mining step. The same situation may occur at high concentration of Thy. Under these circumstances, the radicals will be used up as soon as they diffuse into the interphase, and the boundary conditions at the inner boundary of the interphase of radius, r_i, will be

$$C(i,t) \rightarrow 0 \tag{11}$$

$$-\frac{d[Thy]}{dt} \propto \frac{dC}{dt} = D\frac{\delta^2 C}{\delta r^2} + \frac{2D}{r_i}\frac{\delta C}{\delta r} \tag{12}$$

That is, the reaction is of zero order with respect to Thy at high solution temperatures and thymine concentrations. The experiments reported by Mead et al.[3] were carried out at high concentration of Thy (10 mM), and the reaction was found to be zero order. On the other hand, the results reported by McKee et al.[1] were carried out at low uracil concentration (0.1 mM) and the reaction order was found to be unity. Therefore, the results of previous studies[1,3] as well as the present results can be explained as specific cases of a general theory proposed here.

At high solution temperatures, the number of moles, ΔC, of radicals that diffuse into the interphase during the collapse time of the bubble will be given by the expression

$$\Delta C \simeq 8\pi D r_i \tau C_i \tag{13}$$

where C_i is the radical concentration at the bubble surface. Due to a sharp decrease in intracavity temperature as the solution temperature is raised, D and C_i will be significantly reduced. Therefore, even though r_i and τ will increase, with an increase in solution temperature, the number of moles of free radicals that diffuse into the interphase will be greatly reduced leading to a lower degree of degradation of Thy as shown in Figure 3.

Case III. At moderate temperatures, wherre the two diffusion rates are comparable, the net rate of degradation of Thy will be the sum of the two rates of diffusion, i.e.,

$$-\frac{d[Thy]}{dt} = D\frac{\delta^2 C}{\delta r^2} + \frac{2D}{r_i}\frac{\delta C}{\delta r} + D_s\frac{\delta^2 S}{\delta r^2} + \frac{2D_s}{r_1}\frac{\delta S}{\delta r} \tag{14}$$

In this case, the reaction kinetics will be complex with reaction order between the two limiting cases, i.e., 0 and 1.

Conclusions

The present studies show that there is a change in kinetics of the reaction of Thy with a change in the temperature of the solution. Also, at spatial average ultrasonic intensities as low as 1.7 W/cm^2 there is a detectable chemical change. At an aeration rate of 50 mL/min and 34 °C the concentration of thymine is reduced to half in 30 min. This means an average 3×10^{-8} mol or 1.8×10^{16} molecules of Thy reacted per second per liter. The chemical rate is large enough to produce a substantial chemical change during prolonged sonication of living systems.

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Reactions of Copper(I) with Micellar Porphyrins and Hemes. Spectroscopic Evidence for Copper(I)–Heme Binuclear Ion Formation

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Abstract: Addition of cuprous ion to sodium dodecyl sulfate solubilized porphyrins and ferrihemes containing olefinic substituent groups gives rise to spectral perturbations diagnostic of Cu(I) π complexation. The hemes undergo slow subsequent demetalation in acidic solution, forming porphyrin dications; in the presence of high concentrations of Cu(II) ion the corresponding cupriporphyrins are also formed. For ferriprotoporphyrin IX, the rate of product formation is *inversely* dependent upon the Cu(II) ion concentration; the data are interpreted in terms of a reaction mechanism, the central feature of which is a dynamic equilibrium between oxidized and reduced hemes, i.e., $Fe^{III}PPIX–Cu^{I} + Cu(I) \rightleftarrows Fe^{II}PPIX–Cu^{I} + Cu(II)$. This interpretation is supported by the observation that hemes containing electron-withdrawing substituents in β-pyrrolic positions are extensively reduced to the ferro state by copper(I), but hemes lacking these groups remain primarily ferric when mixed with the cuprous reagent. The reduced Fe(II)–Cu(I) and mixed-valent Fe(III)–Cu(I) binuclear ions are discussed as potential structural models for the oxygen-binding site in cytochrome oxidase.

Cytochrome oxidase is a complex biological particle which acts as the terminal oxidase in mitochondrial respiratory chains. It contains at least seven different protein subunits and associated phospholipids, as well as two distinct heme and copper metal centers, each of which is generally thought to function as a redox carrier in physiological reactions.[1,2] The molecular organization of cytochrome oxidase is presently poorly understood, precisely because of its great structural and dynamic complexity. One conceptual model which is capable of accounting for a wide range of spectroscopic and magnetic data is based upon the hypothesis that the oxygen reduction site is an antiferromagnetically coupled heme *a*–copper(II) binuclear ion.[3] This proposal has stimulated

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(2) An alternative view has been presented (Seiter, C. H. A.; Angelos, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1806–1808) in which it is proposed that one of the copper sites remains univalent during oxygen reduction, the cytochrome a_3 site acting as a two-electron donor.

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considerable activity directed toward synthesizing simple mixed-metal binuclear ions which might serve as structural analogues of the site. Iron–copper complexes formed from cofacial biporphyrins and related ring structures,[4] "picket fence" chelates,[5] and nitrogen heterocyclic bridging ligands[6] have been characterized. In none of the complexes yet described has there been found antiferromagnetic interactions sufficiently large to mimic the postulated heme–copper site in the oxidase.

