Intramolecular Photoredox Reactions in Iron(III) Cytochrome c and its Azide Derivative*

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Abstract

The irradiation of deaerated solutions of horse heart cytochrome c causes the reduction of Fe(III) to Fe(II). The dependence of the photoreaction quantum yield on pH shows that the photoreactive species is a form of cytochrome c which contains methionine-80 and histidine-18 as heme ligands. The primary photochemical event consists of an electron transfer from the sulphur of methionine-80 to iron. The re-oxidation of the photochemically obtained Fe(II) protein gives a Fe(III) cytochrome which exhibits a typical low-spin absorption spectrum, lacking the 695-nm band and indicating that a strong field ligand, other than methionine-80, coordinates to the sixth binding site of the heme iron. Spectrophotometric titration of the photochemically modified Fe(III) cytochrome shows that histidine-18 remains bound in the fifth position.

The substitution of methionine-80 with the more oxidizable azide ligand increases the efficiency of the intramolecular electron transfer. Azide radicals, detected by spin-trapping ESR technique, are formed in the primary act. Visible–UV spectral data indicate that histidine-18 and methionine-80 occupy the fifth and sixth position, respectively, in the photoreaction product. All the results obtained correlate well with those previously obtained in investigations concerning the photoredox behavior of iron porphyrin complexes.

Introduction

The role that cytochrome c, a heme protein from the mitochondrial respiratory chain [2], plays in biological electron transport has been the subject of extensive investigations [3]. Nevertheless, the detailed mechanism by which cytochrome c assists electron transfer is still a matter of discussion.

Experimental evidence indicates that the site which determines the physiological function of cytochrome c is the heme prosthetic group where the iron center can alternate between diamagnetic Fe(II) and low spin Fe(III). It is likely that cytochrome c transfers electrons via an outer sphere mechanism, [4] since its function is accomplished when the prosthetic group, that is an exacoordinated low-spin Fe(III) porphyrin complex, is completely buried in the protein crevice and ligation is sterically hindered.

A large part of the investigation concerning cytochrome c is aimed at clarifying its electron carrier properties through the study of the kinetics and the mechanism of the redox reactions with biological as well as chemical substrates [3b]. Likewise, the study of the chemical modifications of the proteic structure, in particular in proximity of the heme group, proved to be a powerful tool for obtaining information on the relationship between the structure and the biological activity. In this line, the use of light appeared to be a clean and polished method for inducing chemical and/or structural changes in hemoproteins and a number of papers have been published on this subject [5]. On the other hand, in most of the reported investigations little attention was devoted to the photoredox reactions involving the iron center [6].

Some studies on the photoredox reactions of Fe(III) porphyrin complexes, which are known to be very suitable models for hemoproteins, have been published in recent years [7]. The most interesting result obtained in these studies is that, in every case, an intramolecular electron transfer from the axial ligand to iron was the primary photochemical event.

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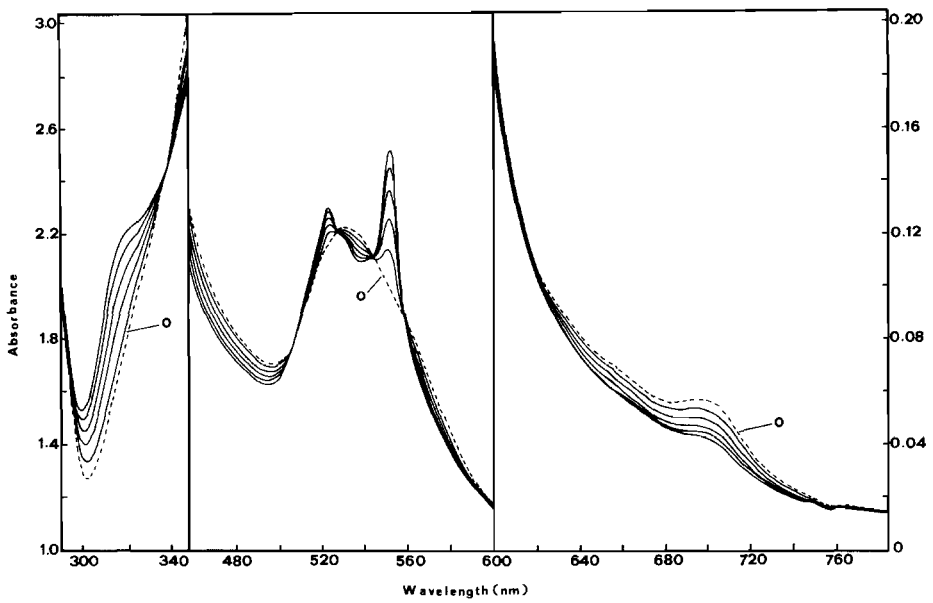


Fig. 1. Spectral variations observed upon irradiation with $\lambda > 300$ nm light of a 1×10^{-4} mol dm^{-3} deaerated solution of Fe(III)-cytochrome c. pH, *ca.* 7; irradiation times, 5 minutes; o, initial spectrum. Central panel, read ordinate on right X10.

