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Lipase catalyzed Cannizzaro-type reaction with substituted benzaldehydes in water

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ABSTRACT

Lipases were found to catalyze Cannizzaro-type reaction of substituted benzaldehydes in aqueous medium at 30 °C without the addition of any external redox reagent. The ratio of alcohol product to acid product varied with nature of the substituted benzaldehyde, enzyme, and the presence of organic co-solvent.

Enzymes are used in both aqueous and non-aqueous media for organic synthesis.[1] These applications of enzymes have been further expanded in recent years by exploiting their biocatalytic promiscuity. Among enzymes, lipases have been used more often for exploring new promiscuous activities. Numerous examples of lipase catalyzed C—C, C—N, and C—S bond formation have been reported.[2] Besides this, lipases have also been reported to catalyze various oxidative reactions in combination with hydrogen peroxide as the oxidant.[3] Cannizzaro reaction is the redox disproportionation of aldehydes that lack α-hydrogen to an equimolar mixture of a primary alcohol and a carboxylic acid salt.[4] While the classical Cannizzaro reaction requires strongly basic conditions along with elevated temperatures, there have been reports in recent years about the use of microwave irradiation, ultrasonication, and Lewis acid catalysts as alternative ways of catalyzing the reaction.[5] Recently, the use of alcohol dehydrogenases (ADHs) for catalyzing a Cannizzaro-type reaction has also been reported.[6] We report here that lipases are able to catalyze the Cannizzaro-type reaction of benzaldehydes without the presence of any external redox reagent such as hydrogen peroxide.

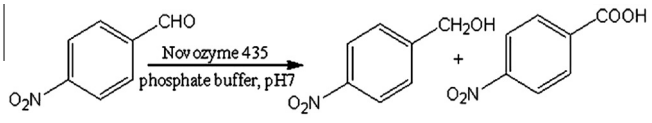
The lipase catalyzed redox reaction is a very simple system for obtaining the reduced as well as oxidized products of *p*-nitrobenzaldehyde. Unlike alcohol dehydrogenases,[6] this system does not require any cofactor, thereby automatically eliminating the need for any cofactor regeneration. Water as an environmentally benign solvent is increasingly being used as the reaction medium for various organic reactions.[7] The lipase catalyzed Cannizzaro-type reaction also uses water as the reaction medium.

Novozyme 435 (commercially available immobilized lipase B from *Candida antarctica*) catalyzed the conversion of *p*-nitrobenzaldehyde to *p*-nitrobenzyl alcohol and *p*-nitrobenzoic acid (Scheme 1). The reaction medium was 100 mM sodium phosphate buffer, pH 7.0 and reaction temperature was 30 °C.

Analysis of the reaction mixture after a period of 24 h showed the formation of these two products in almost equal amounts. A control reaction run in the absence of enzyme did not lead to the formation of any product even after 24 h.

In order to evaluate the scope of this promiscuous lipase catalyzed reaction, benzaldehyde and a few other substituted benzaldehydes were tried as substrates. Table 1 summarizes the product composition obtained after a period of 24 h. In each case, both the redox products were obtained, although the ratio of alcohol: acid showed variation.

Novozyme 435 catalyzed Cannizzaro-type reaction of *p*-nitrobenzaldehyde was further investigated by studying the time course of this reaction by HPLC analysis.[8] The reaction was found



Scheme 1. Lipase catalyzed Cannizzaro-type reaction in water.

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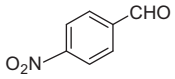
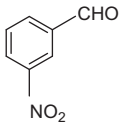
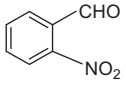
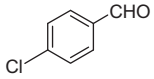
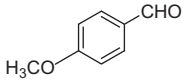
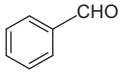
0040-4039/

Table 1
Cannizzaro-type reaction of substituted benzaldehydes catalyzed by Novozyme 435 in aqueous medium[a]

Entry	Substrate	Conversion[b] (%)	Product ratio[c]
1		96	1: 0.81
2		96	1: 1.28
3		90	1: 0.80
4		98	1: 0.63
5		98	1: 1.39
6		78	1: 0.44

[a] Reaction conditions: substrate (1 mM) in 100 mM sodium phosphate buffer, pH 7.0 at 30 °C, 200 rpm.
[b] Total conversion (alcohol + acid) obtained by HPLC analyses after a period of 24 h.
[c] Ratio of the alcohol to the carboxylic acid, after a period of 24 h; the alcohol concentration being arbitrarily set at 1.

to show an unusual pattern of formation of products. Usually in a self-Cannizzaro reaction, the alcohol and acid are expected to be formed in equal amounts, as these result from disproportionation of the same aldehyde. As shown in Figure 1, in the presence of the lipase, *p*-nitrobenzaldehyde underwent a quick conversion to the alcohol; about 60% conversion was achieved in just 6 h. Until this time, the acid appeared only in traces. As mentioned earlier, the two products became almost equal after 24 h. Thereafter, concentration of the alcohol kept decreasing while that of acid kept increasing. This raised the possibility that the *p*-nitrobenzyl alcohol was getting oxidized to *p*-nitrobenzoic acid.

