Catalysis Communications 12 (2011) 580–582

Contents lists available at ScienceDirect

Catalysis Communications

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

Short Communication

Protease-catalyzed direct aldol reaction

Hai-Hong Li, Yan-Hong He, Zhi Guan *

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

ARTICLE INFO

Article history:
Received 7 September 2010
Received in revised form 1 December 2010
Accepted 5 December 2010
Available online 13 December 2010

Keywords:
Enzyme catalysis
Alkaline protease
Enantioselectivity
Aldol reaction

ABSTRACT

A new function of BLAP (alkaline protease from *Bacillus licheniformis*) was first discovered to catalyze direct aldol reactions between aromatic aldehydes and cyclic ketones in an organic medium in the presence of water. The products were obtained in yields of 28–92% with 22–99% ee.

1. Introduction

Since enzymes were discovered to maintain their activities in organic solvents [1], enzymes as biocatalysts have been paid more and more attention. Not only can enzymes work in organic media, but they acquire remarkable properties such as enhanced stability, altered substrate and enantiomeric specificity, molecular memory, and the ability to catalyze unusual reactions which are impossible in aqueous media [2–4]. Although some elegant works on enzyme catalysis in organic solvents have been reported [5–17], more reaction types catalyzed by each available enzyme are still greatly required in order to utilize the enzymes in organic synthesis.

The direct aldol reaction is one of the most important C–C bond-forming reactions in organic chemistry. Berglund and co-workers used mutant CAL-B (lipase from *Candida antarctica*) to catalyze aldol addition in cyclohexane in 2003 [18]. Wang and Yu reported the aldol reaction catalyzed by lipase in "wet" acetone in 2008 [19], and they also found lipase-catalyzed decarboxylative aldol reaction in acetonitrile in 2009 [20]. More recently, we found that nuclease p1 could catalyze direct aldol reaction under solvent-free conditions [21]. However, to the best of our knowledge, protease-catalyzed aldol addition has not been reported, so we decided to investigate this biocatalytic transformation with respect to the synthetic potential and stereochemistry. Herein, we wish to report the discovery that BLAP (alkaline protease from *Bacillus licheniformis* 2709) (EC 3.4.21.14, 200 U/mg) catalyzed direct aldol reactions of aromatic aldehydes with some cyclic ketones in an organic medium in the presence of water.

2. Results and discussion

During screening new activities of existing enzymes in organic solvents, we found that BLAP could catalyze the aldol reaction. Thus, we further investigated this novel activity of the enzyme. Initial studies were undertaken using 4-cyanobenzaldehyde and acetone as a model reaction. A preliminary solvent screen was performed to find out the optimal solvent for this biotransformation (see the supporting information). We found that the reaction gave the best yield in DMSO. However, in the other tested solvents, including DMF, CH_2Cl_2, THF, cyclohexane, TBME (tert-butyl methyl ether), $CHCl_3$, acetone and toluene, the reaction gave lower yields. So DMSO was chosen as the optimum solvent.

To demonstrate the specific catalytic effect of BLAP, control experiments were performed (see the supporting information). Just as we expected, the aldol reaction between 4-cyanobenzaldehyde and acetone showed no adduct in the absence of enzyme in DMSO after 139 h, which indicated that BLAP had a specific catalytic effect on the reaction. Furthermore, in order to prove that the catalytic activity of BLAP for aldol reaction did not arise from unspecific protein-derived activation, the experiment catalyzed by the inhibited enzyme was conducted. A complete inhibition of the catalytic activity of BLAP in aldol reaction was observed by using serine protease inhibitor PMSF (phenylmethanesulfonyl fluoride). In addition, since BLAP is a Ca^{2+}-dependent enzyme, EDTA (ethylenediaminetetraacetic acid) was also used to denature the enzyme. We found that the EDTA-denatured BLAP completely lost its catalytic activity for aldol reaction, which further demonstrated that the catalytic activity of BLAP for aldol reaction did not simply arise from the amino acid sequence of the enzyme. Based on the results of control experiments, we validated that BLAP catalyzed the direct aldol reaction.

