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Reduction of a water-soluble iron(III) porphyrin and an iron(II) nitrosyl porphyrin by benzophenone ketyl radical

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Reactions of benzophenone ketyl radicals with the water-soluble iron(III)porphyrin, $[Fe^{III}(tpps)]^{3-}$ [tpps = *meso*-tetrakis(*p*-sulfonatophenyl)porphyrinate], and a nitric oxide adduct of the iron(II) form of the porphyrin, $[Fe^{II}(tpps)(NO)]^{4-}$, have been studied by laser photolysis in aqueous solutions. Reactions of ferricytochrome c and metmyoglobin with the ketyl radicals were also studied for comparison with the iron(III) porphyrin. Benzophenone ketyl radicals were produced from the reaction of the triplet state of benzophenone with the glucose used as a suitable hydrogen atom donor. The transient absorption spectrum observed after 266 nm laser pulse excitation for the $[Fe^{III}(tpps)]^{3-}$ solution containing benzophenone and glucose shows that the iron(III) porphyrin is reduced by the ketyl radical to the iron(II) form. Ferricytochrome c and metmyoglobin were found also to be reduced to the iron(II) form by the ketyl radical. The rate constants for the reductions of $[Fe^{III}(tpps)]^{3-}$, ferricytochrome c and metmyoglobin were determined to be 3.0×10^9, 4.5×10^8 and 2.2×10^8 dm^3 mol^{-1} s^{-1} respectively. Nitric oxide irreversibly reacts with $[Fe^{II}(tpps)]^{4-}$ to yield $[Fe^{II}(tpps)(NO)]^{4-}$ in an aqueous solution. The transient absorption spectrum observed 500 μs after laser pulsing for the $[Fe^{II}(tpps)(NO)]^{4-}$ solution containing benzophenone and glucose indicates that the ketyl radical reacts with $[Fe^{II}(tpps)(NO)]^{4-}$ to form a new transient species which displays the spectrum with a peak at 418 nm in the Soret band region. The transient species has been identified as a one-electron adduct of $[Fe^{II}(tpps)(NO)]^{4-}$ where the added electron possibly exists on the coordinated NO moiety.

Nitric oxide (NO) has been shown to perform important functions in a variety of physiological processes including vascular smooth muscle relaxation, neurotransmission and immune regulation.[1,2] Even before the physiological functions of NO were noted, haemoproteins as well as synthetic metalloporphyrins were known to react with NO to yield their NO adducts *in vitro*.[3,4] Recently, we reported kinetic and spectroscopic studies on the reactions of NO with ferri- and ferrohaemoproteins as well as synthetic iron porphyrins in aqueous solutions.[5] It has been also recently elucidated that NO binding to the haem iron of haem enzyme activates the inherent enzymatic functions. Examples include soluble guanylate cyclase which catalyses conversion of GTP (guanosine triphosphate) to cGMP (cyclic guanosine monophosphate) required for vascular muscle relaxation and other physiological functions;[1,2] another example is nitric oxide reductase which catalyses reduction of NO to N_2O in the denitrifying process, cytochrome bc complex in bacteria[6–12] or cytochrome P450nor in denitrifying fungus.[13] Possible reaction mechanisms of the NO reduction in the denitrifying process have been proposed most recently, where, in the case of cytochrome bc complex (bacterial nitric oxide reductase), NO anion or NOH is produced by one-electron reduction of the NO–haem moiety and non-enzymatically dimerizes to yield N_2O,[8,14] while in cytochrome P450nor (fungus nitric oxide reductase), a two-electron reduced species of the NO complex of iron(III) P450nor was found as an intermediate during the catalytic cycle and subsequently the intermediate reacts with another free NO on the iron centre to yield N_2O.[15] At present, however, the NO reduction mechanisms catalysed by both nitric oxide reductases are not yet fully apparent. Furthermore, the physiologically important role of NO anion rather than of NO itself is suggested not only in the denitrifying process but also in the process of guanylate cyclase activation.[16]

Therefore, studies on the reduction of NO adducts of iron porphyrins as models of the reactive centre of haemoproteins become highly significant for the elucidation of mechanisms of the reactions involving NO *in vivo*.

Electrochemical studies on the reduction of nitrosyl iron porphyrins in aqueous[17–19] or non-aqueous solutions[20–24] have been published. It is likely, however, to be more useful for elucidation of the reaction mechanism *in vivo* to investigate the reduction of nitrosyl iron porphyrins due to electron transfer from reductants in aqueous solutions.

