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Highly enantioselective double reduction of phenylglyoxal to (*R*)-1-phenyl-1,2-ethanediol by one NADPH-dependent yeast carbonyl reductase with a broad substrate profile

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ABSTRACT

The activity and enantioselectivity of a carbonyl reductase from *Pichia pastoris* GS115 were evaluated with a series of carbonyl compounds including aryl aldehydes, ketones, α- and β-ketoesters. This recombinant enzyme possessed a broad substrate profile with the ability of reducing both aldehydes and ketones. Especially, the enzyme catalyzed the double reduction of phenylglyoxal to (*R*)-1-phenyl-1,2-ethanediol with 99% yield and 99% ee by coupling with D-glucose dehydrogenase for the regeneration of cofactor NADPH, representing the first example of effective reduction of both aldehyde and ketone functional groups in one molecule by using only one enzyme. Furthermore, this study provides valuable information for guiding the future application of this versatile biocatalyst.

1. Introduction

Optically pure alcohols are important intermediates in pharmaceutical, agricultural, and fine chemical industries.[1,2] Chiral metal complexes have been successfully employed in the asymmetric reduction of ketones to prepare chiral alcohols.[3–5] However, these methods usually result in environmental and safety concern in process operation. As an alternative, biocatalytic reactions usually take place in aqueous solution at mild temperature and neutral pH. The high chemo-, regio-, and stereo-selectivity of biocatalysts minimize the side reactions, thus avoiding the protecting and deprotecting steps, which are common in chiral organic synthesis.[6] As such, from both economic and environmental points of view, biocatalysis is preferred and has been become the first choice in chiral alcohol synthesis from ketones.[7–9]

In the course of expanding our carbonyl reductase tool-box for the synthesis of optically pure alcohols,[10–13] a carbonyl reductase from *Pichia pastoris* GS115 was cloned and expressed in *Escherichia coli*.[14] The recombinant protein (PasCR) was found to catalyze the enantioselective reduction of bulky diaryl ketones. Herein we report the substrate profile of this enzyme and its capability of reducing phenylglyoxal to (*R*)-1-phenyl-1,2-ethanediol in one-pot with high conversion and enantioselectivity, which is the first example of highly enantioselective double reduction of α-oxoaldehydes by a single enzyme.

2. Results and discussion

The carbonyl reductase (PasCR) from *P. pastoris* GS115 was produced and purified as previously described.[14] The enzyme was stable at 4 °C for months without significant loss of activity. The activity of purified enzyme was assayed toward a variety of substrates including aryl aldehydes, aryl ketones, and α- and β-ketonesters by spectrophotometrically measuring the oxidation of NADPH at 340 nm. The enantioselectivity for the reduction of various substrates was studied using a NADPH regeneration system consisting of D-glucose dehydrogenase (GDH) and D-glucose. The enantiomeric excess (ee) of the products and conversion were determined by chiral GC or HPLC analysis. The results are presented in Tables 1–3.

PasCR exhibited high activity toward benzaldehyde analogs, affording the corresponding substituted benzyl alcohols (Table 1). The substituent at the benzene ring exerted significant

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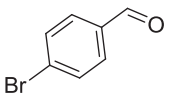
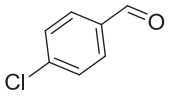
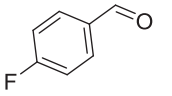
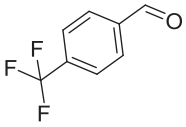
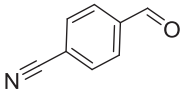
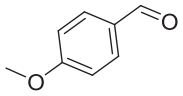
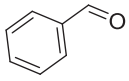
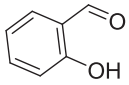
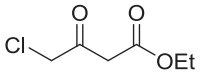
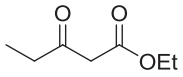
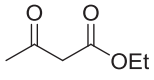
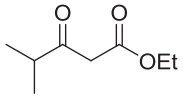
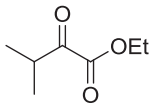
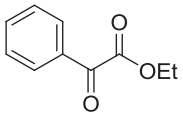
Table 1
Reduction of various aldehyde substrates catalyzed by PasCR