In order to find out whether the oxidation of *p*-nitrobenzyl alcohol by the lipase was responsible for the trend observed during the later part of the reaction, a similar reaction was carried out starting with *p*-nitrobenzyl alcohol and Novozyme 435 under identical reaction conditions. It was found that the lipase indeed brought about the oxidation of *p*-nitrobenzyl alcohol to the corresponding acid (Fig. S1).

The oxidation of *p*-nitrobenzyl alcohol was found to be a much slower reaction as compared to the conversion of *p*-nitrobenzaldehyde to *p*-nitrobenzyl alcohol. Presence of both air as well as enzyme was found to be necessary for the oxidation reaction. Reaction carried out either in the absence of air or in the absence of enzyme led to the formation of less than 10% acid even after 72 h.

Lewis had reported that with sodium hydride as a base, the Cannizzaro reaction with *p*-nitrobenzaldehyde followed two different routes simultaneously.[9] The classical mechanism involving hydride transfer probably dominated in the early phase and provided both alcohol and acid in equal amounts. However, the radical anion mediated route later on converted even the alcohol into acid. [The radical anion intermediate formation in Cannizzaro reaction with benzaldehydes has also been reported in more recent years by Chung[10] (in alkaline aqueous dioxane) and Ashby et al.[11] (in alkaline tetrahydrofuran/hexamethylphosphoramide as the reaction medium)].

To investigate whether there is some involvement of free radical intermediate(s) in the present case, Novozyme 435 catalyzed reaction with *p*-nitrobenzaldehyde was carried out with either ferrous sulfate or diphenylamine added to the reaction medium. Both are known to inhibit free radical mediated reactions.[12] Figure 2 shows the time course of the reaction carried out with each of these additives. While both the additives partially inhibited the formation of *p*-nitrobenzoic acid, no inhibitory effect could be seen on the formation of *p*-nitrobenzyl alcohol. DMSO is known to act as a scavenger of free radicals.[13] Interestingly, addition of 10% DMSO (v/v) as a co-solvent completely inhibited the formation of the carboxylic acid, while the formation of the alcohol remained unaffected (Fig. 2).

It appears that radical anion intermediate(s) is also involved during the lipase catalyzed Cannizzaro-type reaction. The autoxidation of benzyl alcohols (catalyzed by bases) is known to involve radical anion intermediates.[9,14] Hence, it is likely that in the present case *p*-nitrobenzyl alcohol undergoes a slow oxidation to *p*-nitrobenzoic acid. It is interesting to observe that inspite of ESR indicating formation of free radicals, Ashby et al. also found that radical inhibitors or traps did not inhibit the rate of Cannizzaro reaction.[11]

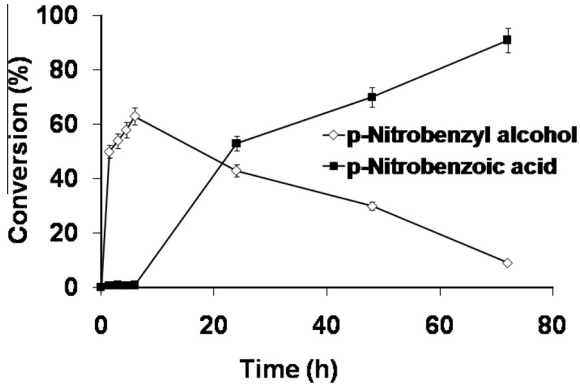


Figure 1. Time course for Cannizzaro-type reaction of *p*-nitrobenzaldehyde catalyzed by Novozyme 435.

