Enzymes responsible for metabolism of $N\alpha$ -benzyloxycarbonyl-L-lysine in microorganisms

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Abstract

The present paper reviews the enzymes catalyzing conversion of *N* α -benzyloxycarbonyl-L-lysine (*N* α -Z-L-lysine) to *N* α -benzyloxycarbonyl-L-aminoadipic acid (*N* α -Z-L-AAA) in fungal and bacterial strains. *Aspergillus niger* AKU 3302 and *Rhodococcus* sp. AIU Z-35-1 converted *N* α -Z-L-lysine to *N* α -Z-L-AAA via *N* α -benzyloxycarbonyl-L-aminoadipate- δ -semialdehyde (*N* α -Z-L-AASA). However, different enzyme combinations were involved in the *N* α -Z-L-lysine metabolism of both strains. *A. niger* strain converted *N* α -Z-L-lysine to *N* α -Z-L-AASA by amine oxidase, and the resulting *N* α -Z-L-AASA was converted to *N* α -Z-L-AAA by an aldehyde oxidase. In the *Rhodococcus* strain, conversion of *N* α -Z-L-lysine to *N* α -Z-L-AASA was catalyzed by L-specific amino acid oxidase. The resulting *N* α -Z-L-AASA was converted to *N* α -Z-L-AAA by an aldehyde dehydrogenase. The present paper also describes characteristics of new enzymes obtained from those strains.

Introduction

L- α -Aminoadipic δ -semialdehyde (L- α -AASA) and L- α -aminoadipic acid (L- α -AAA) are precursors of β -lactam antibiotics biosynthesis, and both compounds and their related compounds provide interesting raw materials for the chemical synthesis of pharmaceuticals or physiologically active peptides. Therefore, some biochemical methods for selective production of those compounds have been developed using enzymes or microorganisms. For example, the methods for the L- α -AASA production were developed using L-lysine-6-aminotransferase or L-lysine-6-dehydrogenase [1, 2]. Recently, simple biochemical methods for selective production of *N* α -Z-L-AASA and *N* α -Z-L-AAA were also reported using *A. niger* AKU 3302 or *Rhodococcus* sp. AIU Z-35-1 [3-6]. In this paper, enzymes

responsible for the production of $N\alpha$ -Z-L-AASA and $N\alpha$ -Z-L-AAA are compared in both strains, and the desirable methods for the selective production of above compounds are viewed.

Identification of metabolites from $N\alpha$ -Z-L-lysine

The cells from *A. niger* AKU 3302 harvested from a *n*-butylamine medium or those from *Rhodococcus* sp. AIU Z-35-1 harvested from a L-lysine medium were incubated with $N\alpha$ -Z-L-lysine, and the reaction products were analyzed by HPLC. In this analysis, $N\alpha$ -Z-L-AASA and $N\alpha$ -Z-L-AAA were identified as reaction products from $N\alpha$ -Z-L-lysine in both strains. It was therefore estimated that $N\alpha$ -Z-L-lysine is converted to $N\alpha$ -Z-L-AAA via $N\alpha$ -Z-L-AASA in both strains [4, 5].

Identification of enzymes catalyzing conversion of $N\alpha$ -Z-L-lysine to $N\alpha$ -Z-L-AASA

Each crude enzyme solution from *A. niger* strain and *Rhodococcus* strain was prepared by disrupting cells harvested from the *n*-butylamine and the L-lysine medium, respectively, and enzyme activities on $N\alpha$ -Z-L-lysine were assayed. Both enzyme solutions exhibited oxidase activity on $N\alpha$ -Z-L-lysine, but the dehydrogenase activity was less than 2% of the oxidase activity. In addition, the produced amounts of H_2O_2 increased in parallel to those of NH_3 and $N\alpha$ -Z-L-AASA. These results indicate that $N\alpha$ -Z-L-lysine is converted to $N\alpha$ -Z-L-AASA by an oxidase in both strains.

