Catalysis of Henry Reaction by Plasmid DNA

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Abstract: This work demonstrates that a plasmid can function as an efficient biocatalyst in mediating the nitroaldol (Henry) reaction at high temperatures in ultrapure water. Compared with ssDNA, the plasmid displayed a remarkable catalytic efficiency (97% yield within 20 min). Aromatic aldehydes which bear strong or moderate electron-withdrawing groups reacted satisfactorily with nitromethane, which afforded the corresponding β-nitroalcohols in good yields within 2 h (up to 98%). By contrast, aliphatic and aromatic aldehydes with electron-donating groups did not react. Moreover, the yield significantly dropped when the size of the carbanion increased, and the yield was only 10% when nitropropane was used as the donor.

Keywords: ssDNA; plasmid; pcDNA3.1; Henry Reaction; C-C bond formation

1. Introduction

Since the 3D structure of the DNA double helix has been discovered by Watson and Crick in 1953[1], the double-helix structure with base pairs has attracted considerable attention as an effective means of storing and transferring genetic information[2]. With the development of science and technology, the study of the DNA double-helix structure has expanded from biology to other science domains [3–7]. Recently, the interest in DNA has focused on the catalysis of organic synthesis[8–10]. Till now, DNA catalysts have four types, namely, ssDNA[11], human telomeric G-quadruplex DNA[12], ctDNA[11] and oligonucleotide[13]. The application of DNA-based hybrid catalysts in asymmetric catalysis in the form of a copper(II)-catalysed Diels–Alder reaction was first reported by Feringa and Roelfes in 2005[14]. Since then, DNA catalysts have become extensively used in the Henry reaction[15], Michael additions[16] and so on.

The Henry reaction is important in forming C-C bonds[17] which are needed in synthesising important compounds[18]. This reaction is usually catalysed by chiral guanidine, chiral (thio) urea derivatives and cinchona alkaloids, but the solvents are mostly organic, which are considerably harmful to the environment. Therefore, the use of environmental-friendly alternatives to these harmful substances is important.

ssDNA has used as a catalyst in the Henry reaction[11]. However, the application of ssDNA has many limitations. For example, consistency in the size and sequence of ssDNAs cannot be guaranteed. Different from ssDNAs, plasmids are stable and can be amplified from generation to generation without mutations. Thus, we attempted to use plasmid DNA to catalyse the Henry reaction. This paper not only presents the capability of plasmid DNA to catalyse the Henry reaction with high yield but also provides a new option for DNA-based catalysis. We demonstrated that plasmid DNA can also be a biomolecular scaffold for chiral transfer and cause an additional rate acceleration.

2. Results and Discussion

The catalytic effect of DNA from different sources (lab-extracted and commercial products) was primarily evaluated (Table 1). The highest yield was 55%, which was achieved when plasmids (pET32a and pcDNA3.1) were used as catalysts for the nitroaldol condensation of 4-nitrobenzaldehyde and

nitromethane (Table 1, entries 4 and 5). By contrast, the commercial ssDNA (deoxyribonucleic acid sodium salt from salmon tests) afforded low yields[11] (only 34%) under 37 ℃ within 2 h (Table 1, entry 1). No significant difference was observed among the four types of plasmids, which were 50%, 53%, and 55% (Table 1, entries 2, 3, 4 and 5). Consequently, plasmid pcDNA3.1 was chosen as a representative in subsequent studies because it can be replicated easily. In consideration that DNA possesses a right-handed helix structure, the chiral information within the DNA may transfer to the organic reaction products[15]. Therefore, we also tested the ee value of the DNA-mediated Henry reaction. However, no enantioselectivity was observed, indicating that the DNA catalyst did not provide chiral selectivity under this condition.

Table 1 Catalytic effect of different DNA for Henry reactions[a]

Entry	DNA		Temperature	Time	Yield[b]
1	ssDNA		37℃	2 h	34%
2	pUC18	E.coli DH5α	37℃	2 h	50%
3	pET20b	E.coli DH5α	37℃	2 h	53%
4	pET32a	E.coli DH5α	37℃	2 h	55%
5	pcDNA3.1	E.coli DH5α	37℃	2 h	55%

[a] Reaction conditions: 4-nitrobenzaldehyde (0.1 mM), nitromethane (2.5 mM), pure water (2 mL), 0.3 mg DNA, 37℃, 2 h. [b] The yields were determined by HPLC using a Chiralpak AD-H chiral column.