It is well known that water plays a major role of "molecular lubricant" in an enzyme resulting in conformational flexibility of the

* Corresponding author. Tel.: +86 23 68254091; fax: +86 23 68254091.
E-mail address: guanzhi@swu.edu.cn (Z. Guan).

1566-7367/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.catcom.2010.12.003

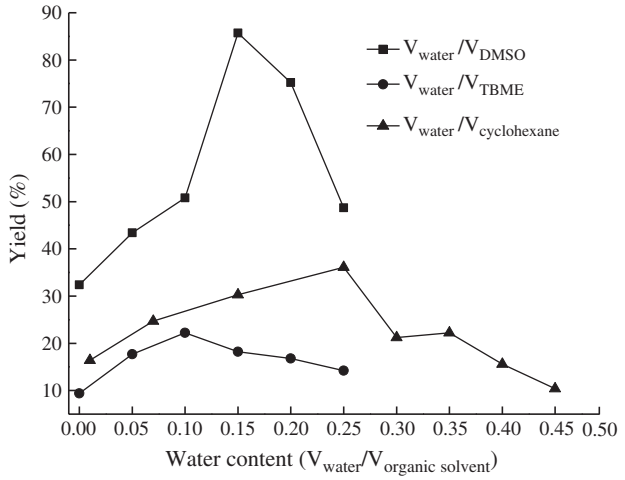


Fig. 1. The influence of water contents in organic solvents on the yield of BLAP-catalyzed aldol reaction. Conditions: BLAP (200 mg), acetone (1.0 mL, 17.0 mmol), 4-cyanobenzaldehyde (200 mg, 1.5 mmol) and solvent (5 mL) at 35 °C for 143 h. Deionized water was added from 0 to 0.45 (water/solvent, v/v).

enzyme and the increased hydration leads to enhanced activity in non-aqueous solvents. There appears to be a critical amount of water necessary for enhancing activity [22,23]. So it is important to confirm the optimal water content in the reaction system. Therefore, we analyzed the effects of the amount of H_2O on the reaction yields of acetone with 4-cyanobenzaldehyde in three different solvents (Fig. 1).

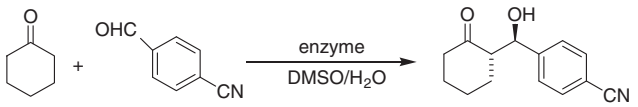
It can be seen that the yield of the reaction could be evidently affected by water content in an organic solvent. There were different optimum water contents in different solvents for BLAP to show the best activity in aldol reaction. The optimum water content in the water-miscible hydrophilic solvent DMSO was 0.15 (V_{water}/V_{DMSO}). Meanwhile, the optimum water content for this reaction in the hydrophobic water-immiscible solvent TBME was 0.10 (V_{water}/V_{TBME}), and in cyclohexane was 0.25 ($V_{water}/V_{cyclohexane}$). In the case of water/DMSO, the enzyme was adequately suspended in the miscible solvent system, which gave a good yield of 86% under the optimum water content conditions. However, the water/TBME and water/cyclohexane were biphasic water–water-immiscible organic solvent systems, and the enzyme was distributed in the aqueous phase to form an emulsion. The enzymatic aldol reaction in these two-phase systems only gave the product in low yields, probably due to the insufficient contact between the enzyme which was distributed in the aqueous phase and the substrates which were mainly distributed in the organic phase. In addition, once the concentration of water in different solvents surpassed the corresponding optimum water content, the yields of aldol product decreased evidently. All the results indicated that water was obviously essential in the enzyme catalyzed aldol reaction in organic solvents. Finally, we chose water/DMSO (0.15, v/v) as the solvent system for aldol reaction.

