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Photoinduced Cross Coupling Reaction of Covalently Linked Tyrosine-Pyropheophorbide a and Tyrosine-9-Desoxomesopyropheophorbide a to 1,4-Benzoquinone

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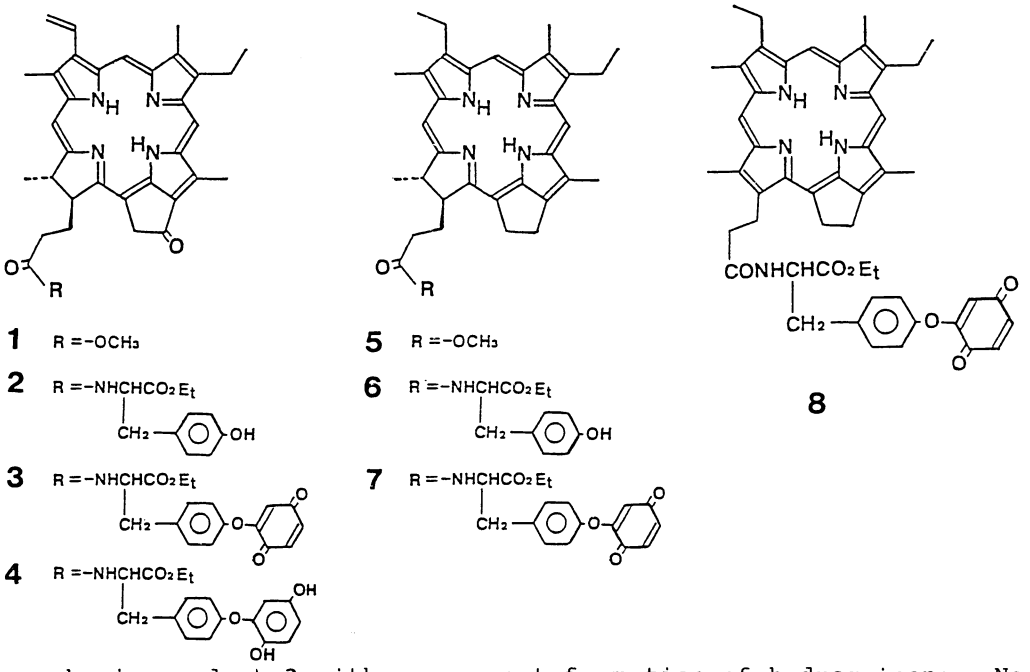
Irradiation of tyrosine-linked pyropheophorbide a and 1,4-benzoquinone (**BQ**) in benzene led to a clean formation of a quinone-linked product, while photoreaction of 9-desoxomesopyropheophorbide a and **BQ** produced a quinone-linked 9-desoxomesopyropheophorbide a and a quinone-linked 9-desoxophylloerythrin a.

Recently the organization of photoactive pigments as well as the surrounding proteins in bacterial photosynthetic reaction center has been revealed by X-ray diffraction technique.[1] These results strongly indicate important roles of amino-acid chains in tuning the primary events in the photosynthetic reaction center. Among these, tyrosine L-162 is located halfway between the special pair and the nearest heme group of cytochrome subunit. This specific arrangement is conserved in Rhodopseudomonas viridis and Rhodobacter sphaeroides, and may play a role in re-reducing the photo-oxidized special pair. Additionally, it has been postulated that tyrosine radical is involved in the photosynthetic oxygen-evolving system, in which the tyrosine acts as electron donor to $P680^{+}$.[2]

We reported recently the photo-induced coupling reaction of tyrosine-linked mesoporphyrins with a variety of quinones.[3] It is of mechanistic interest that the coupling reaction takes place via capture of a photogenerated ion radical pair of porphyrin cation radical and quinone anion radical by the tyrosine residue. We now wish to report here that the photo-induced coupling reaction to quinone occurs similarly for chlorophyll-derived chlorin macrocycles such as a tyrosine-linked pyropheophorbide a **2** and a tyrosine-linked 9-desoxo-meso-pyropheophorbide a **6**.