In the present study, we first examined the reduction of the iron(III) form of the water-soluble porphyrin, $[Fe^{III}(tpps)]^{3-}$ [tpps = *meso*-tetrakis(*p*-sulfonatophenyl)porphyrinate], ferricytochrome c and metmyoglobin (oxidized form of myoglobin) with benzophenone ketyl radical produced *via* the triplet state of benzophenone. Then, reduction of NO adduct of the iron(II) form of the porphyrin with the ketyl radical was studied.

Experimental

The water-soluble iron porphyrin $[Fe^{III}(tpps)]^{3-}$ was synthesized as its sodium salt from the reaction of $Na_4[H_2tpps]$ and iron(II) sulfate in water and purified on a cation exchange column according to the literature.[18,25] Metmyoglobin (skeletal whale) and $Na_4[H_2tpps]$ were purchased from Sigma Chem. Co. Ferricytochrome c (horse heart) was supplied by Wako Pure Chem. Ind. Ltd. Nitric oxide (99.9%) was obtained from Takachiho Chem. Ind. Ltd. The reagents were used without further purification. Benzophenone, purchased from Wako Pure Chem. Ind. Ltd., was recrystallized twice from ethanol. All the sample solutions were prepared with the use of phosphate buffer (2×10^{-3} mol dm^{-3}) to adjust the pH of the solutions. The buffered solutions were degassed on a vacuum line without freezing the solutions. In preparing solutions of the NO adducts, NO gas was introduced into the degassed solutions on the vacuum line. The concentration of NO dissolved in the sample solutions was calculated from the partial pressure of NO with the use of the Bunsen absorption coefficient of NO (4.71×10^{-2} at 1 atm at 293 K).[26] The pressure of NO was measured by a mercury manometer. Optical absorption spectra were recorded on a Hitachi 330 spectrophotometer. Laser photolysis studies were carried out with

266 nm light pulses from a Nd : YAG laser (J. K. Lasers Ltd.): duration = 20 ns, energy *ca.* 100 mJ per pulse. The detection system for the transient spectra has been reported previously.[27]

Results and Discussion

The photo-physical and -chemical properties of the lowest excited triplet state of benzophenone have been extensively studied.[28,29] Triplet benzophenone has been found to react with various kinds of hydrogen-atom donors to yield benzophenone ketyl radical in oxygen-free solutions.[29] Glucose, used as a suitable hydrogen-atom donor in the present study, quenches the triplet state with the rate constant of 1×10^6 dm^3 mol^{-1} s^{-1}.[30] To enable a complete and rapid quenching of the triplet, a high concentration of glucose (0.5 mol dm^{-3}) was employed in the present experiments.

Excitation of benzophenone in an oxygen-free aqueous solution containing glucose gives the transient absorption spectrum due to the benzophenone ketyl radical which has a lifetime of *ca.* 500 μs. The ketyl radical is usually known to behave as a reductant, but it dimerizes to yield benzpinacol in the absence of any reactant which can react with the ketyl radical.[28] It has been found previously that the isopropyl radical, which has a similar structure to the benzophenone ketyl radical at the carbon radical centre, reduces an iron(III) porphyrin to iron(II).[31] This result also supports the reducing ability of the benzophenone ketyl radical. The optical absorption spectrum of the ketyl radical exhibits peaks at 330 nm ($\varepsilon = 1.61 \times 10^4$ dm^3 mol^{-1} cm^{-1}) and 540 (3.22×10^3),[32] and scarcely overlaps with the Soret band absorption of the porphyrin or the haemoproteins used; therefore, absorption changes in Soret bands caused by reaction with the ketyl radicals can be conveniently observed.

Reduction of $[Fe^{III}(tpps)]^{3-}$, ferricytochrome c and metmyoglobin

A dilute aqueous solution of the sodium salt of $[Fe^{III}(tpps)]^{3-}$ (pH < 7) displays an absorption spectrum with a peak at 393 nm in the Soret-band region. In alkaline solution the iron(III) porphyrin forms a μ-oxo dimer, which exhibits an absorption spectrum with a peak at 408 nm for the Soret band.[18] Dimer formation already begins to occurs even around pH 7 at high concentrations of the monomer and high ionic strength. However, there is no complication of dimer formation of the iron(III) porphyrin under the present experimental conditions with the concentration of the monomer $< 1 \times 10^{-4}$ mol dm^{-3} in 2×10^{-3} mol dm^{-3} phosphate buffer at pH 6.0.