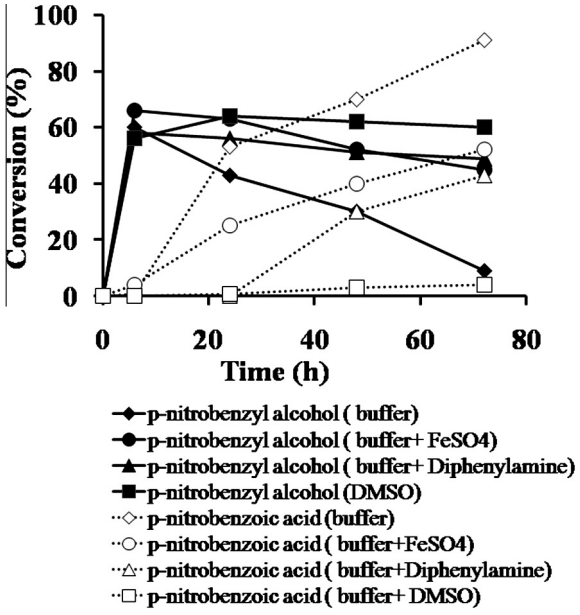


Figure 2. Effect of additives on Novozyme 435 catalyzed Cannizzaro-type reaction of *p*-nitrobenzaldehyde.

Table 2
Lipase screening and control reactions for Cannizzaro-type reaction of *p*-nitrobenzaldehyde[a]

Entry	Catalyst	*p*-Nitrobenzyl alcohol[b]	*p*-Nitrobenzoic acid[b]
1	Novozyme 435	45	54
2	Lipozyme CAL-B	70	25
3	Palatase	66	23
4	Lipozyme RMIM	77	21
5	CAL-A	0	45
6	Triethylamine	0	0
7	Imidazole	0	0.3
8	Urea denatured Novozyme 435[c]	0	6
9	No enzyme	0	2

[a] Reaction conditions: *p*-nitrobenzaldehyde (1 mM) in 100 mM sodium phosphate buffer, pH 7.0, and lipase (20 mg) or imidazole (0.1 mmol), or triethylamine (0.1 mmol) at 30 °C, 200 rpm.
[b] Corresponds to conversion values obtained by HPLC after a period of 24 h.
[c] Enzyme was denatured by incubating overnight with 8 M urea at 100 °C.

Over the years, numerous mechanisms have been proposed for Cannizzaro reaction.[9–11,15] Obviously, a complete mechanistic understanding of lipase catalyzed Cannizzaro-type reaction reported here would need a lot more extensive investigation. Based on the limited evidence that we have, we can only infer that: (a) this enzyme catalyzed Cannizzaro-type reaction does not follow the most widely accepted hydride transfer mechanism alone (b) it looks as if like in the case of Lewis the enzyme catalyzed reaction also follows twin routes simultaneously. Also, it appears as if conversion of alcohol to acid is predominantly carried out via some free radical intermediate. With our limited data, it may not be correct to speculate beyond this.

Some of the other commercially available lipases were also screened for their activity in the Cannizzaro-type reaction of *p*-nitrobenzaldehyde. Table 2 summarizes the product composition obtained after a period of 24 h by HPLC. Lack of formation of either of the reaction products in significant amount in entries 8 and 9 indicates the specific role of the lipase for the Cannizzaro-type reaction. Bases such as triethylamine and imidazole are known to act as catalyst/co-catalyst in various reactions of aryl aldehydes such as Henry reaction,[16] benzoin condensation,[17]and aldol condensation.[18] However, *p*-nitrobenzaldehyde did not show any significant product formation in the presence of these bases under the mild conditions used during biocatalysis (Table 2, entries 6 and 7).

To sum up, we believe that this is the first report of lipases carrying out a Cannizzaro-type reaction without the involvement of any externally added redox reagent. Furthermore, the reaction was carried out under benign conditions using water as the reaction medium (and air as the oxidizing agent for the oxidation step). Lipases are considered to have a very wide specificity among enzymes.[1c,2d] The above results show that the range of reactions which these enzymes catalyze may be even wider than thought so far. This certainly expands their synthetic utility.

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.2014.05.022.

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8. *General procedure for lipase catalyzed redox reaction of p-nitrobenzaldehyde.* A solution of *p*-nitrobenzaldehyde (1 mM) in 100 mM sodium phosphate buffer, pH 7.0 was shaken with 20 mg lipase at 30 °C. Total reaction volume was 1.25 ml. Aliquots were taken at different points of time and analyzed by HPLC. Experiments were done in duplicate and found to have ±5% error. HPLC analysis was carried out on Zorbax C-18 reverse phase column (150 mm × 4.6 mm). The eluent was composed of 25% acetonitrile in water containing trifluoroacetic acid (0.1%), at a flow rate of 1 ml/min. The peaks were detected at 254 nm and the retention times were matched with those of commercially available compounds. The amount of product formed at a given point of time was calculated from the peak area(s) of the product(s).
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