The temperature is another important factor for the enzyme-catalyzed reactions, due to its effects on the selectivity and rate of the reaction, and also on the stability of the enzyme [24]. Thus, a temperature screen was performed (Table 1). The yield of the enzymatic reaction could be increased by raising the temperature from 15 °C to 35 °C. However, the enantiomeric selectivity of the product reached the best ee of 51% at 20 °C, and higher temperature caused an evident decrease of selectivity. Thus, the optimum temperature for the reaction was 20 °C.

Finally, to test the substrate generality of BLAP-catalyzed aldol reaction, various aldehydes and ketones were investigated under the optimized conditions. The enzyme showed a wide range of substrate specificity towards aromatic aldehydes and cyclic ketones (Table 2).

It can be seen that both electron-donating and electron-withdrawing substituents of aromatic aldehydes could be accepted by the enzyme. In general, when cyclohexanone was used as an aldol donor, the aromatic aldehyde containing an electron-donating group gave inferior yield. For instance, 4-methylbenzaldehyde provided the product only in yield of 28% with moderate *anti* diastereoselectivity, but it gave the excellent enantioselectivity (>99% ee) (Table 2, entry 1). In contrast, aromatic aldehydes bearing electron-withdrawing substituents furnished aldol products in good yields (65–92%) with low to good *anti* diastereoselectivities (58:42–95:5) but low enantioselectivities (36–70% ee) (Table 2, entries 2–11). Moreover, substituent positions on the benzene ring of aromatic aldehydes had a great impact on the enantio- and diastereoselectivity of the reaction. The substituent in the 4-position led to a higher ee value than the same substituent in the 2- or 3-position (Table 2, entries 2 and 5). However, the substituent in the 2-position gave higher dr values than the same substituent in the 3- or 4-position (Table 2, entries 3 and 6). The best diastereoselectivity of 95:5 (*anti*/*syn*) was achieved by using 2,6-dichlorobenzaldehyde (Table 2, entry 9). In addition, when cyclopentanone and cycloheptanone were used as the donors, the aldol products were obtained in low yields with low enantioselectivities (Table 2, entries 12 and 13). Interestingly, *anti*-isomers were received as the major products by using cyclohexanone, but reversed diastereoselectivities were observed by using cyclopentanone and cycloheptanone. BLAP-catalyzed aldol reaction seemed to prefer cyclohexanone than cyclopentanone and cycloheptanone. It was also worthy to note that this enzyme showed different degrees of enantioselectivity for *anti* isomers, but low or no enantioselectivity for *syn* isomers. Besides, we found that BLAP did not catalyze the reactions between aliphatic aldehydes and acetone. Also, the enzyme showed poor enantioselectivities for the reactions of aromatic aldehydes and acetone. Maybe the catalytic site of the BLAP had a specific selectivity for the aldol reaction.

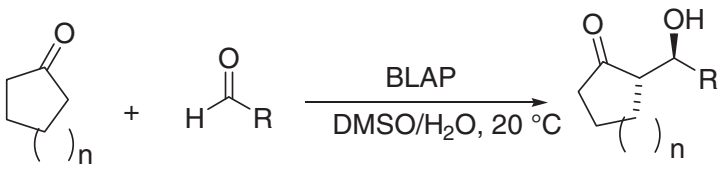
Table 1
The optimization of temperature.[a]



Entry	Temp (°C)	Time [h]	Yield [%][b]	Anti/Syn[c]	ee (%) [d] (anti)
1	15	47	57	65:35	49
2	20	47	59	75:25	51
3	25	22	61	57:43	41
4	30	12	61	51:49	40
5	35	12	67	50:50	21

[a] Reaction conditions: 4-cyanobenzaldehyde (1.0 mmol), cyclohexanone (5.0 mmol), BLAP (200 mg), H_2O (0.15 mL), and DMSO (1.0 mL).
[b] Yield of the isolated product after chromatography on silica gel.
[c] Determined by ^{1}HNMR analysis.
[d] Enantiomeric excess was determined by HPLC analysis using a chiral column; absolute configurations of the products were determined by comparison with the known chiral HPLC analysis.