When the oxygen-free $[Fe^{III}(tpps)]^{3-}$ solution (pH 6.0) containing benzophenone and glucose was subjected to 266 nm laser pulses, a transient spectrum appeared concomitantly with the decay of the ketyl radical. Fig. 1 shows the transient absorption spectrum observed after completion of the radical decay. The transient spectrum is in good agreement with the difference spectrum obtained by subtracting the spectrum of $[Fe^{III}(tpps)]^{3-}$ from that of $[Fe^{II}(tpps)]^{4-}$. This shows that the ketyl radical reduces the iron(III) porphyrin to Fe^{II}. The photoreaction, therefore, is expressed as:

$$Ph_2CO + h\nu \rightarrow {}^3Ph_2CO^*$$

$${}^3Ph_2CO + RH(glucose) \rightarrow Ph_2COH + R^\cdot$$

$$Ph_2COH + [Fe^{III}(tpps)]^{3-} \rightarrow Ph_2CO + [Fe^{II}(tpps)]^{4-} + H^+$$

The formation of $[Fe^{II}(tpps)]^{4-}$ and the decay of the ketyl radical obey first-order kinetics under the conditions that the concentration of the ketyl radical is significantly less than that of $[Fe^{III}(tpps)]^{3-}$. The concentration of ketyl radical was monitored by the absorption at 330 nm and adjusted by controlling the laser intensity. The straight-line plot of the pseudo-first-order rate constant, k_1, *vs.* concentration of

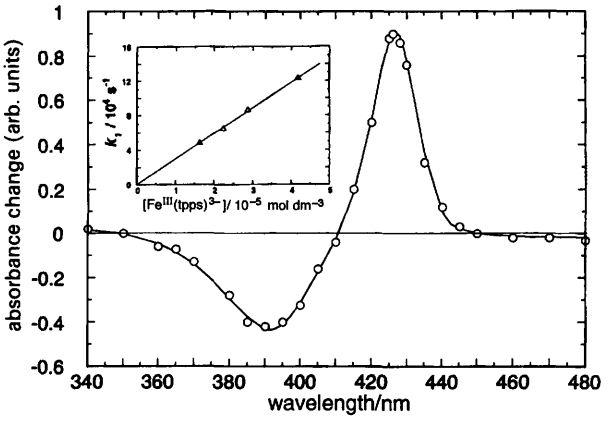

Fig. 1 Transient absorption spectrum observed for a phosphate buffer solution (pH 6.0) of $[Fe^{III}(tpps)]^{3-}$ (2.9×10^{-5} mol dm^{-3}) in the presence of benzophenone (5.1×10^{-5} mol dm^{-3}) and glucose (0.5 mol dm^{-3}), 70 μs after 266 nm laser pulsing. Inset shows a plot of the pseudo-first-order rate constant, k_1, for the reduction of $[Fe^{III}(tpps)]^{3-}$ with benzophenone ketyl radical as a function of concentration of $[Fe^{III}(tpps)]^{3-}$. The time dependence of the absorbance change was analysed mainly at 425 nm.

$[Fe^{III}(tpps)]^{3-}$ is shown in Fig. 1 (insert). From the slope, the rate constant of the reduction of $[Fe^{III}(tpps)]^{3-}$ with the ketyl radical has been determined as 3.0×10^9 dm^3 mol^{-1} s^{-1} in an aqueous phosphate buffer solution at pH 6.0. This value is much larger in comparison with the value of the rate constant for the reduction of ferrideuteroporphyrin by isopropyl radical, determined to be $(2.1 \pm 0.3) \times 10^8$ dm^3 mol^{-1} s^{-1} in pure propan-2-ol.[31]

The reaction of the triplet benzophenone with glucose gives rise to formation of the ketyl and glucose radical. Both radicals are expected to reduce $[Fe^{III}(tpps)]^{3-}$. However, as mentioned above, the formation rate of $[Fe^{II}(tpps)]^{4-}$ strictly follows first-order kinetics. Further, the rate constant for the decay of the ketyl radical is in agreement with that for the formation of $[Fe^{II}(tpps)]^{4-}$. These results indicate that only the ketyl radical is able to reduce $[Fe^{III}(tpps)]^{3-}$. Presumably, the glucose radical undergoes either recombination or disproportionation to give stable products.

