The Kinetics of Flavine Oxidation–Reduction. II. Metal Ion Interactions†

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ABSTRACT: The oxidation–reduction reactions of tetraacetylriboflavine in the presence of various metal ions in dimethylformamide have been investigated using the stopped-flow technique under anaerobic conditions. Dismutation kinetics in the presence of redox-inactive dissociated divalent metal ions such as Cd^{2+}, Zn^{2+}, and Fe^{2+} are typically triphasic. Metal ions act primarily upon an intermediate flavine dimer formed by fast association of flavoquinone and flavohydroquinone, resulting in a parallel formation of neutral and chelated radicals. A competition between metal ions and proton donors, e.g. the neutral flavohydroquinone ($F_{red}H_3$), is observed at the level of this intermediate complex. Small spectral changes occur secondarily as an ill-defined intermediate phase which could correspond to the reorganization of the solvation of radical chelate. The neutral radical is finally chelated at a much slower rate, the yield of total radical formation remaining almost unchanged during this last kinetic phase. The oxidation of flavohydroquinone by ferric ions, either dissociated or strongly coordinated within a porphyrin, is complete and proceeds through biphasic kinetics. The first phase ($F_{red} \rightarrow \dot{F}$) is much faster than the second one ($\dot{F} \rightarrow F_{ox}$). Dismutation resulting from the transient accumulation of neutral flavosemiquinone competes with the direct oxidation with ferric ions for the completion of the second oxidation step. The relative rate of dismutation is essentially limited by acidic-basic reactions in the absence of an excess of ferrous ion. The kinetic analysis of the direct oxidation reactions favors an outer-sphere mechanism for the electron transfer to the ferric ion, either free or strongly coordinated. The formation of a ferrous radical chelate can result from the dismutation reactions only when the amount of ferric ion initially present is not sufficient for complete oxidation.

Metal ions which are redox stable with respect to the three redox states of the flavines may displace the dismutation equilibrium. For example, the general affinity of the flavosemiquinone for divalent metal ions results in a large displacement toward radical chelate formation (Hemmerich, 1964). The mechanism of this reaction will be investigated kinetically in light of the dismutation scheme derived in the previous paper (Favaudon and Lhoste, 1975) for flavines in nonaqueous solvent. The most important biochemical problem is to know whether such a flavine–metal interaction would occur before or after interflavine electron transfer.

The oxidation–reduction reactions of flavines by redox-active ions may also be strongly influenced by the flavine dismutation which could provide, for example, a second and indirect step of oxidation or reduction by metal ions involving one-electron transfer.

The metal ions used in the present investigation were selected in order to investigate these different problems as independently as possible. Divalent cadmium and zinc ions are redox stable and exhibit a high affinity for flavosemiquinone. Ferrous ions participate similarly to the dismutation reaction. The role of dismutation in the oxidation of flavines by ferric ions has been investigated using both dissociated ions and ions strongly coordinated in porphyrin complexes which should interact very differently with the flavines for sterical reasons. Finally, the mechanism of flavine oxidation and chelate formation by monovalent silver ions will be briefly reported since the rather stable complexes formed by these ions with the flavoquinone and the acidic–basic behavior of these complexes have already been discussed as a model for biological electron transfer (Hemmerich et al., 1965; Fritchie, 1972).

Materials and Methods

Solvent. *N,N'*-Dimethylformamide was used as a solvent throughout the present work. It was purified by distillation under a low pressure of dry nitrogen. Each experimental series was carried out using the same solvent sample.

Flavine. Tetraacetylriboflavine (Ac_4RF)[1] was synthesized following the method of McCormick (1970) and then recrystallized from acetic acid–water mixtures and dried in vacuo.

Metallic Salts. Cd^{2+} and Zn^{2+}. Reagent grade anhydrous cadmium iodide (Baker) and zinc perchlorate dihydrate (Fluka) were used without further purification.

Fe^{3+}. Ferric perchlorate, $Fe(ClO_4)_3 \cdot 9H_2O$ (Erba), was recrystallized twice from a dimethylformamide–ethyl acetate mixture as a greenish powdery precipitate. The precipitate was filtered and desiccated in vacuo. The final product in which the water molecules are exchanged by dimethylformamide is not hygroscopic. Its iron content was measured spectrophotometrically in concentrated perchloric acid (Bastian et al., 1956) and polarographically in oxalic acid–oxalate solution (Brezina and Zuman, 1958), using ferric oxalate as reference samples.