### 2.1. Influence of the mole ratio of nitromethane to 4-nitrobenzaldehyde

The Henry reaction is a nucleophilic addition reaction, and nitromethane is a nucleophile which can react with the carbonyl of 4-nitrobenzaldehyde. Therefore, an excessive dosage of nitromethane favours the shift of the reaction equilibrium to the formation of products[18]. Fig. 1 demonstrates that the yields of Henry reaction increase from 1.2% to 98% with the growth of the molar ratio of nitromethane to 4-nitrobenzaldehyde from 1/1 to 25/1. This result is consistent with the reported procedures which typically require an excess of approximately 25-fold mole to perform the biocatalytic Henry reaction and obtain high yields[19]. Thus, we selected 25 equiv. of nitromethane as the optimum molar equivalent for the Henry reaction.
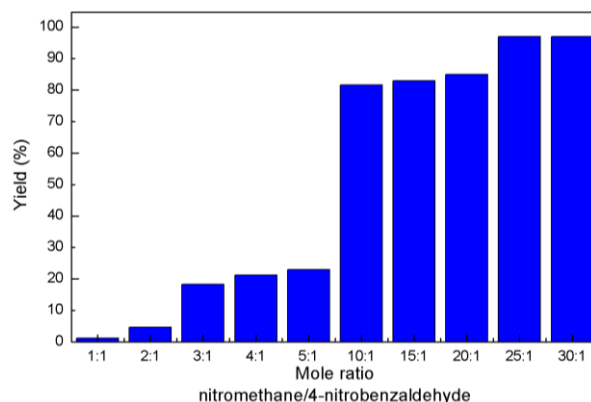
Fig. 1. The effect of mole ratio of nitromethane to 4-nitrobenzaldehyde on the reaction. The Henry reaction was carried out at 70 °C for 2 h using 0.1 mM 4-nitrobenzaldehyde, 0.3 mg of pcDNA3.1.

2.2. Effect of temperature on catalytic efficiency

According to Arrhenius theory, temperature can increase the internal energy of the substrate molecules to overcome the energy barrier and thus enhance the reaction rate and enzyme kinetics[20]. Therefore, the catalytic efficiency of the Henry reaction was evaluated at different temperatures from 20 ℃ to 80 ℃ (Fig. 2). As shown in Fig. 2, the yields of pcDNA3.1 increased with temperature. After peaking at 70 ℃ (98%), the yields dropped slightly. The rising trend was also observed in the yields of ssDNA. Unfortunately, the best catalytic performance of ssDNA in the Henry reaction was only 67%, which was considerably below that of pcDNA3.1.
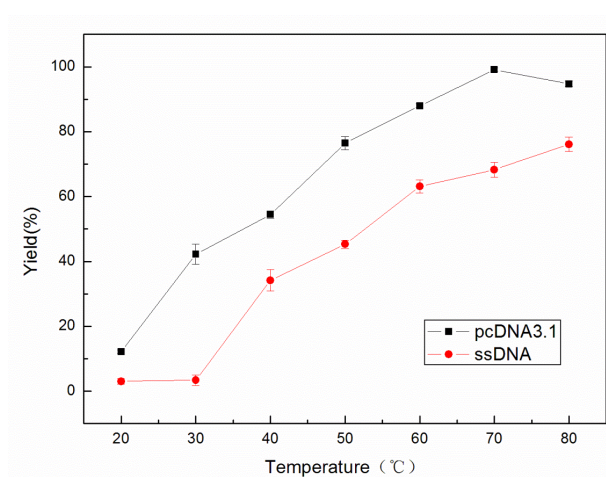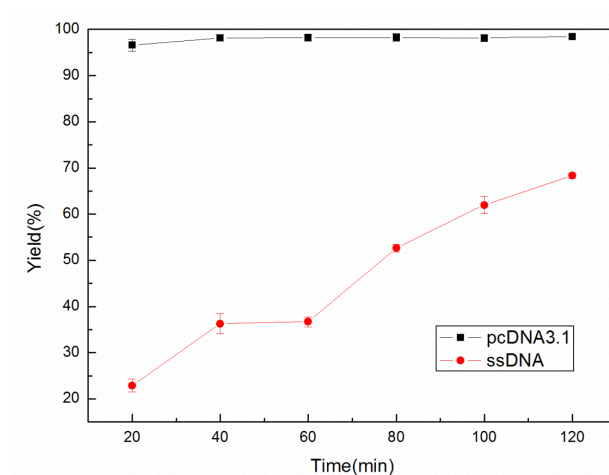

Fig. 2. The effect of temperature on the yields in the Henry reaction catalyzed by pcDNA3.1 and ssDNA. The Henry reaction was carried out at different tenperature for 2 h using 0.1 mM 4-nitrobenzaldehyde, 2.5 mM nitromethane and 0.3 mg DNA

