Biosci. Biotechnol. Biochem., **63** (6), 1063–1069, 1999

Novel Characteristics of *Selenomonas ruminantium* Lysine Decarboxylase Capable of Decarboxylating Both L-Lysine and L-Ornithine

Yumiko TAKATSUKA,[1] Motoko ONODA,[1] Takeyoshi SUGIYAMA,[2] Koji MURAMOTO,[3] Toshio TOMITA,[1] and Yoshiyuki KAMIO[1,†]

Laboratories of the Applied Microbiology,[1] Applied Bioorganic Chemistry,[2] and Applied Phytochemistry,[3] Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumi-dori Amamiya-machi, Aoba-ku, Sendai 981-8555, Japan

Received January 19, 1999; Accepted February 12, 1999

Lysine decarboxylase (LDC; EC 4.1.1.18) of *Selenomonas ruminantium* is a constitutive enzyme and is involved in the synthesis of cadaverine, which is an essential constituent of the peptidoglycan for normal cell growth. We purified the *S. ruminantium* LDC by an improved method including hydrophobic chromatography and studied the fine characteristics of the enzyme. Kinetic study of LDC showed that *S. ruminanitum* LDC decarboxylated both L-lysine and L-ornithine with similar K_m and the decarboxylase activities towards both substrates were competitively and irreversibly inhibited by DL-α-difluoromethylornithine, which is a specific inhibitor of ornithine decarboxylase (EC 4.1.1.17). We also showed a drastic descent of LDC activity owing to the degradation of LDC at entry into the stationary phase of cell growth.

Key words: *Selenomonas ruminantium*; lysine decarboxylase; ornithine decarboxylase; DL-α-difluoromethyllysine; DL-α-difluoromethylornithine

Diamines such as putrescine and cadaverine have been reported to be essential constituents of peptidoglycan and play a significant role in maintenance of the integrity of the cell envelope in *Selenomonas ruminantium*, *Veillonella paruvulla*, and *V. alcalescens*.[1,2] In *S. ruminantium* grown with L-ornithine and L-lysine, putrescine and cadaverine, respectively were found to link covalently to the α-carboxyl group of the D-glutamic acid residue of the peptidoglycan.[1,3,4] Previously, we found that cadaverine is constitutively synthesized from L-lysine[5] and transferred to the D-glutamic acid residue of the lipid intermediate for the synthesis of the cadaverine-containing peptidoglycan by a lipid intermediate: cadaverine transferase.[6] The physiological function of cadaverine in this organism was studied by using DL-α-difluoromethyllysine (DFML), which was shown to be a potent, irreversible, and specific inhibitor of lysine decarboxylase (L-lysine carboxylyase, EC 4.1.1.18) (LDC)[7] and it was found that DFML markedly inhibited the growth of *S. ruminantium* and caused rapid cell lysis owing to the synthesis of the abortive peptidoglycan without cadaverine.[1] The growth inhibition by DFML was completely reveresed by adding 1 mM cadaverine or putrescine to the medium, and cadaverine or putrescine was exclusively incorporated into the peptidoglycan to form a normal cell wall.[1] During study on the inhibitors in the incorporaion of the diamines into the peptidoglycan for the cell growth of *S. ruminantium*, we noticed that 10 mM DL-α-difluoromethylornithine (DFMO), which had been shown to be a specific and irreversible inhibitor of ornithine decarboxylase (L-ornithine carboxylyase, EC 4.1.1.17) (ODC) both *in vivo* and *in vitro*,[8] also completely prevented the growth of *S. ruminantium* and caused formation of an abortive peptidoglycan without cadaveine synthesis. These observations suggested that DFMO as well as DFML inhibits *S. ruminantium* LDC, or that *S. ruminantium* ODC could decarboxylate L-lysine. Taken together with our previous report that the molecular mass of the LDC preparation from *S. ruminantium* is similar to that of eukaryotic ODCs but not prokaryotic LDCs including *Escherichia coli* LDC and *Bacterium cadaveris* LDC,[5] these suggestions prompted us to investigate the substrate specificity of the *S. ruminantium* LDC. We purified LDC from *S. ruminantium* by a 4-step procedure to homogeneity with approximately 10% yield from the cells. The enzyme thus obtained was characterized in its substrate specificity and the mode of inhibition of the enzyme by DFML and DFMO *in vitro*. Here, we demonstrate that the *S. ruminantium* LDC had decarboxylase activities towards both L-lysine and L-ornithine with similar K_m and that the enzyme activities were inhibited by either DFML or DFMO competitively against L-lysine and L-ornithine as substrates. We also show that *S. ruminantium* does not have a typical bacterial ODC with decarboxylase activity only towards L-ornithine. This is the first report on an LDC having a decarboxylase activities towards both L-lysine and L-ornithine and being inhibited by either DFML or DFMO.