On the basis of the results obtained by studying the above models, we report here the results obtained in an investigation on the intramolecular photoredox reactions of cytochrome c and its azide derivative.

Experimental

Materials

Horse-heart cytochrome c, Type VI, was purchased from Sigma Chem. Co. All other chemicals were commercial grade products.

Apparatus and Methods

UV–Vis absorption spectra were recorded with a Jasco Uvidec 650 spectrophotometer. X-band electron spin resonance (ESR) spectra were recorded with a Bruker 220 SE spectrometer which was calibrated using α,α'-diphenyl-picryl-hydrazyl (DPPH). pH measurements were carried out with a Beckman Select Ion 5000 pH-meter. Irradiations were performed with a 250 W Xenon lamp. The required irradiation wavelength ranges were obtained using cut-off glass filters. When necessary, monochromatic light was obtained with an f/3.4 Grating monochromator (Applied Photophysics). Spectrophotometric quartz cells were used as reaction vessels. Irradiations were also carried out in the ESR cavity. In this case, the samples were contained in 0.5-mm flat cells. The protein was dissolved in twice distilled water and the solution obtained was used within 24 hours. Deaerated samples were obtained by bubbling ultrapure argon into the solution through a glass capillary for one hour. The pH was adjusted to the desired value by addition of small amounts of 1/1 HCl or NaOH solutions. The protein solutions were irradiated at room temperature (22 °C) and the photoreaction monitored by UV–Vis spectroscopy. In the chromatographic separations the irradiated solutions were loaded onto a 13 X 2.5 cm CM-52 (Watman) column pre-equilibrated with 5×10^{-2} mol dm^{-3} K-phosphate buffer (pH 7.5) and eluted with a 9×10^{-3} mol dm^{-3} K-phosphate buffer at a flow rate of 50 ml/h at room temperature. 5-ml fractions were collected and spectrophotometrically analyzed at 410 nm or 525 nm depending on the concentration of the sample. The most concentrated fractions were collected and dialysed, first against 100 vols. of 0.5 mol dm^{-3} KCl (pH 7.5) in twice distilled water to remove phosphate anions, and afterwards exhaustively against twice distilled water. Samples treated in this way were employed directly or, when necessary, lyophilised and stored at −20 °C. The quantum yield determination was carried out with monochromatic light and was based on two actinometric measurements [8] performed in the reaction cell before and after each photochemical run. The initial concentration of the reagent was selected so as to obtain the maximum absorption of the excitation light compatible with the spectrophotometric or ESR measurements.

Results

Native Cytochrome c

Aqueous solutions of horse heart Fe(III) cytochrome c in the pH range 4–10 were stable at room temperature for several days.

The irradiation at $\lambda > 300$ nm of 1×10^{-4} mol dm^{-3} deaerated solutions of the native cytochrome c at room temperature and pH 8.5 gave rise to the spectral variations shown in Fig. 1. The absorbance increase at 550 nm together with the decrease of the 695-nm absorption indicate that the irradiation causes the reduction of Fe(III) to Fe(II) [9]. No appreciable changes of pH occurred during the irradiation. In the pH range 4–10, as well as in aerated solutions, qualitatively similar spectral variations were observed. When ferricyanide was added or oxygen was bubbled into the irradiated solutions, the absorption spectrum showed essentially the features of the original Fe(III) cytochrome c, except for the 695-nm band which appeared to be less intense. The decrease in intensity of this band suggests that under irradiation the native Fe(III) cytochrome c underwent other modifications, besides reduction of the heme iron.

The modified Fe(III) cytochrome c was chromatographically separated from the native protein as described in the Experimental section. A two-peak elution pattern was obtained (Fig. 2). On the other hand, a single-peak elution pattern was obtained in the case of a solution of the native protein under similar conditions except that it had been kept in the dark for the whole time of the photochemical run. The solution of the modified Fe(III) cytochrome c, previously purified by dialysis (see Experimental), displays the absorption spectrum typical of the native protein, except for the lack of the 695-nm band.