2.3. Effect of reaction time on yields

We optimised the reaction time using the following coupling conditions: 0.1 mM 4-

nitrobenzaldehyde, 2.5 mM nitromethane, 70 ℃, 0.3 mg DNA for pcDNA3.1 and ssDNA. The results are shown in Fig. 3. The catalytic efficiency of pcDNA3.1 was markedly higher than that of ssDNA. For example, during the first 20 min, pcDNA3.1 yielded four times more β-nitroalcohol than when the reaction was run for the same amount of time using ssDNA as the biocatalyst, and the yields of the Henry reaction catalysed by pcDNA3.1 remained stable until the end. Although the reaction that was catalysed by ssDNA exhibited a steady increase to 67% after 2 h, the figure was still less than that of pcDNA3.1 (98%). Currently reported enzymes which catalyse Henry reaction with high efficiencies of 65% and 75% after 30 min are pancreatic lipase HPL and MJL, respectively[20–22]. However, pcDNA3.1 demonstrated a remarkably higher efficiency than HPL and MJL in catalysing the Henry reaction.

Fig. 3. Time course of yields in the Henry reaction catalyzed by pcDNA3.1 and ssDNA. The Henry reaction was carried out at 70℃ for 2 h using 0.1 mM 4-nitrobenzaldehyde, 2.5 mM nitromethane and 0.3 mg DNA.

2.4. Effect of recycling times on yields

We also investigated the recycling of the catalytic DNA. Fig. 4 depicts the efficiency of the same catalyst used 10 times in the reaction of 4-nitrobenzaldehyde and nitromethane under optimised reaction conditions. Even after the 10th round, no reduction in reaction yield was observed.
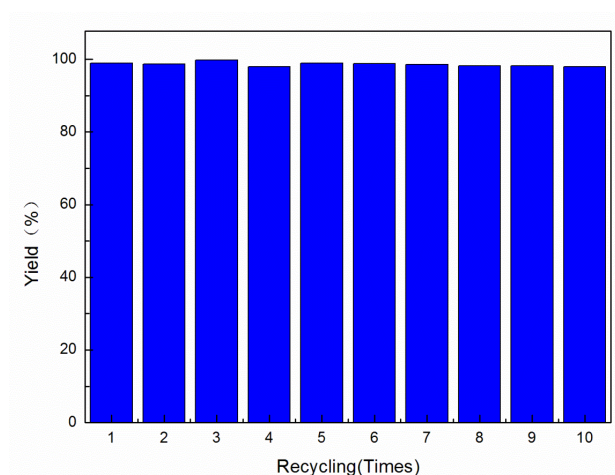
Fig. 4. Effect of recycling times on yields. The Henry reaction was carried out at 70℃ for 2 h using 0.1 mM 4-nitrobenzaldehyde, 2.5 mM nitromethane and 0.3 mg DNA.

2.5. *Effect of substrates with different substituents on pcDNA3.1- catalysed Henry reaction*

Different benzaldehydes with electron-donating or electron-withdrawing substituents were tested under optimised conditions (Table 2). Benzaldehydes with strong or moderate electron-withdrawing substituents can react with nitromethane satisfactorily and result in β-nitroalcohols in good yields within 2 h (Table 2, entries 1, 2, 3 and 4). The position of nitro-substituents also influences the yields of the pcDNA3.1-catalysed Henry reaction. p-Nitrobenzaldehyde reacted more easily with nitromethane than o-nitrobenzaldehyde and m-nitrobenzaldehyde (Table 2, entries 1, 2 and 3). However, aliphatic aldehydes (e.g. benzaldehyde) and aromatic aldehydes which bear electron-rich groups (e.g. 4-methoxybenzaldehyde and 4-hydroxybenzaldehyde) remained unreacted under these conditions.

Table 2 pcDNA3.1-catalyzed Henry reaction of substituted aldehydes with nitromethane[a]

Entry	R(1)	2	Yields
1	2-nitrobenzaldehyde	nitromethane	56%
2	3-nitrobenzaldehyde	nitromethane	43%
3	4-nitrobenzaldehyde	nitromethane	98%
4	2,4-dinitrobenzaldehyde	nitromethane	96%
5	4-methoxybenzaldehyde	nitromethane	Trace
6	4-hydroxybenzaldehyde	nitromethane	Trace
7	benzaldehyde	nitromethane	Trace

[a] The Henry reaction was carried out at 70℃ for 2 h using 0.1 mM benzaldehydes, 2.5 mM nitromethane and 0.3 mg DNA.

Meanwhile, we found that the yields dropped significantly as the numbers of the carbon atoms of nitroalkanes increased, with 44% for nitroethane (Table 3, entry 2) and only 10% for nitropropane (Table 3, entry 3). This phenomenon may be attributed to two reasons. First is water solubility. Nitromethane is partially soluble in water, whereas nitroethane and nitropropane are nearly insoluble in water. The lower the solubility, the lower the chance of having contact with the catalyst. The other reason is steric hindrance[23]. The steric hindrance is large with nitropropane; thus, the reaction with 4-nitrobenzaldehyde is difficult.

