Photoinduced Electron Transfer from a Porphyrin to an Electron Acceptor in an Antibody-Combining Site

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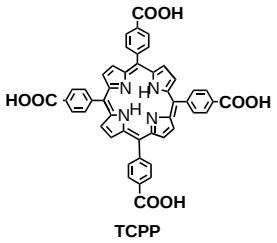
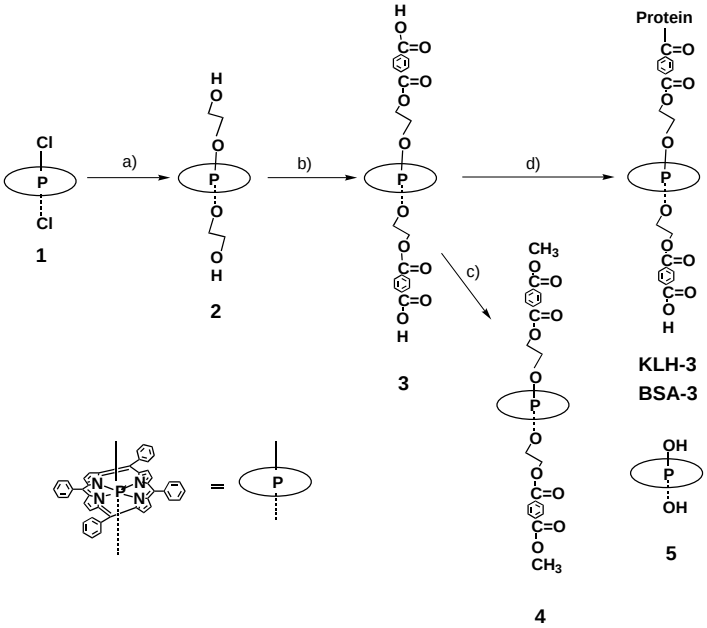
Much effort has been directed toward mimicking the electron transfer of natural photosynthetic systems.[1] Electron transfer in covalently linked donor–acceptor systems has been extensively studied.[2] Recently, attention has been focused on the design of noncovalently assembled donor–acceptor arrays[3, 4] because the chromophores of the in vivo photosynthetic reaction center are not linked covalently through spacer groups but simply held in space by the protein environment. In order to incorporate both artificial porphyrins (electron donors) and electron acceptors into the protein domain, it seemed most appropriate to make monoclonal antibodies for porphyrin–acceptor systems.

Previously, we reported preparation of monoclonal antibodies against *meso*-tetrakis(4-carboxyphenyl)porphyrin (TCPP)[5] and found that one of these antibodies binds not only TCPP but also a TCPP–metal complex (metal = Cu, Zn, Fe).[6] We also found that the monoclonal antibody showed catalytic activities[7] and could control photoinduced electron transfer from a zinc porphyrin to electron acceptor molecules by the formation of the complexes between the antibodies and the zinc porphyrin.[8]



It is more important to design an antibody combining site that can accommodate not only metalloporphyrins but also organic substrates[9] or electron acceptors. Herein, we report the first example of the design and preparation of such an antibody-combining site for both a porphyrin and an electron acceptor. Monoclonal antibodies are raised against an porphyrin which is linked to an electron acceptor by a spacer. A monoclonal antibody was then used to bind the porphyrin and the acceptor without a formal covalent linkage. The complementary combining sites for the porphyrin and acceptor, when linked, were found to have sufficient binding strength to bind the separate moieties with high selectivity. We also found that the antibody binds both porphyrin and an acceptor simultaneously and thereby the antibody facilitates the electron transfer from the porphyrin to an acceptor.

Phosphorus(v) porphyrin was used as a hapten because these complexes are able to form stable axial bonds from the central phosphorus atom.[10] Water-stable, axial dialkoxy hypervalent phosphorus(v) porphyrins were synthesized by efficient substitution of the axial chloride ligands in dichlorophosphorus(v) tetraphenylporphyrin **1**[11] (Scheme 1).



Scheme 1. Synthesis of the antigen. a) Ethylene glycol, b) terephthaloyl chloride/pyridine, c) methanol/pyridine, d) 1) EDC/DMF, 2) protein/phosphate buffer (pH 7). EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Protein = keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA).