Fe^{2+}. The reduction of a solution of oxidized Ac_4RF and ferric perchlorate by H_2/Pd provided both neutral flavohy-

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[1] Abbreviations used are: Ac_4RF, tetraacetylriboflavine; Et_4NCl, tetraethylammonium chloride; $F_{red}H_3$, $\dot{F}H_2$, and $F_{ox}H$, neutral form of flavohydroquinone, flavosemiquinone, and flavoquinone.

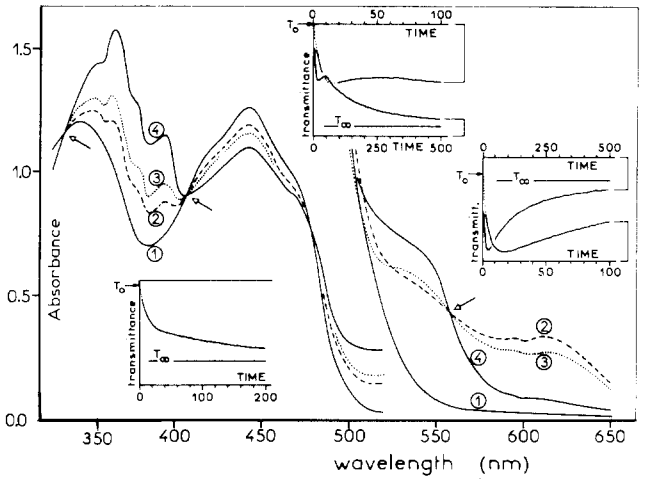


FIGURE 1: Transient absorption spectra of the dismutation reaction of tetraacetylriboflavine in the presence of cadmium ions (1) before mixing, (2) at 15 msec, (3) 50 msec, and (4) infinite reaction times. Isosbestic points corresponding to the slow chelation of the neutral radical are indicated by arrows. The initial flavine and metal ion concentrations were $[F_{red}H_3]_0 = [F_{ox}H]_0 = 1.5 \times 10^{-4}$ *M* and $[Cd^{2+}] = 3 \times 10^{-2}$ *M* for the short-wavelength range, and $[F_{red}H_3]_0 = [F_{ox}H]_0 = 5 \times 10^{-4}$ *M* and $[Cd^{2+}] = 10^{-1}$ *M* for the long-wavelength part. The spectra have been reconstituted from oscillograms taken at wavelengths 5 nm apart from each other. Typical oscillograms recorded at 385 nm (left), 510 nm (top), and 610 nm (right) are shown. The transmittance values at infinite and zero reaction times are indicated as T_∞ and T_0, respectively. The time scale calibration is given as milliseconds.

droquinone and ferrous ions. Protons released stoichiometrically with Fe^{2+} were neutralized with tetrabutylammonium hydroxide diluted in dimethylformamide from a 0.1 *M* alcoholic solution in order to perform the dismutation reactions in initially aprotic conditions. This neutralization must be carried out after total reduction of the flavine and metal ion mixture, in order to prevent irreversible dimerization of the flavine (Hemmerich et al., 1959; Ehrenberg et al., 1967).

Porphyrins. (Deuteroporphyrin IX dimethyl ester)chloroiron(III) (chlorohemine), kindly provided by Dr. M. Momenteau, was taken as a high-spin complex. Dimerization of the chlorohemine was prevented by addition of 2×10^{-2} *M* tetraethylammonium chloride (Et_4NCl)[1] (Momenteau, 1973). It was used in the presence of 4×10^{-2} *M* pyridine, which does not appreciably coordinate the chlorohemine ($\epsilon_{610} = 2800\ M^{-1}\ cm^{-1}$), but forms very rapidly a bispyridine-hemochrome complex ($\epsilon_{610} = 900\ M^{-1}\ cm^{-1}$, $K_{ass} = 1.8 \times 10^5\ M^{-2}$; Brault and Rougée, 1974) after reduction of the iron porphyrin.

Addition of 2×10^{-2} *M* imidazole to chlorohemine results in the formation of a low-spin bisimidazole-hemichrome complex ($\epsilon_{610} = 1100\ M^{-1}\ cm^{-1}$, $K_{ass} = 5.6 \times 10^5\ M^{-2}$; Momenteau, 1973) which does not dissociate after reduction ($\epsilon_{610} = 250\ M^{-1}\ cm^{-1}$, $K_{ass} = 6 \times 10^7\ M^{-2}$; Momenteau, 1973).