Reactions of ferricytochrome c (Cyt^{III}) and metmyoglobin (Mb^{III}) with the benzophenone ketyl radicals have also been studied for comparison with the reaction of $[Fe^{III}(tpps)]^{3-}$. Laser-pulse excitation for oxygen-free solutions of Cyt^{III} or Mb^{III} (pH 7) containing benzophenone and glucose gives transient spectra concurrently with the decay of ketyl radical. The transient spectra are consistent with the difference spectrum between Cyt^{II} and Cyt^{III} or Mb^{II} and Mb^{III}. It is consequently apparent that Cyt^{III} and Mb^{III} are reduced by the ketyl radical to Cyt^{II} and Mb^{II} respectively. The formation of Cyt^{II} or Mb^{II} and the decay of the ketyl radical obey first-order kinetics when the concentration of the ketyl radical is significantly less than that of Cyt^{II} or Mb^{II}. Fig. 2 shows plots of the pseudo-first-order rate constant for the reactions, k_1, as a function of the concentration of Cyt^{III} or Mb^{III}. From the slope, the rate constants of the reactions of ketyl radical with Cyt^{III} and Mb^{III} were determined as 4.5×10^8 and 2.2×10^8 dm^3 mol^{-1} s^{-1}, respectively. The values of the rate constants for Cyt^{III} and Mb^{III} are about one-tenth of that for $[Fe^{III}(tpps)]^{3-}$. This much smaller rate constant for Cyt^{III} or Mb^{III} is probably because of the steric hindrance of the surrounding protein around the haem.

Reduction of $[Fe^{II}(tpps)(NO)]^{4-}$

A solution of $[Fe^{II}(tpps)(NO)]^{4-}$ was prepared by addition of one mol equivalent of NO to $[Fe^{II}(tpps)]^{4-}$ produced by reduction of $[Fe^{III}(tpps)]^{3-}$ with $Na_2S_2O_4$ in air-free solution. The reaction of NO with $[Fe^{II}(tpps)]^{4-}$ has been established

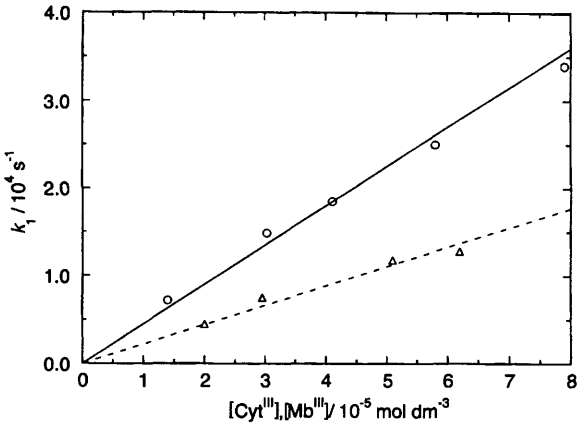

Fig. 2 Plots of the pseudo-first-order rate constants, k_1, for the reduction of Cyt^{III} (○) and Mb^{III} (△) with benzophenone ketyl radical as a function of [Cyt^{III}] or [Mb^{III}]. The time dependence of the absorbance change was analysed mainly at 450 or 550 nm for Cyt^{III} reduction and at 435 nm for Mb^{III} reduction.

to yield $[Fe^{II}(tpps)(NO)]^{4-}$ irreversibly in our previous work.[5] Completion of the reaction at each step was easily confirmed by monitoring the absorption spectrum, since the peak wavelengths and the intensity of the Soret bands for $[Fe^{III}(tpps)]^{3-}$, $[Fe^{II}(tpps)]^{4-}$ and $[Fe^{II}(tpps)(NO)]^{4-}$ are at 393 ($\varepsilon = 1.55 \times 10^5$ dm^3 mol^{-1} cm^{-1}), 426 (2.83×10^5) and 412 (1.55×10^5) nm, respectively.

Fig. 3 shows the transient absorption spectra observed for an aqueous buffered solution (pH 6.0) of $[Fe^{II}(tpps)(NO)]^{4-}$ containing benzophenone and glucose for time delays of 100 ns and 500 μs after 266 nm laser pulses. There are apparent differences in their isosbestic points and peak wavelengths between those two transient spectra, *i.e.*, initially spectrum A (observed after 100 ns) exhibits isosbestic points at 416 and 443 nm, and a peak at 428 nm, while spectrum B (observed after 500 μs) subsequently appears with isosbetic points at 410 and 443 nm, and with a peak at 420 nm. This suggests that the photoreaction of $[Fe^{II}(tpps)(NO)]^{4-}$ solution containing benzophenone and glucose gives two kinds of transient species. Spectrum B decays during a few seconds to give the original spectrum of $[Fe^{II}(tpps)(NO)]^{4-}$ solution, *i.e.*, no permanent product was detected spectrophotometrically.

