Highlight Review

Functionalized Antibodies as Biosensing Materials and Catalysts

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Abstract

Monoclonal antibodies have been prepared against water-soluble porphyrins, viologen derivatives, and transition-metal complexes, respectively. These monoclonal antibodies were utilized to devise biosensing and catalytic systems.

♦ Introduction

The immune system has an ability to generate antibodies against virtually any molecule of interest. Recently, much attention has been directed toward antibodies not only in the field of biology but also in the field of chemistry because of their unique structures and functions. Antibodies, immunoglobulins, have been studied as sensors,[1] diagnostics,[2] DDS,[3] catalysts (catalytic antibodies),[4] and components for nanotechnology.[5] With the advent of cell technology,[6] it has become possible to prepare individual immunoglobulins that are called "monoclonal antibodies" in large amounts and in homogeneous form. We have focused our attention on the special behavior of antibodies, especially monoclonal antibodies, because they can recognize a larger and more complex compound with higher specificity than enzymes can. We have prepared monoclonal antibodies for porphyrins,[7] viologens,[8] and transition-metal complexes[9] to construct supramolecular materials[10] and to use these complexes as biosensing materials or specific catalysts (Scheme 1).

The first topic in this review is the construction of antibody supramolecules and their application for biosensing systems. Antibodies have been widely used as efficient reagents to detect target molecules. Based on the principle that an antibody reacts with an antigen specifically and by non-covalent bonds, several procedures have been developed in the immunosorbent assay.[11] Labeled antibodies or antigens are used for the detection, localization, and quantification of biological constituents. More recently, an optical technique based on surface plasmon resonance (SPR)[12] or a microgravimetric quartz-crystal-microbalance (QCM)[13] technique has been found to be useful for measuring and characterizing macromolecular interactions in the increasingly expanding area of biosensor technology. SPR in particular has great potential for macromolecular interaction analysis in terms of sensitivity and signal translation. The use of biosensors based on SPR has made it possible to determine kinetic parameters in real time and without any labeling of biomacromolecules for detection. However, the SPR response reflects a change in mass concentration at the detector surface as molecules bind or dissociate; the specific sensing of substrates with low molecular weight is difficult. In such a case, functional molecules with a high molecular weight such as antibodies have a great potential for amplification of the response signals. Here, we designed linear and dendritic antibody supramolecular complexes. These supramolecular formations are utilized for the amplification of detection signals for biosensor techniques.

The second topic is concerned with functionalized catalysts prepared by the combination of monoclonal antibodies with cofactors. The development of general strategies for introducing catalytic activity into antibody combining sites could lead to a new class of enzyme-like catalysts with tailored specificities. Especially, strategies that allow incorporation of cofactors into antibody combining sites should expand the scope of antibody catalysis. We used porphyrins or transition-metal complexes as a cofactor. Peroxidase activity of iron porphyrin–antibody complexes has been investigated. The electron-transfer reaction from porphyrin molecules to electron acceptors could be controlled by the binding of the antibodies to porphyrin molecules. We have constructed a hydrogen evolution system using porphyrin–antibody complexes.

Transition metals such as Rh, Pd, Pt, and Ru have been extensively used as heterogeneous catalysts[14] for various transformations of molecules. A great number of transition-metal complexes have been prepared and used as homogeneous catalysts,[15] because they are considered as an intermediate of metal-catalyzed reactions. However, complexes of transition metals such as Rh, Pd, Pt, and Ru have not been found in enzymes. If these complexes can be used as if they are cofactors of enzymes, the scope of the catalysts will be revolutionarily broadened. Asymmetric catalyses[16,17] have attracted much attention because of the importance of chirality for living systems. In recent years, water-soluble complexes of transition metals with proteins or DNAs have played an important role in synthetic chemistry as environmentally benign catalysts.[18] In all cases, the metal complexes were incorporated into biomolecules by *non-direct* methods, for example the utilization of avidin–biotin interactions during the complex formation of avidin with a biotinylated metal complex.[19] The most important method to *directly* incorporate transition-metal complexes into proteins is thought to be the preparation of monoclonal antibodies[20] against transition-metal complexes. Now we have succeeded in preparing monoclonal antibodies for a transition-metal complex.

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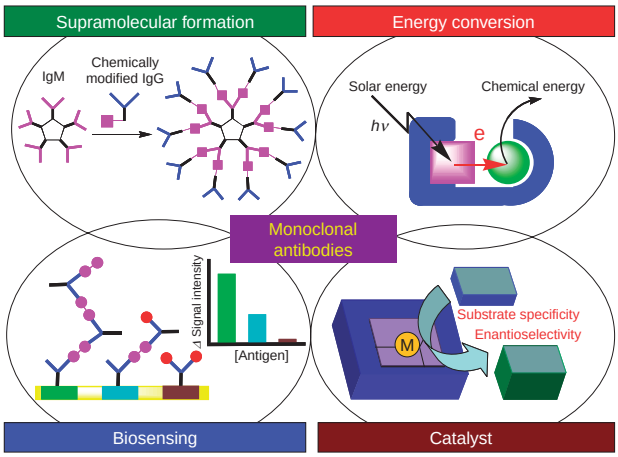


Scheme 1. Functionalization of monoclonal antibodies.

