Biocatalytic promiscuity: the first lipase-catalysed asymmetric aldol reaction

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It was first observed that PPL, lipase from porcine pancreas, and several other lipases have a promiscuous ability to catalyse asymmetric aldol reactions between acetones and aldehydes in the presence of water.

Biocatalytic promiscuity, a new frontier extending the use of enzymes in organic synthesis, has attracted much attention and expanded rapidly in recent years.[1] It focuses on the enzyme catalytic activities with unnatural substrates and alternative chemical transformations, such as the side ability harbored by decarboxylase to catalyse acyloin condensation.[2] Exploiting enzyme catalytic promiscuity might lead to improvements in existing catalysts and provide novel synthesis pathways that are currently not available. Some elegant works have been done in the last decades.[3]

Among the promiscuous enzymes, hydrolases (such as lipase, protease and esterase) undoubtedly play an important role due to their high stability, wide sources and broad range of substrates.[4] Recently, several promiscuous hydrolase-catalysed reactions have been reported.[5] For instance, Wu *et al.* demonstrated that penicillin G acylase, a hydrolase which is widely used as a biocatalyst in the enzymatic synthesis of β-lactam antibiotics, can catalyse Markovnikov addition of allopurinol to vinyl ester.[6] A further example is reported by the group of Gotor.[7] They found an unprecedented lipase catalysed Michael addition of secondary amines to acrylonitrile. These cases and other relevant reports encouraged us to believe that the catalytic activities for addition reaction rather than the well-known hydrolytic function may also have a natural role in hydrolase evolution.

Aldol addition is one of the most useful methods for carbon–carbon bond formation in organic synthesis.[8] Berglund and co-workers once used mutant CAL-B (lipase from *Candida antarctica*) to catalyse aldol addition in 2003.[9] Although the Ser105Ala mutant CAL-B exhibited an increased reaction rate as compared with the wide type in their experiments, both of them showed quite low activities (reaction time more than 50 days). Besides, the enzymatic process is not enantioselective and only simple aliphatic aldehydes, such as propanal and hexanal, had been used. Generally, practical lipase-catalysed aldol reactions hadn't been developed in organic synthesis. To the best of our knowledge, other lipase-catalysed aldol additions, especially asymmetric aldol reactions have never been reported.

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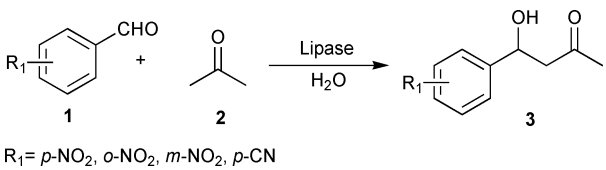
Herein, we surprisingly found that several lipases display observable activities and enantioselectivities for aldol addition in a "wet" reaction condition, where lipase always presents a hydrolytic function (Scheme 1). During our continued work on the new catalytic promiscuity, lipase from porcine pancreas (PPL, EC 3.1.1.3), which is triacylglycerol acylhydrolase and was already commercially available long before, showed a special promiscuous catalytic activity, such as considerable reaction activity and higher enantioselectivity. To clarify the enzymatic process, we performed some experiments to tentatively hypothesize the mechanism of this new biocatalytic promiscuouity. Since this novel catalytic promiscuity is enantioselective and can especially tolerate a wide range of substrates, it not only could extend the enzymatic reaction specificity, but might be practically utilised in organic synthesis.



Scheme 1 Aldol reaction catalyzed by lipase in the presence of water.