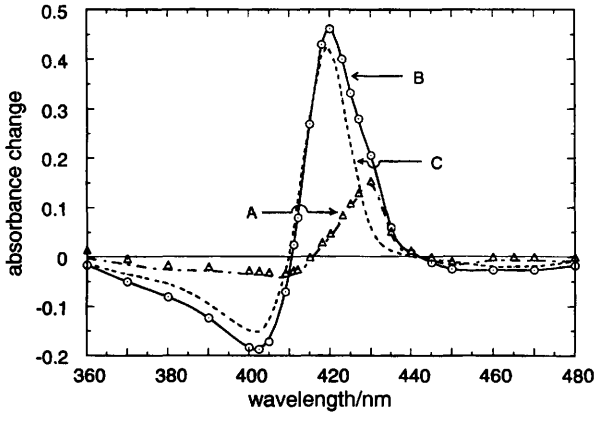

Fig. 3 Transient absorption spectra observed for a phosphate buffer solution (pH 6.0) of $[Fe^{II}(tpps)(NO)]^{4-}$ (6.3×10^{-6} mol dm^{-3}) in the presence of benzophenone (3.5×10^{-5} mol dm^{-3}) and glucose (0.5 mol dm^{-3}): spectrum A at 100 ns (△), and spectrum B at 500 μs (○) after 266 nm laser pulsing. The broken line on spectrum A is corrected for the weak absorption due to benzophenone ketyl radical. Spectrum C (dotted line) shows the difference spectrum obtained by subtracting spectrum A from B.

Spectrum A is in good agreement with the difference spectrum obtained by subtracting the spectrum of $[Fe^{II}(tpps)(NO)]^{4-}$ from that of $[Fe^{II}(tpps)]^{4-}$. The decay of benzophenone ketyl radical was not observed within the time range of 100 ns. These facts indicate that $[Fe^{II}(tpps)]^{4-}$ was produced directly from photoreaction of $[Fe^{II}(tpps)(NO)]^{4-}$ without participation of the ketyl radicals, expressed as

$$[Fe^{II}(tpps)(NO)]^{4-} + h\nu \rightarrow NO + [Fe^{II}(tpps)]^{4-} \quad (1)$$

The quantum yield for the photodissociation of NO from $[Fe^{II}(tpps)(NO)]^{4-}$ was determined to be 0.16 upon laser excitation at both wavelengths of 355 and 532 nm[5] The yield of NO dissociation upon excitation at 266 nm thus has the same value as upon excitation at 355 or 532 nm. This rather high quantum yield caused the direct photodissociation of NO from $[Fe^{II}(tpps)(NO)]^{4-}$, despite the fact that the absorbance of $[Fe^{II}(tpps)(NO)]^{4-}$ at the excitation wavelength (266 nm) is about four times smaller than that of benzophenone. Actually, the amount of $[Fe^{II}(tpps)]^{4-}$ produced by the direct photodissociation was calculated to be *ca.* 10% of the initially present $[Fe^{II}(tpps)(NO)]^{4-}$; this calculation was based on $\Delta(A) = 0.10$ and $\Delta\varepsilon = 1.95 \times 10^5$ dm^3 mol^{-1} cm^{-1}, which are the differences of the absorption and the molar absorption coefficients between $[Fe^{II}(tpps)(NO)]^{4-}$ and $[Fe^{II}(tpps)]^{4-}$ at 426 nm, respectively.

Spectrum B has a shoulder around 428 nm where spectrum A showed a peak. The absorption intensity around the shoulder was found to decrease during a few ms after laser pulsing. This absorbance change is related to recombination of NO and $[Fe^{II}(tpps)]^{4-}$ which are produced by reaction (1), because the recombination process is estimated to occur over almost the same time-range as the absorbance change around the shoulder occurs. This estimation was made by using the rate constant for the recombination determined previously (1.8×10^9 dm^3 mol^{-1} s^{-1})[5] and the initial concentration of $[Fe^{II}(tpps)]^{4-}$ observed at 100 ns after laser pulsing. These findings indicate that spectrum A scarcely starts to decay at 500 μs after the laser pulse when spectrum B was observed. Therefore, we concluded that spectrum B superimposes upon A; when spectrum A is subtracted from spectrum B the spectrum of the transient species resulting from the reaction of $[Fe^{II}(tpps)(NO)]^{4-}$ with benzophenone ketyl radical is obtained. The substantial transient spectrum, shown as spectrum C in Fig. 3, is definitely different from any of the difference spectra among $[Fe^{II}(tpps)(NO)]^{4-}$ and $[Fe^{II}(tpps)^{4-}$, $[Fe^{III}(tpps)]^{3-}$ or $[Fe^{III}(tpps)(NO)]^{3-}$. These facts indicate that benzophenone ketyl radical reacts with $[Fe^{II}(tpps)(NO)]^{4-}$ to produce a new transient species.