Substrate	Specific activity[a] (%)	Conversion[b] (%)
	10.9	>99
	6.3	>99
	7.0	>99
	3.1	>99
	4.2	>99
	0.5	>99
	3.6	>99
	0.4	>99

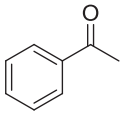
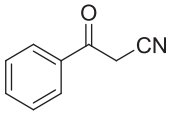
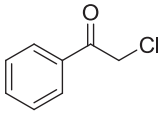
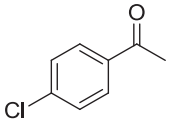
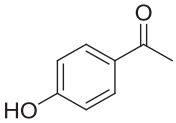
[a] The specific activity was defined as μmol min^{-1} mg^{-1}.
[b] Determined by HPLC analysis using Eclipse XDB-C18 column, a mixture of water and methanol was used as eluent.

Table 2
Reduction of various ketoesters by PasCR

Substrate	Specific activity[a]	Conversion[b] (%)	ee (%)
	1.502	>99	92 (*R*)
	0.522	>99	>99 (*S*)
	0.039	>99	>99 (*S*)
	0.015	45	87.8 (*S*)
	0.135	90	87.5 (*R*)
	0.432	76	>99 (*R*)

[a] The specific activity was defined as μmol min^{-1} mg^{-1}.
[b] Determined by chiral HPLC analysis.

Table 3
Reduction of substituted acetophenone analogs by PasCR

Substrate	Specific activity[a]	Conversion[b] (%)	ee[b] (%)
	0.073	60	>99 (*S*)
	0.015	31	70 (*S*)
	0.047	54	>99 (*R*)
	0.074	86	>99 (*S*)
	n.d.[c]	74	>99 (*S*)

[a] The specific activity was defined as μmol min^{-1} mg^{-1}.
[b] Determined by chiral HPLC analysis.
[c] n.d.: activity was not determined for this substrate, due to the high absorbance at 340 nm.

effect on the enzyme activity. For example, the benzaldehydes with fluoro, chloro, and bromo groups at *para*-position had one or two times higher activity than benzaldehyde, while the activity for *para*-methoxybenzaldehyde was only one seventh of that for benzaldehyde. Although specific activity was different for these aryl aldehydes, PasCR effectively catalyzed their reduction to give the corresponding benzyl alcohols with excellent conversion (>99%).

For the reduction of aromatic and aliphatic ketoesters (Table 2), the enzyme activity and enantioselectivity were affected by the substrate structures. PasCR exhibits the highest activity toward ethyl 4-chloro-3-oxobutyrate, affording ethyl (*R*)-4-chloro-3-hydroxybutyate with 92% ee, which is an important chiral building block. When chloromethyl group was substituted by ethyl, the enzyme activity was reduced to about one third of that for ethyl 4-chloro-3-oxobutyrate. Further decrease in activity was observed for ethyl 3-oxobutyrate and ethyl 4-methyl-3-oxopentanoate, suggesting that the size of one pocket of the substrate-binding cavity was suitable for chloromethyl and ethyl groups, but both the smaller methyl and larger *iso*-propyl did not fit well in the pocket. PasCR catalyzed the reduction of ethyl phenylglyoxylate to give optically pure (*R*)-2-hydroxy-2-phenylacetate, while the enantioselectivity for the reduction of 3-methyl-2-oxobutyrate was lower.

The yeast carbonyl reductase PasCR catalyzed the reduction of substituted acetophenones to the corresponding (*S*)-configurated alcohols with >99% ee (Table 3). This enzyme also reduced α-chloroacetophenone to optically pure (*R*)-2-chloro-1-phenylethanol, but the enantioselectivity for the reduction of α-cyanoacetophenone was much lower (70% ee). These chiral alcohols are important intermediates for the syntheses of β-adrenergic receptor agonists and other drugs.

Since carbonyl reductase PasCR could reduce both aldehydes and ketones, it would be interesting to examine its ability of reducing