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Stereoselective reduction of 4-benzoylpyridine in the heart of vertebrates

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Abstract

The stereoselectivity in the reduction of 4-benzoylpyridine (4-BP) was examined in the cytosolic fractions from the heart of 9 vertebrates (pig, rabbit, guinea pig, rat, mouse, chicken, soft-shelled turtle, frog and flounder). 4-BP was stereoselectively reduced to *S*(−)-α-phenyl-4-pyridylmethanol [*S*(−)-PPOL] in the cytosolic fractions from the heart of pig, rabbit and guinea pig. However, of mammalian heart cytsol tested, only rat heart cytosol had little ability to reduce stereoselectively 4-BP. In an attempt to elucidate this reason, amino acid sequence of rat heart carbonyl reductase (RatHCR) was deduced from the cloned cDNA and compared with that of pig heart carbonyl reductase (PigHCR), which shows a high stereoselectivity in the reduction of 4-BP to *S*(−)-PPOL. RatHCR showed a high identity with PigHCR in amino acid sequence. Furthermore, recombinant RatHCR was confirmed to reduce stereoselectively 4-BP to *S*(−)-PPOL with a high optical purity comparable to recombinant PigHCR. It is possible that in the cytosolic fraction from the heart of rat, constitutive reductase other than RatHCR counteracts the stereoselective reduction of 4-BP to *S*(−)-PPOL, by catalyzing the reduction of 4-BP to the *R*(+)-enantiomer.

Keywords: Stereoselective reduction; Carbonyl reductase; 4-Benzoylpyridine; Species difference; Heart

Introduction

Carbonyl reductase (EC 1.1.1.184) catalyzes the reduction of endogenous and exogenous carbonyl compounds to their corresponding alcohols (Forrest and Gonzalez, 2000; Oppermann and Maser, 2000; Rosemond and Walsh, 2004). Many carbonyl reductases have been purified from various tissues such as the brain, liver and kidney in humans and animal species (Wermuth, 1981; Ikeda et al., 1984; Usui et al., 1984; Imamura et al., 1993). In general, carbonyl reductases are monomeric and cytosolic enzymes with molecular weight of around 34 kDa. The enzymes have been considered as a member of the aldo-keto reductase family based on their functional porperties (Maser, 1995). However, recent structural investigations including amino acid sequences have demonstrated that most of carbonyl reductases belong to the short chain dehydrogenase/reductase (SDR) family (Forrest and Gonzalez, 2000; Oppermann et al., 2001).

We have purified a tetrameric form of carbonyl reductase from cytosolic fractions of rabbit and pig heart, using 4-benzoylpyridine (4-BP) as a substrate (Imamura et al., 1999; Usami et al., 2003). The enzyme purified from pig heart (pig heart carbonyl reductase, PigHCR) belongs to the SDR family, and has the ability to reduce efficiently alkyl phenyl ketones, α-dicarbonyl compounds and all-*trans* retinal. Furthermore, recombinant PigHCR isolated from the cell extract of *Escherichia coli* (*E. coli*) expressing its cDNA, like native PigHCR, is demonstrated to catalyze the stereoselective reduction of 4-BP to *S*(−)-α-phenyl-4-pyridylmethanol [*S*(−)-PPOL] (Fig. 1) (Shimada et al., 2003). As evident from the chemical structure of 4-BP, the phenyl group shows a structural resemblance to the pyridyl group in size and stereochemical characteristics. Furthermore, α-phenylpyridylmethanols produced from benzoylpyridine derivatives including

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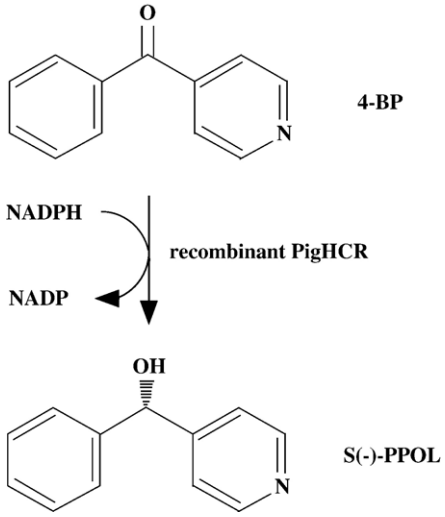

Fig. 1. Stereoselective reduction of 4-BP to *S*(−)-PPOL by recombinant PigHCR.

4-BP are of pharmacological interest as intermediates (Barouh et al., 1971; Takemoto et al., 1995). Thus, it is interesting that PigHCR has the ability to reduce stereoselectively 4-BP.

