Journal of Molecular Catalysis B: Enzymatic 97 (2013) 1–4

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Ficin-catalyzed asymmetric aldol reactions of heterocyclic ketones with aldehydes

Jian-Ping Fu, Na Gao, Yang Yang, Zhi Guan*, Yan-Hong He*

School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

ARTICLE INFO

Article history:
Received 28 January 2013
Received in revised form 23 June 2013
Accepted 26 June 2013
Available online xxx

Keywords:
Enzymatic promiscuity
Ficin
Asymmetric aldol reaction
Heterocyclic ketones

ABSTRACT

Ficin from fig tree latex displayed a promiscuous activity to catalyze the direct asymmetric aldol reactions of heterocyclic ketones with aromatic aldehydes. Ficin showed good substrate adaptability to different heterocyclic ketones containing nitrogen, oxygen or sulfur. The enantioselectivities up to 81% ee and diastereoselectivities up to 86:14 (*anti*/*syn*) were achieved under the optimized reaction conditions.

1. Introduction

Enzymatic reactions are appreciated to be an efficient and green tool for modern organic synthesis due to its high selectivity, mild conditions and potential use of inexpensive regenerable resources, which has attracted much attention and expanded rapidly in recent years. Enzymatic promiscuity focuses on the enzyme catalytic activities with unnatural substrates and alternative chemical transformations [1–5]. Some elegant works on promiscuous enzyme catalysis have been reported [2,4,6–9]. Among those promiscuous enzymes, hydrolases are considered to be some of the most useful due to their good stability, broad range of substrate compatibility and high efficiency in forming various chemical bonds. They have been used to catalyze Michael additions [10–12], Markovnikov additions [13,14], direct Mannich reactions [15,16] and Henry reactions successfully [17].

Aldol reaction is a powerful synthetic strategy for the construction of new carbon-carbon bonds. There are many reports about enantioselective aldol reactions catalyzed by small organic molecules and metal complexes [18–21]. In recent years, a few successful hydrolase-catalyzed direct aldol reactions have been reported. Berglund and co-workers used mutant CAL-B (Lipase from *Candida antarctica*) to catalyze aldol additions in 2003 [22]. Wang and Yu et al. reported lipase-catalyzed asymmetric aldol reaction in "wet" acetone in 2008 [23]. Recently, our group has reported direct asymmetric aldol reactions catalyzed by nuclease p1 from *Penicillium citrinum*, alkaline protease from *Bacillus licheniformis*, chymopapain from *Carica papaya*, and acidic protease from *Aspergillus usamii* respectively [24–26]. However, up to now, the range of aldol donors has remained narrow in the hydrolase-catalyzed aldol reactions. More recently, our group reported the lipase from porcine pancreas catalyzed aldol reactions of aromatic aldehydes and heterocyclic ketones [27], which is the only report about hydrolase-catalyzed aldol reactions of heterocyclic ketones. Herein, we wish to report another example of hydrolase-catalyzed direct asymmetric aldol reactions of heterocyclic ketones with aldehydes in organic medium. Ficin from fig tree latex, a plant cysteine proteinase, has been exploited commercially in food industry for meat tenderizing, brewing and cookie baking, as well as the production of protein hydrolysates [28]. We found that ficin displayed catalytic promiscuity to catalyze the direct asymmetric aldol reactions of nitrogen, oxygen or sulfur containing heterocyclic ketones with aldehydes in organic medium.

2. Materials and methods

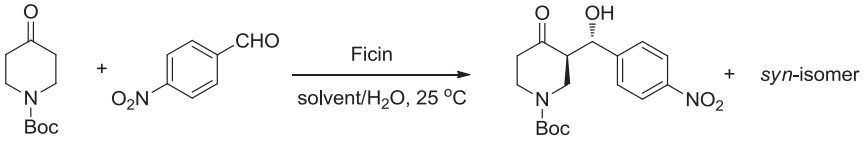
2.1. Materials

Ficin from fig tree latex (≥0.1 unit/mg, Product Number: F4165) was purchased from Sigma–Aldrich Co. All reagents were obtained

* Corresponding authors. Tel.: +86 23 68254091; fax: +86 23 68254091.
E-mail addresses: guanzhi@swu.edu.cn (Z. Guan), heyh@swu.edu.cn (Y.-H. He).