Materials and Methods

Bacterial strains and cultivation. *S. ruminantium* subsp. *lactilytica*, described previously,[9] was used. For

† To whom correspondence should be addressed. Tel: +81-22-717-8779; Fax: +81-22-717-8780; E-mail: ykamio@biochem.tohoku.ac.jp

Abbreviations: LDC, L-lysine decarboxylase; ODC, L-ornithine decarboxylase; DFML, DL-α-difluoromethyllysine; DFMO, DL-α-difluoromethylornithine; PLP, pyridoxal-5′-phosphate; TCA, trichloroacetic acid

preparation of LDC from the cells, the organism was grown in a tryptone (Difco)-yeast extract (Difco)-sodium lactate medium (TYL medium) or a tryptone-yeast extract-glucose medium (TYG medium) at 37°C under anaerobic conditions.[9] Large-scale cultivation of *S. ruminantium* in 60 liters of TYG medium was started by adding cultured bacteria at an initial concentration of 2×10^7 cells/ml. When the cell density reached 7×10^8 cells/ml, the cells were harvested quickly by continuous centrifugation at 4°C. The packed cells were stored at −80°C until preparation of the enzyme.

Reaction mixture and enzyme assay. LDC was assayed by measuring cadaverine converted from L-lysine by LDC. The reaction mixture for measuring LDC activity contained 0.5 M sodium acetate buffer (pH 6.0), 5 mM L-lysine, 50 μM PLP, and the enzyme fraction in a total volume of 100 μl. The reaction mixture was incubated at 40°C for 20 min. The reaction was stopped by adding 34 μl of 20% trichloroacetic acid (TCA), and the precipitate was removed by centrifugation at 10,000 × *g*. The amount of cadaverine in the supernatant was measured by HPLC using a TSK-gel Polyaminepak column (Tosoh, Tokyo) and a cellulose thin-layer electrophoresis using a cellulose thin-layer plate (Art. 5716, Merk) by the method described previously.[10] For measurement of cadaverine or putrescine in a reaction mixture, a spectrophotometric assay was used as described previously.[11]

Specific activity of LDC. One katal (kat) of enzyme activity was defined as 1 mole of cadaverine formed from L-lysine at 40°C in 1 second. The specific activity is given as kat per kilogram of protein.

Protein measurement. Protein was measured by the method of Bradford,[12] with bovine serum albumin used as the standard.

Purification of LDC from S. ruminantium. The previous purification procedure was composed of 7 steps including affinity chromatography.[5] In this study, we improved the purification procedure as follows. The cells (125.7 g) derived from 60 liters of culture were suspended in 50 mM potassium phosphate buffer (pH 6.7, buffer A) containing 10 mM 2-mercaptoethanol and 0.1 mM PLP, and disrupted them in a French pressure cell. After centrifugation at 17,500 × *g* for 20 min, the supernatant was collected. Ammonium sulfate (3 M) in buffer A was added to the enzyme fraction at a final concentration of 1 M with stirring. After 2 h, the supernatant was collected by centrifugation and was put on a TSKgel butyl-Toyopearl 650M column (4.0 by 24 cm). The column was washed with 3.5 liters of buffer A containing 1 M ammonium sulfate and 50 μM PLP. The enzyme was then eluted with a linear gradient created by mixing 750 ml of 1 M ammonium sulfate in buffer A with 750 ml of buffer A. The fractions which were eluted from 0.80 to 0.85 M ammonium sulfate were pooled. After 3 M ammonium sulfate in buffer A was added at the final concentration of 0.8 M, the enzyme preparation was put on a TSKgel phenyl-5PW column (21.5 by 150 mm; Tosoh, Tokyo). The column was washed with 450 ml of buffer A containing 0.8 M ammonium sulfate and then eluted with a linear gradient of ammonium sulfate in buffer A (0.8 to 0 M). The fractions eluted from 0.54 to 0.56 M ammonium sulfate were pooled. After being dialyzed against 20 mM potassium phosphate buffer containing 50 μM PLP (pH 6.5, buffer B), the enzyme preparation was put on a TSKgel DEAE-5PW column (7.5 by 75 mm; Tosoh) equilibrated with 20 mM potassium phosphate buffer (pH 6.5, buffer C). The column was washed with buffer C and then eluted with a linear gradient of NaCl in buffer C (0 to 0.4 M). The fractions eluted from 0.18 to 0.20 M NaCl were pooled and dialyzed against buffer B containing 20% glycerol.

Kinetic study. Kinetic parameters, K_{cat}, K_m, and K_i were calculated from initial rate measurements for decarboxylation of L-lysine or L-ornithine, by fitting to the Michaelis-Menten equation using a nonlinear regression algorithm.