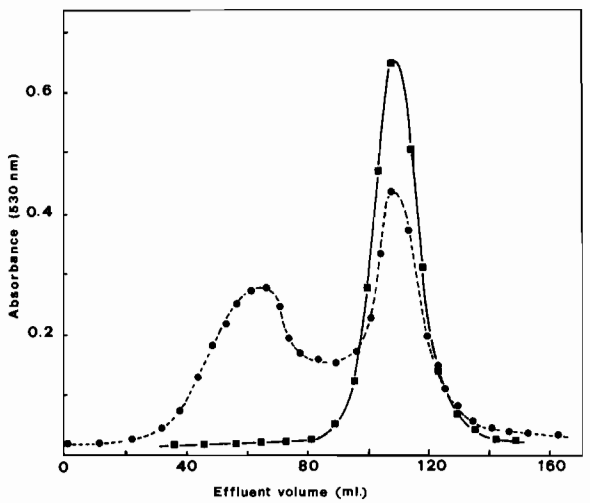


Fig. 2. Elution diagrams of Fe(III)-cytochrome c aqueous solutions before (■) and after (●) irradiation at $\lambda > 300$ nm, obtained by chromatography on Watman CM-52 column (13 × 2.5 cm).

The modified cytochrome exhibits a different behavior with respect to the native species as to the effect of pH on the 620-nm absorption (Fig. 3).

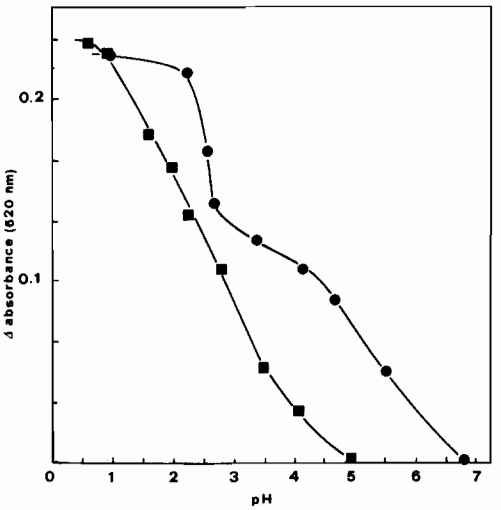


Fig. 3. Spectrophotometric titrations at 620 nm of (■) native and (●) photochemically modified Fe(III)-cytochrome c. Cytochrome concentration, 1×10^{-4} mol dm^{-3}.

The increase in absorbance with pH decrease, which is indicative of the formation of high-spin species, occurs as a two-step process in contrast to the case of the native species which exhibits a single-step transition.

The change in absorbance at 550 nm (Fig. 1) was monitored as a function of irradiation time. A linear plot was obtained which indicates that the photoreaction follows zero-order kinetics for an irradiation time long enough to minimize errors in the quantum yield calculation. The quantum yield values at different pHs and irradiation wavelengths are set out in Table I.

Azide Cytochrome c

The presence of an excess of azide in neutral solutions of Fe(III) cytochrome c is known to produce a stable form of cytochrome in which N_3^- binds to the sixth position of iron in the place of methionine-80 [10].

The addition of 10 mol dm^{-3} azide to a 1×10^{-4} mol dm^{-3} aqueous solution of Fe(III) cytochrome c changed the absorption spectrum as shown in Fig. 4 and increased the pH to *ca.* 9.5. No appreciable spectral variations were observed if the pH was re-adjusted to *ca.* 7, thus suggesting that, in the conditions used, the azido derivative can be considered stable in the pH range 7–9.5. The irra-

TABLE I. Photoredox Quantum Yields of Fe(III)-cytochrome c (ϕ_{nat}) and its Azide Derivative (ϕ_{az}).

pH	ϕ_{nat}[a] × 10^4	λ_{irr}	ϕ_{nat}[b] × 10^4	ϕ_{az}[c] × 10^4
1.7	0.0	275	6.6	
2.1	0.0	280	5.3	
3.1	1.5	290	4.5	
3.6	3.6	295	4.3	
4.5	5.2	310	3.3	
5.0	5.5	315	3.3	
6.0	5.5	330	1.7	
8.5	3.3	340	0.6	4.0
10.1	2.2	360	0.4	1.4
10.7	1.7	400	<0.1	<0.1
11.1	1.2			
12.0	15.0			

[a]λ_{irr} 313 nm. [b]pH 8.5. [c]pH 9.5.

