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RATE-ENHANCING EFFECT OF INTRAMOLECULAR LINKAGE OF FLAVIN-PORPHYRIN ON REDUCTION BY 1,4-DIHYDROPYRIDINE

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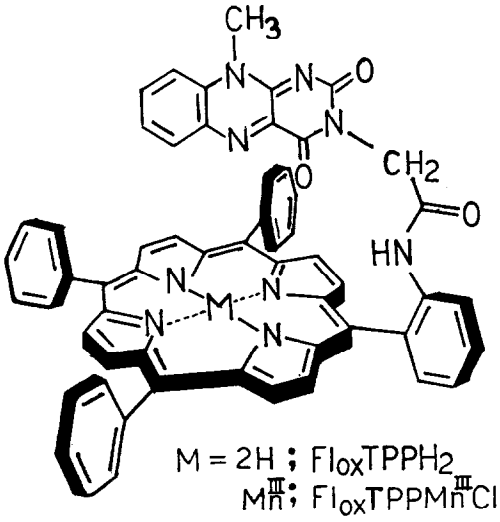
Summary; Intramolecular linked flavin-manganeseporphyrin has been synthesized. This flavin-metalloporphyrin was efficiently reduced by N-benzyl-1,4-dihydronicotinamide(BzNADH) compared with these intermolecular system.

In biological electron transfer, flavocoenzymes accept two electrons from organic reductants such as NAD(P)H and transport one electron to a metal-containing acceptor such as heme, iron-sulfur and molybdenum sites in protein; in other words they serve as two-electron/one-electron transfer carriers .[1] It is known that several flavocoenzymes exist as metalloflavoproteins,[2] but the redox mechanism of these enzymes is obscure, since the component system of chromophores is complicated. Recently, several model systems have been used in attempts to elucidate the nature of the $2e^-/1e^-$ transfer process and the transient metal-flavin interactions,[3] but these models are very different from the biological system of flavin and metal.



We report here the results of kinetic studies on the electron transfer reaction of flavin-linked manganese-tetraphenylporphyrin ($Fl_{ox}TPPMn^{III}Cl$).

$Fl_{ox}TPPH_2$ was synthesized[4] by condensing mono-(o-aminophenyl)triphenyl-porphyrin[5] and 10-methylisoalloxazine-3-acetyl chloride, which was obtained from its acid derivative[6] and thionyl chloride.

The structure was confirmed by the ^{1}H-NMR, IR, UV and mass spectra and elemental analysis.[7] Insertion of manganese(III) was carried out with $Mn(OAc)_2$ in DMF at 130°C.[8] The cyclic voltammetric study in DMF showed two reversible redox couples at $E_{1/2}^1$ = -0.21 V vs. SCE (Mn^{III}/Mn^{II}) and $E_{1/2}^2$ = -0.655 V ($Fl_{ox}/Fl^{\cdot-}$). The first redox potential, $E_{1/2}^1$ was almost the same value compared with $TPPMn^{III}Cl$[9] (-0.20 V), but the second redox potential, $E_{1/2}^2$, was positively shifted compared with Fl_{ox}[9] (-0.74 V).

The electron transfer from BzNADH to $Fl_{ox}TPPMn^{III}Cl$ was investigated spectrophotometrically by following the decrease of the absorption (466 nm) of the Mn^{III} moiety in $Fl_{ox}TPPMn^{III}Cl$ under anaerobic conditions in ethanol solution. Introduction of air into the cuvette after complete reduction regenerated at least 90% of the starting $Fl_{ox}TPPMn^{III}Cl$ as determined by spectrophotometry. The UV-VIS spectral change accompanying this reaction has clear isosbestic points at 406, 449, 540, 560 and 578 nm (Fig. 1). Pseudo-first-order plots, $\ln(A_0-A_\infty/A_t-A_\infty)$ vs. time, indicated that $Fl_{ox}TPPMn^{III}Cl$ reduction by BzNADH followed pseudo-first-order kinetics up to more than three half-lives (Fig. 2). The observed pseudo-first-order rate constant k_{obsd} (the slope in Fig. 2) was linearly dependent on BzNADH concentration in the range of 0 - 7.5×10^{-3} M.