♦ *Linear Antibody Supramolecules: Application for a Novel Biosensing Method*

In this study, methyl viologen is selected as one of the target molecules to be detected. Although viologens are harmful, they are well-known functional molecules which act as herbicides and electron acceptors. Methyl viologen (MV) has been suggested as a potential etiologic factor in Parkinson's disease because of the structural similarity to the known dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.[21] Anti-viologen antibodies[22] may be expected to be useful not only as a highly sensitive reagent for viologens but also as a functional material to control the electron transfer from electron donors to viologens. However, the detection of viologens at low concentrations is difficult owing to the low sensitivity (small response) in a common SPR biosensor technique using corresponding antibodies. To improve the sensitivity, it is important to detect MV as a large response signal caused by the antibody bindings. A solution to this problem is thought to be the inhibition of the supramolecular formation between the anti-viologen antibody and viologen dimer by MV.

Monoclonal antibody 10D5 (IgG_1) has been elicited for the viologen derivative **1** and was found to bind MV with a dissociation constant $K_d = 2.0 \times 10^{-7}$ M. We investigated the complex formation of antibody 10D5 with MV or viologen dimer **2** using the SPR biosensor. The specific binding of the antibody and **2** produces supramolecules such as linear or cyclic antigen–antibody oligomers. Figure 1 shows the sensorgram of the repeated injection of the aqueous viologen dimer and antibody solutions to the sensor chip on which the antibody 10D5 was precoated. The signal intensity was enhanced by the binding of the antibody to the **2**–antibody complex. The viologen dimer molecule is considered to act as a connector between antibodies. It is suggested that the phenomena observed in the SPR measurement are ascribable to higher-order complex formation between antibodies and viologen dimer.

We found that the supramolecular formation of antibody 10D5 and **2** effected an SPR signal enhancement of the biosensor. The additional binding of the antibody to the **2**–antibody complex gave a remarkable increase in signal intensities. On the other hand, the addition of MV (viologen monomer) instead

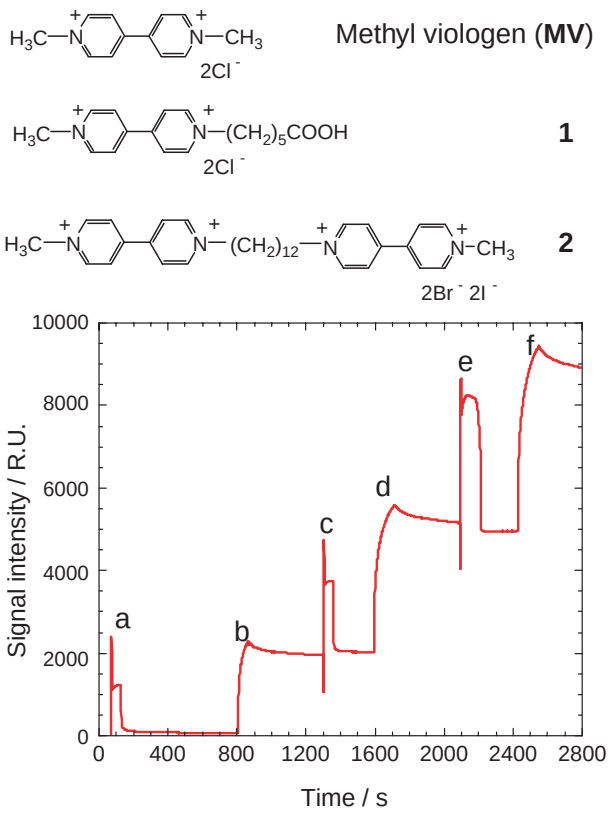
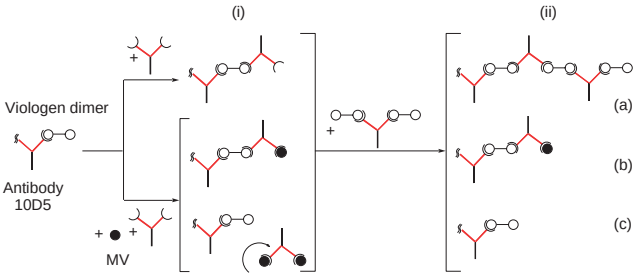


Figure 1. The sensorgram of repeated injection of aqueous viologen dimer **2** (a, c, and e) and antibody (b, d, and f) solutions. [Viologen dimer **2**] = 2.0 μM and [antibody] = 2.0 μM in phosphate borate buffer. Injection period 60 s for a–c and 120 s for d–f. A solution of viologen dimer **2** or the antibody passes over the surface of the sensor chip for 60 or 120 s at a constant flow rate of 20 μL/min. The surface of the sensor chip was subsequently washed with buffer.



Scheme 2. Strategy for the highly sensitive detection of MV based on SPR biosensor technique. Inhibition of the complex formation of the antibody with viologen dimer **2** by MV and the signal enhancement due to the supramolecular formation between the antibody and viologen dimer. Antibody 10D5–**2** complex is immobilized onto the surface of the sensor chip. An aqueous solution of antibody 10D5 and a sample including MV or that without MV is injected into the flow cell (i) before the addition of the complex between antibody 10D5 and viologen dimer **2** (ii). The supramolecular formation between antibody 10D5 and **2** without MV (a), and that in the presence of MV (b) and (c). [MV] < [antibody combining site]: (b) and [MV] ≫ [antibody combining site]: (c).