The formation rate of the transient species obeys first-order kinetics when the concentration of benzophenone ketyl radical is significantly larger than that of $[Fe^{II}(tpps)(NO)]^{4-}$. The first-order rate constant, k_1, can be formulated as $k_1 = k_2[Ph_2COH]$ under the above conditions, where k_2 is the second-order rate constant for the reaction of $[Fe^{II}(tpps)(NO)]^{4-}$ with the ketyl radical. The value of k_2, 9.6×10^8 dm^3 mol^{-1} s^{-1}, was obtained by dividing the observed value of k_1 by the concentration of benzophenone ketyl radical evaluated by monitoring the absorption at 330 nm ($\varepsilon = 1.61 \times 10^4$ dm^3 mol^{-1} cm^{-1}).

We observed that the absorption due to the ketyl radical still remains after completion of the reaction of $[Fe^{II}(tpps)(NO)]^{4-}$. Thus, we concluded that all $[Fe^{II}(tpps)(NO)]^{4-}$ in the reaction are expected to react with the ketyl radical to produce the new transient species. Therefore, addition of the spectra of $[Fe^{II}(tpps)(NO)]^{4-}$ and the new transients was attempted. The resultant spectrum, shown in Fig. 4, displays an absorption peak at 418 nm in the Soret band. The peak wavelength of the resultant spectrum is shifted from any of those in the spectrum of $[Fe^{II}(tpps)]^{4-}$ (426 nm), $[Fe^{III}(tpps)]^{3-}$ (393 nm), $[Fe^{II}(tpps)(NO)]^{4-}$ (413 nm) or

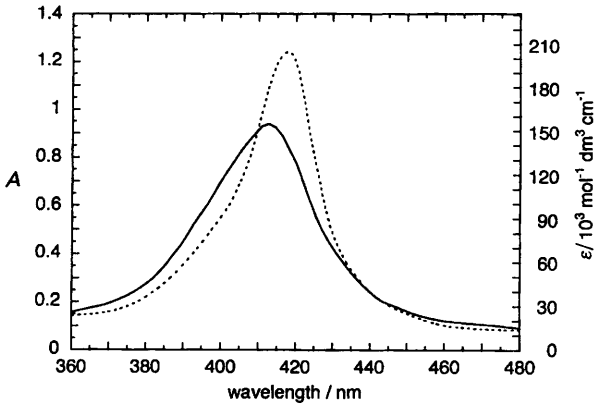

Fig. 4 Absorption spectrum (dotted line) for the one-electron adduct of $[Fe^{II}(tpps)(NO)]^{4-}$ estimated from addition of the spectrum of $[Fe^{II}(tpps)(NO)]^{4-}$ and spectrum C of Fig. 3. The spectrum of $[Fe^{II}(tpps)(NO)]^{4-}$ (solid line) is also shown for comparison.

$[Fe^{III}(tpps)(NO)]^{3-}$ (420 nm), although the shape of the spectrum in the Soret band region is similar to that of $[Fe^{II}(tpps)(NO)]^{4-}$ or $[Fe^{III}(tpps)(NO)]^{3-}$.

Reaction of the ketyl radical with $[Fe^{II}(tpps)]^{4-}$ was also examined, and no absorption change was found in the Soret band region after laser pulsing a $[Fe^{II}(tpps)]^{4-}$ solution containing benzophenone and glucose. This fact indicates that no reaction occurs between the ketyl radical and $[Fe^{II}(tpps)]^{4-}$ to produce either a reduction product or an adduct of the ketyl radical to $[Fe^{II}(tpps)]^{4-}$. The ketyl radical in this case may react with itself to give benzpinacol. Therefore, the reaction between the ketyl radical and $[Fe^{II}(tpps)(NO)]^{4-}$ presumably does not produce the ketyl radical adduct of $[Fe^{II}(tpps)(NO)]^{4-}$.