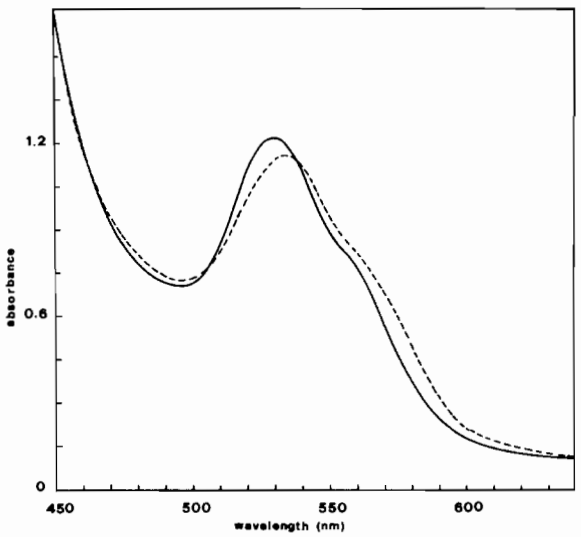


Fig. 4. Absorption spectra of Fe(III)-cytochrome c in aqueous solution at pH 9.5: (– – – –) in 10 mol dm^{-3} sodium azide; (———) without sodium azide. Cytochrome concentration, 1 × 10^{-4} mol dm^{-3}.

diation of the above oxygen-free solutions with light of $\lambda > 335$ nm gave, at pH 8.5–9.5, spectral variations which indicate the formation of Fe(II) cytochrome c*. At pH 6.5–8.0 no appreciable amount of Fe(II) protein was formed upon irradiation. The photoproduct formed in basic solution was stable in the absence of oxygen and underwent complete re-oxidation within 24 hours when air was let into the reaction vessel. On the other hand, when the pH was lowered below 8, the photoproduct underwent fast oxidation both in the presence and in the absence of air.

Irradiated solutions which were dialyzed against distilled water in order to remove excess azide exhibited an absorption spectrum identical to that of the native Fe(III) cytochrome c at neutral pH.

Photochemical experiments have been carried out by irradiating inside the ESR cavity solutions of azide–Fe(III) cytochrome derivative containing phenyl-*tert*-butyl-nitrone (PBN) as spin trap [11]. The pH of the solution was in the range 6 to 9.5 and 350-nm irradiation light was used in order to avoid photolysis of PBN. Irradiated solutions displayed an ESR spectrum which is typical of an N_3 adduct of PBN [12].

The observed good zero-order kinetics followed by the photo-redox reaction of the azide-cytochrome derivative has allowed the calculation of the quantum yields using the same procedure as that described above for the photoredox of the native protein. The values obtained at different irradiation wavelengths are reported in Table I.

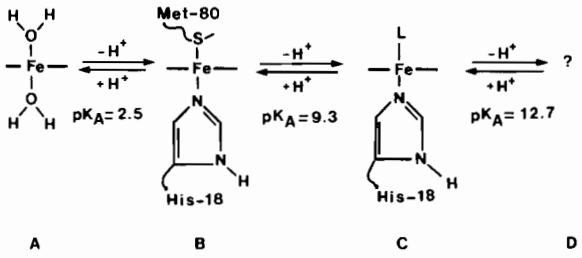
Discussion

The product formed as a consequence of the irradiation of Fe(III) cytochrome c solutions exhibits an absorption spectrum very similar to that of Fe(II) cytochrome c (Fig. 1). This product, after re-oxidation and separation from the unreacted native protein, displays an absorption spectrum which is typical of a low-spin Fe(III) cytochrome with the 695-nm band missing. Since this band is known to be an index of the stability of the heme iron–methionine-80 bond, its absence in the new spectrum indicates the breaking of the iron–sulfur bond in the photoredox process.