Stopped-Flow Apparatus. The anaerobic stopped-flow apparatus used in the present study has been described in the preceding paper (Favaudon and Lhoste, 1975). The mixing dead time of 2.5 ± 0.2 msec was lowered to 2.0 ± 0.2 msec in some experiments using a new mixing-jet made in the laboratory.

The reduction of the flavine was carried out in pure dimethylformamide using H_2/Pd, which results in neutral flavohydroquinone, $F_{red}H_3$, without any excess of protons. All experiments were carried out at controlled temperature (20°C).

Results and Discussion

Dismutation in the Presence of Redox-Stable Divalent Ions (Cd^{2+} and Zn^{2+}). The chemical system was investigated following several different initial conditions, in order to clarify the kinetics or the competition of the various chelation and acid–base reactions.

Dismutation at Half-Reduction. A large excess of the divalent metal ion was introduced with the flavoquinone. Under such conditions, the oxidized flavine is partially chelated before mixing with the fully reduced flavine. The absorption spectra of equilibrium and transient species have been reconstituted for the triphasic reaction course (Figure 1).

A radical chelate, characterized by its absorption in the 370–410 nm and 480–570 nm wavelength ranges (Müller et al., 1968), appears first in stoichiometric yield with a neutral radical characterized by its long-wavelength absorption (Müller et al., 1972). The initial rate of the reaction is about 100 times that measured in the absence of metal ion.

The second phase, characterized by a spectral modification in the 480–520 nm range, can correspond to a reorganization of the solvation sphere of radical chelate, as suggested by the appearance of a weak and poorly defined absorption band in the near-infrared (Müller et al., 1968).

The last phase of the reaction corresponds to a slow and isosbestic conversion of the neutral radical into radical chelate, leading to an equilibrium ratio depending upon the relative metal ion to flavine concentration.

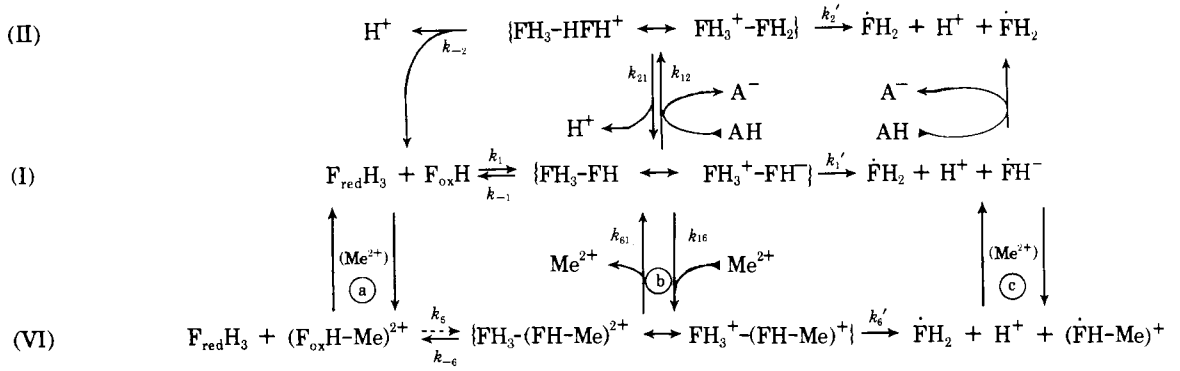
Thus, the radical chelate formation results from two processes, the slower one corresponding to the secondary chelation of the neutral radical. The primary chelation could occur at three different stages of the general reaction scheme of dismutation in the absence of metal (Scheme I).

The large increase in the initial reaction rate upon addition of metal ion does not agree with a simple chelation of the radical anion (path c), since such a mechanism for primary chelation should be kinetically limited by the rate of flavine dismutation as observed in the absence of metal ($k \simeq 560\ M^{-1}\ sec^{-1}$). On the other hand, the kinetics at various flavine concentrations and reduction degrees allow one to eliminate also path a, i.e. the chelation of the flavoquinone, as a main pathway for primary radical chelation.

