Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 1887–1893

TETRAHEDRON: *ASYMMETRY*

Catalytic hydrogenation of itaconic acid in a biotinylated Pyrphos–rhodium(I) system in a protein cavity

Chi-Ching Lin, Ching-Wen Lin and Albert S. C. Chan *

Open Laboratory of Chirotechnology and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China

Received 16 March 1999; accepted 13 May 1999

Abstract

The construction of a chiral catalyst system embedded at a specific site in a protein has been studied. The preparation of the biotinylated Pyrphos–Rh(I) complex attached to the binding site in avidin and its application to the asymmetric hydrogenation of itaconic acid have been investigated. By introducing the chiral Pyrphos–Rh(I) moiety into the constrained environment of the protein cavity it was found that the enantioselectivity of the system was significantly influenced by the tertiary conformation within the avidin cavity. The effects of reaction conditions such as temperature, hydrogen pressure, and the pH value of the buffer on enantioselectivity are reported.

1. Introduction

Catalytic enantioselective reactions have received much attention during the past three decades and have played a crucial role in modern synthetic chemistry.[1] Transition-metal catalysts containing various chiral ligands have been extensively studied for this purpose.[2] In addition to the use of chiral metal complexes, one strategy used to design selective catalysts is to incorporate nonspecific achiral catalytic groups into chiral cavities.[3] The general method is to modify a known protein or enzyme at a defined site with a cofactor or new functional group to create a semisynthetic system with novel properties.[4–13] The significant advantage offered by this strategy is the avoidance of the arduous synthesis of the chiral ligands. However, most of these systems are usually limited to those substrates which specifically bind to the native enzymes. Only a few systems developed recently based on a protein cavity can accommodate a variety of substrates.[14] Since transition-metal complexes catalyze a large variety of chemical reactions, the development of new catalysts by introducing a chiral catalytic functionality into the tertiary restricted environment of a protein cavity is of great interest. For the convenient test of this concept, we decided

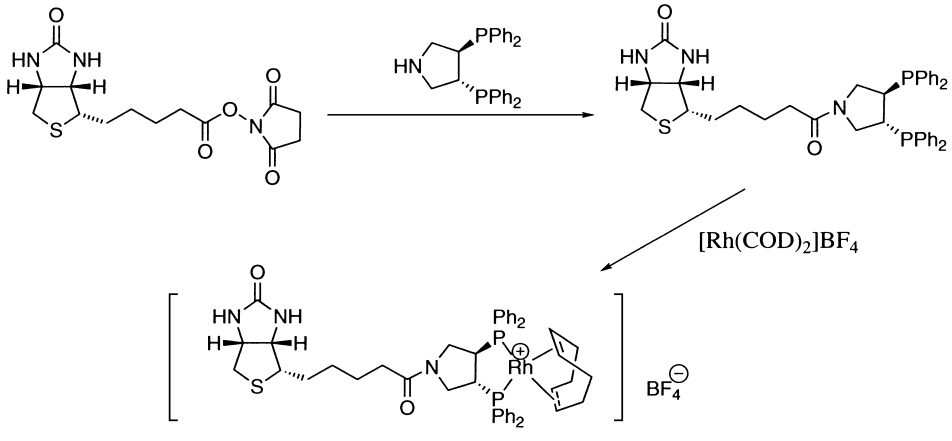
* Corresponding author. Tel: (852) 27665607; fax: (852) 23649932; e-mail: bcachan@polyu.edu.hk

0957-4166/99/$ - see front matter
PII: S0957-4166(99)00193-7

to use a carrier which binds strongly to specific sites of the target protein to introduce the catalyst to the protein cavity like a 'guided missile'. If the combination is properly optimized, it may possess not only better compatibility with different substrates but also a multiplicity of various catalytic reactions. Moreover, the protein-based catalysts may offer the possibility of easier catalyst recovery and reuse. In this study, we examined the effect of the interaction of a chiral catalyst with a protein cavity by converting a Pyrphos ligand to its corresponding biotinylated derivative and attaching the biotinylated Pyrphos–Rh(I) complex to the specific binding site of avidin. The catalytic hydrogenation of itaconic acid with this new type of catalyst was investigated. For the purpose of comparison, hydrogenation reactions catalyzed by the biotinylated Pyrphos–Rh(I) complex, both with and without the association with avidin, were carried out. Both *R* and *S* forms of Pyrphos ligands were linked to the avidin cavity in order to find a suitable match of chirality between the chiral catalyst and the avidin cavity. The effects of reaction conditions such as temperature, hydrogen pressure, and the pH of the solution on the enantioselectivity have also been studied.