Preparation of antiserum against S. ruminantium LDC. Rabbit antiserum raised with recombinant *S. ruminantium* LDC was prepared by a following method. The recombinant LDC was prepared from *Escherichia coli* DH5α having a plasmid, pTLDC, which contained the *S. ruminantium* LDC gene (Takatsuka *et al.*, unpublished data). One ml of a purified recombinant LDC preparation (1 mg/ml) was emulsified with 1 ml of Freund's complete adjuvant (Wako Pure Chemical Industry, Osaka) and injected subcutaneously into a female NZW rabbit (2.5 kg). Two weeks later, the second injection of 1 mg of enzyme preparation (1 mg/ml) emulsified with an equal volume of Freund's incomplete adjuvant (Wako Pure Chemical Industry, Osaka) was given in the same way. Every two weeks, injections of 1 mg enzyme with Freund's incomplete adjuvant were repeated 5 times. Two weeks after the seventh injection, the rabbit was anesthetized and killed for preparation of antiserum.

Chemicals. DL-2,7-diaminoheptanoic acid dihydrochloride was synthesized chemically by the method using diethyl acetamidomalonate.[13] 5-Carbethoxypentyl tosylate was condensed with diethyl acetamidomalonate to give ethyl 2-carbethoxy-2,7-diacetamidoheptanoate. This ester was hydrolyzed directly to DL-2,7-diaminoheptanoic acid dihydrochloride by treatment at reflux temperature with concentrated hydrochloric acid. The structure of the product was confirmed spectroscopically.

DFML and DFMO were kind gifts from Dr. McCann of Merrel Daw Research Institute (Cincinnati, OH, U.S.A). Other chemicals used were of the best grade commercially available.

Results and Discussion

Decision of the timing of harvest of cells for preparation of the crude extract

Figure 1 shows fluctuation in LDC activity during growth of the *S. ruminantium* cells in the medium upon

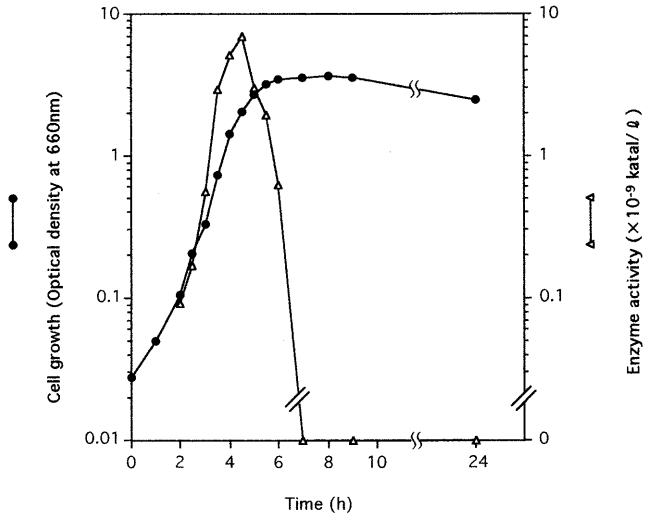

Fig. 1. Fluctuation in the Activity of LDC during Growth of *S. ruminantium*.

S. ruminantium cells were grown in 1 liter of TYG medium at 37°C under anaerobic conditions, and the cell growth (●) and the LDC activity (△) were measured. To measure LDC activity, at the time indicated in this figure, 4 ml of the culture was taken up and centrifuged. The cells, suspended in 20 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM PLP, were sonicated. The LDC activities in the cell extracts were measured by the amount of cadaverine formed from lysine by HPLC using a TSK-gel Polyaminepak column (Tosoh, Tokyo, Japan). The incubation mixture contained 0.5 M sodium acetate buffer (pH 6.0), 5 mM L-lysine, 50 μM PLP, and the cell extracts in a total volume of 50 μl. The reaction mixture was incubated at 40°C for 60 min. The reaction was stopped by adding TCA at a final concentration of 5%, and the precipitate removed by centrifugation. The amount of cadaverine in the supernatant was measured. One katal (kat) of enzyme activity was defined as 1 mole of cadaverine formed from L-lysine at 40°C in 1 second.

inoculation of cells that were grown overnight with an initial cell density of 2×10^7 cells/ml. The sonicated extracts from the cells taken at various times pointed out in this figure were prepared and the decarboxylase activity towards L-lysine was assayed. The enzyme activity in total cells in a culture increased up to 4.5 h and reached approximately 7×10^{-9} katal/liter of culture and hereafter a sudden decrease of the total enzyme activity happened to occur. Accordingly, the timing of cell harvest for the preparation of the crude extract from the cells was decided to be during 3.5–4.0 h ($O.D._{660}=1.0$) after inoculation. The fluctuation of LDC will be discussed later.

Enzyme purification

Through the four steps shown in Table 1, the enzyme was purified about 1,100-fold to electrophoretic homogeneity with a specific activity of 0.198 kat/kg of protein. The N-terminal amino acid sequence of the enzyme was MKNFRLSEKEVKTLAKRIPXPFLV, in which X represents unidentified amino acid residue, and it was identical with that deduced from the DNA sequence of a LDC gene (*ldc*) cloned from the chromosonal DNA of *S. ruminanitum* (Y. Takatsuka *et al.*, DDBJ/EMBL/Genbank nucleotide sequence databases with the accession No. AB011029). Although the purified LDC lost its activity within two weeks even at −80°C, it can be stored at −80°C for one month without any appreciable loss in the presence of 20% glycerol.