Recently, the reduction of 4-BP in the cytosolic fraction of pig heart has been reported to exhibit a stereoselectivity comparable to that in recombinant PigHCR (Shimada et al., 2004). In our preliminary experiment, however, such a high stereoselectivity was not observed for the reduction of 4-BP in the cytosolic fractions from the heart of several vertebrates. One of the most important aspects of drug metabolism, particularly in relation to preclinical safety evaluation, is species difference in metabolic pathways (Fournel and Caldwell, 1986; Suckow et al., 1986; Takasaki et al., 1999). However, information about species difference in the stereoselective reduction of drugs containing a ketone group within their chemical structures has been very limited.

The present study was designed to evaluate the stereoselectivity in the reduction of 4-BP in cytosolic fractions from the heart of 9 vertebrates (pig, rabbit, guinea pig, rat, mouse, chicken, soft-shelled turtle, frog and flounder). Furthermore, amino acid sequence of rat heart carbonyl reductase (RatHCR) was deduced from the cloned cDNA and the stereospecificity of recombinant RatHCR for 4-BP reduction was compared with that of recombinant PigHCR.

Materials and methods

Materials

4-Benzoylpyridine (4-BP) was purchased from Wako Pure Chemicals (Osaka, Japan). *S*(−)- and *R*(+)-α-phenyl-4-pyridylmethanol [*S*(−)-PPOL and *R*(+)-PPOL] were synthesized from 4-BP as reported previously (Shimada et al., 2003). NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Oriental Yeast (Tokyo, Japan). All other chemicals were of reagent grade.

Animals

Pig hearts were supplied from a slaughterhouse and stored at −20 °C. Male rabbits at 15 weeks of age (Japanese White) and guinea pigs at 10 weeks of age (Hartley) were purchased from KBT Oriental (Saga, Japan). Male rats at 8 weeks of age were obtained from the following sources: Fischer 344 (Fischer), Wistar, Sprague-Dawley (SD) and WKY (Japan SLC, Shizuoka, Japan); Long-Evans (LE) [KBT Oriental (Saga, Japan)]; Wistar-Imamichi (WI) [Imamichi Institute for Animal Reproduction (Ibaraki, Japan)]. Male mice (ddY) at 10 weeks of age were purchased from Japan SLC. Male chickens were supplied from Kumamoto Prefectural Agricultural Research Center (Kumamoto, Japan). Male frog (*Rana catesbeiana*) was purchased from Kyudo (Saga, Japan). Male soft-shelled turtle (*Pelodiscus sinensis*) and flounder (*Paralichthys olivaceus*) were supplied from Hattori SST (Kumamoto, Japan) and Kumamoto Prefectural Fisheries Research Center (Kumamoto, Japan), respectively. All animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kumamoto University.

Preparation of cytosolic fraction

The hearts were excised from animals anesthetized. The tissues were homogenized in three volumes of 10 mM sodium potassium phosphate buffer containing 1.15% KCl (pH 6.0). The homogenates were centrifuged at 105,000 g for 60 min to obtain the cytosolic fraction.

Stereoselective reduction of 4-BP

The stereoselective reduction of 4-BP was estimated by measuring *S*(−)- and *R*(+)-PPOL formed from 4-BP in the reaction mixture (Shimada et al., 2003). The reaction mixture consisted of substrate (0.5 mM 4-BP), NADPH-generating system (50 μM NADP, 1.25 mM glucose-6-phosphate, 50 munits glucose-6-phosphate dehydrogenase and 1.25 mM $MgCl_2$), the cytosolic fraction (or recombinant RatHCR) and

Table 1
Species difference in the stereoselective reduction of 4-BP in the cytosolic fractions from the heart of vertebrates

Species	Enantiomers formed (nmol/mg protein)	
	S(−)-PPOL	*R*(+)-PPOL
Pig	59.3±5.4 (94.4)	3.5±1.5 (5.6)
Rabbit	30.9±1.6 (80.5)	7.5±0.7 (19.5)
Guinea pig	2.5±0.9 (83.3)	0.5±0.1 (16.7)
Rat	2.7±0.6 (45.8)	3.2±0.3 (54.2)
Mouse	6.0±0.9 (66.7)	3.0±0.5 (33.3)
Chicken	15.0±3.8 (56.4)	11.6±1.8 (43.6)
Soft-shelled turtle	1.9±0.1 (42.2)	2.6±0.2 (57.8)
Frog	3.1±0.4 (45.6)	3.7±0.5 (54.4)
Flounder	3.7±0.1 (45.7)	4.4±0.1 (54.3)

The value represents the mean±S.D. of three to six experiments. The parenthesis is the percentage of *S*(−)-PPOL or *R*(+)-PPOL in enantiomers formed.