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Biocatalytic asymmetric aldol reaction in buffer solution

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ABSTRACT

A green and convenient protocol has been developed for asymmetric cross-aldol reaction. In this Letter, bovine pancreatic lipase (BPL) was first reported to catalyze the aldol reaction and acidic buffer was first used for promiscuous enzymatic aldol reaction.

Biocatalysis is considered to be one of the available approaches to achieve green chemistry owing to its high selectivity, mild conditions, low energy requirements, and few by-products. Especially, in recent years, some hydrolytic enzymes have demonstrated high activity for unnatural substrates and alternative chemical transformations, namely, biocatalytic promiscuity, which provides a new tool for organic synthesis and largely extends the application of enzymes.[1] Several novel examples of catalytic promiscuity of hydrolases have been reported for their ability to catalyze carbon–carbon or carbon–heteroatom bond formations in the last decade, such as aldol reaction,[2] Michael addition,[3] Mannich reaction,[4] Henry reaction,[5] and Knoevenagel reaction.[6] Humble and Berglund reviewed various aspects of enzyme catalytic promiscuity from the biocatalytic perspective[1c] Kapoor and Gupta also summarized the promiscuity of lipase and its applications in a biochemical process more recently.[7]

The aldol reaction is considered one of the most important C–C bond-forming reactions and widely used in synthetic organic chemistry for constructing the hydroxyl ketones frequently found in many biologically active compounds and drugs. The development of asymmetric aldol reaction catalysts remains an active area in recent years,[8] and some enzymes have been described. In 2003, the first promiscuous aldol reaction catalyzed by CAL-B (lipase from *Candida antarctica*) was reported by Berglund.[9] Our previous studies showed some lipases and proteases could catalyze aldol addition in an organic medium, and reported the first lipase-catalyzed asymmetric aldol reaction in 2008.[2b,10] In the past two years, several enzyme-catalyzed aldol reactions were also reported by Guan and co-workers.[11] To the best of our knowledge, all of the hydrolase-catalyzed aldol reactions were performed in organic media, and the harm from organic solvent was nearly unavoidable. Therefore, it is significant to expand environmentally benign media for biocatalytic aldol reaction and other processes.

In our previous study, we have been aware of the fact that lipase-catalyzed aldol reaction could be performed in pure water. Then, as a part of our continuing interest in enzymatic synthesis and green synthetic methodology, we wish to report a green and efficient biocatalytic route for asymmetric aldol reaction. Here, an eco-friendly medium, buffer solution was first used in promiscuous enzymatic aldol reaction and BPL (bovine pancreatic lipase) was first reported to catalyze asymmetric aldol reaction. Happily, the pH had a significant impact on the stereoselectivity and most of the tested substrates gave high yields and moderate stereoselectivity.

Based on our previous research, initial efforts were performed in pure water using 4-nitrobenzaldehyde and cyclohexanone as a model reaction and some hydrolases as catalysts. To our delight, a few examined enzymes could promote the aldol reaction efficiently and BPL gave a better result (data not shown). We also understand the catalytic activity of enzyme is strongly dependent on the pH value of the medium. Then, the BPL-catalyzed reactions were carried out in phosphate–citrate buffer with different pH (4.0–8.0), and a series of control experiments were performed to demonstrate the specific catalytic effect of BPL. As expected, pH has a significant influence on the yield and stereoselectivity (Table 1). Generally, higher pH inclined to give the products in better yield, but slightly acidic conditions were better for improving the stereoselectivity. Finally, pH 5.6 was chosen as the optimal pH in terms of efficiency and selectivity of BPL. Additionally, some organic solvents were also

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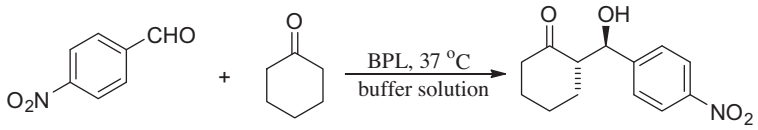
Table 1
The effect of pH on the BPL-catalyzed aldol reaction in phosphate–citrate buffer[a]