The spectral titration curves at 620 nm (Fig. 3) show that the photochemically modified cytochrome is converted to a high-spin form in a two-step process with pK 2.5 and 5.3, respectively, whereas the native protein undergoes a one-step transition with pK 2.5. A similar pH dependence was previously observed and discussed by Kaminsky *et al.* [5d] for the methionine-sulfoxide derivative of cytochrome c. The two transitions were taken as an indication of the replacement of the fifth and sixth ligands of heme iron respectively. The fact that the pK of 2.5 did not vary after the modification of the native protein was taken by the above authors as evidence that histidine-18 was the fifth ligand in the methionine-sulfoxide cytochrome c. We propose a similar explanation for the spectrophotometric titration reported in Fig. 3, *i.e.*, histidine-18 is also the fifth ligand in the photochemically modified Fe(III) cytochrome c.

*Irradiation at wavelengths shorter than 335 nm gave complicated spectral variations indicating that photoprocesses other than photoredox were occurring.

Fe(III) cytochrome c is known to have, in aqueous solution, different pH dependent states which differ from each other in the nature of the amino-acid residues bound to the two axial positions of the heme group [2]. The equilibria involving these states are reported in Scheme 1:



Scheme 1

The nature of the heme ligands in the 'basic' states (C and D) is presently an object of discussion. The substitution of methionine-80 with lysine-79 in state C was previously proposed by Dickerson *et al.* [2]. However, a detailed work by Bosshard [13] clearly shows that lysine-79 is not ligated in the sixth coordination site of heme iron in state C of Fe(III) cytochrome c. The situation is more complicated in state D: at pH > 12 the protein does not retain a closed conformation and the heme is no longer completely buried in the proteic crevice. As a consequence, OH^- and H_2O are likely to compete with amino-acid residues in binding to the axial positions of Fe(III).

As is apparent from Table I, the highest quantum yield is obtained at pH 12. However, due to drastic conformational changes occurring in the proteic moiety at these pHs [2], it is not possible to present a clear picture of the photoredox process. On the other hand, in the pH range 2–11 the maximum quantum yield value is obtained near pH 5, where state B is by far the most abundant species and thus the one absorbing most of the excitation light. This is an indication that the state which contains methionine-80 and histidine-18 as heme ligands (B) is the only photoreactive species and that the replacement of methionine-80 with other ligands (states C and A) prevents the photoredox reaction.

In Fig. 5, the experimental quantum yield values are reported as a function of pH and compared with a plot obtained by assuming that state B is the only photoreactive species. Theoretical plots of quantum yield *versus* pH can be constructed using the concentration of state B evaluated from the equilibrium constants of Scheme 1. Both the experimental and the calculated points lie on bell-shaped curves which have a maximum at about pH 6. This provides further evidence that state B is the actual photoreactive species. The fact that at pH > 9 and < 4 the experimental points do not fit the theoretical plot can be explained in the following way. At pH > 9 the concentration of species D, which is likely to be responsible for the high quantum yield obtained at pH 12, is no longer present in a negligible amount, thus causing the experimental values to be higher than the calculated ones. At pH < 4 the photoreac-

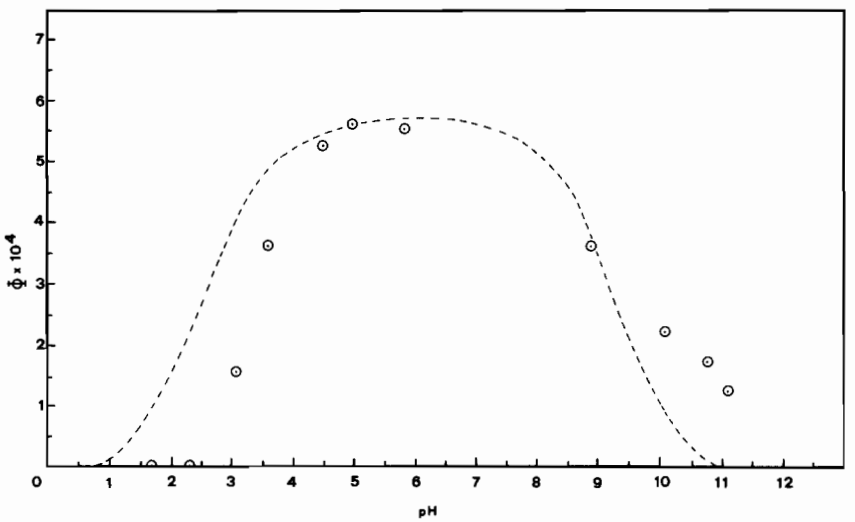
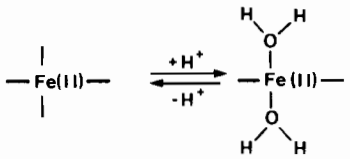


Fig. 5. Dependence of the photoreaction quantum yield on pH. Irradiation wavelength, 313 nm; ⊙, experimental points; ------ calculated plot.

tion product is likely to be in equilibrium with a reduced cytochrome state which contains two water molecules bound to Fe(II) eqn.:



(1)

This species, the concentration of which increases with decreasing pH, is known to be very unstable with respect to oxidation [2] even in deaerated solution; this provides a plausible explanation for the experimental quantum yields being progressively lower than the calculated ones as pH is lowered (Fig. 5).