Dismutation at High Degree of Reduction. At high reduction ratios, metal ions were introduced at low concentration with the flavoquinone. Thus, the product $[F_{ox}H]_0[Me^{2+}]_0$ was too low to allow any detectable chelation of the flavoquinone (chelate formation constant $\simeq 3\ M^{-1}$ in dimethylformamide). Under such conditions, the rate of formation of the neutral and the chelated radicals is still high when compared to that measured in the absence of metal ion. It depends linearly on the metal ion concentration (Figure 2) and on the product of initial concentrations of flavoquinone and flavohydroquinone. However, the negative catalytic effect due to the neutral flavohydroquinone acting as proton donor is not suppressed by the metal and results in a decrease of the initial rate proportional to the concentration of reduced flavine. Thus, the initial rate equation can be written as:

$$V_i = k_i[F_{red}H_3]_0[F_{ox}H]_0[Me^{2+}]_0\,(1/[F_{red}H_3]_0) = k_i\,[F_{ox}H]_0[Me^{2+}]_0 \quad (1)$$

Scheme I: Reaction Scheme of the Initial Flavine Dismutation in the Presence of Divalent Metal Ions.[a]



[a] The pathways corresponding to the direct and mixed backward dismutation reactions following the accumulation of the neutral and chelated radicals are not described.

The rate constant k_i is in the range of 4.4 to 8.9 × 10^5 M^{-1} sec^{-1} for Cd^{2+}, depending upon water contamination. This kinetic behavior suggests that the metal ion and the neutral flavohydroquinone compete with each other at the level of the primary intermediate complex following path b for the primary metal chelation and subsequent release of neutral and chelated radicals (see microfilm edition of this issue, eq 27-30; see Supplementary Material Available paragraph).

The transient equilibrium observed at the end of the first phase shows that the yield of radical formation increases much more slowly than the initial rate as a function of the metal ion concentration (Figure 2). Therefore, the rate of the backward dismutation reactions among neutral and chelated radicals should also be increased by the presence of the metal. However, the influence of the metal ion on these dismutation reactions cannot be precisely estimated since the presence of protons released in a stoichiometric amount with the neutral flavosemiquinone changes the conditions.

Fate of the Neutral Radical. The secondary chelation of the neutral radical could start with the backward dismutation of this radical species. However, this should require a dismutation rate of the neutral semiquinone much faster than that of the radical chelate or that for a mixed reaction of the two radicals. This would be in disagreement with the nearly equal yield of the two radicals observed at the transient equilibrium. Furthermore, the overall yield of radicals remains practically constant during the final phase of the reaction, indicating that the secondary chelation is mostly a direct reaction. It is slow due to the stability of the neutral flavosemiquinone in dimethylformamide. Upon addition of a strong base, such as piperidine, the radical chelate formation is complete and the total reaction appears monophasic, corresponding to a direct and fast chelation of radical anion. However, the reaction kinetics are complicated by metal chelation with the nitrogeneous base. The dismutation of the radical chelate with itself should be slow since the presence of two metal ions must hinder the formation of an intermediate dimer, both for steric and electrostatic reasons. Thus, it appears that the mixed disproportionation of the two types of radicals, neutral and chelate, is the most efficient process for the backward reaction.

This behavior has been observed for both Cd^{2+} and Zn^{2+} ions, the former one corresponding to slightly higher initial rates and equilibrium radical yields. However, differences in the water content of the used salts (see Materials and Methods) may well explain these observed kinetic and equilibrium differences.

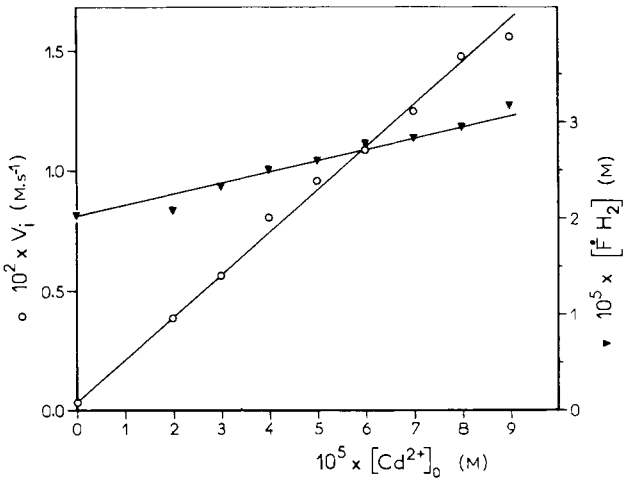


FIGURE 2: Initial formation rate (○) and transient equilibrium concentration (▼) of the neutral flavosemiquinone measured at 610 nm in the presence of the cadmium ion. The initial flavohydroquinone and flavoquinone concentrations were 2 × 10^{-2} and 2 × 10^{-4} M, respectively.