It is reasonable that the reaction of the ketyl radical with $[Fe^{II}(tpps)(NO)]^{4-}$ produces a one-electron reduction product, judging from the results of the reactions of the ketyl radical with $[Fe^{III}(tpps)]^{4-}$, Cyt^{III} and Mb^{III}. An anion radical of the porphyrin π-ring system is excluded as a candidate for the reduction product, because, in spite of the fact that the spectrum of a porphyrin π-anion generally has its absorption band around 450 nm and at wavelengths higher than 800 nm,[33,34] the spectrum of the reduction product has no absorption peak at such wavelengths. We, consequently, concluded that the reduction product resulting from the reaction with benzophenone ketyl radical, which gives the spectrum shown in Fig. 4, is a one-electron adduct of $[Fe^{II}(tpps)(NO)]^{4-}$ where the added electron mainly exists on a hybrid Fe—NO orbital.

Although the decay process of the electron adduct of $[Fe^{II}(tpps)(NO)]^{4-}$ was not fully investigated, we found that the electron adduct reoxidizes to $[Fe^{II}(tpps)(NO)]^{4-}$ with a half-life of about 2 s. It has been reported that iron(I) deuteroporphyrin, produced from reduction of the iron(II) state with Me_2CO radical anion, reoxidizes to the iron(II) state with a rate of constant of $(1.35 \pm 0.15) \times 10^{-3}$ s^{-1} in alkaline aqueous propan-2-ol solution.[35] This means that the half-life of the iron(I) porphyrin is about 500 s. It is noteworthy that the stability of the iron(I) porphyrin is much greater than that of the electron adduct of $[Fe^{II}(tpps)(NO)]^{4-}$, although the pHs of the solutions were different so preventing a fully valid comparison.

It has already been reported that electrochemical reduction of some types of iron(II) nitrosyl porphyrins, $[Fe^{II}(por)(NO)]$, where por = tpp (*meso*-tetraphenylporphyrinate), tpc (*meso*-tetraphenylchlorinate), oep (2,3,7,8,12,13,17,18-octaethylporphyrinate), tpps *etc.*, in aqueous[17–19] or non-aqueous[20–24] solutions produces one-electron adducts of the respective iron(II) nitrosyl porphyrins. The respective electron adducts are rather stable in benzonitrile or tetrahydrofuran, and their visible spectra have been measured by using optically transparent thin-layer electrochemical cells.[20,23] The visible spectra of the electron adducts show only slight changes of the Soret band position and intensity in comparison with those of the original nitrosyl porphyrins, although there is a rather large difference in the Q band position and intensity between the original and reduced species. The resonance-Raman spectra of $[Fe^{II}(tpp)(NO)]$ and its one-electron adducts in tetrahydrofuran were also measured by Choi *et al.*[23] Their results that the reduction of $[Fe^{II}(por)(NO)]$ caused a decrease in ν_{NO} and an increase in ν_{Fe-N} were consistent with the addition of the electron to the half-filled orbital of $(d_{z^2} + \sigma_N)$.

In aqueous solution, however, neither the visible nor the resonance-Raman spectra of one-electron adducts of iron(II) nitrosyl porphyrins have been yet reported, possibly due to their instability in aqueous solution. Our present methods using laser photolysis made it possible to measure their visible spectra easily even in aqueous solutions. The Soret peak position of the electron adduct $[Fe^{II}(tpps)(NO)]^{4-}$ in aqueous solution (418 nm) is only slightly shifted from that of the original nitrosyl porphyrin (413 nm), as noted above. The small change of the Soret band upon reduction is analogous to the behaviour in the case of the electrochemical reduction in non-aqueous solutions.[20,23] This analogous behaviour in the spectral change upon reduction also supports our above conclusion that the electron transfer from the ketyl radical to $[Fe^{II}(tpps)(NO)]^{4-}$ occurs to produce the one-electron adduct of $[Fe^{II}(tpps)(NO)]^{4-}$ where the added electron possibly exists on the coordinated NO moiety. In order to elucidate detailed structure of the electron adduct of $[Fe^{II}(tpps)(NO)]^{4-}$, we are preparing to measure the resonance-Raman spectrum of this adduct.