In order to demonstrate the influence of equilibrium 1 on the quantum yields, these were made independent of the equilibria of Scheme 1 by dividing the experimentally obtained values by the intensity of the excitation light absorbed by the photoreactive species B. The quantum yields obtained in this way are reported as a function of pH in Fig. 6. In

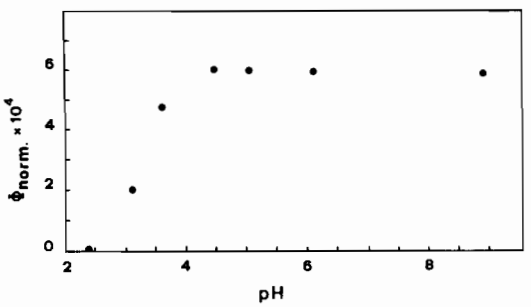
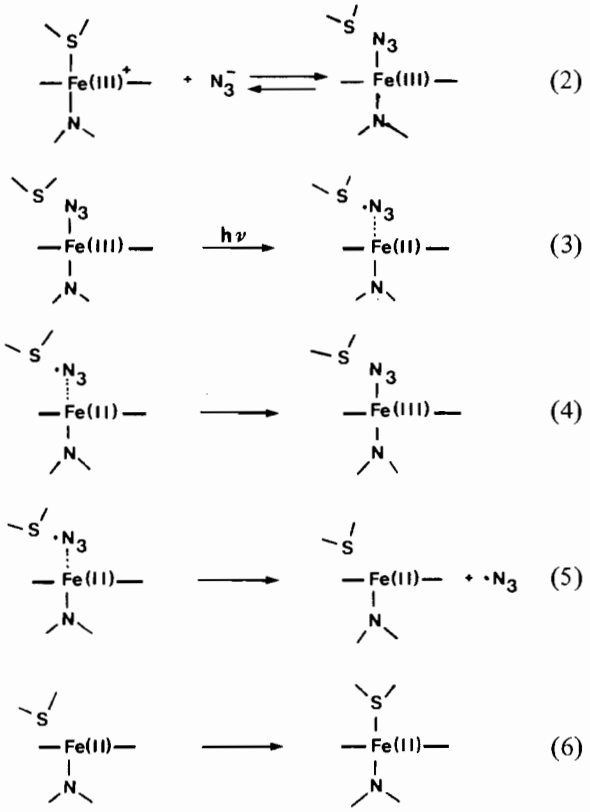


Fig. 6. Dependence of the corrected photoreaction quantum yield ($\phi_{norm.}$) on pH. The corrected values have been calculated using the intensity of excitation light (313 nm) absorbed by the photoreactive species.

one portion of this plot (low pH) the quantum yield strongly increases with pH, in agreement with the difference between the theoretical and experimental quantum yield values in the same pH region of Fig. 5. At high pHs, where the concentration of the easily oxidizable Fe(II) cytochrome species becomes negligible, the corrected quantum yields do not depend on pH.

Azide has been chosen as an exogenous ligand in the present study, since it proved to be frequently very useful in spectroscopic investigations on hemoproteins, particularly with regard to charge transfer transitions involving axial heme-iron ligands [14].

The experimental results clearly indicate that the irradiation of solutions of Fe(III) cytochrome c containing an excess of azide, at pH 9.5, causes the reduction of heme iron and the formation of $\cdot N_3$ radicals. The photoreaction product is Fe(II) cytochrome c containing methionine-80 and histidine-18 bound in the two axial binding sites of the heme iron. This behavior can be interpreted in terms of Scheme 2, where S is the sulphur of methionine-80 and N the nitrogen of histidine-18.



Scheme 2

Equilibrium 2 is strongly displaced toward the right due to the presence of a large excess of N_3^-. The primary electron transfer from axial N_3 to Fe(III) (eqn. 3) is followed by a back electron transfer (eqn. 4) which competes with the N_3 radical diffusion (eqn. 5). The free binding site of the Fe(II) intermediate is rapidly occupied by methionine-80 (eqn. 6) which is known to be a good ligand for Fe(II) cytochrome species.