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of **2** was expected to block the antigen-binding sites and to inhibit the additional antibody binding. A small amount of MV can be detected as a decrease in signal enhancement due to the inhibition of the complex formation between viologen dimer and antibody by MV, compared with the signal intensity of complete supramolecular formation between the antibody and **2**. Scheme 2 shows the strategy for the amplification of detection signals for MV based on the signal enhancement by the supramolecular formation between the antibody and **2** using the biosensor technique. This system includes a two-step procedure as follows. (i) The aqueous solution of antibody 10D5 with MV is injected into the sensor chip, whose surface is modified with the antibody–**2** complex, and then (ii) an antibody–**2** (1:2) complex is added to the previous state. The changes in the signal intensities in the presence of MV are compared with those in the absence of MV. The amount of antibody immobilized to the sensor chip decreased as the concentration of MV increased. To enlarge the difference in the signal intensities in the presence of a small amount of MV, a solution of the antibody–viologen dimer complex was added to the previous state (i). The sensitivity in this system was 140-fold larger than that in the simple addition of MV to the antibody immobilized to the surface of the sensor chip. It was found to be clear that this system can be utilized for the quantitative detection of MV. Amplification of MV sensing processes was realized by the inhibition of complex formation between the antibody and **2**–antibody complex and signal enhancement due to the supramolecular formation of the antibody and **2**. The amount of MV ($M_w = 257$) was expressed as the amount of the antibody ($M_w = 150000$) that could not form the supramolecules between **2** and the antibody.

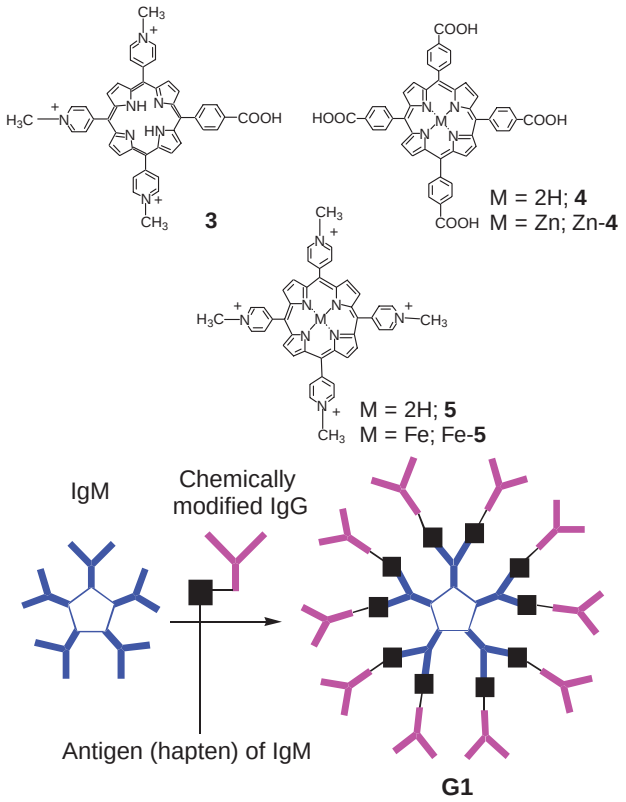
♦ Dendritic Antibody Supramolecules

IgG is a basic type of antibody, consisting of two heavy peptide chains with a molecular weight of about 50000 and two light chains with a molecular weight of about 25000. There are two identical binding sites at the top of Fab fragments of IgG that are bound by flexible hinges with a single constant stem. IgG takes a Y or T shape.[23] IgG is generated in a final stage of immunization, so it is matured and highly selective. IgM has a pentameric structure of IgG and ten antigen binding sites in a single molecule. The presence of ten antigen binding sites enables IgM to bind tightly to antigens containing multiple identical epitopes. However, IgM is generated in an initial stage of immunization, so it is unmatured and less specific for the antigen than IgG. In order to design an antibody system with a high specificity and a high affinity, a combination of the functions of both IgG and IgM seems to be important. We designed and prepared dendritic antibody supramolecules, in which IgM is placed in a core and many IgGs are bound around the IgM. Scheme 3 shows the route for the construction of the dendritic supramolecules. A monoclonal antibody (IgM) for cationic porphyrin **3** was prepared. IgG specific for anionic porphyrin **4** was also prepared. The cationic porphyrin **3** was attached to the IgG via activation of carboxylic acid in **3** using the condensation agent, carbonyldiimidazole. The IgG–**3** conjugate was purified by column chromatography using Sephadex G-150 to remove the porphyrins that did not react with the antibody. The characteristic binding ability and specificity of IgG were found to remain during the chemical modification of IgG with **3**. When IgM for **3** is treated with the IgG–**3** conjugate, IgM binds **3** attached on the IgG to give a dendritic antibody supramolecule "antibody dendrimer" (**G1** in Scheme 3). The binding property of the antibody dendrimer **G1** with a cationic or anionic porphyrin was measured by enzyme-linked immunosorbent assay (ELISA). Figure 2a shows the binding properties of IgG, IgM, and **G1** with the cationic porphyrin **5**. Although IgG did not bind **5** and IgM bound **5**, the dendrimer did not bind **5**. These results show that the cationic porphyrin attached to IgG occupies the binding sites of IgM in the dendrimer; thus there are no free binding sites against **5** on IgM. Figure 2b shows the binding of IgG, IgM, and **G1** to **4**. The IgM used in this study can bind both anionic and cationic porphyrins, owing to the low specificity of IgM against porphyrins. Both IgM and IgG bound **4**, while **G1** bound **4** more efficiently than IgM or IgG. The increase in affinity of **G1** for **4** indicates that many IgG molecules attach to the surface of the IgM molecule.

