Asymmetric hydrogenation with antibody-achiral rhodium complex†

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Monoclonal antibodies have been elicited against an achiral rhodium complex and this complex was used in the presence of a resultant antibody, 1G8, for the catalytic hydrogenation of 2-acetamidoacrylic acid to produce *N*-acetyl-L-alanine in high (>98%) enantiomeric excess.

Transition metals such as Rh, Pd, Pt, Ru *etc.* have been extensively used as heterogeneous catalysts[1,2] for various transformations of molecules. A great number of transition metal complexes have been prepared and used as homogeneous catalysts,[3,4] because they are considered as an intermediate of metal catalyzed reactions. However, the complexes of transition metals such as Rh, Pd, Pt, 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 have attracted much attention because of the importance of chirality for living systems.[5,6] 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.[7] 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.[8] The most important method to *directly* incorporate transition metal complexes into proteins is thought to be the preparation of monoclonal antibodies[9] against transition metal complexes. Now we have succeeded in preparing monoclonal antibodies for transition metal complex **1** (Fig. 1) and found that the antibody–Rh complex catalyzed the hydrogenation of amino acid precursors to give L-amino acid ((*S*)-enantiomer) with >98% enantiomeric excess (ee).

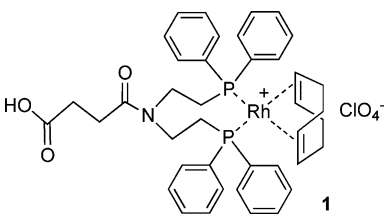
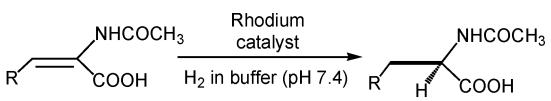


Fig. 1 Structure of achiral Rh complex **1** used as hapten.

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, isomerization, and so on.[10] Achiral Rh complex **1**, [(1,5-cyclooctadiene){bis(2-diphenylphosphinoethyl)succinamido} rhodium(I)] perchlorate, was synthesized[8a,11] and characterized by ^{1}H-, ^{31}P-NMR and FAB-MS measurements. The Rh complex **1** 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–**1** and BSA–**1** were purified by size exclusion chromatography and used as an antigen to immunize mice and in enzyme-linked immunosorbent assays (ELISA), respectively. Balb/c mice were immunized with KLH–**1** conjugate in saline emulsified 1 : 1 in Freund's complete adjuvant four times at two week intervals. The hybridomas secreting anti-**1** antibodies were cloned twice by limiting dilution. Four monoclonal antibodies (1B11, 1C5, 1G8, 1H5) specific for Rh complex **1** were chosen and their subclasses were found to be immunoglobulin M (IgM). The dissociation constant (K_d) of the complex between one of the antibodies (1G8) and **1** was found to be 2.3×10^{-7} M by ELISA.

The hydrogenation of amino acid precursors **2–4** was examined (Table 1). The Rh complex was added to the aqueous solution of the monoclonal antibody under argon atmosphere at room temperature. The substrate was added to the mixture and H_2

Table 1 Results of Rh-catalyzed hydrogenation of amino acid precursors[a]



R = Ph (**2**), $CH(CH_3)_2$ (**3**), H (**4**)

Entry	Catalyst	Substrate	ee (%)	Yield (%)
1	**1**	**2**	0	14
2	**1**–Antibody 1G8	**2**	—[b]	0
3	**1**–Antibody 1G8	**3**	—[b]	0
4	**1**	**4**	0	11
5	**1**–Antibody 1G8	**4**	>98 (*S*)	23
6	**1**–Antibody 1H5	**4**	<10 (*S*)	10
7	**1**–Antibody 1C5	**4**	<10 (*S*)	<5
8	**1**–BSA[c]	**4**	0	12

[a] Rh complex **1** (21 nmol) was dissolved in 5 mL of phosphate buffer (0.1 M, pH 7.0) with or without monoclonal antibodies (4.2 nmol, [antigen binding site] = 42 nmol). The substrates **2–4** (78 μmol) were added into a Schlenk containing an aqueous solution of Rh complex **1** or the antibody–Rh complexes. The flask was purged with argon at first for 1 h, and then hydrogen was introduced into the solutions through a needle. After stirring for 12 h at 37 °C, the conversions and the ee values of the reaction products were determined by HPLC or GC. [b] No products were obtained. [c] The conjugate of Rh complex **1** with BSA (21 nmol) was used. It contains 1 mol of **1** per mol of BSA.