It was reported earlier that *A. niger* AKU 3302 produced amine oxidase (MAO, EC 1.4.3.6), which catalyzed oxidation of aliphatic and aromatic amines but not amino acids [7]. However, recently Isobe *et al.* revealed that $N\alpha$ -Z-L-lysine was also oxidized by the MAO, although L-lysine was not oxidized [3]. Thus, it was revealed that the oxidase from *A. niger*

strain is MAO, and it catalyzes the oxidative deamination of the ϵ -amino group of $N\alpha$ -Z-L-lysine, but not of the α -amino group of L-amino acids.

In contrast, the oxidase from *Rhodococcus* strain exhibited oxidase activity on $N\alpha$ -Z-L-lysine and $N\epsilon$ -acetyl-L-lysine, but not on aliphatic and aromatic amines. L-Amino acids except for glycine, L-proline and L-cysteine were also oxidized by this enzyme, but $N\alpha$ -Z-D-lysine, $N\epsilon$ -acetyl-D-lysine and D-amino acids were not [8]. These results indicate that the oxidase from *Rhodococcus* strain is a L-specific amino acid oxidase (L-AAO, EC 1.4.3.2), and catalyzes an oxidative deamination of not only the ϵ -amino group of $N\alpha$ -acyl-L-lysine but also the α -amino group of $N\epsilon$ -acyl-L-lysine.

Thus, it was concluded that $N\alpha$ -Z-L-lysine is oxidized to $N\alpha$ -Z-L-AASA by MAO in *A. niger* AKU 3302 and L-AAO with broad substrate specificity in *Rhodococcus* sp. AIU Z-35-1, respectively (Figs 2 and 3).

Characteristics of enzymes catalyzing conversion of $N\alpha$ -Z-L-lysine to $N\alpha$ -Z-L-AASA

Isobe *et al.* [3] revealed that MAO from *A. niger* strain oxidized not only $N\alpha$ -Z-L-lysine but also $N\alpha$ -Z-D-lysine. In contrast, L-AAO from *Rhodococcus* strain oxidized $N\alpha$ -Z-L-lysine and L-amino acids, but did not oxidize $N\alpha$ -Z-D-lysine and D-amino acids. Thus, both oxidases were differentiated by substrate specificity and stereospecificity. Both enzymes were further differentiated by other characteristics. The L-AAO from *Rhodococcus* strain is a flavoprotein with a molecular mass of 100 kDa, while MAO contains copper/Topa quinone and the molecular mass is 150 kDa [9]. When the N-terminal amino acid sequence similarity of L-AAO from *Rhodococcus* sp. AIU Z-35-1 was searched, no similarity was found to MAO from *A. niger* [9], while more than 50% of the amino acids were identical to that of flavin-containing MAOs (EC 1.4.3.4) [10-12]. The N-terminal amino acid sequence of

L-AAO from *R. opacus* DSM 43250 [13] and a putative L-AAO from *Rhodococcus* sp. RHA 1 [14] also exhibited high similarity to that of L-AAO from *Rhodococcus* sp. AIU Z-35-1 (Fig. 1), although oxidation of $N\alpha$ -Z-L-lysine by the *R. opacus* L-AAO has not been recognized. It was therefore concluded that $N\alpha$ -Z-L-lysine is converted to $N\alpha$ -Z-L-AASA by new L-AAO in *Rhodococcus* sp. AIU Z-35-1.

Identification of enzymes catalyzing conversion of $N\alpha$ -Z-L-AASA to $N\alpha$ -Z-L-AAA

The crude enzyme solution from *A. niger* strain exhibited $N\alpha$ -Z-L-AASA oxidase activity, but the dehydrogenase activity was not detected. In addition, the produced amount of H_2O_2 increased in parallel to that of $N\alpha$ -Z-L-AAA. The oxidase reaction of this enzyme was also confirmed by the formation of $N\alpha$ -Z-L-AAA from $N\alpha$ -Z-L-lysine using a mixture of MAO and this enzyme [15].