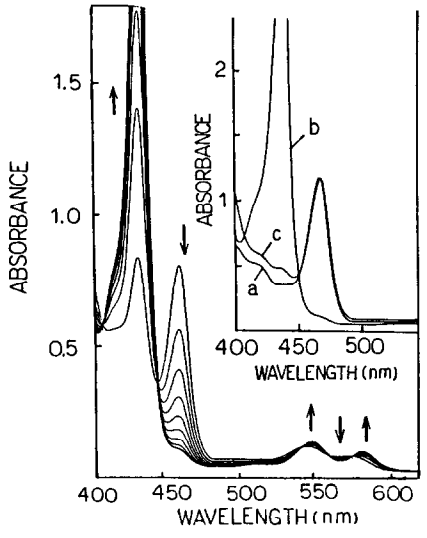


Fig.1 Repeative scans for the intramolecular electron transfer reaction of [BzNADH] = 1.36×10^{-3} M with [$Fl_{ox}TPPMn^{III}Cl$] = 1×10^{-5}M in EtOH at 30°C. Arrows indicate progression of the reaction. Inset ; Vis.spectra of a) oxidized b) rednced and c) re-oxidized with air.

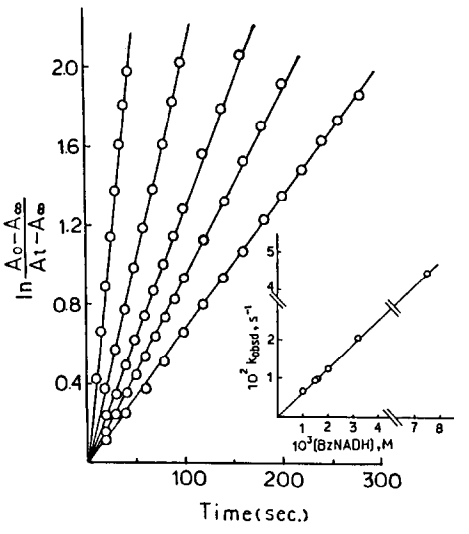


Fig.2 Pseudo-first-order plots for the intramolecular electron transfer reaction of[BzNADH] with $Fl_{ox}TPPMn^{III}Cl$ in EtOH at 30°C. Inset ; Dependence of BzNADH upon k_{obsd}.

In the intermolecular electron transfer system (BzNADH, Fl_{ox}, $TPPMn^{III}Cl$), the observed electron transfer rate was zero-order with respect to $TPPMn^{III}Cl$ under pseudo-order conditions, $[BzNADH]_0 >> [Fl_{ox}]_0 \sim [TPPMn^{III}Cl]$, and the pseudo-zero-order rate constant was linearly dependent upon both $[BzNADH]_0$ and $[Fl_{ox}]_0$. The rate constant of direct electron transfer from BzNADH to $TPPMn^{III}Cl$ (in the absence of flavin) was negligible (Fig. 3).

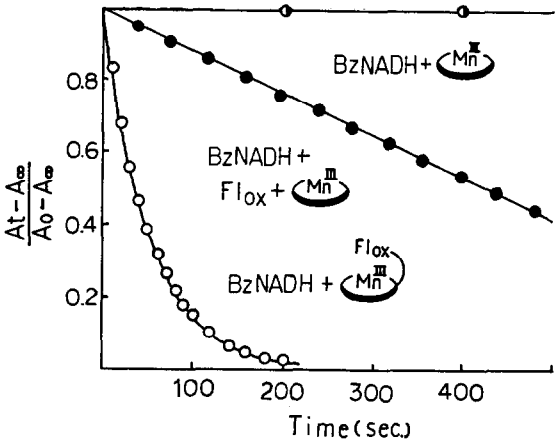


Fig.3 Absorbance change, (A_t-A_∞ / A_0-A_∞) at 466 nm, vs. time plots for the electron transfer reactions in EtOH at 30°C.