The biosensor technique based on SPR shows that the antibody dendrimer has an advantage in its amplification of detection signals for antigens. A solution of **G1** was added to the sensor chip on which **4** was precoated by the coupling with hexamethylenediamine as a spacer. The signal intensity increased by the injection of the antibody dendrimer was sufficiently larger than that of simple addition of IgG. Taking into account the change of the binding property of the antibody dendrimer for porphyrins with the increase in the amount of bound antibody to **4** on the SPR biosensor, the antibody dendrimer had many IgG molecules successively bound to IgM molecule. The structural observation of the antibody dendrimer was carried out by using atomic force microscopy (AFM).[24] The sample surface was observed under suitable conditions such that any damage caused by scanning the cantilever is minimized and that any nonspecific assembly among antibodies does not occur. Figure 3 shows AFM images of starting IgM and the dendrimer. The image of the dendrimer was twice as large as that of starting IgM. Some branches (IgGs) can be seen outside of the IgM core. Such an assembled structure was not observed in a chemically modified IgG solution or an IgM solution alone. The characteristic features of the antibody dendrimer are that they (1) are composed of proteins, (2) are composed of noncovalent bonds, and (3) bind antigens strongly with high specificity.

♦ Peroxidase Activity of Antibody–Fe-Porphyrin Complex

Monoclonal antibody 12E11G, which was also obtained by the immunization of the conjugate between cationic porphyrin **3** and a carrier protein to mice, bound not only to **5** but also to iron complex of **5** (Fe–**5**). The dissociation constant of the complex between antibody 12E11G and Fe–**5** was found to be 2.6×10^{-7} M. The catalytic effects of the complex of Fe–**5** with antibody 12E11G on the oxidation of substrates, which are oxidized by horseradish peroxidase (HRP), were investigated. The complex of antibody 12E11G with Fe–**5** (0.5 μM) was dissolved in tris-acetate buffer (90 mM, pH 8.0) and incubated for 2 days at room temperature. Hydrogen peroxide (50 mM) was then added, followed by a substrate. The changes of absorbance at λ_{max} for the oxidation product of each substrate were monitored. Results showed that the Fe–**5**–antibody 12E11G complex had catalytic effects on catechol, guaiacol, and pyrogallol. In particular, the complex markedly catalyzed the oxidation of pyrogallol.



Scheme 3. Preparation of antibody dendrimers using IgM and chemically modified IgGs. An ideal structure of the dendrimer is shown as **G1**.

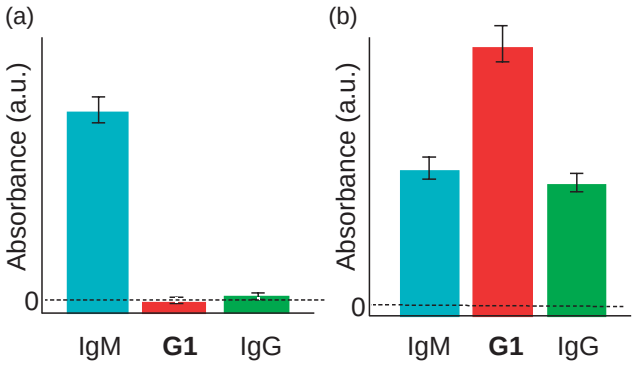


Figure 2. Binding affinities of IgG, IgM, and the antibody dendrimer **G1** with the cationic porphyrin **5** (a) and those with the anionic porphyrin **4** (b) estimated by ELISA.

Peroxidation catalyzed by the antibody–Fe–**5** complex was faster than oxidation in the presence of Fe–**5** alone. Further addition of the substrate caused a further catalytic reaction in the presence of the antibody–Fe–**5** complex, indicating that the catalyst was still active. On the other hand, the catalytic activity of Fe–**5** alone disappeared. It was suggested that the porphyrin catalyst in the absence of the antibody would be destroyed by an excess amount of hydrogen peroxide. The complex of the antibody with Fe–**5** accelerated the oxidation of smaller substrates such as catechol, guaiacol, and pyrogallol; however, it had no effect on the oxidation of the substrates with a large molecular size such as

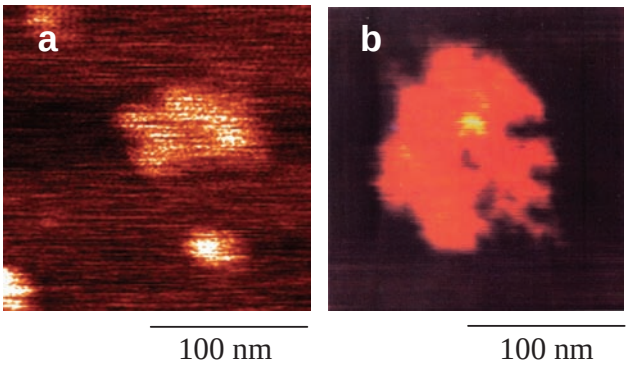


Figure 3. AFM images of IgM (a) and dendritic antibody supramolecule **G1** (b) on surface of graphite plate.