In the case of *Rhodococcus* strain, $N\alpha$ -Z-L-AASA dehydrogenase activity was obtained, but the oxidase activity was not. In addition, the produced amount of NADH increased in parallel to that of $N\alpha$ -Z-L-AAA [16].

Thus, it was concluded that $N\alpha$ -Z-L-AASA is converted to $N\alpha$ -Z-L-AAA by aldehyde oxidase (ALO) in *A. niger* AKU 3302 and aldehyde dehydrogenase (ALDH) in *Rhodococcus* sp. AIU Z-35-1, respectively (Figs 2 and 3).

Characteristics of enzymes catalyzing conversion of $N\alpha$ -Z-L-AASA to $N\alpha$ -Z-L-AAA

The ALO from *A. niger* AKU 3302 exhibited oxidase activity on $N\alpha$ -Z-L-AASA but not on short-chain aliphatic aldehydes, aromatic aldehydes and alcohols [15]. The molecular mass of this enzyme was 215 kDa, which is different from that of other microbial ALOs (EC 1.2.3.1) [17-20].

The ALDH from *Rhodococcus* sp. AIU Z-35-1 exhibited dehydrogenase activity on $N\alpha$ -Z-L-AASA, $N\alpha$ -Z-D-AASA and short-chain aliphatic aldehydes. In addition, this enzyme preferred β -NAD⁺ rather than β -NADP⁺ as a cofactor, which was therefore classified into a group of NAD⁺-dependent ALDH (Aldehyde: NAD⁺ oxidoreductase, EC 1.2.1.3) [16]. When the N-terminal amino acid sequence similarity of ALDH from *Rhodococcus* sp. AIU Z-35-1 was searched, similarity was obtained in a putative ALDH from *Rhodococcus* sp. RHA 1 [14] and piperidine-6-carboxylate dehydrogenase (P6CDH) from *Streptomyces clavuligerus* [21], which catalyzes the conversion of piperidine-6-carboxylate to α -aminoadipic acid (Fig. 4), but not in other microbial ALDHs. The P6CDH was also purified from *Flavobacterium lutescens* IFO 3084 [22], but the N-terminal amino acid sequence was not similar to that of ALDH from *Rhodococcus* sp. AIU Z-35-1. In addition, dehydrogenase activity on $N\alpha$ -Z-L-AASA and $N\alpha$ -Z-D-AASA has not been reported by P6CDH from *S. clavuligerus* [23] and *F. lutescens*. It was therefore concluded that ALO from *A. niger* AKU 3302 and ALDH from *Rhodococcus* sp. AIU Z-35-1, which catalyze the conversion of $N\alpha$ -Z-L-AASA to $N\alpha$ -Z-L-AAA, are new enzymes.

Outlook

Recently, new biochemical methods for selective production of $N\alpha$ -Z-L-AASA and $N\alpha$ -Z-L-AAA from $N\alpha$ -Z-L-lysine were developed using bacterial or fungal strains, and enzymes responsible for the production of both compounds were identified. Therefore, characteristics of those enzymes are compared, and desirable methods are viewed. The production of $N\alpha$ -Z-L-AASA from $N\alpha$ -Z-L-lysine is catalyzed by MAO or L-AAO, and $N\alpha$ -Z-L-AAA was produced from $N\alpha$ -Z-L-lysine by the combination of MAO and ALO in *A. niger* AKU 3302, and L-AAO and ALDH in *Rhodococcus* sp. AIU Z-35-1. In the analysis of

enzymes for production of $N\alpha$ -Z-L-AAA, it was revealed that the ALDH reduced the speed of $N\alpha$ -Z-L-AAA production in the cell reaction with the *Rhodococcus* strain, since the dehydrogenase requires the NAD regeneration system, while ALO does not require the cofactor regeneration system. Thus, oxidase methods have an advantage in that the methods require oxygen but not the second substrate and/or cofactor regeneration system to oxidize the substrate. In addition, the oxidase methods do not produce any by-products except hydrogen peroxide which is easily converted to H_2O by catalase. Therefore, it is concluded that oxidase methods are much better than other biochemical methods for selective production of oxidative products in the future.