Analogous mechanisms have previously been proposed for the photoredox reactions of Fe(III) porphyrin complexes [7a, 7c] and the detection of radical species provided in some cases [7e] direct evidence for an electron transfer from axial ligand to Fe(III) being the primary photochemical act. These results, together with those reported above for the azido cytochrome derivative, suggest that the photoredox reaction of the native Fe(III) cytochrome c occurs according to a mechanism where the primary photochemical process is an inner sphere electron transfer from axially bound methionine-80 to heme

iron. Although it is reasonable to suppose that a methionyl radical cation is actually formed in the primary electron transfer, its fate is not easily predictable. The formation of a sulfoxide derivative, previously proposed by Jori *et al.* [5b] on the basis of results obtained in a study concerning the photochemical behavior of cytochrome c in the absence of photosensitizers, was not subsequently confirmed [5d] and on this basis it can also be ruled out in the present case.

The photochemically modified cytochrome c exhibits, in its oxidized state, a low-spin type absorption spectrum. This suggests that the modification of the methionine-80 residue yields the sixth binding position of heme available for ligation of a strong field ligand. This might be either an aminoacid residue which is drawn closer to the heme group as a consequence of conformational changes induced by the photochemical process, or a new species formed through reactions of the methionyl radical cation.

The involvement of methionine-80 in the photoreaction of native Fe(III) cytochrome as well as the involvement of N_3^- in the azide derivative, implies that an axial ligand-to-metal charge transfer excited state is responsible for the primary photoprocesses. The possibility of charge transfer transitions to or from axial ligands in metallo-porphyrins and metalloproteins was postulated on the basis of Polarized Electronic and Resonance Raman Spectroscopy investigations [14]. Unfortunately, the intense $\pi-\pi^*$ transitions which dominate the spectrum of these compounds make the location of the ligand-to-metal or metal-to-ligand charge transfer transitions very difficult.

The comparison of the photoreaction quantum yield values obtained for the native Fe(III) cytochrome c with those obtained for the azide derivative indicates that the latter photoreacts at irradiation wavelengths longer than 360 nm where the native species appears to be quite unreactive. This allows one to consider the possibility that the $N_3 \rightarrow$ Fe(III) charge transfer transition is shifted toward lower energies, in agreement with the higher oxidizability of N_3^- with respect to methionine. The location of the absorption bands corresponding to these transitions is hazardous because of the complicated nature of the spectrum of cytochrome c. However, the depencence of the quantum yield on the irradiation wavelength allows one to locate them in the near UV spectral region.

The results obtained in the present investigation demonstrate that the excitation of Fe(III) cytochrome c leads to an inner sphere electron transfer from heme axial ligand to iron. This behavior is quite similar to that previously observed for iron porphyrin complexes which can be considered good models for a number of heme proteins. The efficiency of the photoredox depends, as expected, on the nature of the axial ligand. However, a correlation between the oxidizability of the ligands and the photoredox quantum yield should be of little significance because the quantum yield value depends on a number of different parameters such as excited state lifetime, efficiency of the back electron transfer and reactivity of intermediates formed in the primary photochemical act. It should be noted, however, that state C (Scheme 1) of the native cytochrome c, which is known to be capable of undergoing oxidation and reduction of its heme iron in physiological conditions, is the only photoreducible species. Despite the difficulties involved in this correlation, it gives further evidence of the fundamental role of the binding of methionine-80 to iron in the redox processes of cytochrome c.

The finding that the light induced oxidation of the methionine-80 residue of Fe(III) cytochrome c proceeds via an intramolecular electron transfer from the methionyl sulfur to iron is not in agreement with the results obtained by Jori and co-workers [5b] on the photo-oxidative modification of heme ligands of Fe(III) cytochrome c. These authors did not observe photoreduction of Fe(III) to Fe(II) during the irradiation and ascribed the formation of methionyl sulfoxide to a heme photosensitized oxidation of methionine-80 residue. In a successive investigation Kaminsky and co-workers [5d] were able to perform the oxidation of the methionine-80 residue of horse heart cytochrome c to sulfoxide in the presence of methylene blue as sensitizer. On the contrary, any attempt to obtain methionine sulfoxide by irradiation of the Fe(III) protein without external sensitizer failed in the hands of the same author as well as in those of a number of other researchers in different laboratories. As a matter of fact, the inability of the heme group to work as sensitizer is in good agreement with the known very short lifetime of Fe(III) porphyrin excited states [15] in fluid solution which is presumably due to radiationless decay to low lying d–d states. In spite of the evident disagreement between our results and those of Kaminsky *et al.* [5d], a common indication is that the photo-oxidation of the methionine-80 residue of Fe(III) cytochrome c occurs in the presence as well as in the absence of external sensitizers. While in the former case methionyl sulfoxide is formed without changing the oxidation state of iron, in the latter case an intramolecular electron transfer from methionine-80 to iron produces a methionine oxidation product other than the sulfoxide, simultaneously reducing Fe(III) to Fe(II).