2. Results and discussion

Avidin is a basic glycoprotein and a stable tetramer with twofold symmetry.[15] The strong binding between biotin and avidin is characterized by a dissociation constant of the order of 10^{-15} M. Moreover, it is found that only the intact bicyclic ring of biotin is required for the strong interaction between avidin and biotin. The carboxylic group on the valeric acid side chain of biotin is not involved in this interaction.[16] Consequently, biotin derivatives reactive to a variety of functional groups can be prepared simply by modifying this carboxylic group. As early as 1978, Whitesides et al. elaborated an elegant approach to this kind of semisynthetic enzymes by the use of a modified biotin molecule.[17] Our initial effort was focused on the biotinylated 3,4-bis(diphenylphosphino)pyrrolidine, abbreviated as 'biotin-Pyrphos', as the moiety to bind with avidin. The rhodium(I) catalysts were prepared by the complexation of bis(1,5-cyclooctadiene)rhodium tetrafluoroborate, $[Rh(COD)_2]BF_4$, with Pyrphos ligand and its biotinylated derivatives. A synthetic scheme is outlined in Scheme 1.



Scheme 1. The synthetic route of biotinylated Pyrphos–rhodium(I) complex

The catalysts prepared for this study are shown in Fig. 1, and were used to catalyze the hydrogenation of itaconic acid. The initial results of the hydrogenation are summarized in Table 1.

From the data in Table 1, it is obvious that the incorporation of biotin with Pyrphos–Rh(I) complex has an effect on enantioselectivity. This effect is not surprising because biotin itself is chiral. The change

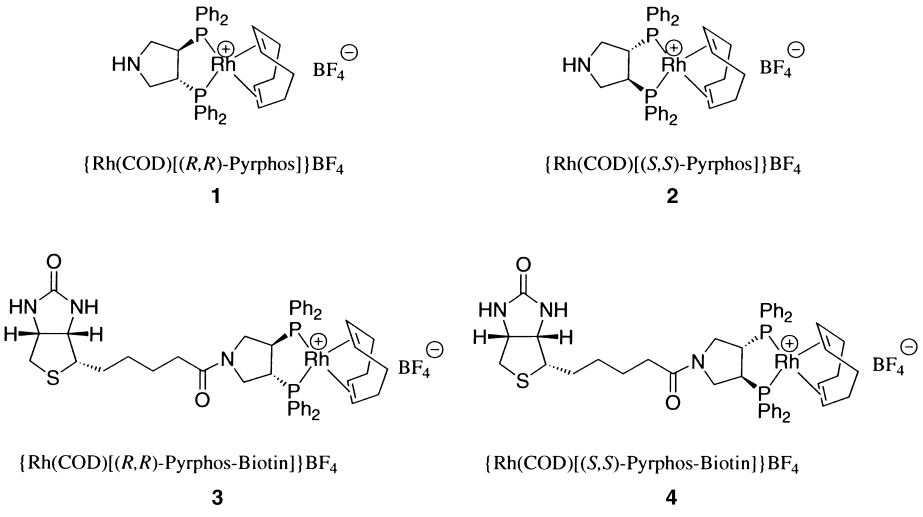


Figure 1. The catalysts prepared for the study of the hydrogenation of itaconic acid