1381-1177/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molcatb.2013.06.016

Table 1
Solvent screening and control experiments of ficin-catalyzed direct asymmetric aldol reaction.[a]



1a **2a** ***anti*-3a**

Entry	Solvent	Yield (%)[b]	dr (*anti*:*syn*)[c]	% ee (*anti*)[d]
1	MeCN	28	40:60	57
2	THF	34	34:66	29
3	*i*-PrOH	11	32:68	29
4	DMSO	26	35:65	5
5	DMF	23	39:61	3
6	Isopropyl ether	Trace	—	—
7	1,4-Dioxane	Trace	—	—
8	Butyl acetate	Trace	—	—
9	$CHCl_3$	Trace	—	—
10	MeCN (no enzyme)	No reaction	—	—
11	MeCN (bovine serum albumin)	43	37:63	3
12	MeCN (ficin denatured with urea[e])	5	35:65	1
13	MeCN (ficin inhibited with MMTS[f])	Trace	—	—
14	$NaHCO_3$[g]	48	31:69	0

[a] Reaction conditions: ketone **1a** (0.25 mmol), 4-nitrobenzaldehyde **2a** (0.50 mmol), and ficin (50 mg) in solvent (0.90 mL) and deionized water (0.10 mL) at 25 °C for 120 h.
[b] Isolated yield after silica gel chromatography.
[c] Determined by chiral HPLC.
[d] Determined by chiral HPLC, and the absolute configuration was assigned by comparison with literatures.
[e] Pretreated with urea (0.83 M, 50 mg urea in 1 mL water) at 100 °C for 24 h, water was removed before use.
[f] Pretreated with MMTS (0.40 M, 50 mg MMTS in 1 mL MeCN) at 25 °C for 24 h, MeCN was removed before use.
[g] The reaction was performed by employing ketone **1a** (0.25 mmol), 4-nitrobenzaldehyde **2a** (0.25 mmol), $NaHCO_3$ (20 mg) in EtOH (1 mL) for 48 h.

from different commercial suppliers and were used without further purification.

2.2. General methods

Routine monitoring of reaction was performed by TLC using precoated Haiyang GF254 silica gel TLC plates. All the column chromatography separations were done by using silica gel (100–200 mesh) at increased pressure. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AMX-300 MHz spectrometer. TMS (^{1}H) and $CDCl_3$ (^{13}C) were used as internal standards. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz.

2.3. General experimental procedure for the ficin-catalyzed aldol reactions

A mixture of aldehyde (0.50 mmol), ficin (75 mg) and ketone (0.25 mmol) in MeCN (0.85 mL) and deionized water (0.15 mL) was stirred for the specified time at 30 °C. The reaction was terminated by filtration to remove the enzyme. CH_2Cl_2 was used to wash the filter paper to assure that products obtained were all dissolved in the filtrate. Then the filtrate was washed three times with water. The organic phase was dried over anhydrous Na_2SO_4, and the solvents were removed under reduced pressure. The crude products were purified by column chromatography with petroleum ether/ethyl acetate as eluent.

3. Results and discussion

In initial research, the aldol reaction between 4-nitrobenzaldehyde and N-Boc-piperidone was used as a model reaction. Since reaction medium has been recognized to be one of the most important factors influencing the enzymatic reactions [29,30], the catalytic activity of ficin in the model aldol reaction was evaluated in different solvents (Table 1). The catalytic activity and stereoselectivity of ficin were significantly influenced by the reaction media. The reaction gave product in the best yield of 34% with a poor enantioselectivity of 29% ee in THF (Table 1, entry 2), while the best enantioselectivity of 57% ee with a yield of 28% was received in MeCN (Table 1, entry 1). In the tested polar solvents including *i*-PrOH, DMSO, and DMF, ficin showed lower activity and enantioselectivity (Table 1, entries 3–5). The reaction in other tested solvents only gave trace amounts of product (Table 1, entries 6–9). Thus, to optimize selectivity, we selected MeCN as the solvent for the ficin-catalyzed aldol reaction.