A common structural feature of these complexes is that copper(II) is axially aligned with respect to the iron macrocycle. We report here the formation of unique heme–copper binuclear ions in which copper(I) is bound to olefin substituents on the periphery of the porphyrin ring. As described in the text, such compounds are potential models for reduced Fe(II)–Cu(I) or intermediate Fe(III)–Cu(I) redox states of the oxygen reductase site.

Experimental Section

Reagents. Heme *a* was isolated from bovine beef hearts according to the methods of Caughey et al.[7] The absorption spectrum of the stannous chloride reduced heme in pyridine closely resembled published spectra,[7] on the basis of the atomic absorption analysis for iron: λ_{max} (ϵ, mM^{-1} cm^{-1}) 583 (30.7), 532 (7.0), 428 (142); lit. 583 (32.7–34.2). Porphyrin *a* was prepared in diethyl ether from heme *a* by using the ferrous sulfate method.[8] A typical oxorhodo-type spectrum was obtained that was nearly identical with its published spectrum.[9] Cupric protoporphyrin IX was prepared by adding cupric acetate in methanol to refluxing chloroform solutions containing the porphyrin.[10] Hemin and protoporphyrin IX were commercial samples; other porphyrins and metalloporphyrins used were the gift of Peter Hambright (Howard University).

Aqueous acidic solutions of detergent-solubilized hemes and porphyrins were generally prepared by dissolving the compound in an appropriate solvent (methanol, chloroform, diethyl ether), adding sodium dodecyl sulfate, evaporating to dryness under vacuum or by using a stream of nitrogen gas, and then adding the aqueous medium. Because Cu^{II}PPIX[11] isolated as a solid dissolved with difficulty in organic solvents, reagent solutions were prepared directly from reaction product solutions obtained in its synthesis. The product solutions were washed with water to remove excess cupric acetate and then added to SDS dissolved in methanol which also contained some benzene to ensure complete removal of residual water in the subsequent solvent evaporation step. In other instances where the compounds were not completely soluble in organic solvents, insoluble matter was removed by filtration. In all cases, however, the final aqueous dispersions were optically clear. Reagent aqueous micellar solutions of hemin were prepared by diluting aliquots of standardized solutions of the hemin μ-oxo dimer, the concentrations of which had been determined spectrophotometrically by using $\epsilon_{384} = 10.5$ mM^{-1} cm^{-1}. In other instances, reagent concentrations were determined by weight. Given the inherent inaccuracies associated with this method, as well as the presence of small amounts of insoluble matter in several samples, the concentrations were determined to only about ±10%. This degree of error was deemed acceptable because the information sought from these compounds was qualitative in character. The visible absorption spectra of the various hemes and porphyrins in 0.1 M trifluoroacetic acid, 2% SDS, are given in Table I; where accurately known, millimolar extinction coefficients are also recorded. Our spectral parameters compare well with values reported in the literature (Table I). The solutions were generally fairly stable with only a few percent loss in Soret intensity occurring after 1 week. Both Cu^{II}PPIX and the porphyrin dication appeared less stable, however, losing appreciable intensity within 24 h. An additional instability was found in acidic solutions containing just transition-metal ions (Cu(II), Zn(II), Cr(III)) and SDS. Over a period of several days, absorption bands slowly developed in the ultraviolet region (280, 320 nm). The products of these reactions were reactive toward porphyrins, giving rise to bleaching or spectral shifts in the Soret region detectable by difference spectroscopy. These degradative side reactions, by preventing accurate base-line determinations, caused some difficulty in kinetic analyses of the slowest rates associated with copper(I)–heme reactions described below. To minimize complications associated with heme instabilities, we always prepared reactant solutions immediately before use.

Table I. Spectral Maxima for Hemes and Porphyrins in Aqueous Acidic SDS[a]

	Soret,[b] nm		visible,[b] nm	
	A. Porphyrins[c]			
H_4PPIX[d]	412 (267)	556 (15.4)	600 (6.63)	
H_4MesoIX[e]	403 (471)	547 (18.5)	591 (7.93)	
H_4DeutIX[f]	402 (420)	547 (17)	589 (6.0)	
H_4DaDeutIX[g]	418 (37)*	534 (1.6)*	565 (2.5)*	612 (1.0)*
H_4Iso-DME	416 (31)*	562 (1.9)*	611 (1.0)*	
porphyrin *a*	413 (26)*	562.5 (1.7)*	588 (1.6)*	613 (1.0)*
	B. Hemes[c]			
Fe^{III}PPIX[h]	393 (92.5)	504 (9.08)	632 (2.64)	
Cu^{II}PPIX	403 (5.0)*	528 (0.82)*	567 (1.0)*	
Fe^{III}DeutIX-DME	382 (36)*	495 (2.8)*	621 (1.0)*	
Fe^{III}DaDeutIX	410 (19)*	510 (1.8)*	608 (1.0)*	
heme *a*	403 (9.4)*	550 (1.0)*		

[a] In 0.1 M HTFA, 2% SDS at 23 °C. [b] λ_{max} (ϵ, mM^{-1} cm^{-1}) or λ_{max} (relative intensity)*. [c] Abbreviations given in ref 11. [d] Lit.[20] H_2PPIX-DME, 412 (282), 557 (15.1), 602 (6.84). [e] Lit.[20] H_4MesoIX-DME, 404 (406), 549 (15.2), 592 (6.56). [f] Lit.[20] H_4DeutIX-DME, 402 (394), 549 (14.7), 589 (15.7). [g] Lit.[20] H_3DaDeutIX-DME, 414.5 (108), 538 (8.4), 570 (10.8), 616 (4.4); H_4DaDeutIX-DME, 421.5 (192), 565 (11.4), 610 (4.6). [h] Lit.[17] 393 (90).

