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Enzyme-catalyzed Henry (nitroaldol) reaction

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ABSTRACT

Transglutaminase was first used to catalyze Henry reactions of aliphatic, aromatic and hetero-aromatic aldehydes with nitroalkanes. The reactions were carried out at room temperature, and the corresponding nitro alcohols were obtained in yields up to 96%.

1. Introduction

The Henry (nitroaldol) reaction is a powerful and atom economical C–C bond forming reaction in synthetic organic chemistry [1–3]. The resulting β-nitro alcohols are often used as valuable synthetic intermediates in the synthesis of numerous products [4], such as 2-aminoalcohols, 2-nitroketones and nitroalkenes [5–8], which are useful for the synthesis of biologically important compounds [9,10]. In recent years, the development of new chiral catalysts for this important reaction has attracted the interest of many groups [11–18]. On the other hand, enzymes as practical catalysts have been increasingly exploited for synthetic transformations due to their simple processing requirements, high selectivity and mild reaction conditions [19,20]. However, to the best of our knowledge, there was only one group that had reported the asymmetric catalytic Henry reaction using Hydroxynitrile Lyase from *Hevea brasiliensis* (*HbHNL*) (EC 4.1.2.39) [21,22]. Therefore, the development of new enzymatic catalysts is still in great demand. Herein, we wish to report a novel discovery that the cheap and readily available transglutaminase (TGase) efficiently promotes the Henry reaction (nitroaldol) of nitroalkanes with aliphatic, aromatic and hetero-aromatic aldehydes resulting in moderate to good yields.

TGase (protein-glutamine γ-glutamyltransferase; EC 2.3.2.13) are a family of enzymes that are widely distributed in mammals [23], plants [24], fishes [25] and micro-organisms [26], and their enzymatic and physiological properties have been extensively studied [26,27]. It is well known that TGase catalyses an acyl transfer reaction between a γ-carboxyamide group of glutamine residue and ε-amino group of lysine residue or other primary amines. In this report, however, TGase was first used to catalyze Henry (nitroaldol) reaction. It shows the ability of the enzyme to catalyze synthetic reaction which varies from its natural role [28]. This is known as biocatalytic promiscuity, a new frontier which has emerged recently and largely extended the application of enzymes. Some elegant reports have addressed the importance and wide application of biocatalytic promiscuity in organic synthesis [29–46]. Herein, we report this TGase-catalyzed Henry reaction as another example of biocatalytic promiscuity.

2. Experimental

2.1. General remarks

Immobilized lipase from *Thermomyces lanuginosus* (0.25 U/mg. One unit corresponds to the amount of enzyme producing 1 μmol methyl oleate from triolein per minute at 35 °C) was purchased from Novozymes (China) Investment Co., Ltd. TGase from *Streptorerticillium griseoverticillatum* (0.06 U/mg. The activity was determined by the colorimetric hydroxamate procedure. One unit generates 1 μmol hydroxamic acid per minute at 37 °C), Pancreatin from porcin pancreas (4 U/mg. One unit of activity was defined as the amount of the enzyme to produce TCA-soluble hydrolysis products from casein, which gives an absorbance value equivalent to 1 μg of tyrosine at 275 nm per minute at 40 °C and pH 7.5), Papain from fruit jam from *chaenomeles* (650 U/mg. One unit

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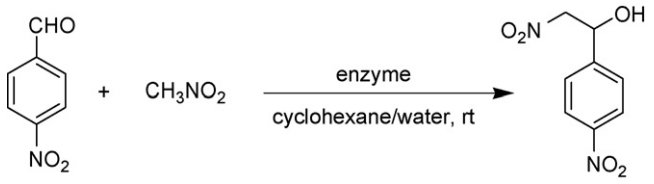
of activity was defined as the amount of the enzyme to produce TCA-soluble hydrolysis products from casein, which gives an absorbance value equivalent to 1 μg of tyrosine at 275 nm/min at 37 °C and pH 7.0), Lysozyme from hen egg white (20,000 U/mg. The activity determination was according to the procedure described by Shugar [47]. One unit of activity was defined as the amount of enzyme that lowers 0.001 absorbance at 450 nm/min), Chymosin from fruit jam from *chaenomeles* (20 U/mg. The activity determination was according to the procedure described by Lowry et al. [48]. One unit of milk-clotting activity was defined as the amount of enzyme required to clot 1 ml of milk in 40 min at 35 °C), Nuclease from *Penicillium citrinum* (5 U/mg. The activity determination was according to the procedure described by Eaves and Jeffries [49]. One unit of activity was defined as the amount of the enzyme which liberates the digestion product not precipitated by the ammonium molybdate-perchloric acid reagent and gives an extinction change of 1 at 260 nm), Bromelain from pineapple peduncle (500 U/mg. One unit of activity was defined as the amount of the enzyme to produce TCA-soluble hydrolysis products from casein, which gives an absorbance value equivalent to 1 μg of tyrosine at 275 nm/min at 37 °C and pH 7.0), and Cellulase from *Trichoderma* (10 U/mg. One unit of activity was defined as the amount of enzyme which released 1.0 mg of glucose from cellulose in 1 h at 40 °C and pH 4.8) were purchased from Guangxi Nanning Pangbo Biological Engineering Co. Ltd. (Nanning, China). Alkaline proteinase from *Bacillus licheniformis* No 2709 (200 U/mg. One unit of activity is the amount of enzyme that liberates 1.0 μequiv. of tyrosine from casein per minute at 40 °C and pH 10.5), Acidic proteinase from *Aspergillus usamii* No 537 (50 U/mg. One unit of activity is the amount of enzyme that liberates 1.0 μequiv. of tyrosine from casein per minute at 40 °C and pH 3.0), Neutral proteinase from *Bacillus subtilis* A.S.1.398 (130 U/mg. One unit of activity is the amount of enzyme that liberates 1.0 μequiv. of tyrosine from casein per minute at 30 °C and pH 7.5), and Trypsin from porcin pancreas (4 U/mg. One unit of activity was defined as the amount of the enzyme to produce TCA-soluble hydrolysis products from casein, which gives an absorbance value equivalent to 1 μg of tyrosine at 275 nm/min at 40 °C and pH 8.0) were purchased from Wuxi Xuemei Enzyme Co. Ltd. (WuXi, China). Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. All reactions were monitored by thin-layer chromatography (TLC) with Haiyang GF254 silica gel plates. Flash column chromatography was carried out using 100–200 mesh silica gel at increased pressure.