References

1 S. Moncada, R. M. J. Palmer and E. A. Higgs, *Pharmacol. Rev.*, 1991, **43**, 109.
2 A. R. Butler and D. L. H. Williams, *Chem. Soc. Rev.*, 1993, 233.
3 T. Yonetani and H. Yamamoto, in *Oxidases and Related Redox Systems, Vol. I*, ed. T. E. King, S. Mason and M. Morrison, University Park Press, Baltimore, 1973, p. 278.
4 G. Palmer, in *The Porphyrins, Vol. IV, Physical Chemistry, Part B*, ed. D. Dolphin, Academic Press, New York, 1979, ch. 6.
5 M. Hoshino, K. Ozawa, H. Seki and P. C. Ford, *J. Am. Chem. Soc.*, 1993, **115**, 9568.
6 B. Heiss, K. Frunzke and W. G. Zumft, *J. Bacteriol.*, 1989, **171**, 3288.
7 G. J. Carr and S. J. Ferguson, *Biochem. J.*, 1990, **269**, 423.
8 M. Dermastia, T. Turk and T. C. Hollocher, *J. Biol. Chem.*, 1991, **266**, 10899.
9 W. G. Zumft, *Arch. Microbiol.*, 1993, **160**, 253.
10 A. M. Jones and T. C. Hollcher, *Biochim. Biophys. Acta*, 1993, **1144**, 359.
11 D. H. W. Kastrau, B. Heiss, P. M. H. Kroneck and W. G. Zummft, *Eur. J. Biochem.*, 1994, **222**, 293.
12 R. W. Ye, B. A. Averill and J. M. Tiedje, *Appl. Environ. Microbiol.*, 1994, **60**, 1053.
13 K. Nakahara, T. Tanimoto, K. Hatano, K. Usuda and H. Shoun, *J. Biol. Chem.*, 1993, **268**, 8350.
14 T. Turk and T. C. Hollocher, *Biochem. Biophys. Res. Commun.*, 1992, **183**, 983.
15 Y. Shiro, M. Fujii, T. Iizuka and S. Adachi, K. Tsukamoto, K. Nakahara and H. Shoun, *J. Biol. Chem.*, 1995, **270**, 1617.
16 M. E. Murphy and H. Sies, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 10860.
17 M. H. Barley, K. Takeuchi, W. R. Murphy, Jr. and T. J. Meyer, *J. Chem. Soc., Chem. Commun.*, 1985, 507.
18 M. H. Barley, K. Takeuchi and T. J. Meyer, *J. Am. Chem. Soc.*, 1986, **108**, 5876.
19 M. H. Barley, M. R. Rhodes and T. J. Meyer, *Inorg. Chem.*, 1987, **26**, 1746.
20 D. Lancon and K. M. Kadish, *J. Am. Chem. Soc.*, 1983, **105**, 5610.
21 E. Fujita and J. Fajer, *J. Am. Chem. Soc.*, 1983, **105**, 6743.
22 I-K. Choi and M. D. Ryan, *Inorg. Chim. Acta*, 1988, **153**, 25.

23 I-K. Choi, Y. Liu, D. Feng, K. Paeng and M. D. Ryan, *Inorg. Chem.*, 1991, **30**, 1832.
24 J. N. Younanthan, K. S. Wood and T. J. Meyer, *Inorg. Chem.*, 1992, **31**, 3280.
25 E. B. Fleischer, J. M. Palmer, T. S. Srivastava and A. Chatterjee, *J. Am. Chem. Soc.*, 1971, **93**, 3162.
26 *Kagakubinran*, Japan Chem. Soc., Maruzen, Tokyo, 1984, vol. II, p. 158.
27 H. Seki, *J. Chem. Soc., Faraday Trans.*, 1992, **88**, 35.
28 M. Hoshino and H. Shizuka, in *Photoinduced Electron Transfer, Part C, Photoinduced Electron Transfer Reactions: Organic Substrates*, ed. M. A. Fox and M. Chanon, Elsevier, Amsterdam, 1988, p. 313 and references therein.
29 M. Hoshino and H. Shizuka, in *Radiation Curing in Polymer Science and Technology, Vol. II, Photoinitiating Systems*, ed. J. P. Fouassier and J. F. Rabek, Elsevier Applied Science, London and New York, 1993, ch. 15.
30 M. V. Encinas, E. A. Lissi and A. F. Olea, *Photochem. Photobiol.*, 1985, **42**, 347.
31 C. Bizet, P. Morliere, D. Brault, O. Delgado, M. Bazin and R. Santus, *Photochem. Photobiol.*, 1981, **34**, 315.
32 R. V. Bensasson and J-C. Gramain, *J. Chem. Soc., Faraday Trans. 1*, 1980, **76**, 1801.
33 J-H. Fuhrhop, in *Struct. Bonding (Berlin)*, 1974, **18**, 1.
34 R. H. Felton, in *The Porphyrins, Vol. V, Physical Chemistry, Part C*, ed. D. Dolphin, Academic Press, New York, 1978, ch. 3.
35 D. Brault, R. Santus, E. J. Land and A. J. Swallow, *J. Phys. Chem.*, 1984, **88**, 5836.

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