Reagent solutions of cupric trifluoroacetate were prepared from cupric perchlorate and potassium trifluoroacetate. After filtration to remove precipitated potassium perchlorate, the solutions were standardized either as the cupric thiocyanate complex[12] or by iodometric titration. Chromic trifluoroacetate was prepared from potassium dichromate by reduction with hydrogen peroxide in acidic solution, sufficient perchloric acid being added to just precipitate the potassium ion. The chromic solutions were analyzed by oxidation with alkaline peroxide to chromate.[13] Chromous trifluoroacetate was prepared by reduction of chromic ion over amalgamated zinc in an argon atmosphere. Metastable solutions of cuprous trifluoroacetate were prepared by anaerobic reaction of cupric ion by chromous ion.[14] To prevent disproportionation, cupric ion was maintained in excess; cuprous reagent solutions also contained Zn(II) and Cr(III) ions formed in the redox reactions generating copper(I). Sodium dodecyl sulfate was recrystallized from ethanol to remove UV-absorbing impurities. Other chemicals were reagent quality and were used without further purification. Water was purified by a reverse-osmosis ion-exchange system.

Methods. Optical spectra were recorded on a Cary Model 16 spectrophotometer equipped with a scan drive and recorder interface; the temperature was maintained by circulating water from a thermostated bath through the sample chamber. Oxygen was purged from reactant solutions with argon; reactants were mixed by using syringe-transfer techniques. Introduction of some adventitious oxygen during these manipulations was apparent at the lowest reagent concentrations used, $[Cu(I)] < 10^{-4}$ M; to minimize error the optical cell was fitted with a double-septum antechamber which was swept with argon. Any oxygen introduced in piercing the outer septum was removed before reagents were mixed in the inner chamber. Difference spectra were generally recorded by using septum-stoppered tandem 1.0-cm optical cells. In early attempts to obtain reaction stoichiometries by using the varying mole ratio method, the effects of oxygen contamination were clearly seen at very low cuprous ion concentrations. These studies were therefore made at higher reactant concentrations by using matched 0.01-cm cells. The

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(11) Abbreviations used: PPIX, protoporphyrin IX; MesoIX, mesoporphyrin IX; DeutIX, deuteroporphyrin IX; DaDeutIX, diacetyldeuteroporphyrin IX; DeutIX-DME, deuteroporphyrin IX dimethyl ester; Iso-DME, 2-vinyl-4-formyldeuteroporphyrin IX dimethyl ester, (*Isospirographis*); HTFA, trifluoroacetic acid; SDS, sodium dodecyl sulfate.
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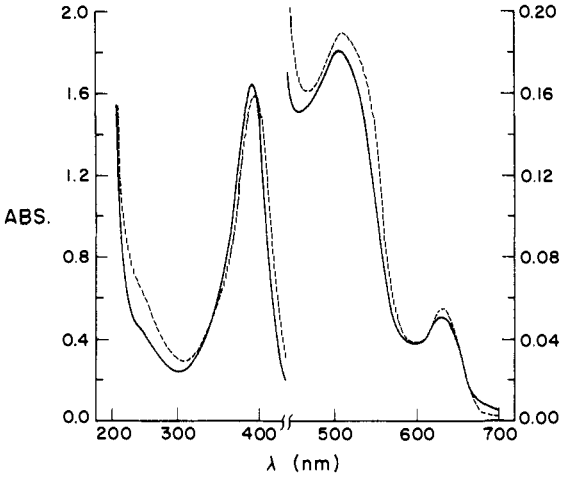

Figure 1. Absorption spectrum of hemin in SDS at pH 4.0. Conditions: solid line, [Fe^{III}PPIX] = 0.018 mM, 2% SDS, 0.1 M NaTFA, 23 °C; dashed line, [Cu(I)] = 0.22 mM, [Cu(II)] = 0.38 mM, other conditions same.

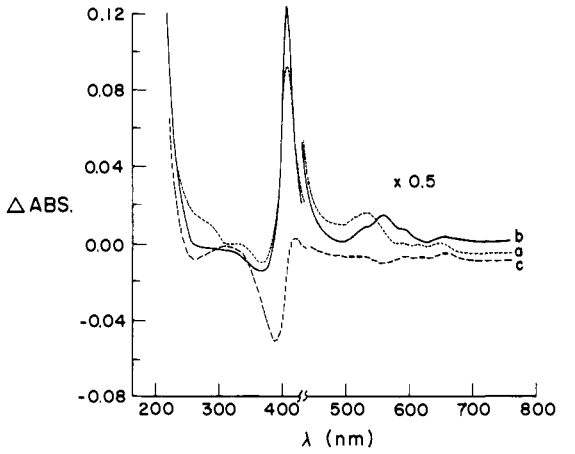

Figure 2. Optical difference spectrum of Fe^{III}PPIX and Cu(I). Conditions: [Fe^{III}PPIX] = 0.6 mM, [Cu(I)] = 1.6 mM, [Cu(II)] = 13 mM, in 0.1 M HTFA, 0.8% SDS, 23 °C; 0.01-cm path length; sample cell, Fe^{III}PPIX + Cu(I); reference, cell, Fe^{III}PPIX. Trace a taken immediately upon mixing reactants, trace b, after 18 h; and trace c, solutions oxygenated after mixing. Base lines are displaced slightly to provide clarity. Note scale change in visible region.