Five monoclonal antibodies have been obtained by immunization of KLH-conjugated porphyrin **3** (**KLH-3**)[12] to Balb/c mice. One of these antibodies, 74D7A, binds the antigen strongly (dissociation constant $K_d = 2.2 \times 10^{-7}$ M). The antibody also shows a high selectivity. Antibody 74D7A binds **BSA-3** but not TCPP, as shown by an enzyme-linked immunosorbent assay (ELISA) as well as by the absorption and emission spectra. Although antibody 74D7A does not bind TCPP, it binds **2**, **3**, and **5** with K_d values of 7.5×10^{-7}, 2.2×10^{-7}, and 2.1×10^{-6} M, respectively. The differences of dissociation constants for the complexes between antibody 74D7A and these porphyrins indicate that the antibody recognizes not only the porphyrin moiety but also the axial ligands.

The antibody was found to bind terephthalic acid. Figure 1 shows Klotz plots for benzoic acid and phthalic acid derivatives. Table 1 shows the dissociation constants of the complexes between antibody 74D7A and various substrates. Although the dissociation constant of the antibody with benzoic acid is 1.1×10^{-2} M, that of the antibody with terephthalic acid is 1.2×10^{-5} M; the affinity of the antibody for terephthalic acid is about 1000 fold greater than that for benzoic acid. The antibody shows an excellent specificity to the part of the axial ligands.

Terephthalic acid and its derivatives act as an electron acceptor[13] for porphyrins. Fixation of terephthalic acid to the

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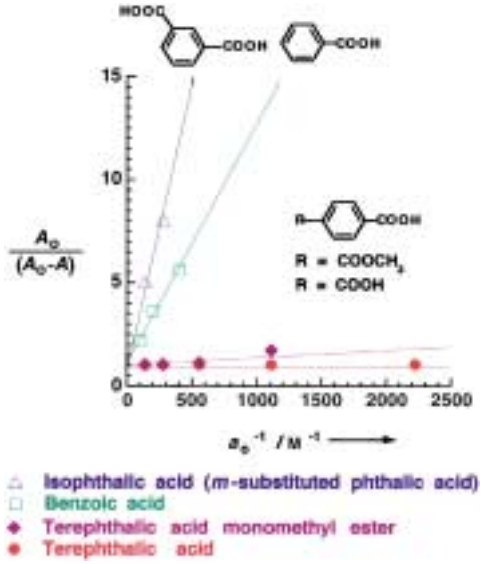

Figure 1. Binding of benzoic acid and phthalic acid derivatives as shown by a Klotz plot. Klotz equation: $A_0/(A_0-A)=1+K_d/a_0$, where A_0 or A are the ELISA absorbances at 405 nm in the absence or presence of substrates, a_0 is the total substrate concentration, and K_d is the dissociation constant.

Table 1. Dissociation constants of the complexes between antibody 74-D7A and benzoic acid or phthalic acid derivatives.

Substrate		Dissociation constant K_d [M]
HOOC-⬡		1.1×10^{-2}
HOOC-⬡(COOH)	*ortho*	$<10^{-1}$
	meta	3.0×10^{-2}
	para	1.2×10^{-5}
HOOC-⬡-$COOCH_3$		4.5×10^{-5}

axis of porphyrin induces the changes of photochemical properties of phosphorus(v) porphyrin. For example, the lifetime of the singlet excited state of **KLH-3** is shorter than that of porphyrin **2** (**KLH-3**: 0.4 ns; **2**: 4.4 ns; in phosphate borate buffer/CH_3CN). The fluorescence quenching and the shortened excited state lifetime of **KLH-3** are considered to be due to an intramolecular electron transfer between the porphyrin and the terephthaloyl moiety. When the complex between porphyrin **2** and antibody 74D7A is formed, it is expected that the antibody may provide a space where acceptor molecules may enter. Table 2 shows the degree of fluorescence quenching of porphyrins on addition of terephthalic acid or isophthalic acid in the presence of the antibody. A 20 % fluorescence quenching of **2** in the presence of antibody 74D7A was observed on addition of terephthalic acid. This degree of quenching on addition of terephthalic acid is ten-fold greater than that of added isophthalic acid. Under the same conditions (**2** (8.0×10^{-7} M) and terephthalic acid (1.6×10^{-6} M)), the quenching behavior was not observed in the absence of the antibody. The titration experiments of fluorescence quenching of **2** with terephthalic acid showed that terephthalic acid–antibody binding is at a 1:1 molecular ratio.