Besides, some control experiments were performed in order to verify the specific catalytic effect of ficin on the aldol reaction. As can be seen from Table 1, in the absence of enzyme, no reaction was observed between 4-nitrobenzaldehyde and N-Boc-piperidone in MeCN at 25 °C even after 5 days (Table 1, entry 10), indicating that catalyst was essential for the reaction. The urea-denatured ficin almost lost its activity in the model aldol reaction, and only 5% yield was obtained with 1% ee (Table 1, entry 12), suggesting that the tertiary structure of ficin is responsible for its activity and selectivity. In addition, a complete inhibition of the catalytic activity of ficin in aldol reaction was observed when treated with the cysteine protease inhibitor MMTS (methyl methanethiosulfonate) [31] (Table 1, entry 13), indicating that the active site of ficin contributed to this enzymatic promiscuity. The reaction catalyzed by non-enzyme protein bovine serum albumin gave product in 43% yield with 3% ee (Table 1, entry 11), which showed that a non-enzyme protein also had the ability to catalyze the aldol reaction, but almost did not exhibit enantioselectivity for aldol product. This experiment suggested that the catalytic activity of ficin in aldol reaction did not simply arise from the amino acid sequence of the enzyme. Thus, we confirmed that the aldol reaction must take place in a specific fashion on the catalytic site of ficin.

Water content has been considered as a very important factor in enzymatic reactions for it affects both the enantioselectivity and activity of enzymes [32–34]. The control of this parameter was proven to be vital. Thus, we designed some experiments to optimize the percentage of water for this reaction. The effect of water concentration on the ficin-catalyzed model aldol reaction was illustrated in

Table 2
Effect of water content on the ficin-catalyzed aldol reaction.[a]

Entry	Water content	Yield (%)[b]	dr (*anti*:*syn*)	% ee (*anti*)
1	0	21	33:67	43
2	0.05	25	37:63	45
3	0.10	28	40:60	57
4	0.15	32	41:59	59
5	0.20	34	44:56	53
6	0.25	30	43:57	48
7	0.30	33	41:59	41

[a] Reaction conditions: ketone **1a** (0.25 mmol), 4-nitrobenzaldehyde **2a** (0.50 mmol), ficin (50 mg) in 1 mL mixed solvents [$H_2O/(H_2O + MeCN)$ = 0–0.30, v/v] at 25 °C for 120 h.
[b] Isolated yield after silica gel chromatography.

Table 3
Effect of the temperature on the ficin-catalyzed aldol reaction.[a]

Entry	Temperature (°C)	Yield (%)[b]	dr (*anti*:*syn*)	% ee (*anti*)
1	15	20	41:59	56
2	20	24	40:60	58
3	25	28	40:60	57
4	30	35	41:59	60
5	35	37	41:59	55
6	40	38	39:61	43

[a] Reaction conditions: ketone **1a** (0.25 mmol), 4-nitrobenzaldehyde **2a** (0.50 mmol), ficin (50 mg) in MeCN (0.85 mL) and deionized water (0.15 mL) at 15–40 °C for 120 h.
[b] Isolated yield after silica gel chromatography.