In order to confirm the catalytic activity of the lipases, we performed some experiments to focus on the specific catalytic effect of enzymes. As shown in Table 1, several lipases had been screened in our experiments. In the case of PPL, the yield increased with time progress, up to 96.4% after 144 h (entries 2, 3, 4 and 5). Two lipases from *Mucor* (MJL and MML) also showed the ability to catalyse the asymmetric aldol reaction, while their catalytic activities were less efficient (entries 8 and 12). CAL-B showed very low but detectable activity in the same condition (entry 11). Other lipases (CRL, PCL, PSL) displayed no activity for aldol reaction in the reaction conditions (entries 10, 14 and 15). The control experiment was carried out in the absence of any biocatalyst (entry 1). No product had been detected, even after 72 h. When the reaction was incubated with denatured PPL, denatured MJL, denatured MML or bovine serum albumin (BSA), respectively, the reaction rate was nearly equal to the control reaction, suggesting that the specific structure of lipase was necessary to carry out the aldol addition (entries 7, 9, 13 and 16). Interestingly, when the water concentration was controlled to 1%, the degree of enantioselectivity rose up to 43.6% (entry 6). All the results suggest that the special spatial conformation of these lipases is responsible for the aldol reaction.

To improve the activity of the enzyme, we carried out some experiments focusing on the influence of water concentration,

Table 1 The catalytic activities and stereoselectivities of the aldol reaction between 4-nitrobenzaldehyde and acetone[a]

Entry	Catalyst	Reaction time/h	Yield (%)	E.e (%)
1	No enzyme	72	< 0.5	—[b]
2	PPL	24	25.6	17.7
3	PPL	48	32.2	18.5
4	PPL	72	55.7	15.8
5	PPL	144	96.4	14.7
6	PPL	72	11.7	43.6[c]
7	Denatured PPL[d]	48	< 0.5	—
8	MJL	24	14.5	12.9
9	Denatured MJL[d]	48	< 0.5	—
10	CRL	24	< 0.5	—
11	CAL-B	24	2.3	9.4
12	MML	24	9.8	9.6
13	Denatured MML[d]	48	< 0.5	—
14	PCL	24	< 0.5	—
15	PSL	24	< 0.5	—
16	BSA	24	< 0.5	—

[a] Reaction conditions: lipase 20 mg, 4-nitrobenzaldehyde 0.12 mmol, acetone 1 ml, deionized water 0.25 ml, 30 °C. [b] Not determined. [c] Reaction conditions: lipase 20 mg, 4-nitrobenzaldehyde 0.12 mmol, acetone 1 ml, deionized water 0.01 ml, 30 °C. [d] Pretreated with urea at 100 °C for 8 h.