Characteristics of S. ruminantium LDC

(*i*) *The presence of the enzyme molecule as a dimeric form with the decarboxylase activity.* The apparent molecular mass of the purified LDC preparation is 88 kDa, as judged by gel filtration analysis, and 42 kDa by SDS-PAGE. It has an isoelectric point of 4.7. On gel filtration, the monomeric form of LDC of 42 kDa was also separated with no enzyme activity (data not shown). The data show that the active LDC comprises two identical monomeric subunits.

(*i*) *Substrate specificity and inhibitors.* To examine the substrate specificity of the enzyme preparation, the enzyme concentrations used for the enzyme reaction were around 8–100 nM. The purified LDC preparation decarboxylated both L-lysine and L-ornithine to the same extent. The general chemical structure of diaminocarboxylic acid is shown as $NH_2(CH_2)_nCH(NH_2)COOH$; ornithine and lysine correspond to n=3 and 4, respectively. We examined whether or not the other diaminocarboxyric acids could act as substrates. We used L-2,4-diaminobutyric acid dihydrochloride (n=2, ACROS) and DL-2,7-diaminoheptanoic acid dihydrocloride (n=5) which was synthesized chemically, but both chemicals were not decaboxylated by the enzyme preparation. Neither D-lysine, DL-2,6-diamimopimelic acid, L-arginine, L-glutamine, L-histidine, nor ε-aminocapronic acid was able to act as substrate (decarboxylase activity towards the substrates were below 4% compared with that towards L-lysine, data not shown). The kinetics of

Table 1. Purification of the Lysine Decarboxylase from *S. ruminantium*

Purification steps	Total vol. (ml)	Total protein ($\times 10^{-6}$ kg)	LDC		Yield (%)	Purification (Fold)
			Total activity ($\times 10^{-9}$ kat)	Specific activity (kat/kg)		
Crude extract*	429	5990	1081	0.000181	100	1
Butyl-Toyopearl 650M	118	85.7	532	0.00621	49.2	34.5
Phenyl-5PW	15.8	2.59	141	0.0544	13.0	302
DEAE-5PW	2.75	0.531	105	0.198	9.71	1100

* Prepared from the cells of 60-liter culture (125.7 g wet cells).