Table 2. Fluorescent quenching of porphyrins on addition of terephthalic acid or isophthalic acid in the presence of antibody 74D7A.

Porphyrin	Substrate	Quenching[a] [%]
2	HOOC-⬡-COOH	20
2	HOOC-⬡(COOH)	2
TCCP	HOOC-⬡(COOH)	5

[a] Fluorescent quenching = $(1-I/I_0)\times100$. I_0 = fluorescent intensity of the porphyrin in the presence of antibody 74D7A; I = as I_0 but with substrate present.

The fluorescence decay of porphyrin **2** in the presence of the antibody was expressed by a monoexponential curve with a lifetime of 4.5 ns. Addition of terephthalic acid shortened the lifetime to 0.3 ns.[14] On the other hand, the lifetime of **2** with terephthalic acid was 4.5 ns in the absence of antibody 74D7A. These results show that the electron transfer from porphyrin to an electron-accepting molecule occurred in the antibody combining site. The rate constant for the electron transfer from the porphyrin to terephthalic acid in the combining site was estimated to be $3.1\times10^9\ s^{-1}$. Figure 2 shows a schematic representation of donor–acceptor system in the antibody combining site.

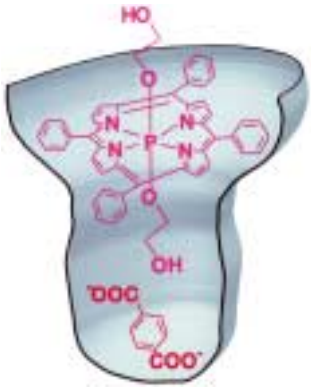

Figure 2. Representation of the donor–acceptor system in the antibody combining site.

In conclusion, one of the antibodies elicited by an antigen containing both phosphorus(v) porphyrin and terephthalic acid positioned axially shows high selectivity for both porphyrins and axial parts. Moreover, the antibody was found to bind both the porphyrin and terephthalic acid simultaneously and that the specific insertion of terephthalic acid as an electron acceptor into the antibody combining site makes it possible to facilitate the electron transfer from the porphyrin to the acceptor molecule. Detailed investigation of the mechanism is underway.

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[1] a) J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel, *J. Mol. Biol.* **1984**, *180*, 385–398; b) J. Deisenhofer, H. Michel, *Angew. Chem.* **1989**, *101*, 872–892; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 829–847; c) D. Gust, T. A. Moore, *Science* **1989**, *244*, 35–41; d) R. Huber, *Angew. Chem.* **1989**, *101*, 849–871; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 848–869.

[2] a) A. Osuka, K. Maruyama, N. Mataga, T. Asahi, I. Yamazaki, N. Tamai, *J. Am. Chem. Soc.* **1990**, *112*, 4958–4959; b) M. R. Wasielewski, *Chem. Rev.* **1992**, *92*, 435–461; c) A. Giraudeau, L. Ruhlmann, L. El Kahef, M. Gross, *J. Am. Chem. Soc.* **1996**, *118*, 2969–2979; d) A. Osuka, S. Marumo, N. Mataga, S. Taniguchi, T. Okada, I. Yamazaki, Y. Nishimura, T. Ohno, K. Nozaki, *J. Am. Chem. Soc.* **1996**, *118*, 155–168; e) K. Uosaki, T. Kondo, X.-Q. Zhang, M. Yanagida, *J. Am. Chem. Soc.* **1997**, *119*, 8367–8368; f) K. Tsukahara, N. Sawai, S. Hamada, K. Koji, Y. Onoue, T. Nakazawa, R. Nakagaki, N. Nozaki, T. Ohno, *J.*

  1433-7851/00/3921-3830 $ 17.50+.50/0

Phys. Chem. B **1999**, *103*, 2867 – 2877; g) S. Tsuchiya, *J. Am. Chem. Soc.* **1999**, *121*, 48 – 53; h) H. Tsue, H. Imahori, T. Kaneda, Y. Tanaka, T. Okada, K. Tamaki, Y. Sakata, *J. Am. Chem. Soc.* **2000**, *122*, 2279 – 2288.