spectral curves obtained were corrected for ultraviolet absorption by cuprous ion by subtracting calculated values based upon previously determined extinction coefficents.[15] Kinetic runs were made under pseudo-first-order conditions with cuprous ion held in excess. For the slowest reactions studied, involving transmetalation or demetalation of iron porphyrins, first-order kinetic plots often showed upward curvature after several half-lives, attributable to the continued slow degradation of reaction products mentioned above. Rate constants reported are based upon initial slopes of the first-order plots.

Results

Reactions between Fe^{III}PPIX and Cu(I). Hemin is solubilized by SDS in aqueous acidic media, forming micelle-bound monomeric diaquoferriprotoporphyrin IX.[16] Upon addition of cuprous ion, the Soret band undergoes a bathochromic shift of about 3 nm, accompanied by loss of intensity and peak broadening. A pronounced shoulder appears at about 540 nm on the β band at 504 nm and a lesser shoulder at 650 nm on the α band at 632 nm (Figure 1). These effects are clearly seen in the Fe^{III}PPIX–Cu^{I} difference spectrum (Figure 2); the spectral changes accompanying intermediate formation are complete within the time of mixing of reactants. The spectrum then slowly changes over a period of several hours (Figure 3), giving a final product that is

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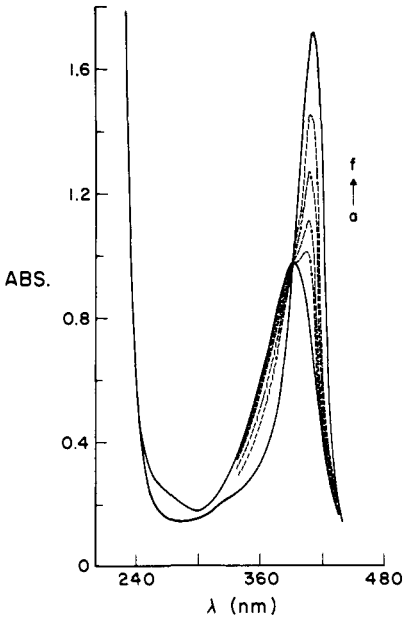

Figure 3. Ultraviolet absorption spectra of Fe^{III}PPIX–Cu(I) solutions. Conditions: [Fe^{III}PPIX] = 0.012 mM, [Cu(I)] = 0.22 mM, [Cu(II)] = 0.38 mM, in 0.1 M HTFA, 0.2% SDS, 23 °C; 1.0-cm optical pathlength; trace a, 30 s; trace b, 8 min; trace c, 15 min; trace d, 30 min; trace e. 1.0 h; trace f, 22 h after mixing.

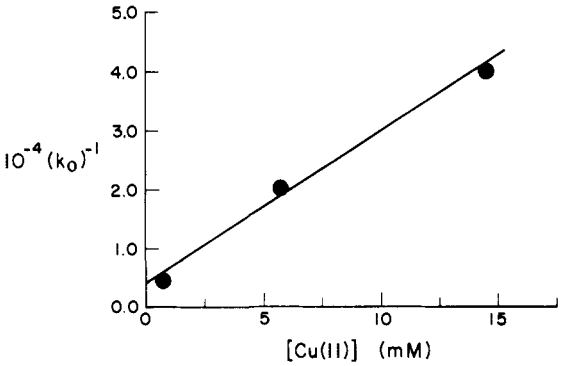

Figure 4. Cu(II) ion dependence of intermediate decomposition. [Fe^{III}PPIX] = 0.012 mM and other reaction conditions are given in the text. Rate constant units are s^{-1}; data points are averages of six individual runs; average deviations from the mean values are ±20%. The solid line is the linear least-squares fit to the data.

dependent upon the Cu(II) ion concentration in the medium. With [Cu(II)] < 10^{-3} M, the final absorption spectrum corresponds to that of the SDS-solubilized porphyrin dication, H_4PPIX; quantitative calculations based upon measured extinction coefficients (Table I) indicate greater than 90% conversion to the dication. With [Cu(II)] > 10^{-2} M, the final spectrum was that of Cu^{II}PPIX. Intensities and spectral shapes of the bands at intermediate Cu(II) concentration levels are consistent with the simultaneous presence of both product ions. The ferriheme is unreactive toward Cu(II), Cr(III), and Zn(II) ions under the experimental conditions. In 0.1 M HTFA, the net reaction stoichiometries are therefore given by eq 1 and 2.