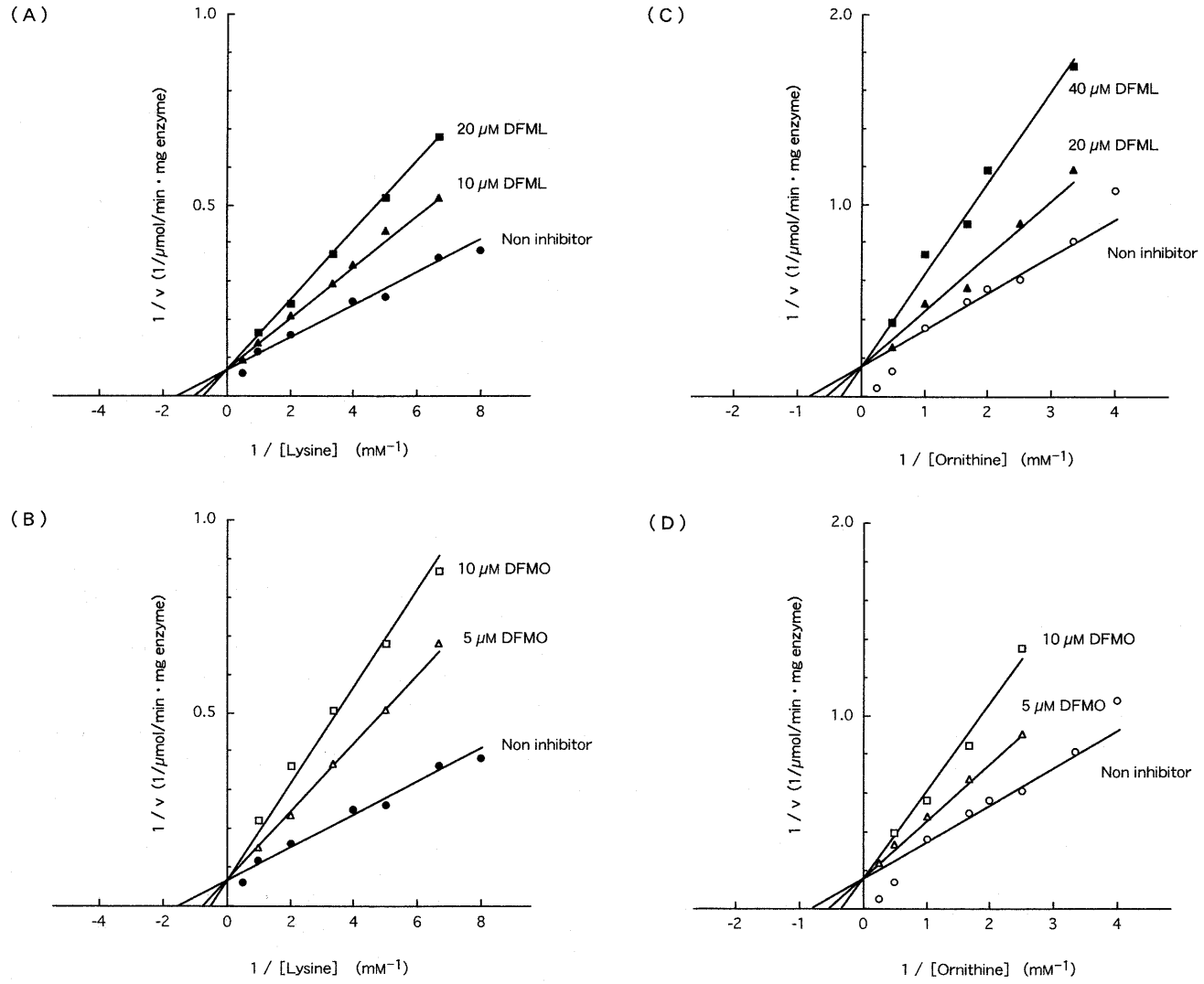

Fig. 2. Lineweaver-Burk Plots of Lysine Decarboxylase (A, B) and Ornithine Decarboxylase (C, D) Activities of the Purified LDC Preparation with or without Inhibitors. Inhibitors; DFML and DFMO were used for A and C, and B and D, respectively.

decarboxylation of both L-lysine and L-ornithine by the LDC preparation were analyzed by measuring initial velocities over a range of the substrates concentrations (0.125 to 4 mM). Over the concentration range used, both L-lysine and L-ornithine behaved as Michaelis-Menten-type substrates (Fig. 2). A double-reciprocal plot of the initial velocities demonstrated that the K_m for L-lysine and L-ornithine were 0.63 mM and 1.2 mM, respectively and the V_{max} were 14.7 and 6.71 μmol min^{-1} mg^{-1} of protein for L-lysine and L-ornithine, respectively (Table 2). To ascertain that the *S. ruminantium* LDC had the novel characteristic of decarboxylating both L-lysine and L-ornithine, the *ldc* was cloned from the *S. ruminantium* chromosomal DNA and expressed in a *ldc*-lacking *E. coli* mutant which was constructed previously.[14] When the recombinant LDC (rLDC) was purified and assayed for the decarboxylase activity towards L-lysine and L-ornithine, the rLDC decarboxylated both substrates with the K_m identical with that of the intact LDC from *S. ruminantium* (Takatsuka, Y. *et al.*, unpublished data).

It is well known that ODC preferentially decarboxylates L-ornithine and that the enzyme activity is inhibited competitively by DFMO.[15] Taken together with the evidence, the novel characteristic of our enzyme having decaboxylase activities towards both L-lysine and L-ornithine led us to examine, by using DFML and DFMO, whether or not the catalytic domains of our LDC for recognition of both substrates are identical. If the catalytic domain(s) in LDC for decarboxylating both L-lysine and L-ornithine is/are identical, the decarboxylation of L-lysine and L-ornithine should be inhibited by DFML and/or DFMO. Accordingly, we examined the inhibitory effects of both inhibitors on the decarboxylase activities towards L-lysine and L-ornithine. As shown in Table 2, both DFML and DFMO inhibited the decarboxylation of L-lysine by the LDC preparation with K_i of 17.5 μM and 5.25 μM, respectively. In the same manner, both DFML and DFMO prevented decarboxylation of L-ornithine by the LDC preparation with K_i of 28.0 μM and 8.20 μM, respectively. In the ranges from 0 to 40 μM DFML and 0 to 10 μM DFMO, plots of

Table 2. Kinetic Analysis and Inhibition of Lysine Decarboxylase from *S. ruminantium*

Substrate	K_m (mM)	V_{max} (μmol/min·mg enzyme)	k_{cat} (sec^{-1})	k_{cat}/K_m ($M^{-1}\,sec^{-1}$)	Inhibitor	K_i (μM)	Type of inhibition
L-lysine	0.63	14.7	10.6	17,000	DFML	17.5	Competitive
					DFMO	5.25	Competitive
L-ornithine	1.2	6.71	4.83	4,000	DFML	28.0	Competitive
					DFMO	8.20	Competitive

Table 3. Comparison of *S. ruminantium* LDC with Bacterial and Eukaryotic ODCs Including ODCs from *E. coli*, *Lactobacillus* sp. 30a, Mouse, *Trypanosoma brucei*, and *Neurospora crassa*

