calculated to be 18.1 and 0.439 kK. The value of B_{35} is significantly greater than that for $Cr(BzAc)_3$, 0.358 kK.[1] The increase in B_{35} for $Cr(o\text{-}MeBzAc)_3$ compared to $Cr(BzAc)_3$ is attributed to the loss of resonance interaction between the phenyl ring and the chelate ring. In view of these data, it is reasonable to assume that the phenyl group is acting as an electron withdrawer and that the effect is greatly enhanced by resonance.

For complexes with no π^* orbitals, FeF_6^{3-} and $Fe(H_2O)_6^{3+}$, the electronegativity of the π orbitals is, of course, undefined. It is, therefore, consistent with the above argument that B and F^2 for these complex ions are very close to the free-ion values. The presence or absence of t_{2g}–π type interaction between the metal and ligands appears very critical in determining the values B and F^2. In this respect, the mechanism for reduction of B and F^2 from the free-ion values is closely related to the symmetry-restricted covalency discussed by Jørgensen.[5]

Attaching a physical significance to the trend in F^4 is more difficult. It is tempting to postulate that F^4 is a function of repulsions in the e_g subset and that σ-orbital electronegativity is greatest for $Fe(tert\text{-}BuPDO)_3$ and least for $Fe(DBM)_3$. In light of the value of F^4 for FeF_6^{3-}, however, this approach is undoubtedly overly simplistic.

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Iron(II) and Iron(III) Complexes of Penicillamine[1]

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Admission of oxygen to neutral solutions containing Fe(II) and excess penicillamine yields a bis red complex that is relatively stable in aqueous solutions at room temperature. Quantitative determination shows that 1 mol of H_2O_2 as the oxidizing agent is required for each Fe(II) for maximal development of the red color in solutions containing 3 equiv of penicillamine. This result indicates that for each mole of Fe(II) 1 mol of sulfhydryl compound also undergoes oxidation and suggests a link with the Fe(III) catalyzed oxidation of sulfhydryl compounds by oxygen. The red complex may also be formed by direct addition of Fe(III) to penicillamine in neutral solutions. It disappears more quickly in the presence of O_2 or H_2O_2 than with a N_2 atmosphere. A less stable blue complex is formed in acidic solutions containing equimolar amounts of Fe(III) and penicillamine.

Nonheme iron proteins frequently contain iron–divalent sulfur linkages. Attempts to employ cysteine as a model compound for the sulfhydryl group of proteins in interactions with iron require nonaqueous solvents and low temperatures in order to inhibit rapid oxidation–reduction reactions in these systems.[2] A blue complex exhibiting an absorption maximum at 620 nm with no optical activity is formed upon mixing equimolar amounts of $FeCl_3$ and cysteine in acidic solutions of 90% ethanol at −78°. The blue color was shown to be due to a 1:1 complex, and it was concluded that chelation occurs through S and O donor atoms. An optically active red complex absorbing at 525 nm was prepared at −78° in water-free ethanol and assigned as a bis-Fe(III) complex with chelation through S and O donors.[2]

In earlier work from this laboratory it was reported that the fleeting violet color formed upon mixing Cu(II) and cysteine is stable for hours when penicillamine is employed as a ligand.[3] The two β-methyl groups in penicillamine inhibit oxidation–reduction reactions and polynuclear complex formation. This paper describes the behavior of penicillamine with both Fe(II) and Fe(III) at several pH values in aqueous solutions at room temperature. Blue and red complexes of Fe(III) and penicillamine are easily observable under these conditions.

Polynuclear complex formation is also inhibited in formation of Co(II)[4] and Ni(II)[4,5] complexes of penicillamine compared to cysteine. With Ni(II) both ligands form bis, diamagnetic, tetragonal, red complexes in neutral or basic aqueous solutions.[6] A strong absorption peak at 375 nm, perhaps indicative of polynuclear complex formation, occurs more easily with cysteine than with penicillamine at nonintegral molar ratios of ligand to Ni(II). In basic solutions, Co(II) forms a well-defined, bis, hexacoordinate, pink-purple

(1) This research was supported by a grant from the National Science Foundation.

(2) A. Tomita, H. Hirai, and S. Makishima, *Inorg. Chem.*, **7**, 760 (1968); **6**, 1746 (1967).

(3) E. W. Wilson, Jr., and R. B. Martin, *Arch. Biochem. Biophys.*, **142**, 445 (1971).

(4) Unpublished work performed in this laboratory by Dr. Peter Morris, who initiated the experiments described in this paper.