2.2. Representative procedure for enzyme-catalyzed Henry reactions (products **2a–s**, **3a–d**, **4a–d**)

For **2a–s**: a 25 ml round-bottomed flask was charged with the enzyme (200 mg), deionized water (3 ml) and CH_2Cl_2 (5 ml), to which the aldehyde (200 mg) and nitromethane (2 g, 32 mmol) were introduced. The resulting solution was stirred for the specified amount of time at rt (20–30 °C).

For **3a–d** and **4a–d**: a 25 ml round-bottomed flask was charged with the enzyme (200 mg), deionized water (3 ml) and CH_2Cl_2 (5 ml), to which the aldehyde (1 mmol) and nitroalkane (25 mmol) were introduced. The resulting solution was stirred for the specified amount of time at 30 °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. 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 solvent was then removed under reduced pressure. The crude products were purified by column chromatography with petroleum ether/ethyl acetate as eluent.



Scheme 1. Enzyme-catalyzed Henry reaction of 4-nitrobenzaldehyde and nitromethane.

3. Results and discussion

Initial studies were undertaken using 4-nitrobenzaldehyde and nitromethane as a model reaction. We chose a cyclohexane/water 10:1 (v/v) system as the reaction medium. The reaction was performed at room temperature (20–30 °C) (Scheme 1). In order to select the appropriate enzymes, by using the model system several commercially available enzymes were screened (Table 1).

As shown in Table 1, the best result (93% yield) was achieved by using TGase as catalyst after 32 h (Table 1, entry 1). Immobilized lipase and Pancreatin also showed moderate catalytic activities (Table 1, entries 2 and 3), while some tested enzymes including Papain, Lysozyme, Chymosin, Alkaline proteinase, and Nuclease presented low activities in this reaction (Table 1, entries 4–8). In addition, in the presence of Acidic proteinase, Neutral proteinase, Trypsin, Bromelain and Cellulase, respectively, only trace product was observed on TLC (Table 1, entries 9–13).

We also performed some control experiments to demonstrate the specific catalytic effect of the TGase. Just as we expected, the Henry reaction of 4-nitrobenzaldehyde and nitromethane in the absence of enzyme showed low yield adduct (10%) even after 5 days (Table 1, entry 14). Moreover, the reaction catalyzed by non-enzyme protein bovine serum albumin (B.S.A.) gave product only

Table 1
The catalytic activities of different enzymes in Henry reaction between 4-nitrobenzaldehyde and nitromethane.[a].

Entry	Catalyst	Time (h)	Yield (%)[b]
1	TGase from *Streptorerticillium griseoverticillatum*	32	93
2	Immobilized lipase from *Thermomyces lanuginosus*	48	64
3	Pancreatin from porcin pancreas	48	41
4	Papain from fruit jam from *chaenomeles*	48	15
5	Lysozyme from hen egg white	48	13
6	Chymosin from fruit jam from *chaenomeles*	48	13
7	Alkaline proteinase from *Bacillus licheniformis* No 2709	48	12
8	Nuclease from *Penicillium citrinum*	48	11
9	Acidic proteinase from *Aspergillus usamii* No 537	48	Trace
10	Neutral proteinase from *Bacillus subtilis* A.S.1.398	48	Trace
11	Trypsin from porcin pancreas	48	Trace
12	Bromelain from pineapple peduncle	48	Trace
13	Cellulase from *Trichoderma*	48	Trace
14	No enzyme	120	10
15	Bovine serum albumin (B.S.A.)	48	16
16	TGase denatured with EDTA [c]	120	0
17	TGase inhibited with NBS[d]	48	12

[a] All reactions were carried out using 4-nitrobenzaldehyde (200 mg, 1.3 mmol), nitromethane (2 g, 32 mmol), deionized water (0.5 ml), cyclohexane (5 ml) and enzyme (200 mg) at rt (20–30 °C).
[b] Yield of the isolated product after chromatography on silica gel.
[c] Pre-treated with EDTA at 100 °C for 24 h.
[d] Pre-treated with NBS at 25 °C for 24 h.