Entry	pH	Yield[b] (%)	*dr*[b] (*anti*/*syn*)	*ee*[b] (%) (*anti*)
1	4.0	18	55:45	33
2	4.6	35	62:38	43
3	4.8	41	63:37	45
4	5.0	48	63:37	46
5	5.2	53	64:36	46
6	5.4	57	64:36	46
7	5.6	65	63:37	45
8	5.8	70	61:39	42
9	6.0	77	59:41	38
10	6.4	81	57:43	33
11	7.0	88	51:49	10
12	8.0	91	48:52	2
13	4.0 (No enzyme)	Trace	—	—
14	5.6 (No enzyme)	Trace	—	—
15	7.0 (No enzyme)	44	65:35	0
16	8.0 (No enzyme)	99	60:40	0
17	BSA[c,e]	24	46:54	10
18	Denatured BPL[d,e]	15	54:46	14

[a] Reaction conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (1 mmol), and BPL (10 mg) in phosphate–citrate buffer (1.0 mL, pH 4.0–8.0) at 37 °C for 48 h.
[b] Yield, *dr*, and *ee* were determined by HPLC using AD-H chiral column.
[c] Bovine serum albumin.
[d] Pretreated with guanidine hydrochloride solution (6 mol/L).
[e] In phosphate–citrate buffer (1.0 mL, pH 5.6).

chosen as the reaction media for the purpose of comparison, but all the examined solvents only gave poor results from the point of yield or selectivity (Table S2).

And we incline to attribute the above phenomena to the following reasons. On the one hand, it was not hard to see that the reaction could carry out freely in the absence of enzyme in pH 7.0, but no enantioselectivity was observed (Table 1, entry 15). So we believed that BPL only made a part of contribution to the aldol reaction in such pH, which corresponds to the higher yield and lower stereoselectivity. In pH 8.0, the reaction nearly became a base-catalyzed process, therefore, extremely low enantioselectivity and higher yield were obtained (Table 1, entries 12 and 16). On the other hand, the selectivity improved under acidic conditions which might be due to the following two aspects. Firstly, BPL made greater contribution to the reactions at lower pH because the blank control reactions did not happen at all in pH 4.0 or 5.6 (Table 1, entries 13 and 14). Secondly, the conformational changes of BPL under acidic conditions might play a certain role, which could be demonstrated through the fluorescence emission spectra of BPL (Fig. 1). Fluorescence spectra are usually employed for conformational characterization of enzymes, and have been used to correlate changes in the stability and activity of *Candida antarctica* lipase B, α-chymotrypsin with their secondary structure.[12] Because most of proteins such as BPL contain fluorescent residues (e.g., Trp, Tyr or Phe), structural variations can be reflected by changes in the maximal intensity of fluorescence (I_{max}) or the maximal emission wavelength (λ_{max}), both of which result from altered polarity of the microenvironment of these residues. Generally, a red shift of λ_{max} in an aqueous system corresponds to unfolding of the enzyme, which enhances the exposure of fluorescent residues to the bulk solvent.[12a,b] As shown in Figure 1, compared with pH 7.0, the λ_{max} was decreased from 358 to 347 nm in pH 5.6, which implies a more compact conformation in pH 5.6. And the compact conformation may be beneficial to improve the selectivity of BPL.

At the same time, it was easy to see that the reactions in acidic buffer were BPL-catalyzed processes from some control experiments (Table 1, entries 13 and 14, 17 and 18). In pH 4.0 and 5.6, no corresponding aldol products were produced in the absence of BPL (Table 1, entries 13 and 14). Though denatured-BPL and non-enzyme protein BSA could catalyze the reaction to produce small amount of target products, both of them only showed poor enantioselectivity (Table 1, entries 17 and 18), which suggested that the tertiary structure of BPL was essential in the process.