Oxidoreduction Reactions of Flavines in the Presence of Iron Ions. The redox potentials of the iron ions relative to the flavine in dimethylformamide are comparable to those measured in aqueous solutions. The polarography of the perchloric salt, completely dissociated in dimethylformamide, exhibits a monoelectronic and reversible Fe^{III}/Fe^{II} wave ($E_{1/2}$ = +0.37 V vs. SCE) on a rotating platinum electrode, and an irreversible Fe^{II}/Fe^{0} bielectronic wave ($E_{1/2}$ = −1.26 V vs. SCE) on the dropping mercury electrode. Under similar conditions, a Ac_4RF solution was found to exhibit a partly irreversible wave ($E_{1/2}$ = −0.78 V vs. SCE) as previously reported by Sawyer et al. (1971). Thus, ferric ions are potentially good oxidants for the reduced flavines but the ferrous ions should be redox inactive relative to the flavines.

Dismutation in the Presence of Fe^{II} Perchlorate. Ferrous ions initially added to the flavoquinone solution are redox stable in the course of the dismutation reaction as expected from the electrochemical data. The reactions are comparable to those observed in the presence of other divalent ions. The poorly defined second phase, attributed to the reorganization of the solvation sphere of radical chelate with Cd^{2+}, was not observed with the ferrous ion. The radical yields at the transient and final equilibria were comparable to those measured with Cd^{2+} or Zn^{2+}, but the initial rate constant

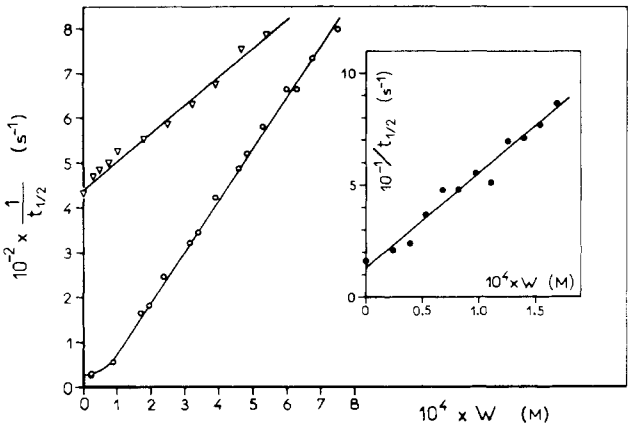


FIGURE 3: Kinetic test (Capellos and Bielski, 1972) for the oxidation of neutral radical measured at 610 nm during the second oxidation step of the flavohydroquinone by the ferric ion (second-order reaction 3 in text). The concentration function W is defined as $W = \{[Fe^{III}]_1 - [F]_0\}/\ln\{2 - [F]_0/[Fe^{III}]_1\}$ where $[F]_0$ is the initial concentration of reduced flavine and $[Fe^{III}]_1 = [Fe^{III}]_0 - [F]_0$ is the ferric ion concentration at completion of the first oxidation step: (○) ferric perchlorate, $[F]_0 = 2.5 \times 10^{-5}$ M; (▽) bisimidazole hemichrome, $[F]_0 = 2 \times 10^{-5}$ M; (●) chlorohemine, $[F]_0 = 10^{-5}$ M.

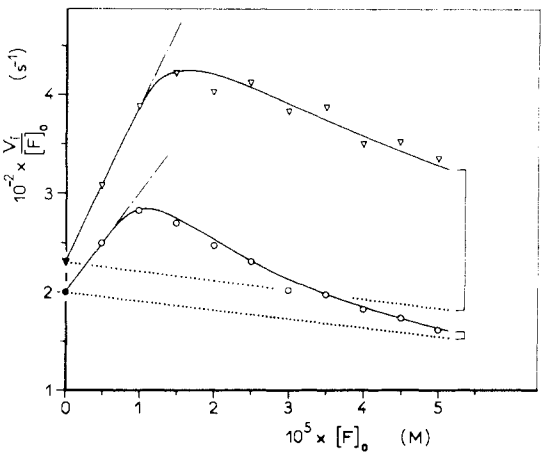


FIGURE 4: Initial rate analysis following eq 4b (in text) of the neutral flavosemiquinone decay as a function of the total flavine concentration in the presence of ferric perchlorate (○) and of bisimidazole-hemichrome (▽), 2.5×10^{-4} M. The initial rate was measured for the second oxidation step of the fully reduced flavine from the log plots of the absorbance variation at 610 nm. The dotted lines represent the linear variation of $k_b[Fe^{III}]_1$ at the beginning of this second step, i.e. the contribution from the direct oxidation by the ferric ion alone.

k_i (eq 1 above) measured at high reduction ratios and low ferrous ion concentration ($k_i = 3.9$ to 5.7×10^4 M^{-1} sec^{-1}, depending on the solvent sample used) is somewhat lower. The chelation rate of the intermediate dimer of path I must be slower by one order of magnitude because of the solvation of the ferrous ion.