ABTS or *o*-dianisidine. The substrate specificity of the antibody–Fe–**5** complex on the catalytic oxidation might be due to the limitation of space around the active site by the binding of the antibody to the porphyrin molecule. The complex of Fe–**5** with antibody 83B5D, which was also obtained by the immunization of the **3**-conjugate, had no catalytic effect on the oxidation of pyrogallol and the other substrates. Antibody 12E11G was suggested to have catalytic residues in its antigen-combining site and antibody 83B5D was thought to have no binding space for the substrates or no catalytic residues. The increase of the catalytic activity of the Fe–**5**–antibody 12E11G complex was ascribable mainly to the high reactivity of the cationic Fe–porphyrin itself and stabilization of Fe–porphyrin by the binding of the antibody. The differences in k_{cat} values of the Fe–**5**–antibody complex and HRP are found to be within a factor of three. Although the k_{cat}/K_m value was $2.2 \times 10^6\,M^{-1}\,min^{-1}$ for HRP, being 28 times higher than that of the Fe–**5**–antibody complex, the Fe–**5**–antibody complex was highly reactive (Table 1). The catalytic activity of HRP decreased at higher concentrations of H_2O_2, however that of the antibody–porphyrin complex was retained. Naturally occurring catalyst, HRP, catalyzes the oxidations of various substrates, not only pyrogallol but also hydroquinone, catechol, resorcinol, guaiacol, ABTS, and *o*-dianisidine. The reactions promoted by HRP are nonspecific. In contrast, the catalytic oxidation by the Fe–**5**–antibody complex was selective for small molecular substrates such as catechol, guaiacol, and pyrogallol.

Table 1. Kinetic parameters for the oxidation of pyrogallol by Fe–**5** in the absence and presence of antibody 12E11G or HRP

Catalysts	K_m/mM	k_{cat}/min^{-1}	$k_{cat}K_m^{-1}$/M^{-1} min^{-1}
Fe–**5**		83	
Fe–**5**–12E11G	8.6	680	7.9×10^4
HRP	0.81	1750	2.2×10^6

♦ *Hydrogen Evolution System Using Antibody–Porphyrin Complex as Photosensitizer*

One of the complexes between monoclonal antibody 2B6 for **4** and Zn–**4**[25] was used to construct an energy conversion system. The antibody 2B6 could bind Zn–**4** with a dissociation

constant of 2.1×10^{-8} M, estimated by ELISA. When antibody 2B6 was added to an aqueous solution of Zn–**4**, the lifetime of its singlet exited state was lengthened from 1.7 to 2.1 ns. The triplet state lifetime of Zn–**4** was also lengthened from 0.5 to 1.2 ms.

Continuous light irradiation of Zn–**4** was performed in the presence of the antibody and MV. The color of the solution turned blue and a product with a maximum absorbance at 602 nm appeared.[26] Methyl viologen cation radical ($MV^{+\bullet}$) was obtained by the photoinduced electron transfer from porphyrin in the binding site of the antibody to MV. The half-life of $MV^{+\bullet}$ was over 15 min. No color changes were observed without antibodies. The antibody was found to catalyze the electron transfer to give a stable $MV^{+\bullet}$. We found that the $MV^{+\bullet}$ obtained in the antibody–porphyrin complex system could be utilized for producing chemical energy, hydrogen.[27] The antibody–porphyrin complex, MV, and colloidal Pt were equipped for the construction of a hydrogen evolution system. An increase of the concentration of hydrogen was observed in the solution of the antibody–porphyrin complex. The electron transfer could take place efficiently from porphyrin in the antibody binding site to MV outside of the binding pocket (Figure 4).

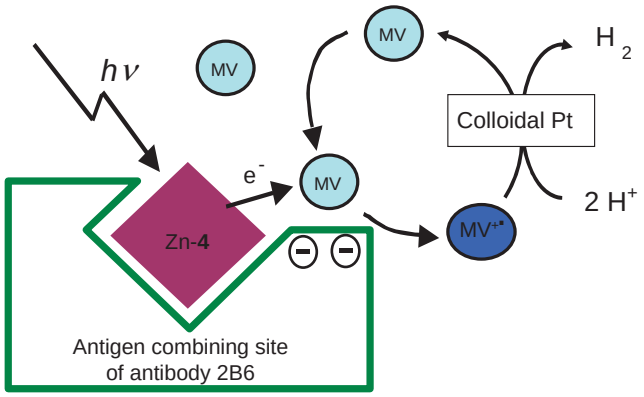
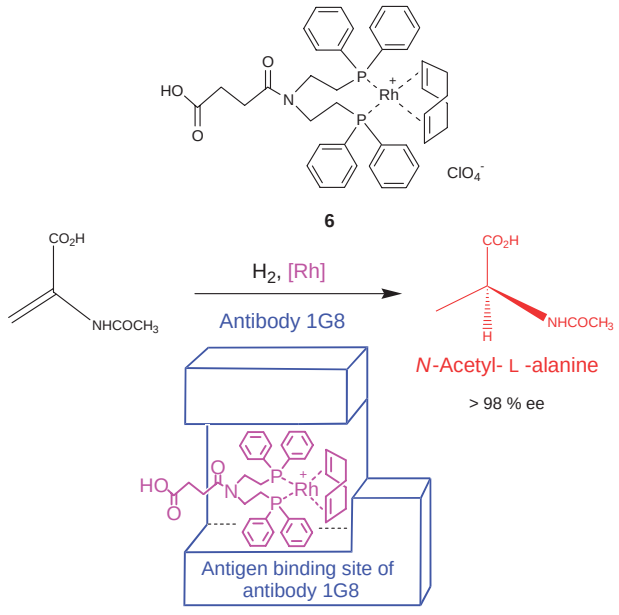