$$Fe^{III}PPIX + Cu(I) \rightarrow Fe(II) + Cu(II) + H_4PPIX \quad (1)$$

$$Fe^{III}PPIX + Cu(I) \rightarrow Fe(II) + Cu^{II}PPIX \quad (2)$$

The rate of conversion from intermediate to final product(s) was followed by measuring spectral changes occurring in the Soret region (Figure 3). In 0.1 M HTFA, 0.2–2.0% SDS, and 0.22 mM Cu(I), the reaction rate is *inversely* dependent upon Cu(II) ion concentration; rate data were fitted to the law $(k_0)^{-1} = a + b$ [Cu(II)] with a correlation coefficient of $r = 0.997$ (Figure 4). Individual first-order rate constants (k_0) are independent of SDS concentration over the range investigated. At pH 4, in 0.1 M

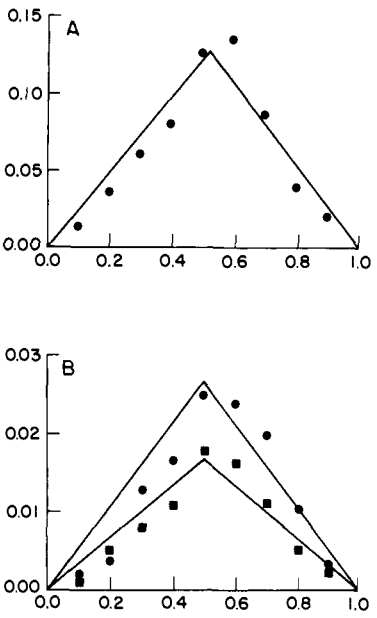

Figure 5. Job diagrams for $Fe^{III}PPIX$–Cu^{I} solutions: ordinate, deviation from Beer's law in absorbancy units; abscissa, solute mole fraction Cu(I); conditions, ([$Fe^{III}PPIX$] + [Cu(I)]) = 1.7 mM, in 0.1 M HTFA, 23 °C; other conditions given in the text; a, 412 nm; b; circles, 260 nm; squares, 540 nm. Data points are based on one to three individual determinations.

NaTFA, intermediate conversion did not occur, but rather the ion underwent slow chromophoric degradation. The reactant hemin spectrum is clearly that of the diaquo ion (Figure 1); hydrolysis constants reported for formation of the monohydroxy ion are pK_a = 5.5 at 25 °C.[17,18]

Oxygenation of solutions containing the intermediate causes immediate reversion to the ferriheme spectrum, although with a few percent loss in intensity. This chromophoric bleaching is most evident in difference spectra taken in the Soret region (Figure 2) and was always observed upon oxygenation of various solutions containing hemes or porphyrins and copper(I).

The stoichiometry for intermediate formation was examined by measuring difference absorption maxima at varying molar ratios of reactants;[15,19] the results (Figure 5) indicate predominantly 1:1 interaction. In addition to the spectral changes occurring in the Soret (412-nm) and visible (504-nm) regions, there appears a prominent broad absorption in the ultraviolet region below 300 nm which is not accountable by absorption from the Cu(I) aquo ion and which disappears upon oxygenation (Figure 2). Job analysis of this band (Figure 3) indicates that it also appears maximally at a 1:1 $Fe^{III}PPIX$–Cu^{I} stoichiometry and must therefore be associated with the intermediate. The Cu(II)/Cu(I) ratio was maintained constant at 8.2 for these measurements; the reaction half-time for subsequent demetalation–transmetalation reactions was calculated to be 105 min, based upon our proposed mechanism (below). The SDS concentration varied from 0.2 to 2.0% with increasing Cu(I) concentrations; in the absence of Cu(I), Beer's law was obeyed by the ferriheme absorption bands over this range.

Addition of excess sodium dithionite under anaerobic conditions to the ferriheme in 0.1 M HTFA, 2% SDS, caused immediate spectral changes which were quite similar to those observed upon Cu(I) addition, i.e., red shift, broadening, and 5% loss in maximal Soret intensity, with appearance of a low-energy shoulder on the β band. Within a few minutes after mixing, spectral features corresponding to formation of the H_4PPIX cation were evident. The latter reactions did not go to completion, however, presumably because dithionite rapidly decomposes in this medium. The porphyrin dication does not react with dithionite.

Reactions between Porphyrins and Cu(I). On the basis of the spectral comparisons given in Table I, most of the porphyrins exist in 0.1 M HTFA–SDS solutions as the dications;[20] consistent with the base weakening properties of electron-withdrawing peripheral substituents,[21] formation of both mono- and dications is indicated from the spectrum of 2,4-diacetyldeuteroporphyrin (and possibly porphyrin *a*).

Addition of Cu(I) to porphyrin cations possessing vinyl β-substituent groups (PPIX, Iso-DME, porphyrin *a*) gives rise to immediate loss of intensity and slight red-shifting of the Soret bands (Figure 6). The effect was studied most carefully with H_4PPIX, which gave no subsequent reaction. In addition to changes in the Soret region, the α and β visible bands were red-shifted and an absorption band appeared in the ultraviolet region below 300 nm which was not accountable by normal Cu(I) absorptions. Oxygenation caused reversion to the original porphyrin spectrum with some chromophoric bleaching. No spectral change was observed when Cu(II) alone (0.7 mM) was mixed with H_4PPIX in either the presence or absence of oxygen.