Flavine Oxidation by Fe^{III} Perchlorate. Ferric ions interact very weakly with flavoquinone in nonaqueous solvents in which they cannot form stable chelates as observed for most divalent ions including Fe^{II} (Lauterwein et al., 1975). On the other hand, the oxidation of reduced flavine is fast and complete in the presence of an excess of ferric ion, and three distinct reactions can be observed kinetically in the stopped-flow experiments.

The first one-electron step of the oxidation of neutral flavohydroquinone by ferric ion is fast, at the limit of the 2.5 msec time of resolution of the apparatus. However, at very low flavine concentration and with a fivefold excess of Fe^{III} perchlorate, it was possible to observe the completion of the first oxidation reaction:

$$F_{red}H_3 + Fe^{III} \xrightarrow{k_a} \dot{F}H_2 + H^+ + Fe^{II} \qquad (2)$$

and to measure its rate constant ($k_a \simeq 2.7 \times 10^7$ M^{-1} sec^{-1}). The oxidation of the neutral radical proceeds more slowly and appears pseudo-first-order at large enough excesses of ferric ion. Under such conditions, the extrapolation at zero reaction time of the first-order log plots indicates that the oxidation of the fully reduced flavine to yield neutral flavosemiquinone is completed during the mixing time. Ferrous ions should be formed simultaneously but no radical chelate was observed. Incidently, this reaction provided a precise measurement of the molar extinction coefficients of the neutral radical in the 400–700 nm wavelength range ($\epsilon_{610} = 5000 \pm 100$ M^{-1} cm^{-1}).

The neutral flavosemiquinone can be oxidized directly by the ferric ion:

$$\dot{F}H_2 + Fe^{III} \xrightarrow{k_b} F_{ox}H + H^+ + Fe^{II} \qquad (3)$$

with a rate constant $k_b = 8.0$ to 11.3×10^5 M^{-1} sec^{-1} depending upon the solvent sample used. This reaction becomes important only at high ferric ion concentrations because of the competition of the backward dismutation.

The kinetic test used for the analysis of the direct oxidation of the neutral radical corresponds to the integral form of the differential equation for the second-order reaction (3) at constant flavine concentration (Figure 3). The slope of the curves is a direct measurement of k_b and the ordinate intercept, corresponding to zero ferric ion concentration after completion of reaction 2, reflects directly the importance of the dismutation reaction.

The dismutation reaction can also be analyzed kinetically by varying the initial concentration of the fully reduced flavine in the presence of a large excess of ferric perchlorate. This corresponds to a variation of the concentration of neutral radical, of protons, and of ferrous ions after completion of the first oxidation step (2). The rate equation for the decay of the neutral radical is:

$$V_i = k_b[\dot{F}H_2]_1[Fe^{III}]_1 + 2\alpha[\dot{F}H_2]_1^2 \qquad (4a)$$

where the subscript 1 refers to the concentration obtained after completion of the first step. At this reaction time the radical concentration is equal to the initial flavohydroquinone concentration, $[F]_0$. Then:

$$V_i/[F]_0 = k_b[Fe^{III}]_1 + 2\alpha[F]_0 \qquad (4b)$$

Under strictly aprotic conditions, the intrinsic probability of dismutation 2α is larger than k_b. It is measured as the initial slope in the plot of Figure 4 ($2\alpha_0 = 1.05 \times 10^7$ M^{-1} sec^{-1}) and is found equal to the value obtained from dismutation experiments at low reduction degree in the absence of metal ion. When the flavine concentration is increased the dismutation probability decreases because of the protons released in the first oxidation step. At high enough flavine concentration the neutral radical decays only through the direct oxidation (eq 3) and the ratio $V_i/[F]_0$ decreases slowly because of the partial reduction of the ferric ion in the first step. The amount of ferrous ion produced in this reaction is not sufficient to influence the rate of radical dismutation. Therefore, the dismutation pathway for the radical decay can be important only under aprotic conditions,

i.e. at low flavine concentration or in solutions buffered at slightly alkaline pH values.