Property	*S. ruminantium* LDC	Bacterial ODC[a]		Eukaryotic ODC[a]		
		E. coli (constitutive)	*Lactobacillus* sp. 30a (inducible)	Mouse	*T. brucei*	*N. crassa*
Subunit M_r	42,000	82,000	85,000	53,000	45,000	53,000
Native M_r	88,000	146,000	1,040,000	100,000	90,000	110,000
Subunit structure	Dimer	Dimer	Dodecamer, Dimer	Dimer	Dimer	Dimer
p*I*	4.7		4.55	4.8	5.0	5.25 & 5.5
Cofactor	PLP	PLP	PLP	PLP	PLP	PLP
Substrate specificity						
K_m L-lysine (mM)	0.63			5.2	21	
K_m L-ornithine (mM)	1.2	5.6	1.7	0.09	0.24	0.35
Inhibitor	DFML & DFMO	not inhibited by DFMO		DFMO	DFMO	DFMO
K_i for LDC activity (μM)	18 5.3					
K_i for ODC activity (μM)	28 8.2			39	220	
Optimal pH	6.0	8.1	5.8	7.4	7.3	7.1
Optimal temperature	45°C					
pH stability	5.5-8.0 (>80%)					
Thermal stability for 30 min						
with PLP	50°C (50%)					
without PLP	35°C (50%)					

[a] Data are derived from Refs. 15-21.

the reciprocals of reaction velocities against both L-lysine and L-ornithine gave a series of straight lines intercepting a single point on the 1/v axis (Fig. 2, A-D), indicating that the inhibition type of both inhibitors is competitive. Thus, it was concluded that *S. ruminantium* LDC has decarboxylase activities towards both L-lysine and L-ornithine with similar K_m and that catalytic domains of the enzyme activities towards both substrates are identical. When the enzyme was incubated for 60 min at 4°C with DFMO (500 μM) and dialyzed against buffer C, about 97% of the decarboxylating activities towards both substrates was irreversibly lost (data not shown), indicating that DFMO as well as DFML is a irreversible inhibitor of *S. ruminantium* LDC. This is the first report on the existence of LDC with decarboxylase activities tawards L-lysine and L-ornithine. Table 3 shows a comparison of properties of the *S. ruminantium* LDC with that of ODCs from *E. coli* and *Lactobacillus* sp. 30a, representative of prokaryotic ODC, and that of eukaryotic ODCs, such as ODCs from mouse, *Trypanosoma brucei*, and *Neurospora crassa*, which have been extensively studied.[18-21] There are several similarities between the enzymes from *S. ruminantium* and three kinds of eukaryotes, including subunit structure, p*I*, cofactor requirement, inhibition by DFMO, and decarboxylase activity towards L-ornithine. In contrast, the molecular structure of the enzyme in *S. ruminantium* is markedly different from that in *Lactobacillus* sp. 30a which has been analyzed crystallographically.[22] The molecular weight of the native enzyme of the former is 88,000, and it consists of two identical subunits, whereas the latter has a dodecameric structure with a molecular weight of 1,040,000, consisting of 12 identical subunits. The ODC from *E. coli* and *Lactobacillus* sp. 30a were also found to have a strict substrate specificity for L-ornithine, with a very weak activity towards L-lysine. The *E. coli* ODC is not inhibited by DFMO.[16]

The entire amino acid sequence of *S. ruminantium* LDC was found to be 34% identical with that of ODCs from mammalian species (Takatsuka, Y. *et al.*, unpublished data). This prompted us to examine the occurrence of ODC which is specifically involved in decarboxylating only L-ornithine in *S. ruminantium*. While purifying the ODC preparation from *S. ruminantium* using L-ornithine as a substrate, the elution profile of ODC preparation in any chromatography was identical with that for LDC, and the purified ODC preparation

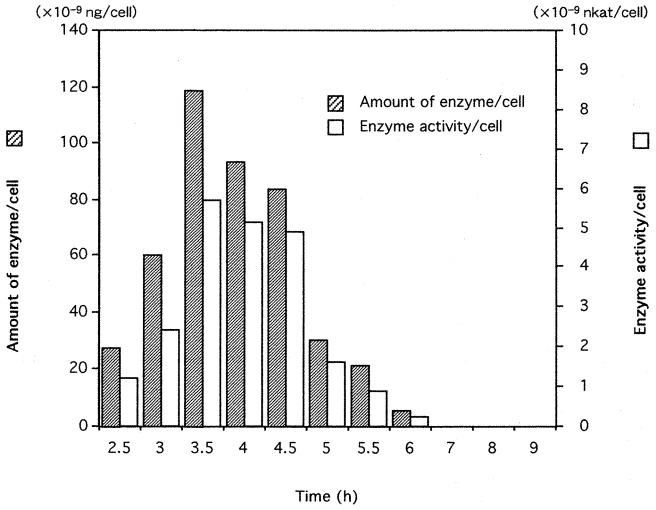

Fig. 3. Fluctuation in the Quantity of LDC during Growth of *S. ruminantium*.