To further optimize reaction conditions, the effects of mole ratio (Table 2), enzyme loading (Table S3), and temperature (Table S4) on the BPL-catalyzed aldol reaction were investigated successively. The data indicated that these factors had very significant impacts on the yield and *ee* value, but the changes of *dr* value were less obvious. Consequently, we chose mole ratio 1:20, 30 °C, and enzyme

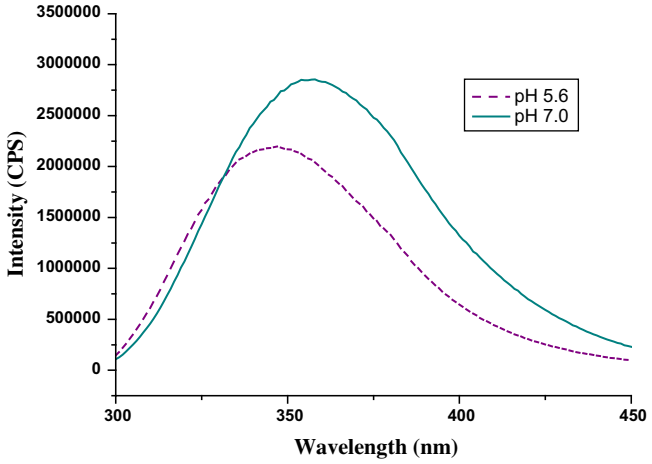
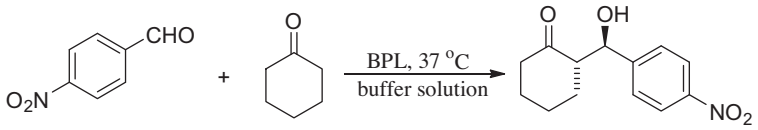


Figure 1. Fluorescence emission spectra of BPL[a] in different pH[b]. [a]BPL (5 mg) was dissolved in phosphate–citrate buffer (5 mL) and shaken at 37 °C for 5 h. [b]Conditions: λ_{exc} = 292 nm, slits = 3 nm/3 nm.

Table 2
Effect of mole ratio on the model reaction in phosphate–citrate buffer[a]



Entry	Mole ratio[b]	Yield[c] (%)	*dr*[c] (*anti*/*syn*)	*ee*[c] % (*anti*)
1	1:1	4	55:45	27
2	1:5	43	59:41	39
3	1:10	68	62:38	44
4	1:15	71	64:36	47
5	1:20	71	65:35	51
6	1:25	68	65:35	51
7	1:30	66	65:35	52

[a] Reaction conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (0.1–3.0 mmol), and BPL (10 mg) in phosphate–citrate buffer (1.0 mL, pH 5.6) at 37 °C for 48 h.
[b] Mole ratio = 4-nitrobenzaldehyde/cyclohexanone.
[c] Yield, *dr*, and *ee* were determined by HPLC using AD-H chiral column.

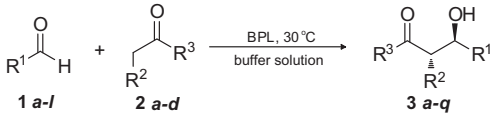
concentration of 30 mg/mL as the best conditions after weighing each side of the model reaction.

Next, more aldehydes and ketones were used to expand the BPL-catalyzed aldol reaction to show the generality and scope of this enzymatic asymmetric process. The results are given in Table 3, it can be seen that a wide range of aromatic aldehydes can effectively react with cyclic ketones under the identified conditions, and the best yield of 99% has been obtained. In general, the corresponding aldol products were obtained in higher yields when ketones reacted with aromatic aldehydes bearing an electron-withdrawing substituent such as a nitro group (Table 3, entries 8–10). And heteroaromatic aldehydes, for instance, 3-pyridinecarboxaldehyde had much higher activity than benzaldehyde (Table 3, entries 1, 11, and 12), which should owe to the electron-withdrawing effect of the nitrogen atom of pyridine ring. On the other side, five-, six-membered cyclic ketones were better donors relative to cycloheptanone and acetone.