Table 2
The scope of BLAP-catalyzed aldol reactions.[a]



1 2 3

Entry	R	Product	n	Time [h]	Yield [%][b]	Anti/Syn[c]	ee [%][d] (anti)
1	4–Me–C_6H_4	3a	2	72	28	70:30	>99
2	4–NO_2–C_6H_4	3b	2	72	91	68:32	70
3	2–NO_2–C_6H_4	3c	2	72	81	80:20	44
4	3−NO_2−C_6H_4	3d	2	72	87	58:42	51
5	4–Cl–C_6H_4	3e	2	72	74	75:25	55
6	2–Cl–C_6H_4	3f	2	72	72	80:20	46
7	3–Cl–C_6H_4	3 g	2	72	73	72:28	50
8	2,4–Cl_2–C_6H_3	3 h	2	72	82	62:38	51
9	2,6–Cl_2–C_6H_3	3i	2	76	92	95:5	36
10	4–Br–C_6H_4	3j	2	72	65	73:27	53
11	4–CF_3–C_6H_4	3 k	2	76	91	68:32	50/22[e]
12	4–CN–C_6H_4	3 l	1	65	61	33:67	22/47[f]
13	4–NO_2–C_6H_4	3 m	3	120	35	32:68	24

[a] Reaction conditions: aldehyde (1.0 mmol), ketone (5.0 mmol), BLAP (200 mg), H_2O (0.15 mL), and DMSO (1.0 mL) at 20 °C.
[b] Yield of the isolated product (*anti* + *syn*) after chromatography on silica gel.
[c] Determined by ^{1}HNMR analysis.
[d] Enantiomeric excess was determined by HPLC analysis using a chiral column; absolute configurations of the products were determined by comparison with the known chiral HPLC analysis [25–29]. (See the supporting information).
[e] *Anti* (50% ee), and *syn* (22% ee).
[f] *Anti* (22% ee), and *syn* (47% ee).

3. Conclusion

We reported an enzyme-catalyzed direct aldol reaction in an organic medium in the presence of water. BLAP as a novel biocatalyst showed a wide range of substrate tolerance towards aldol reactions of aromatic aldehydes and cyclic ketones. The products were obtained in yields of 28–92% with 22–99% ee without using any additive. The influence of reaction conditions including solvents, water content and temperature was also investigated. The specific catalytic effect of BLAP was demonstrated by some control experiments. This BLAP-catalyzed direct aldol reaction provided a novel case of unnatural activities of existing enzymes in an organic medium. Further studies focusing on the improvement of enantioselectivity of this enzyme-catalyzed transformation are currently under investigation.

4. Experimental

General procedure for aldol reactions (products, **3a–m**): A 25 mL round-bottomed flask was charged with the enzyme (200 mg), DMSO (1 mL) and aldehyde (1.0 mmol), to which the deionized water (0.15 mL) and ketone (5.0 mmol) were introduced. The resulting mixture was stirred for a specified amount of time at 20 °C. The reaction was terminated by filtering the enzyme. CH_2Cl_2 was used to wash the filter paper to assure that products obtained were all dissolved in the filtrate. 20 mL of water was then added to the filtrate, and the filtrate was extracted three times with 20 mL of CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4 and the solvents were then removed under reduced pressure. The crude products were purified by column chromatography with petroleum ether/ethyl acetate as eluent.