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References

1 A. Maldotti, C. Bartocci, O. Traverso and V. Carassiti, *Inorg. Chim. Acta, 79,* 174 (1983).
2 R. E. Dickerson and R. Timkovich, in P. D. Boyer (ed.), 'The Enzymes, Vol. XI', Academic Press, New York, 1975, Part A, p. 397.
3 (a) S. Ferguson-Miller, D. L. Brautigan and E. Margoliash, in D. Dolphin (ed.), 'The Porphyrins, Vol. VII', Academic Press, New York, 1979, p. 149;
(b) G. Moore, Z-X Huang, G. C. S. Eley, H. A. Barker, G. Williams, M. N. Robinson and R. J. P. Williams, *Faraday Discuss. Chem. Soc., 74,* 311 (1982).
4 R. Timkovich, in D. Dolphin (ed.), 'The Porphyrins, Vol. VII', Academic Press, New York, 1979, p. 277.
5 (a) G. Jori, G. Gennari, G. Galiazzo and E. Scoffone, *FEBS Lett., 6,* 267 (1970);
(b) G. Jori, G. Gennari, M. Folin and G. Galiazzo, *Biochim. Biophys. Acta, 229,* 525 (1971);
(c) M. Folin, A. Azzi, A. M. Tamburro and G. Jori, *Biochim. Biophys. Acta, 285,* 337 (1972);
(d) K. M. Ivanetich, J. J. Bradshaw and L. S. Kaminsky, *Biochemistry, 15,* 1144 (1976).
6 C. Yu. Y. C. Chang, L. Yu and T. E. King, *J. Biol. Chem., 250,* 6218 (1975).
7 (a) C. Bartocci, F. Scandola, A. Ferri and V. Carassiti, *J. Am. Chem. Soc., 102,* 7067 (1980);
(b) C. Bizet, P. Morliere, D. Brault, O. Delgado, M. Bazin and R. Santus, *Photochem. Photobiol., 34,* 315 (1981);
(c) C. Bartocci, A. Maldotti, O. Traverso, C. A. Bignozzi and V. Carassiti, *Polyhedron, 2,* 97 (1983);
(d) P. Zanello, C. Bartocci, A. Maldotti and O. Traverso, *Polyhedron, 2,* 791 (1983);
(e) A. Maldotti, C. Bartocci, R. Amadelli and V. Carassiti, *Inorg. Chim. Acta, 74,* 275 (1983).
8 S. L. Murov, in 'Handbook of Photochemistry', Marcel Dekker, New York, 1973, p. 119.
9 H. A. Harbury and R. H. L. Marks, in G. L. Eichhorn (ed.), 'Inorganic Biochemistry, Vol. II', Elsevier, Amsterdam, 1973, p. 907.
10 R. K. Gupta and G. Redfield, *Biochem. Biophys. Res. Commun., 41,* 273 (1970).
11 E. G. Janzen, *Acc. Chem. Res., 4,* 31 (1971).
12 D. Rehorek, P. Thomas and H. Hennig, *Inorg. Chim. Acta, 32,* L1 (1979);
(b) E. G. Janzen, H. J. Stronks, D. E. Nutter, Jr., E. R. Davis, H. N. Blount, J. L. Poyer and P. B. McCay, *Can. J. Chem., 58,* 1596 (1980).
13 H. R. Bosshard, *J. Mol. Biol., 153,* 1125 (1981).
14 T. G. Spiro, in A. B. P. Lever and H. B. Gray (eds.), 'Iron Porphyrins', Addison–Wesley, Reading, 1983, p. 89.
15 M. Gouterman, in D. Dolphin (ed.), 'The Porphyrins, Vol. III', Academic Press, New York, 1978, p. 69.