Table 1
The hydrogenation of itaconic acid with catalysts **1**, **2**, **3**, and **4**[a]

entry	cat.	pressure of H_2 (psi)	ee[b] (%)	config.[c]	conv.[b] (%)
1	**1**	100	2.2	*R*	80.5
2	**1**	15	11.8	*R*	6.4
3	**3**	100	3.2	*R*	56.2
4	**3**	15	28.6	*R*	11.8
5	avidin + **3**[d]	100	15.5	*S*	>99
6	avidin + **3**[d]	15	11.2	*R*	7.6
7	**2**	100	6.5	*S*	96.7
8	**2**	15	7.9	*S*	23.0
9	**4**	100	11.1	*S*	74.0
10	**4**	15	25.5	*S*	51.6
11	avidin + **4**[d]	100	44.2	*R*	>99
12	avidin + **4**[d]	15	48.1	*R*	40.2

a). The hydrogenations were carried out at 22 °C in a 0.1 M NaH_2PO_4 buffer solution (pH 7.0) with S/C=80 (molar ratio) for 48 h. b). The ee values and conversions were determined by GC analyses with a 50m Chrompack Chiralsil-DEX CB capillary column. c). The absolute configurations were determined by comparing the optical rotations with literature values. d). 1.1 eq. avidin based on the biotinylated Pyrphos-Rh(I) complex was added.

of enantioselectivity for catalyst **3** and **4** in the presence of avidin is clearly observed and supports our hypothesis. The enhancement of enantioselectivity by introducing avidin to the catalyst **4** system was most significant. It is also of interest to note that the introduction of avidin induced the reverse chiral preference of the catalyst while maintaining comparable conversion rates (entries 9, 11 and 10, 12). The most remarkable feature is the consistence of chiral preference achieved by the association of avidin with **3** and **4** under 15 psi H_2 (entries 6, 12). This phenomenon implied that the peripheral asymmetric environment within the avidin cavity was the dominant factor responsible for the enantioselectivity. These results also revealed the possibility of the Pyrphos–Rh(I) complex encapsulated completely within the globular tertiary structure of avidin. Because the protein folding process involves many complicated kinetic and thermodynamic variables, it is possible that the ensuing conformation through protein folding

Table 2
The effect of the pH values of the buffered solutions on the enantioselectivity of the catalyst in the hydrogenation of itaconic acid[a]

entry	cat.[b]	pressure of H_2 (psi)	pH of Buffer	ee[c] (%)	config.	conv.[c] (%)
1	avidin+**3**	100	6	12.4	*S*	>99
2	avidin+**3**	15	6	5.1	*S*	59.1
3	avidin+**3**	100	7	15.5	*S*	>99
4	avidin+**3**	15	7	11.2	*R*	7.6
5	avidin+**3**	100	8	2.9	*S*	49.1
6	avidin+**3**	15	8	3.3	*S*	46.5
7	avidin+**4**	100	6	19.6	*R*	>99
8	avidin+**4**	15	6	3.1	*R*	47.7
9	avidin+**4**	100	7	44.2	*R*	>99
10	avidin+**4**	15	7	48.1	*R*	40.2
11	avidin+**4**	100	8	20.4	*R*	77.9
12	avidin+**4**	15	8	17.0	*R*	64.3

a). The hydrogenations were carried out at 22 °C in a 0.1 M NaH_2PO_4 buffered solution with S/C=80 (molar ratio) for 48 h. b). 1.1 eq. of avidin based on the biotinylated Pyrphos-Rh(I) complex was added. c). The ee values and conversions were determined by GC analyses with a 50m Chrompack Chiralsil-DEX CB capillary column.

Table 3
The effects of temperature and H_2 pressure on the enantioselectivity in the hydrogenation of itaconic acid

entry	cat.[e]	pressure of H_2 (psi)	temp. (°C)	ee[f] (%)	config.	conv.[f] (%)
1[a]	avidin+**3**	1000	40	8.6	*S*	89.4
2[b]	avidin+**3**	1000	22	36.8	*S*	>99
3[c]	avidin+**3**	1000	0	12.0	*S*	46.1
4[c]	avidin+**4**	1000	0	32.4	*R*	94.9
5[c]	avidin+**4**	100	0	21.4	*R*	75.8
6[d]	avidin+**4**	15	0	1.5	*S*	62.3
7[b]	avidin+**4**	15	22	48.1	*R*	40.2
8[a]	avidin+**4**	15	40	20.3	*S*	82.0