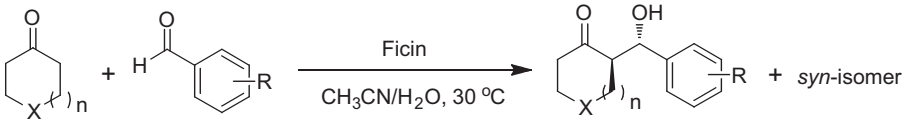
Table 2. When water content was at 0.15 [$H_2O/(H_2O + MeCN)$ = 0.15, v/v], the reaction gave the best enantioselectivity of 59% ee with 32% yield (Table 2, entry 4). Thus, in order to obtain the best enantioselectivity, the water content of 0.15 was chosen for the following research.

It was confirmed that temperature affects the selectivity and rate of the reaction in enzyme-catalyzed reactions [35]. So the effect of temperature on the model aldol reaction was studied. As seen from Table 3, the ficin-catalyzed model aldol reaction reached the best enantioselectivity of 60% ee at 30 °C (Table 3, entry 4). Once the temperature surpassed 30 °C, the ee value decreased sharply (Table 3, entries 5 and 6). Moreover, the by-product (which was formed by the elimination of a water molecule from the corresponding aldol product) increased along with temperature rising. Thus, we selected 30 °C as the optimum temperature for the reaction.

Next, effects of the molar ratio of substrates, enzyme concentration, and pH (buffer) on the ficin catalyzed model aldol reaction were investigated. The molar ratio of aldehyde to ketone 2:1 and the enzyme concentration of 75 mg/mL were chosen as the optimum conditions for the reaction. However, no better results were obtained with buffer in comparison with deionized water. Thus, the mixed solvents of $MeCN/H_2O$ were still used as the optimum reaction medium. In addition, the time course of the reaction was also investigated. (For details about effects of the molar ratio of substrates, enzyme concentration, and pH (buffer) on the ficin catalyzed model aldol reaction, and the time course of the reaction, please refer to the Supplementary Data.)

Having established optimum reaction conditions, the generality of this ficin-catalyzed aldol reaction was examined. The reactions between different aromatic aldehydes and different cyclic ketones under the optimized conditions were conducted, and the results were summarized in Table 4. It can be seen that the desired products were obtained in yields of 21–44% with enantioselectivities up to 81% ee. The steric effect and electronic effect of the substituents in aromatic aldehydes played important role in the reaction. For instance, among the 2-, 3- and 4-nitrobenzaldehydes, the most sterically hindered 2-nitrobenzaldehyde reacting with tetrahyropyran-4-one provided the best enantioselectivity and diastereoselectivity in lowest yield (Table 4, entries 5–7). Moreover, no product was observed between 2-nitrobenzaldehyde and N-Boc-piperidin-4-one. It was speculated that the large steric hindrance obstructed the attack of ketone. Otherwise, the procedure worked smoothly when benzaldehydes bearing various electron-withdrawing groups were employed (Table 4, entries 1–14). On the contrary, only trace

Table 4
Substrate scope[a].



1 **2** *anti*-**3**

Entry	*n*	*R*	*X*	Product	Time (h)	Yield (%)[b]	dr (*anti*:*syn*)[c]	% ee (*anti*/*syn*)[d]
1	1	4-NO_2	NBoc	**3a**	120	36	41:59	60/14
2	1	3-NO_2	NBoc	**3b**	120	29	51:49	59/45
3	1	4-Br	NBoc	**3c**	120	35	75:25	67/36
4	1	3-Br	NBoc	**3d**	120	25	47:53	51/45
5	1	4-NO_2	O	**3e**	120	31	53:47	25/17
6	1	3-NO_2	O	**3f**	120	28	54:46	57/28
7	1	2-NO_2	O	**3g**	144	21	42:58	60/24
8	1	4-Br	O	**3h**	120	29	77:23	71/27
9	1	3-NO_2	S	**3i**	120	26	86:14	77/1
10	1	4-CN	S	**3j**	144	23	84:16	76/53
11[e]	1	4-NO_2	NCH_3	**3k**	72	44	19:81	5/1[f]
12[g]	1	3-NO_2	CH_2	**3l**	117	39	86:14	81/14
13	1	4-CN	CH_2	**3m**	117	21	79/21	77/5
14[e]	0	4-NO_2	S	**3n**	120	31	40:60	45/23[f]