To measure the amount of the LDC protein in the cells immunologically, *S. ruminantium* cells were grown as described in the legend of Fig. 1, and at the time indicated in the figure, 4 ml of the culture was taken up and centrifuged. The cells were suspended in 0.4 ml potassium phosphate buffer described in the legend to Fig. 1. The suspension (10 μl) was then treated with SDS (2%) in the presence of 2-mercaptoethanol (2%) at 100°C for 5 min and analyzed on 12.5% SDS-polyacrylamide gel. The proteins on the gel were transferred to nitrocellulose membrane (Hybond-C pure, Amersham), and cross-reacted with anti-LDC antibodies. Antigen-antibody complex was detected with anti-rabbit IgG (Fc)-alkaline phosphatase conjugate (Seikagaku Kogyo Co., Tokyo). The LDC band was developed with Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as the substrate.

decarboxylated L-lysine as well as L-ornithine and its activity was completely blocked by both DFML and DFMO with the K_m and K_i identical with that of the LDC preparation described above. Thus, we conclude that the ODC which is involved in decarboxylation of L-ornithine alone is lacking in *S. ruminantium* and that LDC acts in the biosynthesis of both cadaverine and putrescine from L-lysine and L-ornithine, respectively, in this organism.

Fluctuation of the amount of LDC during cell growth

The result from Fig. 1 suggests that the drastic vanishing of the LDC activity after 7 h of incubation of the cells might be due to a degradation of the LDC or the inactivation of LDC by an unknown inhibitor(s). To examine these possibilities, the amount of the LDC was measured using specific antibodies raised against an LDC preparation. As shown in Fig. 3, the accumulation of the enzyme protein in the cells proceeded up to 3.5 h and reached approximately 120×10^{-9} ng/cell. Thereafter, the amount of LDC in the cells decreased with a gradual slope within one hour, and a dramatic descending of the LDC was observed between 4.5 to 6 h of inoculation. After 7 h of inoculation, the LDC was not detected in the cells (Fig. 3). At 7 h after inoculation, mRNA for LDC still remained in 29% maximal amount at 4 h of inoculation (data not shown). The data indicate that the vanishing of the enzyme activity after 4.5 h of inoculation is due to the degradation of LDC in the growing cells. To detect the degradation products of LDC, total protein preparation from the cells through the growth was analyzed by SDS-PAGE and immunoblotting using the specific antibodies raised against *S. ruminantium* LDC. No significant protein band except for intact LDC could be detected by the Western immunoblotting (data not shown), suggesting that the LDC molecule is degraded into small peptide fragments. Recently, the antizyme-mediated ODC degradation mechanism in mammalian cells has been established.[23] According to the mechanism, active ODC, comprising two identical monomer subunits, is in rapid equilibrium with the inactive monomeric form. Antizyme, which is induced by the accumulation of putrescine in the cells preferentially, binds with an inactive ODC monomer to form an ODC-antizyme complex. This binding causes a conformational change of the ODC subunit allowing it to be attacked by protease, and the ODC is broken down by the protease to small peptides of 5–11 amino acid residues. Although the presence of an LDC-antizyme complex in *S. ruminantium* has not been demonstrated, the degradation mechanism of the mammalian ODC might be applicable to that in our *S. ruminantium* LDC, *i.e.* during entry into the stationary phase of *S. ruminantium* cells, the α-carboxyl group of the D-glutamic acid residue of the peptidoglycan has been saturated with cadaverine,[1,2] and an excess of free cadaverine were accumulated constitutively in the cells. The accumulation of free cadaverine in *S. ruminantium* might prompt the induction of the synthesis of a negative regulation factor like antizyme in the cells.

Acknowledgment

Y. Takatsuka is a recipient of a Research Fellowship (No. 1853) of the Japan Society for the Promotion of Science for Young Scientists.