(5) D. D. Perrin and I. G. Sayce, *J. Chem. Soc. A*, 53 (1968). Reduction of the number of coordinating groups from six in the aqueous ions to four in the cysteine and penicillamine complexes of Zn(II) and Ni(II) is the major factor in the greater magnitude of the second formation constants compared to the first in each case. The tendency of at least the intrinsic formation constants to exhibit this order is generally observed for Zn(II). The inverse order occurs with Ni(II) for ligands such as sulfur that stabilize tetragonal, low-spin complexes.[6] The low spin state of Ni(II) requires two sulfur atoms for stabilization giving rise to a cooperative effect which further increases the relative magnitude of the second formation constant.

(6) J. W. Chang and R. B. Martin, *J. Phys. Chem.*, **73**, 4277 (1969). Solutions containing 1:1:1 molar ratios of Ni(II), cysteine (or its methyl ester), and ethylenediamine disproportionate to give 2:1 complexes of each ligand with Ni(II); the complex with two sulfur atoms is tetragonal and the other complex octahedral.

complex with penicillamine that exhibits a split absorption peak with maxima at 494 nm (ϵ 56) and 516 nm (ϵ 58). In contrast similar solutions with 2:1 or 3:1 molar ratios of cysteine to Co(II) give spectra that are dependent on total concentrations.[4]

Experimental Section

All reagents were high-quality products: both D- and D,L-penicillamine were Calbiochem grade A, while both $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ were Mallinckrodt analytical reagents. In typical experiments the ligand was dissolved in 25 ml of degassed distilled water in a vessel connected to a Radiometer TTT1A-SBR2b Titrator-Titrigraph so that the pH could be set and maintained by addition of acid or base. Ultrahigh-purity nitrogen that had been bubbled through vanadium chloride scrubbers was passed through the solution.[7] Metal ions were added as solid salts most often at a final concentration of 5 m*M* but also in 10 times greater or lesser amounts. In the Fe(II) plus oxidizing agent experiments the addition was followed by bubbling of oxygen through the solution or addition of a 0.3% solution of H_2O_2. The red complexes were studied at molar ratios of ligand to metal ion from 2:1 to 10:1. Samples were removed from the reaction vessel and transferred to evacuated cells without exposure to air for recording of spectra on a Cary 14R spectrophotometer and Jasco J10B circular dichroism instrument. All (differential) molar absorptivities are based on total iron content. All experiments were performed at room temperature, about 24°.

Results

Titration under nitrogen of a solution containing 3 mol of protonated penicillamine ($HSRH_2^+$) for 1 mol of Fe(II) indicates that 1 equiv of ligand titrates freely and that only two penicillamine molecules are bound to Fe(II) by pH 9 to form a complex $[Fe(^-SR^-)_2]^{2-}$ containing two tridentate ligands. This conclusion is consistent with a formation constant study where only a 2:1 complex is found with 4:1 molar ratios of ligand to Fe(II).[8]

A wide variety of experiments were conducted by admitting oxygen to solutions containing D-penicillamine and Fe(II) at several ratios over the pH range 5.5–10.5 in order to determine maximum molar absorptivities and the equivalents of acid or base needed to maintain constant pH. Based on Fe(II) molar concentration the maximum molar absorptivity of ϵ 2500 near 525 nm of the red (-violet) complex is obtained in solutions containing at least a 3:1 molar ratio of penicillamine to Fe(II) from pH 7 to 9. In order to maintain constant pH during the admission of oxygen over this pH range, about 1.2 equiv of base was required at pH 7, while no base or acid was required near pH 7.7, and 1.0 equiv of acid was required at pH >9. With a 2:1 molar ratio of penicillamine to Fe(II), about 0.7 of the absorption intensity was attained and 0.7 equiv of acid was required to maintain constant pH at pH >9. Solutions containing a 3:1 molar ratio of penicillamine to Fe(II) and held at pH 8.5 upon admission of oxygen rapidly take up about 1 equiv of hydrogen ion and then cease. The red color produced rapidly diminishes in intensity if oxygen is continued but holds relatively stable when the gas is switched to nitrogen. This result indicates that Fe(II) in the complex is more easily oxidized than most of the sulfhydryl compound.