[a] Reaction conditions: aldehyde (0.50 mmol), ketone (0.25 mmol), ficin (75 mg) in MeCN (0.85 mL) and deionized water (0.15 mL) at 30 °C.
[b] Isolated yield after silica gel chromatography.
[c] Determined by chiral HPLC.
[d] Determined by chiral HPLC, and the absolute configurations were assigned by comparison with literatures (for details, please refer to the Supplementary Data).
[e] Reaction conditions: aldehyde (0.25 mmol), ketone (0.50 mmol), ficin (75 mg) in MeCN (0.85 mL) and deionized water (0.15 mL) at 30 °C.
[f] The absolute configuration was not determined.
[g] Reaction conditions: aldehyde (0.25 mmol), ketone (2.50 mmol), ficin (75 mg) in MeCN (0.85 mL) and deionized water (0.15 mL) at 30 °C.

amounts of products were observed when benzaldehydes bearing electron-donating groups were used. This can be explained in that electron-withdrawing groups enhance the electrophilicity of carbonyl carbons in aldehydes which facilitates the reaction, while electron-donating groups lessen the electrophilicity. The reaction between 4-nitrobenzaldehyde and 1-methylpiperidin-4-one gave the best yield of 44% with reversed diastereoselectivity of 19:81 (*anti*:*syn*), but almost no enantioselectivity (Table 4, entry 11). When reacting with 3-nitrobenzaldehyde, tetrahydrothiopyran-4-one gave better diastereoselectivity and enantioselectivity than N-Boc-piperidone and tetrahydropyran-4-one (Table 4, entries 2, 6 and 9). The reaction of dihydrothiophen-3(2*H*)-one with 4-nitrobenzaldehyde gave 2-position aldol condensation compound as the major product in a yield of 31% with 45% ee for *anti* isomer (Table 4, entry 14), and 4-position condensation product was only obtained in a yield of 13%. Besides heterocyclic ketones, cyclohexanone was also used as an aldol donor, which gave enantioselectivities up to 81% ee (Table 4, entries 12 and 13). Finally, it is notable that all the investigated reactions gave better enantioselectivities for *anti* isomers than *syn* isomers.

In summary, we have succeeded in obtaining enantiomeric aldol products of cyclic and heterocyclic ketones by using ficin from fig tree latex as a new biocatalyst. A wide range of ketones and substituted benzaldehydes could be accepted by the enzyme. Although the yields and stereoselectivities are not satisfactory, the ficin-catalyzed direct asymmetric aldol reaction is more economically feasible and sustainable by using inexpensive regenerable resources compared with current chemical technologies. This work provided another example of biocatalytic promiscuity that widens the application of ficin.

Acknowledgements

Financial support from the National Natural Science Foundation of China (No. 21276211) and the Doctoral Foundation of Southwest University (SWU112019) are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2013.06.016.