For the porphyrins containing β-carbonyl substituents (DaDeutIX, Iso-DME, porphyrin *a*), Cu(I) addition caused a moderately rapid reaction, not reversed by oxygenation, which was characterized by loss of Soret intensity and the appearance of one (porphyrin *a*, 600 nm; Iso-DME, 592 nm) or two (DaDeutIX, 542, 582 nm) relatively sharp bands in the visible region. The reaction products have not yet been identified, although the spectral changes suggest chlorin formation.[22] Reaction rates were first order, with k(DaDeut) = 3.5×10^{-4} s^{-1} and k(Iso–DME) = 2.1×10^{-4} s^{-1} in 0.1 M HTFA, 2% SDS, [Cu(I)] = 0.22 mM and [Cu(II)] = 0.38 mM at 23 °C. For the 2,4-diacetyldeuteroporphyrin cations, no faster spectral changes were observed upon mixing with Cu(I); for the other porphyrins these slower changes followed the rapid reactions associated with the presence of vinyl substituents. No reaction was observed if other metal ions (Cu(II), Zn(II), Cr(III)) were added in the absence of Cu(I).

Apart from the effects of the very slow degradative reactions common to all of the porphyrin and heme solutions, the mesoporphyrin and deuteroporphyrin dications exhibited no spectral changes when mixed with Cu(I).

Reactions between Other Hemes and Cu(I). The very similar spectral curves of $Fe^{III}PPIX$, Fe^{III}DeutIX–DME, and Fe^{III}DaDeutIX ions (Table I) suggest that the latter hemes are also monomeric in acidic SDS solutions. Ferriheme *a* is reported to be monomeric in neutral solutions of ionic detergents, but dimeric in nonionic detergents, based upon sequential spectral changes accompanying micellarization and quantitative ligand-binding studies.[23] We have found that acid-base titration of hemin *a* in 2% SDS gives two distinct species, at pH 0–2.5 and pH 4.5–11; the lack of isosbestic behavior in the transition region (pH 3) indicates the simultaneous presence of several forms. The absorption spectrum of the alkaline species is qualitatively identical with that previously reported;[23] the absorption spectrum of the acidic species (Table I) appears unique. The state of aggregation of hemin *a* should not be taken as firmly established, however, since the absorption spectrum of its alkaline form in SDS is also nearly identical with the published spectrum of μ-oxo-bishemin *a* (monoethyl ether)–DME in benzene.[7]

Ferrideuteroporphyrin IX–DME gives no immediate spectral change when mixed with copper(I). A moderately rapid reaction occurs which is first order in the ferriheme, with $k = 2.4 \times 10^{-4}$ s^{-1} in 0.1 M HTFA, 2% SDS, [Cu(I)] = 0.22 mM, and [Cu(II)] = 0.38 mM, at 23 °C. The reaction product is identified as H_4DeutIX–DME from the similarity of its absorption spectrum

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(λ_{max} (relative intensity) 402 (58), 545 (2.5), 588 (1.0)) to those of closely related dications (Table I, H_4DeutIX, H_4MesoIX).

Ferri-2,4-diacetyldeuteroporphyrin IX reacts with Cu(I) in 0.1 M HTFA, 2% SDS, within the time required for mixing and sampling to give an immediate product which is characterized by bathochromic shifts of Soret (412 → 415 nm) and the major visible (520 → 545 nm) bands. Slower conversion occurs to yield a more strongly absorbing product with Soret maximum at 420 nm and visible bands at 543 and 582 nm. Because the spectrum is qualitatively identical with that obtained from the reaction of DaDeutIX cations with Cu(I), it appears that the reactions have proceeded to a common product. Spectra taken at intermediate stages of reaction are quite complex, consistent with concurrent demetalation and the "carbonyl" reaction.

Hemin *a* reacts with Cu(I) in 0.1 M HTFA, 2% SDS, within the time of mixing to give an immediate product characterized by a somewhat broadened and red-shifted Soret band (405 → 410 nm) and the appearance of a new, fairly broad band at 600 nm. Oxygenation causes loss of the 600-nm band and bleaching in the Soret region. The intermediate spectrum slowly changes to one qualitatively identical with the product obtained from reaction of porphyrin *a* with Cu(I), i.e., intensification and further shift of the Soret band to 412 nm, as well as intensification and sharpening of the 600-nm peak with loss of spectral intensity over the rest of the visible region. Oxygenation causes no change in the final product spectrum. Dithionite reduction of hemin *a* causes appearance of a new visible band at 600 nm and loss of intensity and broadening of the Soret band. Because the Soret maximum undergoes no spectral shift, it appears that the intermediate formed by dithionite reduction is distinct from the one produced with Cu(I).