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Figure legends

Fig. 1 Alignment of N-terminal amino acid sequence of L-AAOs and MAOs.

The N-terminal amino acid sequence of L-AAO from *Rhodococcus* sp. AIU Z-35-1 was aligned to those of L-AAOs, putative L-AAO and MAOs obtained by BLAST search. Identical amino acid residues are boxed. *Rhodococcus* sp. Z-35-1 (*R. sp.* Z-35-1) and *R. opacus* DSM 43250 are L-AAO; *R. sp.* RHA 1 is a putative AAO; *D. radiodurans* R1, *P. fluorescens* pf-5 and *Synechococcus* sp. JA-2-3'a (*S. sp.* JA-2-3'a) are putative MAOs.

Fig. 2 Conversion of $N\alpha$ -Z-lysine to $N\alpha$ -Z-L-AAA by *A. niger* AKU 3302.

$N\alpha$ -Z-L-lysine, $N\alpha$ -benzyloxycarbonyl-L-lysine; $N\alpha$ -Z-L-AASA, $N\alpha$ -benzyloxycarbonyl-L-aminoadipic- δ -semialdehyde; $N\alpha$ -Z-L-AAA, $N\alpha$ -benzyloxycarbonyl-L-aminoadipic acid.

Fig. 3 Conversion of $N\alpha$ -Z-lysine to $N\alpha$ -Z-L-AAA by *Rhodococcus* sp. AIU Z-35-1.

$N\alpha$ -Z-L-lysine, $N\alpha$ -benzyloxycarbonyl-L-lysine; $N\alpha$ -Z-L-AASA, $N\alpha$ -benzyloxycarbonyl-L-aminoadipic- δ -semialdehyde; $N\alpha$ -Z-L-AAA, $N\alpha$ -benzyloxycarbonyl-L-aminoadipic acid.

Fig. 4 Alignment of N-terminal amino acid sequence of ALDHs.

The N-terminal amino acid sequence of ALDH from *Rhodococcus* sp. AIU Z-35-1 was aligned to those of other ALDHs obtained by BLAST search. Identical amino acid residues are boxed. X indicates an unknown residue.

Fig. 1

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	10	20	30	40	References
<i>R. sp.</i> Z-35-1	G D L I G K V K G N	H S V V I L G G G P S	G L X X A Y E L Q K A G Y K V T V L E	8	
<i>R. opacus</i> DSM 43250	A G D L I G K V K G S	H S V V V L G G G P A	G L C S A F E L Q K A G Y K V T V L E	13	
<i>R. sp.</i> RHA 1	A G D L I G K V K G S	H S V V V L G G G P A	G L C S A F E L Q K A G Y T V T V L E	14	
<i>D. radiodurans</i> R1	K P Q L T G G G K G K -	S V L I L G G G L A G M A S	A Y E M Q K A G Y D V Q I L E	10	
<i>P. fluorescens</i> pf-5	A L P T A A L G K G A -	S V V V I G A G I G G L V S	A Y E L S K A G F K V T L L E	11	
<i>S. sp.</i> JA-2-3'a		M Q S T V V I G A G P A	G L S A A Y E L N K Q G Y P V T V L E	12	

Fig. 2

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Fig. 3

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Fig. 4**K. Isobe**

		10	20	30	40	References
<i>R. sp. Z-53-1</i>	M N L P D P S S L A D R A E R A L A A X G V E W P A - - G D - L S A H T P I T G G Q L					16
<i>R. sp. RHA 1</i>	M N L P A P E E L C R R A E E A L I R C G A I T D A - - G G P L V A R T P I T G T E L					14
<i>S. clavuligerus</i>	M V T A A I S G T D E I R A R A E Q A L T R C G V D L T A V K G D A L T A R T P L T G A D L					21