The steady-state spectroscopic observation of a radical chelate by Hemmerich and Spence (1966) resulted from rather different conditions since the oxidation of the reduced flavines occurred in the presence of 1 equiv of ferric ion.

Dismutation and Oxidation of Flavines in the Presence of Iron Porphyrins. Fe^{II} porphyrins (hemes) do not interact with flavines in dimethylformamide, either reduced or oxidized, as observed spectrophotometrically. Fe^{III} porphyrins are still good oxidants with respect to the reduced flavines whatever their axial complexation could be. The irreversible one-electron wave Fe^{III}/Fe^{II} is observed on a rotating platinum electrode at potential values varying from −0.10 to −0.30 V (vs. SCE) depending upon the nature of the porphyrin ring and of axial ligands. This can correspond to a high-spin or to a low-spin electronic configuration for the ferric ion (chlorohemines and bishistidine complexes for example) (Lexa et al., 1974).

The behavior of the reduced flavines in the presence of Fe^{III} porphyrins is qualitatively identical with that reported using ferric perchlorate. The strong visible absorption of porphyrins, especially of the low-spin chlorohemine, makes difficult the investigation of the first phase of the one-electron oxidation of flavohydroquinone. However, this oxidation step appears fast as compared with the resolution time of the stopped-flow apparatus. The rate constant for the direct oxidation of radical by Fe^{III} porphyrins in the second phase appears smaller for the high-spin chlorohemine ($k_b \simeq 4.2 \times 10^5\ M^{-1}\ sec^{-1}$) than for the low-spin bisimidazole complex (k_b = 6.2 to $9.2 \times 10^5\ M^{-1}\ sec^{-1}$). However, the measured rate constants of dismutation and of oxidation are strongly dependent on the solvent sample used, as observed with ferric perchlorate.

The dismutation rate is not directly affected by the presence of the iron porphyrins, either ferric or ferrous, but the added bases or salts may have a large influence due to buffer effect ($2\alpha_0 = 1.60 \times 10^7\ M^{-1}\ sec^{-1}$ in the presence of $2 \times 10^{-2}\ M$ imidazole) or to changes in the ionic strength ($2\alpha_0 = 3.1 \times 10^6\ M^{-1}\ sec^{-1}$ in the presence of $2 \times 10^{-2}\ M$ Et_4NCl and $4 \times 10^{-2}\ M$ pyridine). In some instances, the dismutation reaction may overcome the direct process (Figures 3 and 4).

Kinetics of the Silver(I)–Flavoquinone Chelate Formation. Flavoquinone may chelate Ag^+ ions in aprotic solvents without release of the N-3 proton, as revealed by nuclear magnetic resonance (NMR) (Lauterwein et al., 1975). The overall equilibrium formation constant of the chelate in pure dimethylformamide ($K \simeq 500\ M^{-1}$) is not large enough to allow the complete formation of the complex at the flavine concentrations used in the spectrophotometric experiments. Full chelate formation can be observed upon addition of 1 equiv (with respect to flavine) of piperidine. This situation appears similar to that analyzed in water by titrimetry and for which the following scheme for metal chelation was proposed (Hemmerich et al., 1965):

$$F_{ox}H + Ag^+ \rightleftarrows (F_{ox}H\text{–}Ag)^+ \rightleftarrows (F_{ox}\text{–}Ag) + H^+ \quad (5)$$

Kinetics of the chelate formation of Ac_4RF in dimethylformamide have been studied both in the presence and in the absence of piperidine. Addition of 1 equiv of base per mol of flavine results in a tenfold increase of the initial chelation rate while, as mentioned above, the chelation equilibrium is completely displaced to the right. The kinetic analysis under various conditions of concentrations indicates that the proton is taken off from the primary complex $(F_{ox}H\text{–}Ag)^+$ and not from the free flavoquinone. As a matter of fact, ionization of the flavoquinone should be negligible since the pK_a of piperidine is about 4 pH units lower than that of flavoquinone in dimethylformamide (Demange-Guérin, 1970). Otherwise, no other modification which could be attributed to charge transfer occurs upon base addition. On the contrary, Ag^+ ions in dimethylformamide oxidize rapidly both the flavohydroquinone and the flavosemiquinone. However, the precipitation of metallic silver resulting from this reaction does not allow one to investigate the kinetics.