Table 3 pcDNA3.1-catalyzed Henry reaction of different nitroalkanes with 4-nitrobenzaldehyde[a]
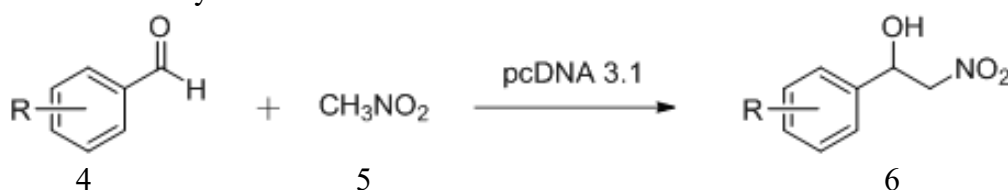
Entry	4	R(5)	Yields
1	4-nitrobenzaldehyde	nitromethane	98%
2	4-nitrobenzaldehyde	nitroethane	44%
3	4-nitrobenzaldehyde	nitropropane	10%

[a] The Henry reaction was carried out at 70℃ for 2 h using 0.1 mM 4-nitrobenzaldehyde, 2.5 mM nitroalkanes and 0.3 mg DNA.

3. Experimental Section

3.1. Materials

Adenine (CAS 73-24-5) was purchased from Sangon Biotech. Aldehydes were supplied by Sangon Biotech. Nitroalkanes were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Plasmid DNA maxiprep kits were purchased from Thermo Fisher Scientific. Silica gel plates were supplied by Branch of Qingdao Haiyang Chemical Co., Ltd. (Qingdao, China). Milli-Q water was used for the experiments in aqueous solutions. Deoxyribonucleic acid sodium salt from salmon sperms (CAS 438545-06-3; Cat. No. D1626) was supplied by Sigma-Aldrich. All reagents and solvents were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Plasmids

pUC18, pET-20b, pET-32a and pcDNA3.1 were conserved in the laboratory. Escherichia coli strain DH5α was used for DNA manipulation and amplification.

3.2. Methods

The plasmid was amplified in the host *E. coli* DH5α. The cell was cultured in 2YT medium (1% yeast extract, 1.6% tryptone and 0.5% NaCl) containing 100 μg/mL ampicillin at 37 ℃ overnight. The cells were collected by centrifugation at 8000 r/min for 15 min (4 ℃) and then stored at –20 ℃. The cells were extracted using the ZymoPURE Plasmid Maxiprep Kit. The obtained DNA was stored at –40 ℃.

^{1}H-NMR spectra were recorded on an AvanceⅢ600 spectrometer (Bruker) or a Mercury 300BB spectrometer (Varian) using $CDCl_3$ as a solvent. MS spectra were obtained by a AB Sciex Triple TOF 6600. Relative configurations of the compounds were assigned by comparison with 1H-NMR data reported in the literature. HPLC analysis was carried on a Waters e2695 equipped with a UV detector and a Chiralpak AD-H chiral column to determine the yield and the potential enantioselectivity. HPLC analysis was performed using 90% of *n*-hexane and 10% of isopropanol as eluents. The flow rate was 1 mL/min, and the run time was 60 min.

3.3. General procedure for DNA-catalyzed Henry reactions

In a glass bottle, 0.3 mg of DNA, 2 mL of ultrapure water containing 4-nitrobenzaldehyde (0.1 mM, 15.1 mg) and nitromethane (2.5 mmol, 134 μL) were mixed. The reaction mixture was gently stirred (200 rpm) at 70 ℃ for 1 h. After completion, 1 mL of water was added. The reaction mixture was washed with EtOAc three times and then dried at 0.1 mbar and 30 °C. The resulting products were purified using column chromatography and petroleum ether/ethyl acetate (5:2 v/v) as the eluent. To determine the NMR yield, the obtained product was dissolved in CDCl3. The reaction progress was monitored by thin-layer chromatography and HPLC analysis.

The spectral data of the compound matched those reported in the literature.

3.4. Typical Recycling Procedure

After the reaction, the aqueous phase was washed with EtOAc three times in the reaction vial. The

organic phase was removed, and the vial with the aqueous phase was subjected to N_2-flow before adding the reaction substrates for the next run.

4. Conclusions

For the first time, the plasmid was used as DNA catalyst for henry reaction. It turns out that, a superior activity (97% yields and 20 mins) could be achieved compared to ssDNA (21% yields within 20 mins) at the temperature of 70℃. Overall, this research not only presents the unmodified plasmid DNA can catalyze Henry reaction with high yield, but also provides a new option for DNA-based catalysis. It demonstrated that plasmid DNA can also be a biomolecular scaffold final rate acceleration.

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