[3] a) A. M. Brun, A. Harriman, V. Heitz, J. P. Sauvage, *J. Am. Chem. Soc.* **1991**, *113*, 8657 – 8663; b) A. M. Brun, S. J. Atherton, A. Harriman, V. Heitz, J. P. Sauvage, *J. Am. Chem. Soc.* **1992**, *114*, 4632 – 4639; c) J. L. Sessler, B. Wang, A. Harriman, *J. Am. Chem. Soc.* **1993**, *115*, 10418 – 10419; d) J. L. Sessler, B. Wang, A. Harriman, *J. Am. Chem. Soc.* **1995**, *117*, 704 – 714; e) A. Berman, E. S. Izraeli, H. Levanon, B. Wang, J. L. Sessler, *J. Am. Chem. Soc.* **1995**, *117*, 8252 – 8257; f) M. Linke, J.-C. Chambron, V. Heitz, J.-P. Sauvage, *J. Am. Chem. Soc.* **1997**, *119*, 11329 – 11330; g) M. D. Ward, *Chem. Soc. Rev.* **1997**, *26*, 365 – 375; h) T. D. Ros, M. Prato, D. Guldi, E. Alessio, M. Ruzzi, L. Pasimeni, *Chem. Commun.* **1999**, 635 – 636; i) A. Berg, Z. Shuali, M. Someda, H. Levanon, M. Fuhs, K. Möbius, R. Wang, C. Brown, J. L. Sessler, *J. Am. Chem. Soc.* **1999**, *121*, 7433 – 7434; j) D. J. Fermín, H. D. Duong, Z. Ding, P.-F. Brevet, H. H. Girault, *J. Am. Chem. Soc.* **1999**, *121*, 10203 – 10210; k) M. Andersson, M. Linke, J.-C. Chambron, J. Davidsson, V. Heitz, J.-P. Sauvage, L. Hammarström, *J. Am. Chem. Soc.* **2000**, *122*, 3526 – 3527.

[4] a) T. Hayashi, T. Takimura, H. Ogoshi, *J. Am. Chem. Soc.* **1995**, *117*, 11606 – 11607; b) K. Shreder, A. Harriman, B. L. Iverson, *J. Am. Chem. Soc.* **1995**, *117*, 2673 – 2674; c) K. Shreder, A. Harriman, B. L. Iverson, *J. Am. Chem. Soc.* **1996**, *118*, 3192 – 3201; d) R. Sadamoto, N. Tomioka, T. Aida, *J. Am. Chem. Soc.* **1996**, *118*, 3978 – 3979.

[5] A. Harada, K. Okamoto, M. Kamachi, T. Honda, T. Miwatani, *Chem. Lett.* **1990**, 917 – 918.

[6] a) A. Harada, K. Okamoto, M. Kamachi, *Chem. Lett.* **1991**, 953 – 956; b) A. Harada, H. Fukushima, K. Shiotsuki, M. Kamachi, *Supramol. Chem.* **1993**, *2*, 153 – 156; c) A. Harada, K. Shiotsuki, H. Fukushima, H. Yamaguchi, M. Kamachi, *Inorg. Chem.* **1995**, *34*, 1070 – 1076; d) A. Harada, H. Yamaguchi, F. Oka, M. Kamachi, *Proc. SPIE Int. Soc. Opt. Eng.* **1999**, *3607*, 126 – 135.

[7] A. Harada, H. Fukushima, K. Shiotsuki, H. Yamaguchi, F. Oka, M. Kamachi, *Inorg. Chem.* **1997**, *36*, 6099 – 6102.

[8] A. Harada, H. Yamaguchi, K. Okamoto, H. Fukushima, K. Shiotsuki, M. Kamachi, *Photochem. Photobiol.* **1999**, *70*, 298 – 302.

[9] S. Nimri, E. Keinan, *J. Am. Chem. Soc.* **1999**, *121*, 8978 – 8982.

[10] a) C. J. Carrano, M. Tsutsui, *J. Coord. Chem.* **1977**, *7*, 79 – 83; b) P. Sayer, M. Gouterman, C. R. Connell, *J. Am. Chem. Soc.* **1977**, *99*, 1082 – 1087; c) C. A. Marrese, C. J. Carrano, *Inorg. Chem.* **1983**, *22*, 1858 – 1862; d) H. Segawa, K. Kunimoto, A. Nakamoto, T. Shimidzu, *J. Chem. Soc. Perkin Trans. 1* **1992**, 939 – 940; e) H. Segawa, A. Nakamoto, T. Shimidzu, *J. Chem. Soc. Chem. Commun.* **1992**, 1066 – 1067; f) H. Segawa, K. Kunimoto, K. Susumu, M. Taniguchi, T. Shimidzu, *J. Am. Chem. Soc.* **1994**, *116*, 11193 – 11194.