Discussion

Copper(I) Ligation. The immediate spectral changes accompanying Cu(I) addition to protoporphyrin IX, *isospirographis* dimethyl ester, and porphyrin *a* cations are indicative of associative interaction. It is inconceivable that Cu(I) coordinates to the pyrrole nitrogen atoms at the ring center under the experimental conditions, where iron porphyrins are rapidly demetalated and cupriporphyrins will not form. Plausible coordination sites include the β-substituent vinyl groups and, for porphyrin *a*, the olefin bonds of the polyisoprenyl chain. Copper(I) coordination to terminal olefin bonds is strong,[15,24] with association constants in polar solvents in the range of $K = 10^4$ M^{-1}; we have shown that Cu(I) coordinates equally well to polyisoprenylpyridine ligands in acidic aqueous ethanol mixtures, forming copper(I)–olefin π complexes with 1:1 stoichiometry.[25] The absence of detectable spectral perturbations when porphyrin cations lacking olefinic substituents are mixed with Cu(I) provides circumstantial evidence that these are the coordination sites. More direct evidence for π bonding is the appearance of broad ultraviolet absorption bands below 300 nm when Cu(I) and H_4PPIX are mixed. Copper(I)–olefin ligation is characterized by Cu(d) → L(π*) charge-transfer excitation in the near-ultraviolet region.[15,25] These transitions are nearly fully allowed, but because of their breadth, molar extinction coefficients for band maxima are found in the 10^{3-4} M^{-1} cm^{-1} range.[15]

Nature of the Redox Intermediates. Possible alternative formulations for intermediate species formed when ferrihemes react with Cu(I) include the reduced ferroporphyrins or ferri- or ferroporphyrin–Cu(I) binuclear ions. In general, Cu(I) π complexation at olefin bonds is little affected by the presence of a second metal ion coordinated to nucleophilic sites on the ligand.[15,27] Strong binding of Cu(I) to the peripheral vinyl groups is evident with porphyrin dications (Figure 6); under the comparable experimental conditions we would anticipate extensive coordination to the corresponding ferriporphyrins as well.

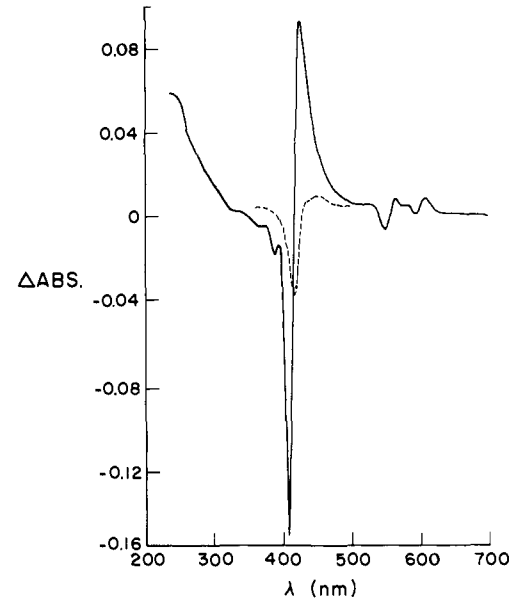

Figure 6. Difference absorption spectra for Cu(I)–porphyrin dication complexes: solid line, [H_4PPIX] = 4.8×10^{-6} M; dashed line, [H_4Iso-DME] = 3.8×10^{-6} M; other conditions, [Cu(I)] = 0.22 mM, [Cu(II)] = 0.38 mM, in 0.1 M HTFA, 2% SDS, 23 °C; 1.0-cm optical path length.

A crucial piece of evidence in identifying the intermediates is the inverse Cu(II) dependence found for formation of the final products in the reactions between ferriprotoporphyrin IX and cuprous ion (Figure 4). Based upon current understanding of demetalation and transmetalation reactions,[21] it is not possible to write an acceptable mechanism for these steps which is inhibitory in Cu(II); i.e., in all cases reported, the reaction is either directly dependent upon or independent of metal ion concentrations. A simple explanation for the unusual Cu(II) dependence is that a rapidly established equilibrium exists between the ferri and ferro redox states which is controlled, in the presence of excess copper ions, by the Cu(II)/Cu(I) ratio, i.e.

$$Fe^{III}PPIX + Cu(I) \rightleftarrows Fe^{II}PPIX + Cu(II) \quad K_3 \qquad (3)$$

$$Fe^{II}PPIX + H^+ \rightarrow Fe(II) + H_4PPIX \quad k_4 \qquad (4)$$

Because demetalation occurs only from the ferrous porphyrin, the overall rate of dication formation is inversely dependent upon the Cu(II) ion concentration. The rate law derived from eq 3 and 4 is $d[H_4PPIX]/dt = k_4[FePPIX]_T/(1 + [Cu(II)]/K_3[Cu(I)])$, where $[FePPIX]_T = ([Fe^{III}PPIX] + [Fe^{II}PPIX])$ and $K_3 = [Fe^{II}PPIX][Cu(II)]/[Fe^{III}PPIX][Cu(I)]$. In buffered media, when [Cu(II)], [Cu(I)] > $[FePPIX]_T$, the reaction is pseudo first order with $(k_0)^{-1} = (k_4)^{-1} + (K_3k_4)^{-1}[Cu(II)]/[Cu(I)]$. From the data in Figure 3, we calculate $K_3 = 8.4$ and $k_4 = 2 \times 10^{-4}$ s^{-1} in 0.1 M HTFA, 2% SDS, at 23 °C.