With D-penicillamine the visible absorption band of the Fe(III) complex produced by oxygenation at pH 8.5 consists of two bands of nearly equal intensity with apparent maxima located at about 500 and 550 nm. When D,L-penicillamine is employed as the ligand, the overall intensity is greater and the band at 560 nm predominates with only a shoulder occurring near 500 nm. Thus stereoselectivity occurs in these complexes. The red complex of D-penicillamine displays a significant circular dichroism spectrum with maxima at 690 nm ($\Delta\epsilon$ −0.64), 502 (+4.5), 436 (−3.9), 388 (−2.4), 348 (+3.0), 318 (−2.4), and 265 (+8.1). The only other absorption band in addition to those described in the visible region occurs at 285 nm (ϵ >7000). This band also undergoes an increase in intensity with a shift to shorter wavelength (280 nm) when racemic ligand is utilized.

Experiments similar to those just described were conducted with measured quantities of H_2O_2 replacing oxygen. With a 3:1 molar ratio of D-penicillamine to Fe(II) the absorption peak at 525 nm gave a maximum value (ϵ 2100) upon addition of 1 mol of H_2O_2 per Fe(II) from pH 7 to 9. The requirements for maintenance of pH are identical with those quoted for the oxygen experiments. The intensity falls off sharply with addition of lesser or greater amounts of H_2O_2, probably accounting for the slightly smaller maximum ϵ value obtained for H_2O_2 than for oxygen. As for the case of oxygen a 2:1 molar ratio of penicillamine to Fe(II) also yields about 0.7 of maximum intensity and requires 0.7 equiv of acid to maintain constant pH.

Titrations under nitrogen of solutions containing Fe(III) and penicillamine yield a sharp end point by pH 4 after the addition of 1 equiv of base due to the redox reaction

$$Fe^{3+} + HSRH \longrightarrow Fe^{2+} + {}^1/_2HRSSRH + H^+ \quad (1)$$

The Fe(II) produced reacts with any excess penicillamine as described for the Fe(II) titration. A blue color is observed at the beginning of the titration before reaction 1 occurs. Little or no red color appears at later stages of the titration.

Mixing of Fe(III) and penicillamine in acidic aqueous or acidic water–alcohol solutions yields, under nitrogen or air, an unstable blue complex absorbing maximally at 605 nm with ϵ >500. We also observe the formation of a blue color upon addition of Fe(III) to acidic solutions of L-cysteine, *N*-acetylcysteine, and *N*-acetylpenicillamine, but not with *S*-methyl-L-cysteine, L-cystine, β-mercaptoethylamine, and cysteine methyl or ethyl esters. Since the blue color has also been observed with thioglycolic acid,[2] not only the sulfur but also the carboxylate group seems essential for its appearance under these conditions. With D-penicillamine the blue complex yields only a weak CD with extrema at 650 nm ($\Delta\epsilon$ ~−0.1) and 515 nm ($\Delta\epsilon$ ~+0.15). Racemic and optically active penicillamine yield blue complexes with identical visible absorption spectra. The weaker CD and lack of stereoselectivity suggest that the ligand of the blue complex is chelated through at least one less donor group than that of the red complex.

Though Fe(III) is reduced to Fe(II) near the beginning of the titration in the presence of penicillamine, it is possible to obtain the intense red color by direct addition under nitrogen of $FeCl_3$ to slightly alkaline solutions of penicillamine, where some loss of Fe(III)

(7) L. Meites, "Polarographic Techniques," Interscience, New York, N. Y. 1955, p 34.

(8) D. A. Doornbos, *Pharm. Weekbl.*, **108**, 1213 (1968).

might be expected. Evidently at higher pH the more strongly bound Fe(III) does not undergo reduction as rapidly as the more weakly bound metal ion at lower pH despite the fact that the overall reaction (eq 1) is favored in less acidic solutions. Full development of the red color is nearly complete in solutions containing 2.5 mol of ligand for 1 mol of Fe(III), a result indicating that the red color is not due to a tris but to a bis complex. A similar situation occurs with penicillamine and Cu(II) where the 2:1 complex is more stable in 0.1 *N* base than in neutral solutions.[3]

Discussion

Whether Fe(II) and an oxidizing agent or Fe(III) is added to solutions of penicillamine a similar red color is obtained. In the preceding section evidence has been presented from experiments with both metal ions to indicate that the red color is due to a bis complex of Fe(III). The occurrence of stereoselectivity in this complex suggests that the penicillamine molecules are bound as tridentate ligands.[9]