Acknowledgements

Financial support from the Natural Science Foundation Project of CQ CSTC (2009BA5051) and the Selected Project in Scientific and Technological Activities in 2007 for Returned Scholars by the State Personnel Ministry is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.catcom.2010.12.003

References

[1] K. Griebenow, A.M. Klibanov, J. Am. Chem. Soc. 47 (1996) 11695–11700.
[2] A.M. Klibanov, Trends Biochem. Sci. 14 (1989) 141–144.
[3] S. Tawaki, A.M. Klibanov, J. Am. Chem. Soc. 114 (1992) 1882–1884.
[4] A.M. Klibanov, Nature 409 (2001) 241–246.
[5] K. Hult, P. Berglund, Trends Biotechnol. 25 (2007) 231–238.
[6] U.T. Bornscheuer, R.J. Kazlauskas, Angew. Chem. Int. Ed. 43 (2004) 6032–6040.
[7] R. Kourist, S. Bartch, L. Fransson, K. Hult, U.T. Bornscheuer, Chembiochem 9 (2008) 67–69.
[8] P. Carlqvist, M. Svedendahl, C. Branneby, K. Hult, T. Brinck, P. Berglund, Chembiochem 6 (2005) 331–336.
[9] R.J. Kazlauskas, Curr. Opin. Chem. Biol. 9 (2005) 195–201.
[10] P. Berglund, S. Park, Curr. Org. Chem. 9 (2005) 325–336.
[11] G. Hasnaoui-Dijoux, M. Majerić Elenkov, J.H. Lutje Spelberg, B. Hauer, D.B. Janssen, Chembiochem 9 (2008) 1048–1051.
[12] J.M. Xu, F. Zhang, B.K. Liu, Q. Wu, X.F. Lin, Chem. Commun. 20 (2007) 2078–2080.
[13] S.P. Yao, D.S. Lu, Q. Wu, Y. Cai, S.H. Xu, X.F. Lin, Chem. Commun. 17 (2004) 2006–2007.
[14] W.B. Wu, J.M. Xu, Q. Wu, D.S. Lv, X.F. Lin, Adv. Synth. Catal. 348 (2006) 487–492.
[15] F.W. Lou, B.K. Liu, Q. Wu, D.S. Lv, X.F. Lin, Adv. Synth. Catal. 350 (2008) 1959–1962.
[16] Q. Wu, J.M. Xu, L. Xia, J.L. Wang, X.F. Lin, Adv. Synth. Catal. 351 (2009) 1833–1841.
[17] R.C. Tang, Z. Guan, Y.H. He, W. Zhu, J. Mol, Catal. B: Enzym. 63 (2010) 62–67.
[18] C. Branneby, P. Carlqvist, A. Magnusson, K. Hult, T. Brinck, P. Berglund, J. Am. Chem. Soc. 125 (2003) 874–875.
[19] C. Li, X.W. Feng, N. Wang, Y.J. Zhou, X.Q. Yu, Green Chem. 10 (2008) 616–618.
[20] X.W. Feng, C. Li, N. Wang, K. Li, W.W. Zhang, Z. Wang, X.Q. Yu, Green Chem. 11 (2009) 1933–1936.
[21] H.H. Li, Y.H. He, Y. Yuan, Z. Guan, Green Chem. (2010), doi:10.1039/C0GC00486C.
[22] A. Zaks, A.M. Klibanov, J. Biol. Chem. 263 (1988) 8017–8021.
[23] G.D. Yadav, P.S. Lathi, J. Mol, Catal. B: Enzym. 27 (2004) 113–119.
[24] A. Zaks, A.M. Klibanov, Science 224 (1984) 1249–1251.
[25] Y.Y. Wu, Y.Z. Zhang, M.L. Yu, G. Zhao, S.W. Wang, Org. Lett. 8 (2006) 4417–4420.
[26] J.R. Chen, H.H. Lu, X.Y. Li, L. Cheng, J. Wan, W.J. Xiao, Org. Lett. 7 (2005) 4543–4545.
[27] N. Mase, Y. Nakai, N. Ohara, H. Yoda, K. Takabe, F. Tanaka, C.F. III Barbas, J. Am. Chem. Soc. 128 (2006) 734–735.
[28] H. Yang, R.G. Carter, Org. Lett. 10 (2008) 4649–4652.
[29] M. Gruttadauria, F. Giacalone, A.M. Marculescu, P.L. Meo, S. Riela, R. Noto, Eur. J. Org. Chem. (2007) 4688–4698.