a). The hydrogenations were carried out at in a 0.1 M NaH_2PO_4 buffered solution (pH 7.0) with S/C=400 (molar ratio) for 2 days. b). The hydrogenations were carried out with S/C=80 (molar ratio) for 2 days. c). The hydrogenations were carried out with S/C=80 (molar ratio) for 4 days. d). The hydrogenations were carries out with S/C=80 (molar ratio) for 7 days. e). 1.1 eq. avidin was added based on the biotinylated Pyrphos-Rh(I) complex. f). The ee values and conversions were determined by GC analyses with a 50m Chrompack Chiralsil-DEX CB capillary column.

depends on the temperature as well as the pH value of the buffer. To evaluate these factors we also altered these two variables in the catalytic hydrogenation so as to explore the matching conformation of avidin. The effect of the pH values of the buffered solutions on the enantioselectivity of the catalyst is summarized in Table 2.

It can be found from Table 2 that the reactions carried out in various buffered solutions with different pH values gave products in different ee values, and the buffered solution of pH 7 gave the best results. The reaction temperature was also found to have a profound effect on the product ees (Table 3).

It is observed from Table 3 that under 1 atm of H_2, the same avidin-based catalyst may change its enantioselectivity to the extent of having an opposite chiral preference when the reaction is carried out at different temperatures (entries 6–8). These results indicate that the structure of the avidin binding site varies significantly with temperature and the influence of the cavity structure on enantioselectivity may be greater than that of the coordinating chiral diphosphine ligand. One possible explanation is that the

size of the avidin binding site may be too tight for the accommodation of the attached Pyrphos–Rh(I) complex. Different magnitudes of distortion of the chiral Pyrphos ligand produced the discrepancies of the ee values in the catalytic reaction.

At this point, it is difficult to establish the exact stereochemistry within the binding site after the incorporation of the Pyrphos–Rh(I) complex moiety. Nevertheless, the binding of the biotinylated chiral complex to avidin and the subsequent influence of the avidin environment on the enantioselectivity of the catalyst is established. In summary, the avidin-biotinylated Pyrphos–Rh(I) system developed in this study gave positive albeit preliminary indications for the potential of the combination of chiral transition-metal complexes and protein cavities. With the rapid progress focused on numerous mutagenesis and protein folding studies, the use of transition-metal complexes attached to specific sites in proteins on selective catalytic reactions is expected to offer more positive potential in the future.

3. Experimental

Unless otherwise noted, all reactions were carried out under nitrogen atmosphere by using standard Schlenk techniques. Melting points were determined using a Büchi melting point B-545 apparatus. Optical rotations were measured on a Perkin–Elmer Model 341 polarimeter. NMR spectra were recorded on a Bruker DPX-400 spectrometer. Mass analyses were performed on a Finnigan Model Mat 95 ST mass spectrometer. The conversions and enantioselectivities of the hydrogenation reactions were determined by GC analyses with a 50 m Chrompack Chiralsil-DEX CB capillary column.

*3.1. Synthesis of (3*R,*4*R*)- and (3*S,*4*S*)-3,4-bis(diphenylphosphino)-pyrrolidine and their rhodium complexes*

The two chiral Pyrphos ligands were prepared according to a method published by Baker et al.[18] Complexes **1** and **2** were synthesized according to a method published by Nagel et al.[19]