intramolecular(—○—)
intermolecular(—●—)
direct(—◑—) system

$[BzNADH]=3.0\times10^{-3}M$
$[TPPMn^{III}Cl]=1\times10^{-5}M$
$[Fl_{ox}]=1\times10^{-5}M$
$[Fl_{ox}TPPMn^{III}Cl]=1\times10^{-5}M$

These results indicate that flavin reduction by BzNADH was the rate-determining step of both the inter- and intramolecular electron transfer reactions and that the subsequent one-electron transfer from reduced and semiqinone flavin to manganese(III)porphyrin was very fast. The reaction rate was enhanced in the intramolecular system: the second-order-rate constants, k^{2nd}, calculated by means of equations (1) and (2) were $k^{2nd}=6.36\pm0.20\ M^{-1}s^{-1}$ for the intramolecular system and $k^{2nd}=0.794\pm0.029\ M^{-1}s^{-1}$ for the intermolecular system.[10] Thus the acceleration factor (the ratio of k^{2nd}) for the intra- vs. intermolecular system was 8.0.

$$v = k^{2nd}[BzNADH]_0[Fl_{ox}TPPMn^{III}Cl] \cdots\cdots\cdots\cdots\cdots (1)$$

$$v = k^{2nd}[BzNADH]_0[Fl_{ox}]_0 \cdots\cdots\cdots\cdots\cdots\cdots\cdots (2)$$

This intramolecular effect can not be interpreted simply, because the complicated factors are probably included. But the two driving forces for the electron transfer can be discussed; i) the positive shift of redox potential for $Fl_{ox}/Fl^{\cdot-}$ which induces the rate enhancement. ii) The transient-state character,that is to say, there is no appreciable interaction between oxidized flavin and manganese(III)tetraphenylporphyrin in the initial state, but the two components must be brought close together. The transient state may be a ternary complex such as $[BzNADH\cdots\overline{Fl_{ox}\cdots TPPMn^{III}Cl}]$ in which the flavin interacts with both BzNADH and $TPPMn^{III}Cl$. The positive shift of the redox potential and the formation of the ternary complex facilitates the electron transfer.

In conclusion, flavin mediated the electron transfer from BzNADH to $TPPMn^{III}Cl$. Intramolecular linkage of flavin and porphyrin accelerated this reaction.

References and Notes

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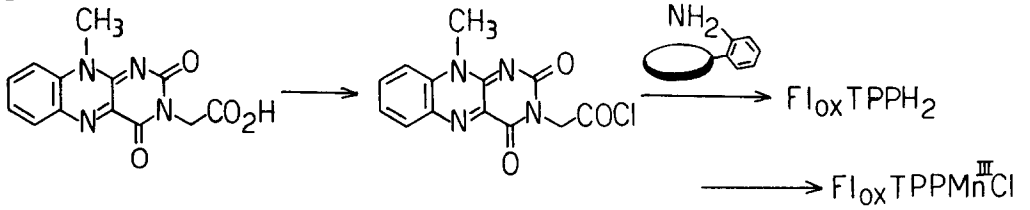
4) Synthetic route.



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7) ^1H-NMR ($CDCl_3$) δ-3.51 (2H, s, internal pyrr.NH), 1.54 (3H, s, $N^{10}CH_3$), 4.25 (2H, s, $-CH_2-$) and aromatic regions. UV-VIS. ($CHCl_3$) λ_{max} 272, 418, 515, 548, 588, 645 (nm). IR.(KBr disk) 1695, 1664, 1555 (cm^{-1}). MS.(FAB) m/z 901 (M^++4). Elemental Anal. Calcd. for $C_{57}H_{39}N_9O_3 3.5H_2O$ C, 71.24; H, 4.82; N, 13.12. Found C, 71.46; H, 4.45; N, 12.54.

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9) Abrreviation; $TPPMn^{III}Cl$, manganese(III)tetraphenylporphyrin chloride. Fl_{ox}, 3,10-dimethylisoalloxazine.

10) The detail discussion will be published elsewhere.

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