This mechanism should not be taken as established in quantitative detail but is only presented to illustrate its central feature, namely, the dynamic equilibrium between the ferro- and ferriheme ions in the reaction intermediates. Several aspects have not been addressed, including formation of $Cu^{II}PPIX$ at high Cu(II) concentrations, the extent of peripheral Cu(I) binding to the hemes and the observation of apparent 1:1 reaction stoichiometry for intermediate formation under conditions where both iron reduction and Cu(I)–heme association are thought to occur. Regarding the latter points, it is evident that formation of mixed-valent Fe^{III}P-PIX–Cu^I ions is extensive from measurements made at high Cu(II)/Cu(I) ratios (maximally 67 in these studies). Here, although less than 10% of the heme is reduced, spectral perturbation is comparable to that seen at much lower ratios. Under the circumstances, the major changes can have arisen only from associative interaction similar to observed for the Cu^I–H_4PPIX

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micellar solutions. Calculations based upon K_3 show that under the experimental conditions of the Job diagram measurements (Figure 5), Fe^{II}PPIX would account for 45% of the total iron at the highest hemin/Cu(I) ratio used; at the midpoint, i.e., 0.5 solute mole fraction, 27% of the iron porphyrin would be ferrous. Because iron reduction and Cu(I) π complexation produce roughly comparable perturbation of the heme spectrum, the difference absorption maximum is expected to be at about 0.45 copper(I) solute mole fraction. The binding data are therefore consistent within experimental error with the kinetic scheme presented. Finally, the magnitude of the ultraviolet absorption seen below 300 nm can be accounted for by copper(I)–olefin π-bonding by assuming typical physical parameters[15,25-27] ($K \simeq 10^4$ M^{-1}, $\epsilon = 3 \times 10^3$ M^{-1} cm^{-1}); π complexation appears to be nearly complete by this criterion. Because the dominant spectral changes are due to Cu(I) ligation of the iron porphyrins, the binding stoichiometries (Figure 5) indicate binuclear ion formation. The porphyrin species listed in eq 3 and 4 are therefore better represented as their Cu(I) complexes, Fe^{III}PPIX–Cu^{I} etc.

This interpretation provides a self-consistent view of the reactions of other ferrihemes with Cu(I). The reduction potential for the micellar diaquoferriheme complex is calculated from K_3 and $E_r^0 = 153$ mV[28] for $Cu^{2+/+}$ to be $E_r \simeq 0.21$ V for the experimental conditions. Reduction potentials for central iron atoms in ferriporphyrins are dependent upon the electronic properties of ring substituent groups, increasing proportionately with the electron-withdrawing character of the group;[29] in particular, introduction of formyl substituents in β-pyrrolic positions is reported to cause anodic shifts of about 50 mV/formyl group.[4] The equilibrium position given by eq 3 for Fe^{III}DeutIX–DME, which does not contain strong electron-withdrawing side-chain groups, lies to the left. Because the porphyrin lacks Cu(I) coordinating groups as well, there is no detectable spectral change accompanying mixing. Nonetheless, some ferroporphyrin is formed, which undergoes relatively rapid demetalation, thus accounting for the Cu(I)-promoted reaction. In contrast, iron reduction is favored in the case of Fe^{III}DaDeutIX, giving rise to spectral changes attributable to ferroporphyrin formation. Heme *a* is probably also more extensively reduced than FePPIX in the intermediate, but in this case Cu(I) coordination also seems to be implicated from the differing intermediate spectra arising from dithionite reduction and reaction with Cu(I). Quantitative assessment of these reaction schemes and structural characterization of the binuclear ions using other methods is in progress.

Oxygenation of solutions containing the binuclear ions causes oxidation of both metal centers, as indicated by reversion to the original ferriheme spectra. The small spectral losses (Figure 2) may be due to coupled oxidation,[30] giving rise to biliverdin formation. It is surprising that oxidation of the Cu^{I}–H_4PPIX ion also occurs with some spectral bleaching since uncomplexed porphyrins are reported to show little tendency toward coupled oxidation.[30] The reaction can possibly be accounted for by the presence of minor amounts of Cu(II)PPIX.[31]

Suitability as Models for Cytochrome Oxidase. The binuclear ions contain Cu(I) peripherally bound to either ferro- or ferriprotoporphyrin IX, depending upon the Cu(II) concentration levels (eq 3); as such, they are potential models for iron–copper sites in cytochrome oxidase in its reduced or partially oxidized states.[3] The suggestion that the heme *a* vinyl substituent might form part of the Cu(I) coordination site in the oxidase is attractive from considerations of both binding strengths and the electron-transfer properties of copper(I)–olefin complexes. We have demonstrated unequivocally that electron transfer occurs through the bridging ligand in the structurally similar $(NH_3)_5Ru^{III}$–4-vinylpyridine–Cu^{I} binuclear ion.[27] By microscopic reversibility, oxidation of the corresponding Ru(II) ion must occur via copper(II)–olefin interaction, even though stable copper(II)–olefin complexes are not formed. Pendant vinyl groups should also provide facile pathways for electron transfer between copper ions and porphyrin centers. Because the vinyl groups are attached to β-pyrrolic sites, this "peripheral" pathway is distinct from the ones usually discussed involving ring methine positions.[32]

Questions regarding the extent of antiferromagnetic coupling between metal centers that might be mediated by ring π interactions and the character of oxygen binding to the binuclear ions have not yet been addressed. The metal centers in the Fe^{II}PPIX–Cu^{I} binuclear ions are separated by about 6 Å, precluding formation of oxygen-bridged intermediates in these complexes. Copper oxidation undoubtedly occurs with loss of olefin-binding sites, however; changes in copper coordination might thereby be linked to redox-dependent conformational changes in the oxidase, for which considerable evidence has accumulated.[1,3]

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