References

1) Kamio, Y., Pösö, H., Terawaki, Y., and Paulin, L., Cadaverine covalently linked to a peptidoglycan is an essential constituent of the peptidoglycan necessary for the normal growth in *Selenomonas ruminantium*. *J. Biol. Chem.*, **261**, 6585–6589 (1986).
2) Kamio, Y. and Nakamura, K., Putrescine and cadaverine are constituents of peptidoglycan in *Veillonella alcalescens* and *Veillonella parvula*. *J. Bacteriol.*, **169**, 2881–2884 (1987).
3) Kamio, Y., Itoh, Y., and Terawaki, Y., Chemical structure of peptidoglycan in *Selenomonas ruminantium*: cadaverine links covalently to the D-glutamic acid residue of peptidoglycan. *J. Bacteriol.*, **146**, 49–53 (1981).
4) Kamio, Y., Structural specificity of diamines covalently linked to peptidoglycan for cell growth of *Veillonella alcalescens* and *Selenomonas ruminantium*. *J. Bacteriol.*, **169**, 4837–4840 (1987).
5) Kamio, Y. and Terawaki, Y., Purification and properties of *Selenomonas ruminantium* lysine decarboxylase. *J. Bacteriol.*, **153**, 658–664 (1983).
6) Kamio, Y., Terawaki, Y., and Izaki, K., Biosynthesis of cadaverine-containing peptidoglycan in *Selenomonas ruminantium*. *J. Biol. Chem.*, **257**, 3326–3333 (1982).
7) Pösö, H., McCann, P. P., Tanskanen, R., Bey, P., Sjoerdsma, A., Inhibition of growth of *Mycoplasma dispar* by DL-α-difluoromethyllsine, a selective irreversible inhibitor of lysine decarboxylase, and reversal by cadaverine (1,5-diaminopentane). *Biochem. Biophys. Res. Commun.*, **125**, 205–210 (1984).
8) Jänne, J. and Alhonen-Hongisto, L., Inhibitors of ornithine

decarboxylase: Biochemistry and applications. In "Ornithine decarboxylase: Biology, enzymology, and molecular genetics", eds. Hayashi, S., Pergamon Press, Inc., New York, pp. 59–85 (1989).
9) Kanegasaki, S. and Takahashi, H., Function of growth factors for rumen microorganisms. I. Nutritional characteristics of *Selenomonas ruminantium*. *J. Bacteriol.*, **93**, 456–463 (1967).
10) Kamio, Y., Itoh, Y., Terawaki, Y., and Kusano, T., Cadaverine is covalently linked to peptidoglycan in *Selenomonas ruminantium*. *J. Bacteriol.*, **145**, 122–128 (1981).
11) Phan, A. P. H., Ngo, T. T., and Lenhoff, H. M., Spectrophotomeric assay for lysine decarboxylase. *Anal. Biochem.*, **120**, 193–197 (1982).
12) Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254 (1976).
13) Albertson, N. F. and Archer, S., The use of ethyl acetamidomalonate in the synthesis of amino acid. The preparation of *dl*-histidine, *dl*-phenylalanine and *dl*-leucine. *J. Am. Chem. Soc.*, **67**, 308–310 (1945).
14) Kikuchi, Y., Kojima, H., Tanaka, T., Takatsuka, Y., and Kamio, Y., Characterization of a second lysine decarboxylase isolated from *Esherichia coli*. *J. Bacteriol.*, **179**, 4486–4492 (1997).
15) Pegg, A. E., Characteristics of ornithine decarboxylase from verious sources. In "Ornithine decarboxylase: Biology, enzymology, and molecular genetics", eds. Hayashi, S., Pergamon Press, Inc., New York, pp. 21–34 (1989).
16) Tabor, C. W. and Tabor, H., Ornithine decarboxylase in microorganisms. In "Ornithine decarboxylase: Biology, enzymology, and molecular genetics", eds. Hayashi, S., Pergamon Press, Inc., New York, pp. 97–106 (1989).
17) Guirard, B. M. and Snell, E. E., Purification and properties of ornithine decarboxylase from *Lactobacillus* sp. 30a. *J. Biol. Chem.*, **255**, 5960–5964 (1980).
18) Seely, J. E., Pösö, H., and Pegg, A. E., Purification of ornithine decarboxylase from kidneys of androgen-treated mice. *Biochemistry*, **21**, 3394–3399 (1982).
19) Phillips, M. A., Coffino, P., and Wang, C. C., *Trypanosoma brucei* ornithine decarboxylase: Enzyme purification, caracterization, and expression in *Escherichia coli*. *J. Biol. Chem.*, **263**, 17933–17941 (1988).
20) Osterman, A. L., Kinch, L. N., Grishin, N. V., and Phillips, M. A., Acidic residues important for substrate binding and cofactor reactivity in eukaryotic ornithine decarboxylase identified by alanine scanning mutagenesis. *J. Biol. Chem.*, **270**, 11797–11802 (1995).
21) DiGangi, J. J., Seyfzadeh, M., and Davis, R. H., Ornithine decarboxylase from *Neurospora crassa*. *J. Biol. Chem.*, **262**, 7889–7893 (1987).
22) Momany, C., Ernst, S., Ghosh, R., Chang, N. L., and Hackert, M. L., Crystallographic structure of a PLP-dependent ornithine decarboxylase from *Lactobacillus* 30a to 3.0 Å resolution. *J. Mol. Biol.*, **252**, 643–655 (1995).
23) Hayashi, S., Murakami, Y., and Matsufuji, S., Ornithine decarboxylase antizyme: a novel type of regulatory protein. *Trans Biochem. Sci.*, **21**, 27–30 (1996).