Conclusion

The kinetics of flavine dismutation in the presence of divalent metal ions, including Fe^{2+}, have shown that the radical chelate formation, responsible for a large displacement of the dismutation equilibrium, arises from two different mechanisms. The first one corresponds to the chelation of the transient dimer formed by the fully reduced and oxidized flavines which results in the parallel formation of a chelated and a neutral radical. The second one is the chelation of the neutral radical. A direct competition between protons and metal ions at the level of the primary intermediate dimer has been evidenced. Such a competition results in kinetically opposite effects. The acids have a negative catalytic influence upon the dismutation rate, but the metal ions accelerate the initial rate of radical formation. This may explain the absence of noticeable radical chelate formation in aqueous solution at pH values lower than 5 (Hemmerich, 1964).

The oxidation of reduced flavines by ferric ions appears as a two-step phenomenon with a rate nearly independent of the coordination state of the ions. This suggests that electron transfer is an outer-sphere mechanism which does not involve a direct coordination of the metal to the flavine for any of the two oxidation reactions. However, the two stages of oxidation appeared kinetically very different and a competition between ferric ions and reduced ferrous ions may occur at the level of the flavosemiquinone. Dismutation of the neutral semiquinone, rapidly formed by one-electron oxidation of the fully reduced flavine, can also be observed independently from metal interaction, providing an indirect pathway for the second step of oxidation through cyclic reactions. The mechanism of overall oxidation of reduced flavine by ferric ions is therefore essentially a kinetic problem strongly dependent upon the conditions.

Monovalent silver in dimethylformamide appeared simply as an oxidative agent with respect to the reduced flavines comparable to the ferric ion. On the other hand, the kinetics of chelation with the flavoquinone in neutral and slightly alkaline conditions indicate that the electronic perturbation observed upon deprotonation of the flavine by NMR experiments (Lauterwein et al., 1975) corresponds to a strictly intramolecular process within the complex which still requires further investigation to confirm whether or not it corresponds to a charge-transfer phenomenon (Hemmerich et al., 1965).

These observations on flavine oxidoreduction in dilute solutions have interrelated stereochemical and functional biochemical implications. The necessity for a direct flavine-flavine contact for dismutation has already been discussed in the previous paper (Favaudon and Lhoste, 1975) and it was shown that flavoproteins could probably better afford

an end-to-end interaction, such as that derived from the investigations in nonaqueous solution, than a plane-to-plane interaction of the quinhydrone dimer type. A direct interaction of metallic ions in the dismutation reactions of flavines in natural systems appears less probable since it should require the formation of a ternary complex. The role of diffusible ions in these reactions has never been evidenced and such a complex could hardly be obtained with an iron atom coordinated in a hemoprotein or even in an iron-sulfur protein, although a flavine coordination model has already been proposed for the latter (Fritchie, 1972). The role of acidic-basic reactions appears more important in the kinetics as well as in the equilibrium of dismutation. Direct flavine-metal interaction does not appear necessary for oxidoreduction reactions involving ferric ions and the stereochemical hindrance present in electron-transferring enzymes may simply modify the rates of the reactions. The complexity of the oxidoreduction reactions and the lack of knowledge of the biochemical structures involving molybdenum ions in metalloflavoproteins (Bray and Swann, 1972) makes it difficult to generalize these conclusions for this metal. The possibility for three valence states did not permit to conclude definitely on the electron-transfer mechanism in model compounds (Kroneck and Spence, 1973).

Functionally, the two-step oxidation of the flavohydroquinone by ferric ions showed that flavine dismutation may occur after formation of the flavosemiquinone, providing an indirect pathway for the second oxidation step. We already proposed (Lauterwein et al., 1972) a scheme for electron transfer in biological chains, providing a link between two-electron carriers such as NAD^+ or NADP and one-electron carriers such as the heme or iron-sulfur. Following this scheme, flavohydroquinone should be the electron donor and the flavine dismutation, which can be controlled by sterical factors and acidic-basic reactions should then provide the pathway for complete oxidation. This has already been observed in enzymatic systems such as the NADPH-sulfite reductase (Siegel et al., 1971, 1974) and the cytochrome b_2 from yeast (Blandin-Capeillère et al., 1975).

Supplementary Material Available

The formal analysis of the general reaction scheme of dismutation in unbuffered dimethylformamide, under alkaline conditions, and in the presence of redox-inactive metal ions, will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number BIO-75-4739.

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