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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 (see ESI†). Without antibodies, 2-acetamidocinnamic acid (phenylalanine precursor, **2**) was converted to racemic *N*-acetyl-phenylalanine by the Rh catalyst in 14% yield (entry 1). However, no catalytic reaction was observed for **2** in the presence of the antibody 1G8–**1** complex (entry 2). A similar result was obtained for the hydrogenation of 2-acetamido-4-methylpentenoic acid (leucine precursor, **3**) in the presence of the antibody–**1** complex (entry 3).

We examined the hydrogenation of 2-acetamidoacrylic acid (alanine precursor, **4**) catalyzed by Rh complex **1** in the absence and presence of antibody 1G8 (entries 4 and 5, respectively). The product obtained by the complex of antibody 1G8 with **1** was (*S*)-enantiomer (*N*-acetyl-L-alanine) with >98% ee. The turnover frequency of the Rh complex in the presence of the antibody was found to be 1.2 min^{-1} for the hydrogenation of **4**. On the other hand, the substrate was converted to racemic *N*-acetyl-alanine 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 (Fig. 2). The enantioselective hydrogenation of the alanine precursor achieved by using this antibody–Rh complex indicates that the H_2 attacks the double bond of the substrate at one face and the other face is blocked by the protein.

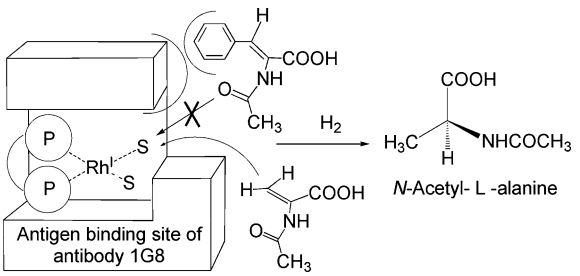

Fig. 2 A schematic representation of the structure of the complex between antibody 1G8 and the Rh catalyst to show the substrate specificity. The characters S and P in the ligands of the Rh complex indicate solvent molecules and diphenylphosphine ligands, respectively.

We have tested the catalytic hydrogenation of **4** by the Rh complex with the other antibodies (entries 6 and 7) and the conjugate of the Rh complex with BSA (entry 8). 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 **1**.

Because of the very low solubility of the substrates to buffer and a decrease of the catalytic activity of Rh complexes in buffer, compared with organic solvents such as methanol, yields of the hydrogenation products were very low in the absence of the antibody. However, conversion of 2-acetamidoacrylic acid to the corresponding hydrogenation product by the antibody 1G8–**1** complex (23%) was higher than that without antibodies (11%). These results suggested that a suitable environment around Rh complex could be introduced by the binding of antibody 1G8 to control the catalytic activity, substrate specificity and enantioselectivity of the hydrogenation reaction.

In conclusion, this work represents the first example of asymmetric hydrogenation of an amino acid precursor catalyzed by the complex of a transition metal with immunoglobulin. Although the hydrogenation reaction of 2-acetamidoacrylic acid by the Rh complex alone was non-selective, one of the antibodies bound achiral Rh complex **1** and the resulting antibody–Rh complex gave an enantiomeric product (*N*-acetyl-L-alanine) with >98% ee. The hapten used to generate the antibodies does not incorporate a substrate mimic. However, the substrate specificity of antibody 1G8 indicated that a smaller substrate such as an alanine precursor could react with the Rh complex immobilized in the antibody binding pocket and larger substrates than the hapten molecules such as leucine and phenylalanine precursors could not bind to the Rh catalyst. Antibody 1G8 could bind substrates with a similar size as the hapten, although we did not design the binding pocket for these amino acid precursors. The antibody 1G8–Rh complex was found to be a precise stereoselective catalyst with substrate specificity through second-sphere coordination.