*3.2. Synthesis of (3*S,*4*S*)- and (3*R,*4*R*)-Pyrphos-d-biotinamide*

A 50 mL flask was charged with *N*-biotinoxysuccinimide (326.8 mg, 1 mmol) and Pyrphos (424.7 mg, 0.966 mmol) dissolved in 14 mL degassed DMF. The mixture was stirred at room temperature for 60 h. The reaction mixture was diluted slowly with 30 mL of degassed water, and then cooled to 0°C. The resulting precipitate was separated by filtration and washed with water (5 mL). The white precipitate was dried in vacuo to give the desired product (610 mg, 95% theoretical yield). Mp: 123.9°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.5–7.1 (m, 20H), 5.4 (s, 1H), 5.0 (s, 1H), 4.5 (d, 1H, *J*=4.9 Hz), 4.3 (d, 1H, *J*=5.2 Hz), 4.0–3.8 (m, 2H), 3.3 (m, 1H), 3.1 (m, 1H), 2.9–2.8 (m, 2H), 2.7 (m, 1H), 2.1 (t, 2H, *J*=14.8 Hz), 1.6–1.4 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 171.9, 163.5, 150.0, 134.2, 134.1, 134.0, 113.9, 130.0, 129.8, 129.7, 129.6, 129.3, 129.2, 129.1, 62.2, 60.6, 55.6, 41.1, 37.7, 34.5, 28.7, 25.0; ^{31}P NMR (160 MHz, $CDCl_3$): δ (ppm) −13.6 (d, J_{P-P}=7.0 Hz), −13.4 (d, J_{P-P}=7.0 Hz); HRMS (ESI) for $C_{38}H_{42}SO_2P_2N_3$: calcd 666.2473; found 666.2478.

3.3. Synthesis of (3S,4S)- and (3R,4R)-Pyrphos-d-biotinamide-cyclooctadiene-rhodium(I) tetrafluoroborate **3** and **4**

A 10 mL Schlenk tube was charged with (3*R*,4*R*)-Pyrphos-d-biotinamide or (3*S*,4*S*)-Pyrphos-d-biotinamide (36 mg, 0.054 mmol) and $[Rh(COD)_2]BF_4$ (20.0 mg, 0.049 mmol) in dichloromethane (5 mL) under a nitrogen atmosphere. The resulting solution was allowed to stir at ambient temperature for 2 h. Diethyl ether (5 mL) was added slowly to the solution to precipitate the desired product as an orange solid. The solid was collected by filtration, washed with diethyl ether (3 mL) and dried in vacuo to give catalyst **3** or **4** (42 mg, 89% of theoretical yield). ^{31}P NMR (160 MHz, $CDCl_3$): δ (ppm) 36.3 (dd, J_{Rh-P}=117.5 Hz, J_{P-P}=7.0 Hz); HRMS (ESI) for $C_{46}H_{54}N_3SO_2P_2Rh$: calcd 877.2467; found 877.2469. {Rh(COD)[(*R*,*R*,)-Pyrphos-biotin]}BF_4: $[\alpha]_D^{25}$=+9.0 (*c* 0.10, MeOH); {Rh(COD)[(*S*,*S*,)-Pyrphos-biotin]}BF_4: $[\alpha]_D^{25}$=+54.5 (*c* 0.17, MeOH).

3.4. General procedure for the hydrogenation reaction

In a nitrogen atmosphere glovebox, a stainless steel reactor was charged with itaconic acid (1.0 mg, 7.7 μmol) in the presence or absence of avidin (2.5 mg, 27.75 units, binds 0.1126 μmol of biotin) followed by the addition of the 0.1 M NaH_2PO_4 buffered solution (1.0 mL, pH 7.0) and the catalyst solution in methanol (40 μL of a 2.5 mM catalyst solution, 0.1 μmol). The reactor was closed and stirred at ambient temperature for 30 min to ensure the complete association of biotin with avidin. The reactor was pressurized to the pre-determined pressure of H_2 and the resulting mixture was allowed to stir at ambient temperature for 2 to 7 days. The reaction mixture was worked up by addition of 2N aqueous HCl solution and filtered through Celite to remove the resulting precipitate. The filtrate was concentrated under reduced pressure and extracted with chloroform. The collected chloroform layer was dried over $MgSO_4$ and concentrated to give a crude solution. The level of conversion and the ee of the hydrogenated products were determined from the crude solution by GC analyses using the following conditions: Chrompack Chiralsil-DEX CB capillary column, 50 m length, 0.25 mm inner diameter, nitrogen as carrier gas (15 psi), 130°C. Retention time for itaconic acid, 10.7 min; *R* form of methylsuccinic acid, 15.6 min; *S* form, 15.9 min.