Figure 4. Schematic representation of a hydrogen evolution system utilizing the porphyrin–antibody complex as a photosensitizer.

♦ *Asymmetric Hydrogenation Catalyzed by Antibody–Transition Metal Complex*

We chose a rhodium cyclooctadiene phosphine complex as a transition-metal complex, because the Rh complex catalyzes a variety of reactions such as hydrogenation, hydroformylation, and isomerization.[28] Achiral Rh complex **6**, (1,5-cyclooctadiene){*N*,*N*-bis[2-(diphenylphosphino)ethyl]succinamido}rhodium(I) perchlorate,[29] was synthesized. The Rh complex **6** was used as a hapten to obtain monoclonal antibodies for the Rh catalyst. The hapten was covalently attached to a keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA) via activation of the carboxyl group in the hapten molecule using carbonyldiimidazole. The conjugates KLH–**6** and BSA–**6** were purified by size exclusion chromatography and used as an antigen to immunize mice and in ELISA, respectively. Balb/c mice were immunized with KLH–**6** conjugate in saline emulsified 1:1 in Freund's complete adjuvant four times at two week intervals. The hybridomas secreting anti-**6** antibodies were cloned twice by limiting dilution. Four monoclonal antibodies specific for Rh complex **6** were chosen and their subclasses were found to be IgM. The dissociation constant of the complex between one of the antibodies (1G8) and **6** was found to be 2.3×10^{-7} M. The hydrogenation of amino acid precursors was examined. The Rh complex was added to the aqueous solution of antibody 1G8 under argon atmosphere at room temperature. The substrate was added to the mixture and H_2 was introduced to the solution at 1 atm, 37 °C for 12 h. The HPLC and GC diagrams of the hydrogenation products of each amino acid precursor were monitored. Without antibodies, 2-acetamidocinnamic acid (phenylalanine precursor) was converted to racemic *N*-acetylphenylalanine by the Rh catalyst in 14% yield. However, no catalytic reaction was observed for phenylalanine precursor in the presence of the antibody 1G8–**6** complex. We examined the hydrogenation of 2-acetamidoacrylic acid (alanine precursor) catalyzed by Rh complex **6** in the absence and presence of antibody 1G8. The product obtained by the complex of antibody 1G8 with **6** was S enantiomer (*N*-acetyl-L-alanine) with >98% ee (Scheme 4). The turnover frequency of the Rh complex in the presence of the antibody was found to be 1.2 min^{-1}. On the other hand, the substrate was converted to racemic *N*-acetylalanine by the achiral Rh complex without antibodies. The substrate specificity of this Rh catalyst–antibody 1G8 complex indicated that the antibody binding to the catalyst could control the accessibility of substrates to the Rh catalyst. We have tested the catalytic hydrogenation of alanine precursor by the Rh complex with the other antibodies and the conjugate of the Rh complex with BSA. In these cases, ee values were less than 10% or 0%. Only antibody 1G8 could catalyze the hydrogenation of 2-acetamidoacrylic acid with high enantioselectivity, although all antibodies tested in this study could bind Rh complex **6**. These results suggested that a suitable environment around Rh complex could be introduced by the binding of anti-



Scheme 4. Asymmetric hydrogenation of alanine precursor catalyzed by the antibody–nonchiral Rh complex.

body 1G8 to control the catalytic activity, substrate specificity and enantioselectivity of the hydrogenation reaction. This work represents the first example of asymmetric hydrogenation of an amino acid precursor catalyzed by the complex of a transition metal with immunoglobulin.

♦ Summary

Linear and dendritic supramolecular formations of monoclonal antibodies have been investigated. These supramolecular assemblies are used to amplify the sensing signals for the target molecules. A series of catalysts with a substrate specificity or a high enantioselectivity have been prepared by the combination of monoclonal antibodies with functional small molecules such as porphyrins or transition-metal complexes. An energy conversion system was also constructed by using an antibody–metalloporphyrin complex. Monoclonal antibodies may become more and more important offering high possibilities as new chemical and tailor-made materials by making the best of their ability.