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Notes and references

1 P. McMorn and G. J. Hutchings, *Chem. Soc. Rev.*, 2004, **33**, 108.
2 J. J. Becker and M. R. Gagné, *Acc. Chem. Res.*, 2004, **37**, 798.
3 M. T. Reetz, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 5716.
4 B. Breit, *Angew. Chem., Int. Ed.*, 2005, **44**, 6816.
5 (*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 and T. Ohkuma, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 5356; (*f*) A. Pfaltz and W. J. Drury, III, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 5723.
6 (*a*) L. C. Hsieh, S. Yonkovich, L. Kochersperger and P. G. Schultz, *Science*, 1993, **260**, 337; (*b*) S. Colonna, N. Gaggero, J. Drabowicz, P. Lyzwa and M. Mikolajczyk, *Chem. Commun.*, 1999, 1787; (*c*) S. Colonna, V. Pironti, J. Drabowicz, F. Brebion, L. Fensterbank and M. Malacria, *Eur. J. Org. Chem.*, 2005, **9**, 1727.
7 (*a*) E. T. Kaiser and D. S. Lawrence, *Science*, 1984, **226**, 505; (*b*) D. Qi, C.–M. Tann, D. Haring and M. D. Distefano, *Chem. Rev.*, 2001, **101**, 3081; (*c*) G. Loelfes and B. L. Feringa, *Angew. Chem., Int. Ed.*, 2005, **44**, 3230; (*d*) R. Krämer, *Angew. Chem., Int. Ed.*, 2006, **45**, 858 and references threrein.
8 (*a*) M. E. Wilson and G. M. Whitesides, *J. Am. Chem. Soc.*, 1978, **100**, 306; (*b*) C.-C. Lin, C.-W. Lin and A. S. C. Chan, *Tetrahedron: Asymmetry*, 1999, **10**, 1887; (*c*) G. Klein, N. Humbert, J. Gradinaru, A. Ivanova, F. Gilardoni, U. E. Rusbandi and T. R. Ward, *Angew. Chem., Int. Ed.*, 2005, **44**, 7764; (*d*) C. M. Thomas and T. R. Ward, *Chem. Soc. Rev.*, 2005, **34**, 337; (*e*) C. Letondor, A. Pordea, N. Humbert, A. Ivanova, S. Mazurek, M. Novic and T. R. Ward, *J. Am. Chem. Soc.*, 2006, **128**, 8320.
9 (*a*) V. A. Roberts, B. L. Iverson, S. A. Iverson, S. J. Benkovic, R. A. Lerner, E. D. Getzoff and 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 and E. Keinan, *Nature*, 1996, **382**, 339; (*c*) D. Blake, P. Chakrabarti, M. Khosraviani, F. M. Hatcher, C. M. Westhoff, P. Goebel, D. E. Wylie and 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 and D. A. Blake, *Bioconjugate Chem.*, 2000, **11**, 267; (*e*) K. M. Nicholas, P. Wentworth, C. W. Harwig, A. D. Wentworth, A. Shafton and K. D. Janda, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 2648; (*f*) R. Ricoux, H. Sauriat-Dorizon, E. Girgenti, D. Blanchard and 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 and D. A. Blake, *Bioconjugate Chem.*, 2004, **15**, 1125; (*h*) A. B. Lindner, Z. Eshhar and D. S. Tawfik, in *Catalytic Antibodies*, ed. E. Keinan, Wiley-VCH, Weinheim, 2004, p. 418.

10 (*a*) E. A. Karakhanov and A. L. Maximov, in *Metal Complexes and Metals in Macromolecules*, ed. D. Wöhrle and A. D. Pomogailo, Wiley-VCH, Weinheim, 2003, p. 457; (*b*) J. M. Brown, in *Modern Rhodium-Catalyzed Organic Reactions*, ed. P. A. Evans, Wiley-VCH, Weinheim, 2005, p. 33.

11 (*a*) R. G. Nuzzo, S. L. Haynie, M. E. Wilson and G. M. Whitesides, *J. Org. Chem.*, 1981, **46**, 2861; (*b*) O. Abril and 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 and T. R. Ward, *J. Am. Chem. Soc.*, 2004, **126**, 14411.