium sulfite). Activity measurements were carried out at 25 °C by using a Uvikon 810 spectrophotometer. The concentration of sulfite oxidase active centers was determined spectrophotometrically by using $\epsilon_{413nm} = 0.0999$ M^{-1} cm^{-1} for the oxidized form of the enzyme.[3] The enzyme activity was 35 enzyme units/mg. A 0.020 M universal buffer containing equal molar concentrations of Bis-Tris, Tris base, and Bis-Tris propane was used for both the low-pH (6.00) and high-pH (9.00) experiments. Adjustment of the pH was performed by the addition of acetic acid. The pH 6.00 buffer and the 0.020 M potassium phosphate buffer contained 0.10 M KCl as electrolyte. The pH 9.00 buffer contained sodium p-toluenesulfonate, a relatively noncoordinating anion (0.10 M), as electrolyte.

Microcoulometry was performed as described previously[9] in the presence of the following mediators (each present at 1.67×10^{-4} M): 2,6-dichlorophenol-indophenol (E_o' = 0.217 V), 1,4-naphthoquinone-2-sulfonate (E_o' = 0.110 V), toluidine blue (E_o' = 0.034 V), pyocyanine (E_o' = 0.060 V), indigo disulfonate (E_o' = -0.124 V), anthraquinone-1,5-disulfonate (E_o' = -0.170 V), anthraquinone-2-sulfonate (E_o' = -0.225 V), safranine T (E_o' = -0.280 V), benzyl viologen (E_o' = -0.360 V), and methyl viologen (E_o' = -0.440 V). Each point was obtained by using duplicate 5.00×10^{-6} or 10.00×10^{-6} L samples of enzyme ($\sim 3.00 \times 10^{-9}$ mol of heme). The reduction potentials were obtained by using a nonlinear least-squares curve-fitting program based on the theoretical Nernst equation for three one-electron reductions: $n = (1 + e^{(E-E_1)F/RT})^{-1} + (1 + e^{(E-E_2)F/RT} + e^{(E_3-E)F/RT})^{-1} + 2(1 + e^{(E-E_3)F/RT} + e^{(2E-E_2E_3)F/RT})^{-1}$ ($E_1 = E$[Fe(III)/Fe(II)]; $E_2 = E$[[Mo(VI)/Mo(V)]; $E_3 = E$[Mo(V)/Mo(IV)]). The SCE reference electrode was checked before and after each run against a SCE and a saturated AgCl electrode used only for calibration and found to be within ±0.002 V of 0.244 V vs NHE. The error in the reduction potentials was estimated to be ±0.015 V.

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Registry No. Mo, 7439-98-7; Cl, 16887-00-6; $H_2PO_4^-$, 14066-20-7; sulfite oxidase, 9029-38-3; heme, 14875-96-8.

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Aqueous Chemistry of Mixed-Amine Cis- and Transplatin Analogues. Intramolecular Preference for a Kinetic Six-Membered Ring over a Thermodynamic Five-Membered Ring Ortho-Platination Product

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A series of mixed-amine cis- and transplatin analogues, containing benzylamine and 2-phenylethylamine functionalities, were synthesized and the aqueous solution chemistry investigated. The *cis*- and *trans*-dichloroplatinum(II) isomers having the neutral ligands ammine and 1,2-bis(4-methoxyphenyl)ethylamine (**1** and **4**), ammine and 2-(4-methoxyphenyl)-1-phenylethylamine (**2** and **5**), and ammine and bis(4-methoxyphenyl)methylamine (**3** and **6**) were synthesized in >98% isomeric purity. With the aim of investigating the pharmacoactivation of this class of compounds, a reversed-phase HPLC assay was developed for determining the rates of Pt-Cl hydrolysis of the cis-configured isomers. The precolumn addition of KBr to the reaction solutions trapped the aquachloro- and diaquaplatinum hydrolysis products as their bromo adducts. The separations of dichloro-, bromochloro-, and dibromoplatinum complexes allowed the quantification of their time-dependent concentrations, and the hydrolysis rate constants for **1-3** could be determined. It was found that, following Pt-Cl hydrolysis, an intramolecular ortho-platination occurred with **1-6**. Proton NMR studies showed that, for the trans-configured **4** and **5**, the kinetically favored, six-membered ring cycloplatinated products were formed specifically over the thermodynamic, five-membered ring ones. For the cis-configurated **1**, the kinetic product was formed selectively. The six-membered cycloplatinated ring could be converted into the five-membered, thermodynamically favored one by heating in dilute KCl. The implications of these novel findings are discussed from both mechanistic chemical and pharmacological points of view.

Introduction

For reasons of their remarkable antineoplastic activity, cis-coordinated diaminedichloroplatinum(II) compounds have come under recent scrutiny. *cis*-Diamminedichloroplatinum(II) (cisplatin) is currently one of the most successful chemotherapeutic agents in the treatment of a variety of tumors, with particular activity against testicular and ovarian carcinomas, as well as squamous cell carcinoma of the head and neck.[2] In an effort to develop more potent and less toxic agents with a broader spectrum of antitumor activity, a wide variety of amine ligands have been substituted for the NH_3 groups of cisplatin.[3] Surprisingly, ligands containing benzylamine or 2-phenylethylamine groups have not received much attention.[4] The lack of antineoplastic data on cisplatin analogues possessing amine ligands with these aromatic functionalities prompted us to synthesize the series of cis and trans mixed-amine dichloroplatinum(II) compounds **1-6**.

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