Pyropheophorbide a[4] was activated with 2-chloro-1-methylpyridinium iodide and triethylamine in CH_2Cl_2, followed by treatment with 5 equiv. of tyrosine ethyl ester hydrochloride to give the tyrosine-linked pyropheophorbide a **2** in 59% yield.[5] Reduction of **2** to the tyrosine-linked 9-desoxomesopyropheophorbide a **6** was accomplished by use of ZnI_2 and $NaCNBH_4$[6] in 54% yield. The absorption and emission properties of tyrosine-linked model compounds are listed in Table 1. In both chromophores, the attached tyrosine residues do not alter the properties of the singlet and triplet excited states.

Irradiation of **2** (0.2 mM) in benzene in the presence of **BQ** (5 mM) with light of wavelength longer than 590 nm under an argon atmosphere produced a



single porphyrin product **3** with concurrent formation of hydroquinone. No other product except unchanged starting materials was detected by HPLC or 400 MHz ^{1}H-NMR analysis. Separation by flash column chromatography yielded **3** in 56% yield. The structure of **3** was fully characterized by the 400 MHz ^{1}H-NMR spectra and the mass spectra (calcd for $C_{50}H_{50}N_5O_7$, M^++1, 832.3713; found 832.3729).[7] The quinone end in **3** appeared at 5.49(d, J=2.4 Hz), 6.60(dd, J=2.4 and 10.4 Hz), and 6.68 ppm(d, J=10.4 Hz). In accord with the quinone-linked structure, the relative fluorescence quantum yield of **3** decreased to 17% of that of **1** and the fluorescence decay profile displayed three components, 0.16 ns (28%), 1.26 ns (67%) and 5.22 ns (6%).[8]

The relative quantum yields for the formation of **3** were dependent upon the quinone concentration, reaching a maximum at [**BQ**]= 2 mM and decreasing with increasing **BQ** concentration. This concentration dependence strongly indicates that the formation of **3** is derived from quenching of the excited triplet state of **2** by **BQ**, since at high **BQ** concentration almost all of the excited singlet state of **2** will be quenched by **BQ**. This mechanism was also supported by observation of photo-CIDNP effects shown in Fig. 1. In addition to the enhanced absorption of **BQ** (H_e) and the emission of $\mathbf{BQH_2}$ (H_d), the CIDNP effects due to

Table 1. Absorption and emission properties of **1**, **2**, **5**, and **6**

Compound	Abosrption (λ_{max}/ nm)	Fluorescence (λ_{em}/ nm)	$f^{a)}$	(τ_f/ ns)$^{b)}$	T_1-T_n absorption (λ_{max}/ nm)	(τ_t/ ms)$^{c)}$
1	414, 510, 540, 609, 669	674	1.0	6.8	460	0.6
2	414, 511, 541, 610, 669	676	0.95	6.6	460	0.5
5	396, 499, 528, 582, 638	641	1.0	5.4	445	0.6
6	396, 499, 528, 582, 638	641	1.0	5.3	445	0.6

a) Relative fluorescence quantum yields of **2** and **6** to those of **1** and **5**, respectively. b) Fluorescence lifetimes measured in air saturated CH_2Cl_2 at 25 °C. c) Triplet lifetimes measured in degassed toluene solution at 25 °C.