References and Notes

1 a) C. Wingren, C. A. K. Borrebaeck, *Curr. Opin. Biotechnol.* **2008**, *19*, 55. b) M. Seidel, R. Niessner, *Anal. Bioanal. Chem.* **2008**, *391*, 1521.
2 P. Holliger, P. J. Hudson, *Nat. Biotechnol.* **2005**, *23*, 1126.
3 a) Y. Kaneda, *Adv. Drug Delivery Rev.* **2000**, *43*, 197. b) M. C. Garnett, *Adv. Drug Delivery Rev.* **2001**, *53*, 171.
4 a) M. C. Feiters, A. E. Rowan, R. J. M. Nolte, *Chem. Soc. Rev.* **2000**, *29*, 375. b) *Catalytic Antibodies*, ed. by E. Keinan, Wiley-VCH, Weinheim, **2004**.
5 J. Wang, *Adv. Biochem. Eng. Biotechnol.* **2008**, *109*, 239.
6 G. Köhler, C. Milstein, *Nature* **1975**, *256*, 495.
7 a) A. Harada, H. Yamaguchi, K. Okamoto, H. Fukushima, K. Shiotsuki, M. Kamachi, *Photochem. Photobiol.* **1999**, *70*, 298. b) H. Yamaguchi, M. Kamachi, A. Harada, *Angew. Chem., Int. Ed.* **2000**, *39*, 3829. c) H. Yamaguchi, K. Tsubouchi, K. Kawaguchi, E. Horita, A. Harada, *Chem.—Eur. J.* **2004**, *10*, 6179. d) H. Yamaguchi, A. Harada, in *Redox Systems under Nano-Space Control*, ed. by T. Hirao, Springer, Heidelberg, **2006**, pp. 263–291. e) T. Onji, H. Ohara, H. Yamaguchi, N. Ikeda, A. Harada, *Chem. Lett.* **2006**, *35*, 1126.
8 H. Yamaguchi, A. Harada, *Biomacromolecules* **2002**, *3*, 1163.
9 H. Yamaguchi, T. Hirano, H. Kiminami, D. Taura, A. Harada, *Org. Biomol. Chem.* **2006**, *4*, 3571.
10 a) A. Harada, H. Yamaguchi, K. Tsubouchi, E. Horita, *Chem. Lett.* **2003**, *32*, 18. b) H. Yamaguchi, A. Harada, *Top. Curr. Chem.* **2003**, *228*, 237.
11 H. Yamaguchi, A. Harada, M. Kamachi, *React. Funct. Polym.* **1998**, *37*, 245.
12 a) S. Löfås, B. Johnsson, *J. Chem. Soc., Chem. Commun.* **1990**, 1526. b) U. Jonsson, L. Fagerstam, B. Ivarsson, B. Johnsson, R. Karlsson, K. Lundh, S. Lofas, B. Persson, H. Roos, I. Ronnberg, S. Sjolander, E. Stenberg, R. Stahlberg, C. Urbaniczky, H. Ostlin, M. Malmqvist, *BioTechniques* **1991**, *11*, 620. c) R. Karlsson, A. Michaelsson, L. Mattsson, *J. Immunol. Methods* **1991**, *145*, 229. d) I. Chaiken, S. Rose, R. Karlsson, *Anal. Biochem.* **1992**, *201*, 197. e) M. Malmqvist, *Nature* **1993**, *361*, 186.
13 a) K. Niikura, K. Nagata, Y. Okahata, *Chem. Lett.* **1996**, 863. b) A. Bardea, A. Dagan, I. Ben-Dov, B. Amit, I. Willner, *Chem. Commun.* **1998**, 839. c) F. Patolsky, A. Lichtenstein, I. Willner, *J. Am. Chem. Soc.* **2000**, *122*, 418.
14 a) P. McMorn, G. J. Hutchings, *Chem. Soc. Rev.* **2004**, *33*, 108. b) J. J. Becker, M. R. Gagne, *Acc. Chem. Res.* **2004**, *37*, 798.
15 a) M. T. Reetz, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5716. b) B. Breit, *Angew. Chem., Int. Ed.* **2005**, *44*, 6816.
16 a) W. S. Knowles, *Angew. Chem., Int. Ed.* **2002**, *41*, 1998. b) R. Noyori, *Angew. Chem., Int. Ed.* **2002**, *41*, 2008. c) K. B. Sharpless, *Angew. Chem., Int. Ed.* **2002**, *41*, 2024. d) B. M. Trost, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5348. e) R. Noyori, M. Kitamura, T. Ohkuma, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5356. f) A. Pfaltz, W. J. Drury, III, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5723.
17 a) L. C. Hsieh, S. Yonkovich, L. Kochersperger, P. G. Schultz, *Science* **1993**, *260*, 337. b) S. Colonna, N. Gaggero, J. Drabowicz, P. Lyzwa, M. Mikolajczyk, *Chem. Commun.* **1999**, 1787. c) S. Colonna, V. Pironti, J. Drabowicz, F. Brebion, L. Fensterbank, M. Malacria, *Eur. J. Org. Chem.* **2005**, 1727.