[11] Characterization of porphyrin **3** was carried out by exchanging of the end groups (acid chlorides) in the product to methyl ester **4**. **4**: ^{1}H NMR ($CDCl_3$): $\delta = -2.17$ (m, 4H), 0.78 (dt, 4H), 1.38 (s, 6H), 7.75 – 8.01 (m, 28H), 9.06 (s, 8H). UV/Vis: $\lambda_{max} = 428$, 520, 559, 599 nm. Elemental analysis calcd for $C_{66}H_{50}N_4O_{10}PCl$: C 70.43, H 4.48, N 4.98; found: C 70.45, H 4.70, N 5.11.

[12] **KLH-3** was used as immunogen and bovine serum albumin conjugate (**BSA-3**) was prepared as the antigen of the ELISA procedure.

[13] N. Ichinose, N. Kitamura, H. Masuhara, *Macromolecules* **1993**, *26*, 2331 – 2339.

[14] Fluorescence lifetimes were measured using a Horiba NAES-550 time-correlated single-photon counting instrument. A solution of terephthalic acid was added to the solution of porphyrin **2** – antibody complex. The concentrations of **2**, terephthalic acid, and antibody 74-D7A combining site were set at 1.6, 3.2, and 3.2 μM, respectively. The error values are less than 0.1 ns.

Hexagonal Layered Materials Composed of $[M_2(O_2CCF_3)_4]$ (M = Ru and Rh) Donors and TCNQ Acceptors**

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Multidimensional assemblies consisting of paramagnetic metal ions and polycyano organic acceptor molecules are being prepared in an effort to access molecule-based magnetic/conducting materials based on dπ – pπ electronic interactions.[1–4] Achieving high conductivity through the backbone of a metalloorganic polymer is one of the most challenging goals, and one that has been realized in only one case, namely in the $[Cu(DCNQI)_2]_\infty$ family of compounds (DCNQI = *N*,*N*′-dicyanoquinonediimine).[5] These materials consist of a three-dimensional skeleton containing tetrahedral mixed-valence Cu^I/Cu^{II} ions connected by partially reduced DCNQI radicals arranged in π-stacked columns throughout the three-dimensional network. Although the latter structural feature is a main contributor to the conducting pathway, it has been demonstrated that delocalization through the Cu – DCNQI skeleton is crucial for stabilizing the metallic state of these materials.

The use of electron-rich dimetal complexes to prepare dπ – pπ delocalized systems began in our laboratories with the isolation of the "dimer-of-dimers" $[\{Re_2Cl_4(dppm)_2\}_2(\mu\text{-TCNQ})]$ (dppm = 1,2-bis(diphenylphosphanyl)methane, TCNQ = 7,7,8,8-tetracyanoquinodimethane). This compound is the first example of a charge transfer complex of TCNQ with a metal – metal-bonded donor. The presence of the oxidized Re – Re species ($\sigma^2\pi^4\delta^2\delta^{*1}$) and reduced $TCNQ^{\bullet-}$ was confirmed by spectroscopic and magnetic studies.[6] In this 2:1 donor – acceptor system, electronic delocalization is favored through good metal dπ/organic pπ overlap; this can be represented by the resonance structures: $[Re_2^{II,III}\text{-}(TCNQ^{\bullet-})\text{-}Re_2^{II,III}]$ (1) $\rightleftharpoons Re_2^{II,III}\text{-}(TCNQ)\text{-}Re_2^{II,III}]$ (2) $\rightleftharpoons [Re_2^{II,II}\text{-}(TCNQ^{\bullet-})\text{-}Re_2^{II,III}]$ (3), with forms 1 and 3 being the main contributors.

A logical extension of the aforementioned chemistry is the construction of networks based on electron-rich Ru^{II}/Ru^{II} complexes. This idea is predicated on the notion that the dπ orbitals on Ru^{II}/Ru^{III} and the pπ orbitals of the TCNQ ligands will be energetically compatible; indeed there is ample

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[**] TCNQ = 7,7,8,8-tetracyanoquinodimethane. H.M. is a recipient of a JSPS Research Fellowship for Young Scientists.

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