Acknowledgements

We thank The Hong Kong Polytechnic University and the Hong Kong Research Grant Council (Project no. HKP 24/95P) for financial support of the study.

References

1. Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1320.
2. (a) *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1985; Vol. 5; (b) Noyori, R. *Asymmetric Catalysis in Organic Synthesis*; Wiley and Sons: New York, 1994; (c) *Catalytic Asymmetric Synthesis*; Ojima, I., Ed.; VCH: New York, 1993; (d) *Advanced Asymmetric Synthesis*; Stephenson, G. R., Ed.; Chapman and Hall: London, 1996; (e) *Advances in Catalytic Processes*; Doyle, M. P., Ed.; JAI Press: Greenwich, CT, 1995; Vol. 1.
3. Collins, A. N.; Sheldrake, G. N.; Crosby, J. *Chirality in Industry: The Commercial Manufacture and Applications of Optically Active Compounds*; Collins, A. N.; Sheldrake, G. N.; Crosby, J., Eds.; John Wiley and Sons: Chichester, 1992.

4. (a) Gilbertson, S. R.; Wang, X.; Hoge, G. S.; Klug, C. A.; Schaefer, J. *Organometallics* **1996**, *15*, 4678. (b) Kaiser, E. T.; Lawrence, D. S. *Science* **1984**, *226*, 505–511. (c) Levine, H. L.; Nakagawa, Y.; Kaiser, E. T. *Biochem. Biophys. Res. Comm*. **1977**, *76*, 64–70.
5. Corey, D. R.; Schultz, P. G. *Science* **1987**, *238*, 1401–1403.
6. (a) Polgar, L.; Bender, M. L. *J. Am. Chem. Soc*. **1966**, *88*, 3153–3154. (b) Neet, K. E.; Koshland, D. E. *Proc. Natl. Acad. Sci. USA* **1966**, *56*, 1606–1609.
7. Wu, Z. P.; Hilvert, D. *J. Am. Chem. Soc*. **1989**, *111*, 4513–4514.
8. Planas, A.; Kirsch, J. F. *Biochemistry* **1991**, *30*, 8268–8273.
9. Wuttke, D. S.; Gray, H. B.; Fisher, S. L.; Imperiali, B. *J. Am. Chem. Soc*. **1993**, *115*, 8455–8456.
10. Imperiali, B.; Roy, R. S. *J. Am. Chem. Soc*. **1994**, *116*, 12083–12084.
11. Suckling, C. J.; Zhu, L. M. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 531–534.
12. Germanas, J. P.; Kaiser, E. T. *Biopolymers* **1990**, *29*, 39–43.
13. (a) Bell, I. M.; Hilvert, D. *New Biocatalysts via Chemical Modification*; Bell, I. M.; Hilvert, D., Eds.; John Wiley and Sons: Chichester, 1994; pp. 73–88. (b) Schultz, P. G. *Science* **1988**, *240*, 426–433.
14. (a) Kuang, H.; Distefano, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 1072–1073. (b) Davies, R. R.; Distefano, M. D. *J. Am. Chem. Soc.* **1997**, *119*, 11643–11652. (c) Kuang, H.; Brown, M. L.; Davies, R. R.; Young, E. C.; Distefano, M. D. *J. Am. Chem. Soc*. **1996**, *118*, 10702–10706.
15. Green, N. M. *Adv. Protein Chem*. **1975**, *29*, 85.
16. *Methods in Enzymology*; Wilchek, M.; Bayer, E. A., Eds.; Vol. 184.
17. (a) Wilson, M. E.; Whitesides, G. M. *J. Am. Chem. Soc*. **1978**, *100*, 306–307. (b) Wilson, M. E.; Nuzzo, R. G.; Whitesides, G. M. *J. Am. Chem. Soc*. **1978**, *100*, 2269–2270.
18. Baker, G. L.; Fritschel, S. J.; Stille, J. R.; Stille, J. K. *J. Org. Chem.* **1981**, *46*, 2954.
19. Nagel, U.; Kinzel, E.; Andrade, J.; Prescher, G. *Chem. Ber*. **1986**, *119*, 3326.