Because only 0.5 mol of the two-electron oxidant H_2O_2 is needed to yield 1 mol of Fe(III) from Fe(II), the observation that 1 mol of H_2O_2 is required for maximal development of the red color demands that 1 mol of sulfhydryl compound also undergo oxidation as part of the overall reaction. Few balanced equations can be written describing all features of the stoichiometry in the H_2O_2 experiments, but the simplest at about pH 9, where penicillamine exists predominantly as the $^-$SRH species is

$$[Fe^{II}(^-SR^-)_2]^{2-} + H_2O_2 + {}^-SRH \longrightarrow [Fe^{III}(^-SR^-)_2]^- + {}^1/_2(^-RSSR^-) + H_2O + OH^-$$

The Fe(III) product complex must possess a single negative charge but could contain bound hydroxide ions if the amino groups were unbound and protonated. The important conclusion from the stoichiometry studies is that a tris Fe(III) complex seems to be excluded by the results so that the red complex is bis. The lesser development of red color in the 2:1 penicillamine to Fe(II) solutions may be related to the redox potentials, which require production of disulfide for a poised solution, or may be a feature of the mechanism of the reaction. In the cysteine system production of a purple color is first order in Fe(II).[10] Since Fe(II) is a one-electron reducing agent and H_2O_2 a two-electron oxidizing agent, the most facile kinetic pathway may involve the additional one-electron reducing agent penicillamine. The radical produced after acceptance of an electron from Fe(II) by O_2 or H_2O_2 may take up rapidly another electron from unbound $^-$SRH to yield $\cdot$SRH, two of which combine to give disulfide. By this mechanism the production of 1 equiv of disulfide for each Fe(II) oxidized is due to the scavenging action of $^-$SRH in cleaning up the radicals produced by addition of an electron to O_2 or H_2O_2. Alternatively the production of these energetically unfavorable radicals may be avoided by an internal redox reaction occurring within the complex followed by expulsion and combination of a sulfhydryl radical

$$[Fe^{II}(^-SR^-)_2]^{2-} + H_2O_2 \rightleftharpoons H^+ + [Fe^{II}(^-SR^-)_2O_2H]^{3-}$$

$$[Fe^{II}(^-SR^-)_2O_2H]^{3-} \xrightarrow{H^+} [Fe^{III}(^-SR^-)(OH^-)_2]^- + \cdot SR^-$$

$$[Fe^{III}(^-SR^-)_2]^- + OH^- + H_2O \xleftarrow{^-SRH} [Fe^{III}(^-SR^-)(OH^-)_2]^-$$

$$\cdot SR^- \longrightarrow {}^1/_2{}^-RSSR^-$$

Coordinated water molecules are not indicated in the mechanism, a similar one of which may be written with O_2 replacing H_2O_2. Under most circumstances the vertical step would be rate limiting. The bleaching of the final red complex to a Fe(II) complex is relatively slow in neutral solutions.

The concomitant oxidation of sulfhydryl groups as well as Fe(II) on reaction with O_2 or H_2O_2 suggests a connection between the above reaction and the Fe(III)-catalyzed oxidation by oxygen of sulfhydryl compounds such as cysteine. An Fe(III) complex of a sulfhydryl compound may also be viewed as the Fe(II) complex of the radical which by acquiring oxygen undergoes a series of intraconversions that might be written as

$$Fe^{III}(RS^-)_2 \rightleftharpoons Fe^{II}(RS^-)(RS\cdot) + RSH$$

$$Fe^{II}(RS^-)(RS\cdot) + O_2 \rightleftharpoons Fe^{II}(RS^-)(RS\cdot)(O_2)$$

$$Fe^{III}(RS^-)_2 + H_2O_2 + RS\cdot \rightleftharpoons Fe^{II}(RS^-)(RS\cdot)(O_2H) + RSH + RS\cdot$$

The hydrogen peroxide produced in the last step then substitutes for oxygen in a similar cycle of reactions. Oxidation-state designations should not be taken literally in such systems. All the species labeled with Fe(II) are present only in low steady-state concentrations. If an equilibrium prevails in the overall reaction and the combination of expelled sulfhydryl radicals to yield disulfide (RSSR) is rate limiting, the two-thirds order dependence of the oxidation rate on the total iron concentration may be accounted for.[11]

(9) P. J. Morris and R. B. Martin, *J. Inorg. Nucl. Chem.*, **32**, 2891 (1970).
(10) A. D. Gilmour and A. McAuley, *J. Chem. Soc. A*, 1006 (1970).
(11) J. E. Taylor, J. F. Yan, and J. Wang, *J. Amer. Chem. Soc.*, **88**, 1663 (1966).