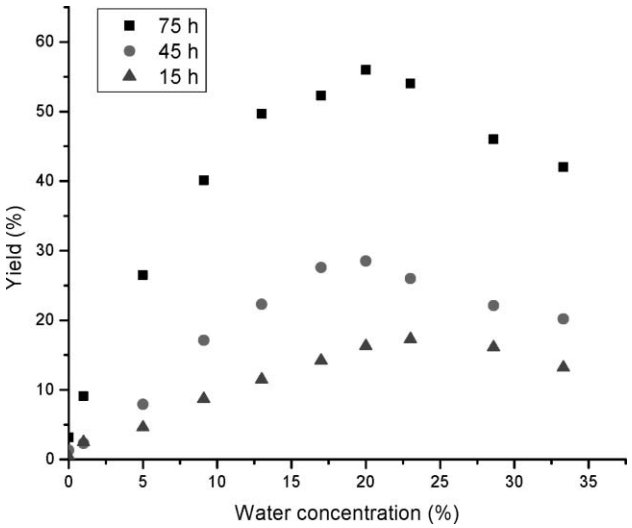


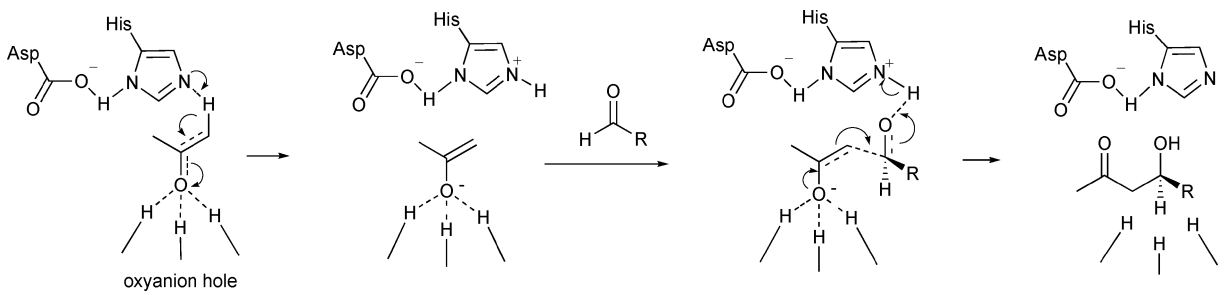
Fig. 1 The influence of water concentration on PPL-catalysed aldol reaction under conditions: lipase 20 mg, 4-nitrobenzaldehyde 0.12 mmol, acetone 1 ml, deionized water from 0% to 35% (water/[water + acetone], v/v), 30 °C.

which commonly exhibits special promotion for many addition reactions.[10] The range of water concentration from 0% to 55% was screened for the lipase-catalysed aldol reaction and the results are shown in Fig. 1. The three hill-shaped curves show that water concentration can greatly influence the reaction rate. The parts of larger percentages are not shown here, as excessive water lead to a serious decrease of the solubility of aldehyde and could change the special conformation of the active site. The optimal water concentration for the PPL-catalysed aldol reaction was about 20%. The results suggest that the special spatial conformation corresponding to the catalytic site of PPL can be mediated by water. A wider range of substrates, such as butanone, cyclohexanone, 4-nitrilbenzadehyde, had been expanded in the wet reaction condition.

Some further experiments were performed to get more information about the catalytic mechanism. It was observed that the reaction rate could be changed by altering the order of the addition of the reactants in our experiments. Firstly, acetone was added into the mixture containing PPL and a proper amount of water, then the mixture was stirred for 12 h. Finally, aldehyde was added into the mixture to initiate the reaction. An observable increase of the initial reaction rate was observed as compared with the typical process (data not shown). Another important observation was that the lipase-catalysed aldol reaction seemed to prefer acetone than any other ketone (such as butanone and cyclohexanone), as other ones lead to the decrease of the reaction rate. These cases indicate that acetone might at first be captured by the specific catalytic site to initiate this lipase-catalysed aldol reaction.†

A generally accepted catalytic mechanism for hydrolase is that the active site for hydrolysis also contributed to the promiscuous catalysis.[11] Combining that viewpoint with our observations described above, we hypothesized the mechanism of the lipase-catalysed aldol reaction and summarized them in Scheme 2. Firstly, the substrate acetone was stablilized by the Asp-His dyad and the oxyanion. Then, a proton was transferred from the acetone to the His residue and enolate ion was formed. Thirdly, another substrate aldehyde accepted the proton and simultaneously connected the acetone with the forming of a carbon–carbon bond. Finally, the aldol adduct was released from the oxyanion hole. This proposed catalytic process is similar with type II aldolase (belongs to lyase), but no metal ion exists.[12]

In conclusion, we described several lipase catalysed asymmetric aldol reactions. Lipase PPL can efficiently catalyse the aldol reaction and presents asymmetric catalytic activity, while another three lipases (MML, MJL, CAL-B) show lower catalytic activities. Interestingly, these lipase-catalysed addition reactions



Scheme 2 Proposed mechanism of lipase-catalysed aldol reaction.

can be greatly promoted by water, which is seldom mentioned in other lipase catalysed promiscuous reactions. The lipase-catalysed asymmetric aldol reaction provides a novel case of catalytic promiscuity and might be a potential synthetic method for organic chemistry.

Acknowledgements

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Notes and references

† A typical enzymatic procedure of aldol rection: the reaction was initiated by adding 200 mg PPL and 1.2 mmol (181.2 mg) aldehyde to a mixture of 5 ml anhydrous ketone and 1.25 ml water. The suspension was maintained at 30 °C and shaken at 200 rpm for 144 h (fomation of products was detected by TLC). The residue was then filtered off and the solvent was evaporated. A single product was prepared by silica gel chromatography with an eluent consisting of petroleum/ethyl acetate (2 : 1, v/v).

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