To our great joy, BPL showed a certain stereoselectivity for most of the substrates, and the best diastereoselectivity of 96:4 *dr* and the best enantioselectivity of 66% *ee* were achieved. Interestingly, moderate to good enantioselectivities were obtained for *anti*-isomers, but *syn*-isomers merely gained negligible results. Moreover, the stereoselectivity was influenced significantly by the structure of either the accepter or the donor. All the tested derivatives of benzaldehyde gave better selectivity when cyclohexanone acted as donor (Table 3, entries 2–10), but benzaldehyde itself and pyridinecarboxaldehyde just showed poor selectivity (Table 3, entries 1, 11, and 12). Unfortunately, no other donors could show better diastereoselectivity except cyclohexanone. In a word, BPL possesses special substrate selectivity as well as stereoselectivity for aldol reaction in acidic buffer.

In short, BPL was first reported to catalyze the asymmetric aldol reaction between aromatic aldehydes and cyclic ketones. More interesting is that a greener medium, namely, acidic buffer was

Table 3
Investigation of the reactant scope of the BPL-catalyzed asymmetric aldol reaction[a]



Entry	R^1	R^2, R^3	Product	Time (d)	Yield[b] (%)	*dr*[c] (*anti*/*syn*)	*ee*[c] (%) (*anti*)
1	C_6H_5	$(CH_2)_4$	**3a**	4.0	13	46:54	14/5[d]
2	4-$F_3CC_6H_4$	$(CH_2)_4$	**3b**	5.0	88	63:37	66
3	4-ClC_6H_4	$(CH_2)_4$	**3c**	5.0	43	67:33	63
4	2,6-$Cl_2C_6H_3$	$(CH_2)_4$	**3d**	4.5	81	96:4	43
5	2-BrC_6H_4	$(CH_2)_4$	**3e**	4.0	44	76:24	58
6	4-BrC_6H_4	$(CH_2)_4$	**3f**	5.0	43	87:13	66
7	4-CNC_6H_4	$(CH_2)_4$	**3g**	5.0	99	67:33	60
8	2-$NO_2C_6H_4$	$(CH_2)_4$	**3h**	5.0	72	67:33	50
9	3-$NO_2C_6H_4$	$(CH_2)_4$	**3i**	4.0	91	72:28	66
10	4-$NO_2C_6H_4$	$(CH_2)_4$	**3j**	2.5	99	70:30	61
11	3-Pyridyl	$(CH_2)_4$	**3k**	3.5	88	54:46	32/6[d]
12	4-Pyridyl	$(CH_2)_4$	**3l**	3.0	86	52:48	35/2[d]
13	2-$NO_2C_6H_4$	$(CH_2)_3$	**3m**	3.0	83	56:44	37/6[d]
14	3-$NO_2C_6H_4$	$(CH_2)_3$	**3n**	3.0	93	57:43	25/14[d]
15	4-$NO_2C_6H_4$	$(CH_2)_3$	**3o**	3.0	95	52:48	21/7[d]
16	4-$NO_2C_6H_4$	$(CH_2)_5$	**3p**	5.0	45	55:45	22/1[d]
17	4-$NO_2C_6H_4$	H, CH_3	**3q**	4.5	26	—	0

[a] Reaction conditions: aromatic aldehyde (0.5 mmol), ketone (10 mmol), and BPL (150 mg) in phosphate–citrate buffer (pH 5.6, 5.0 mL) at 30 °C.
[b] Yield of the isolated product after chromatography on silica gel.
[c] *dr* and *ee* were determined by HPLC using a chiral column.
[d] *ee* of *anti*/*ee* of *syn*.

first used for enzymatic aldol reaction. Under optimized conditions, many aromatic aldehydes and cyclic ketones could participate in the reaction with high yields and moderate stereoselectivity. Though the stereoselectivitives are not satisfactory, this system may be developed into a potentially worthy method for enzyme promiscuity study.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.12.022.

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