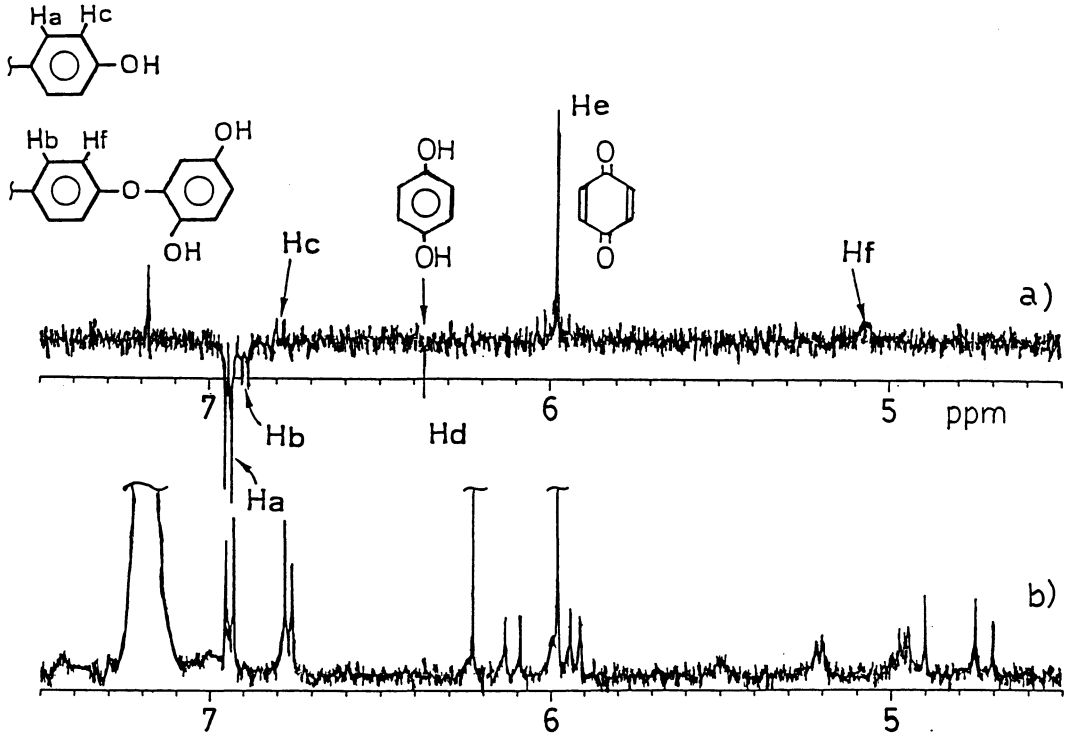


Fig. 1. a, 400MHz photo-CIDNP difference (light-dark) spectrum of **2** (2mM) and **BQ** (5mM)-in benzene-d_6, 32 scans; b, dark spectrum.

the aromatic protons of the tyrosine residue (H_a and H_c) as well as those (H_b and H_f) of hydroquinone-linked pyropheophorbide a **4** were observed. The latter signals were assigned by comparison with the authentic sample of **4**, which was prepared by reduction of **3** with $Na_2S_2O_4$. These CIDNP effects due to the coupling product **4** as well as the starting material **2** clearly indicate the photo-induced coupling reaction takes place via in-cage combination of triplet neutral radical pair composed of tyrosine-derived phenoxy radical and semiquinone radical.[9] The hydroquinone adduct **4** thus formed will be instantaneously photooxidized to **3** by **BQ**. Since the quenching of the excited triplet state of **2** by **BQ** leads to one electron transfer from the pyropheophorbide ring to **BQ**, it may follow that the tyrosine residue acts as proton donor to quinone anion radical and electron donor to pyropheophorbide cation radical, giving the triplet neutral radical pair.

Under similar conditions, irradiation of a benzene solution of **6** and **BQ** led to the formation of two products, a quinone-linked 9-desoxomesopyropheophorbide a **7** (45%) and a quinone-linked 9-desoxophylloerythrin a **8** (22%).[7] The latter photoproduct **8** exhibited the absorption and emission spectra characteristic of porphyrin chromophore, consistent with the assigned structure. Oxidative dehydrogenation through a cation radical intermediate, which may account for the formation of **8**, has never been observed in photoreaction of **2** with **BQ**.

Based on these results, it may be considered that 9-carbonyl group in pyropheophorbide and chlorophyll chromophores contributes the stabilities of their cation radical under photolytic conditions in the presence of quinone.[10]

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References

1) J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, J. Mol. Biol., 180, 385 (1984); H. Michel, O. Epp, and J. Deisenhofer, EMBO J., 5, 2445 (1986).