References

[1] U.T. Bornscheuer, R.J. Kazlauskas, Angew. Chem. Int. Ed. 43 (2004) 6032–6040.
[2] R.J. Kazlauskas, Curr. Opin. Chem. Biol. 9 (2005) 195–201.
[3] P. Berglund, S. Park, Curr. Org. Chem. 9 (2005) 325–336.
[4] K. Hult, P. Berglund, Trends Biotechnol. 25 (2007) 231–238.
[5] P.J. O'Brian, Chem. Rev. 106 (2006) 720–752.
[6] R. Kourist, S. Bartch, L. Fransson, K. Hult, U.T. Bornscheuer, ChemBioChem 9 (2008) 67–69.
[7] G. Hasnaoui Dijoux, M. Majeric Elenkov, J.H. Lutje Spelberg, B. Hauer, D.B. Janssen, ChemBioChem 9 (2008) 1048–1051.
[8] Q. Wu, J.M. Xu, L. Xia, J.L. Wang, X.F. Lin, Adv. Synth. Catal. 351 (2009) 1833–1841.
[9] R.C. Tang, Z. Guan, Y.H. He, W. Zhu, J. Mol. Catal. B Enzym. 63 (2010) 62–67.
[10] M. Svedendahl, K. Hult, P. Berglund, J. Am. Chem. Soc. 127 (2005) 17988–17989.
[11] O. Torre, I. Alfonso, V. Gotor, Chem. Commun. 15 (2004) 1724–1725.
[12] J.F. Cai, Z. Guan, Y.H. He, J. Mol. Catal. B Enzym. 68 (2011) 240–244.
[13] W.B. Wu, N. Wang, J.M. Xu, Q. Wu, X.F. Lin, Chem. Commun. 18 (2005) 2348–2350.
[14] W.B. Wu, J.M. Xu, Q. Wu, D.S. Lv, X.F. Lin, Adv. Synth. Catal. 348 (2006) 487–492.
[15] K. Li, T. He, C. Li, X.W. Feng, N. Wang, X.Q. Yu, Green Chem. 11 (2009) 777–779.
[16] Y. Xue, L.P. Li, Y.H. He, Z. Guan, Sci. Rep. 2 (2012) 761, DOI:10.1038/srep00761.
[17] J.L. Wang, X. Li, H.Y. Xie, B.K. Liu, X.F. Lin, J. Biotechnol. 145 (2010) 240–243.
[18] B. List, A.L. Richard, C.F. Barbas III, J. Am. Chem. Soc. 122 (2000) 2395–2396.
[19] J.T. Suri, D.B. Ramachary, C.F. Barbas III, Org. Lett. 7 (2005) 1383–1385.
[20] Y.M.A. Yamada, N. Yoshikawa, H. Sasai, M. Shibasaki, Angew. Chem. 109 (1997) 1942–1944.
[21] X. Ariza, J. Garcia, P. Romea, F. Urpi, Synthesis 14 (2011) 2175–2191.
[22] C. Branneby, P. Carlqvist, A. Magnusson, K. Hult, T. Brinck, P. Berglund, J. Am. Chem. Soc. 125 (2003) 874–875.
[23] C. Li, X.W. Feng, N. Wang, Y.J. Zhou, X.Q. Yu, Green Chem. 10 (2008) 616–618.
[24] H.H. Li, Y.H. He, Y. Yuan, Z. Guan, Green Chem. 13 (2011) 185–189.
[25] H.H. Li, Y.H. He, Z. Guan, Catal. Commun. 12 (2011) 580–582.
[26] Y.H. He, H.H. Li, Y.Li. Chen, Y. Xue, Y. Yuan, Z. Guan, Adv. Synth. Catal. 31 (2012) 712–719.
[27] Z. Guan, J.P. Fu, Y.H. He, Tetrahedron Lett. 53 (2012) 4959–4961.
[28] S.R. Morcelle, S.A. Trejo, F. Canals, F.X. Avilés, N.S. Priolo, Protein J. 23 (2004) 205–214.
[29] S. Tawaki, A.M. Klibanov, J. Am. Chem. Soc. 114 (1992) 1882–1884.
[30] C.R. Wescott, A.M. Klibanov, Biochim. Biophys. Acta 1206 (1994) 1–9.
[31] M. St-Vincent, M. Dickman, J. Chem. Educ. 81 (2004) 1048–1050.
[32] C. Orrenius, T. Norin, K. Hult, G. Carrea, Tetrahedron: Asymmetry 6 (1995) 3023–3030.
[33] E. Wehtje, J. Kaur, P. Adlercreutz, S. Chand, B. Mattiasson, Enzyme Microb. Tech. 21 (1997) 502–510.
[34] D. Costes, E. Wehtje, P. Adlercreutz, Enzyme Microb. Tech. 25 (1999) 384–391.
[35] A. Zaks, A.M. Klibanov, Science 224 (1984) 1249–1251.