18 a) E. T. Kaiser, D. S. Lawrence, *Science* **1984**, *226*, 505. b) D. Qi, C.-M. Tann, D. Haring, M. D. Distefano, *Chem. Rev.* **2001**, *101*, 3081. c) G. Roelfes, B. L. Feringa, *Angew. Chem., Int. Ed.* **2005**, *44*, 3230. d) R. Krämer, *Angew. Chem., Int. Ed.* **2006**, *45*, 858, and references threrein.
19 a) M. E. Wilson, G. M. Whitesides, *J. Am. Chem. Soc.* **1978**, *100*, 306. b) A. Pordea, M. Creus, J. Panek, C. Duboc, D. Mathis, M. Novic, T. R. Ward, *J. Am. Chem. Soc.* **2008**, *130*, 8085. c) J. Steinreiber, T. R. Ward, *Coord. Chem. Rev.* **2008**, *252*, 751.
20 a) V. A. Roberts, B. L. Iverson, S. A. Iverson, S. J. Benkovic, R. A. Lerner, E. D. Getzoff, J. A. Tainer, *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6654. b) P. Ghosh, D. Shabat, S. Kumar, S. C. Sinha, F. Grynszpan, J. Li, L. Noodleman, E. Keinan, *Nature* **1996**, *382*, 339. c) D. A. Blake, P. Chakrabarti, M. Khosraviani, F. M. Hatcher, C. M. Westhoff, P. Goebel, D. E. Wylie, R. C. Blake, II, *J. Biol. Chem.* **1996**, *271*, 27677. d) M. Khosraviani, R. C. Blake, II, A. R. Pavlov, S. C. Lorbach, H. Yu, J. B. Delehanty, M. W. Brechbiel, D. A. Blake, *Bioconjugate Chem.* **2000**, *11*, 267. e) K. M. Nicholas, P. Wentworth, C. W. Harwig, A. D. Wentworth, A. Shafton, K. D. Janda, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2648. f) R. Ricoux, H. Sauriat-Dorizon, E. Girgenti, D. Blanchard, J.-P. Mahy, *J. Immunol. Methods* **2002**, *269*, 39. g) R. C. Blake, II, A. R. Pavlov, M. Khosraviani, H. E. Ensley, G. E. Kiefer, H. Yu, X. Li, D. A. Blake, *Bioconjugate Chem.* **2004**, *15*, 1125.
21 a) H. H. Liou, M. C. Tsai, C. J. Chen, J. S. Jeng, Y. C. Chang, S. Y. Chen, R. C. Chen, *Neurology* **1997**, *48*, 1583. b) A. I. Brooks, C. A. Chadwick, H. A. Gelbard, D. A. Cory-Slechta, H. J. Federoff, *Brain Res.* **1999**, *823*, 1.
22 a) Z. Niewola, C. Hayward, B. A. Symington, R. T. Robson, *Clin. Chim. Acta* **1985**, *148*, 149. b) P. J. Hogg, S. C. Johnston, M. R. Bowles, S. M. Pond, D. J. Winzor, *Mol. Immunol.* **1987**, *24*, 797. c) M. R. Bowles, S. M. Pond, *Mol. Immunol.* **1990**, *27*, 847. d) M. R. Bowles, D. R. Hall, S. M. Pond, D. J. Winzor, *Anal. Biochem.* **1997**, *244*, 133.
23 A. Harada, H. Yamaguchi, M. Kamachi, *Chem. Lett.* **1997**, 1141.
24 H. Yamaguchi, A. Harada, in *Macromolecular Nano-Structured Materials*, ed. by N. Ueyama, A. Harada, Springer, Berlin Heidelberg New York, **2003**.
25 a) A. Harada, K. Okamoto, M. Kamachi, T. Honda, T. Miwatani, *Chem. Lett.* **1990**, 917. b) A. Harada, K. Okamoto, M. Kamachi, *Chem. Lett.* **1991**, 953. c) A. Harada, K. Shiotsuki, H. Fukushima, H. Yamaguchi, M. Kamachi, *Inorg. Chem.* **1995**, *34*, 1070. d) A. Harada, H. Fukushima, K. Shiotsuki, H. Yamaguchi, F. Oka, M. Kamachi, *Inorg. Chem.* **1997**, *36*, 6099.
26 T. Watanabe, K. Honda, *J. Phys. Chem.* **1982**, *86*, 2617.
27 a) M. Sakamoto, T. Kamachi, I. Okura, A. Ueno, H. Mihara, *Biopolymers* **2001**, *59*, 103. b) Y. Saiki, Y. Amao, *Biotechnol. Bioeng.* **2003**, *82*, 710.
28 a) E. A. Karakhanov, A. L. Maximov, in *Metal Complexes and Metals in Macromolecules*, ed. by D. Wöhrle, A. D. Pomogailo, Wiley-VCH, Weinheim, **2003**, p. 457. b) J. M. Brown, in *Modern Rhodium-Catalyzed Organic Reactions*, ed. by P. A. Evans, Wiley-VCH, Weinheim, **2005**, p. 33.
29 a) R. G. Nuzzo, S. L. Haynie, M. E. Wilson, G. M. Whitesides, *J. Org. Chem.* **1981**, *46*, 2861. b) O. Abril, G. M. Whitesides, *J. Am. Chem. Soc.* **1982**, *104*, 1552. c) M. Skander, N. Humbert, J. Collot, J. Gradinaru, G. Klein, A. Loosli, J. Sauser, A. Zocchi, F. Gilardoni, T. R. Ward, *J. Am. Chem. Soc.* **2004**, *126*, 14411.