2) B. A. Barry and G. T. Babcock, Proc. Natl. Acad. Sci. U.S.A., 84, 7099 (1987); R. J. Debus, B. A. Barry, G. T. Babcock, and L. McIntosh, ibid., 85, 427 (1988).

3) A. Osuka and K. Maruyama, J. Chem. Res., 1987, (S), 286; (M), 2401.

4) K. Iriyama, N. Ogura, and A. Takamiya, J. Biochem., 76, 901(1974).

5) S. G. Boxer and R. R. Bucks, Israel J. Chem., 21, 259 (1981); T. Mukaiyama, M. Usui, and K. Saigo, Chem. Lett., 1976, 49.

6) C. K. Lau, C. Dufresne, P. C. Belanger, S. Pieltre, and J. Scheigetz, J. Org. Chem., 51, 3038 (1986).

7) All new compounds described in this paper gave satisfactory spectral data consistent with the assigned structures. Selected physical properties for **3**; UV (λ_{max}, in CH_2Cl_2; 668, 607, 539, 508, and 414 nm); ^{1}H-NMR($CDCl_3$) 9.48(s, meso, 1H), 9.39(s, meso, 1H), 8.55(s, meso, 1H), 8.00(dd, J=11.7 and 18.1 Hz, 1H), 6.83(d, J=8.3 Hz, 2H), 6.74(d, J=8.3 Hz, 2H), 6.26(dd, J=1.5 and 18.1 Hz, 1H), 6.18(dd, J=1.5 and 11.7 Hz, 1H), 5.51(s, NH, 1H), 5.24 and 5.12(ABq, J=20 Hz, 2H), -1.70(br, NH, 1H), -3.53(br, NH, 1H). **7**; UV (λ_{max} in CH_2Cl_2, 639, 526, 498, and 395 nm); ^{1}H-NMR($CDCl_3$) 9.79(s, meso, 1H), 9.60(s, meso, 1H), 8.91(s, meso, 1H), 6.68(d, J=8.3 Hz, 2H), 6.63(d, J=10.3 Hz, quinone-ring 1H), 6.60(d, J=8.3 Hz, 2H), 6.54(dd, J=2.4 and 10.3 Hz, quinone-ring 1H), 5.38(d, J=2.4 Hz, 1H), -1.68(br, NH, 1H), and -3.52(br, NH, 1H); MS (FAB, m/e, 822 (M^++3)). **8**; UV (λ_{max} in CH_2Cl_2, 640, 618, 565, 499, and 399 nm); fluorescence (λ_{em} in CH_2Cl_2, 619 and 690 nm); ^{1}H-NMR($CDCl_3$) 10.04(s, meso, 1H), 9.94(s, meso, 1H), 9.93(s, meso, 1H), 6.60(d, J=10.3 Hz, quinone-ring 1H), 6.57(dd, J=2.4 and 10.3 Hz, quinone-ring 1H), 5.96(d, J=8.3 Hz, 2H), 5.36(d, J=8.3 Hz, 2H), 4.54(d, J=2.4 Hz, quinone-ring 1H), 3.74(s, Me), 3.66(s, Me), 3.58(s, Me), 3.52(s, Me), -2.88(br, NH, 1H), and -3.50(br, NH, 1H); MS (FAB, m/e, 820(M^++1)).

8) Fluorescence lifetimes were measured by time-correlated single photon counting technique. S. Hirayama and Y. Shimono, J. Chem. Soc., Faraday Trans. 2, 80, 941 (1984).

9) For H_b in **4**, $\Delta g < 0$, $a_H > 0$, $\varepsilon > 0$, $\mu > 0$, and $\Gamma_{ne} < 0$. Consequently, it is concluded that in-cage recombination of triplet radical pair leads to the formation of **4**. R. Kaptein, J. Chem. Soc., Chem. Commun., 1971, 732.

10) Easier dehydrogenation of 9-desoxomesopyropheophorbide than pyropheophorbide was also reported. K. M. Smith, K. C. Langry, and O. M. Minnetian, J. Org. Chem., 49, 4602 (1984)

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