α-Aminoadipate aminotransferase from an extremely thermophilic bacterium, Thermus thermophilus

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INTRODUCTION

Lysine is one of the essential amino acids for higher animals including mammals. However, there are organisms that can synthesize lysine via either of two different lysine biosynthetic pathways: through diaminopimelate as found in plants and bacteria, or through α-aminoadipate (AAA) as found in fungi and yeast (Broquist, 1971; Rico et al., 1995; Vogel, 1964). Our previous studies revealed that the extremely thermophilic bacterium Thermus thermophilus HB27 synthesizes lysine through AAA, following a pathway similar to that in lower eukaryotes up to the step for AAA synthesis (Kobashi et al., 1999; Miyazaki et al., 2001, 2002, 2003; Nishida et al., 1999). However, the latter part of the newly found pathway is different from the pathway of fungi and yeast, which contains saccharopine as a biosynthetic intermediate (Cunin et al., 1986), but similar to the conversion of glutamate to ornithine in arginine biosynthesis (Nishida et al., 1999). We have previously cloned most of the genes for lysine biosynthesis from T. thermophilus HB27 and have provided further support for the idea that lysine biosynthesis through AAA has an evolutionary relationship with other amino acid biosyntheses and the TCA cycle (Miyazaki et al., 2001, 2002; Nishida et al., 1999); this has already been shown in part by Irvin & Bhattacharjee (1998).

In lower eukaryotes, the enzyme α-aminoadipate aminotransferase (AAAAT; EC 2.6.1.39), which catalyses the transamination reaction using 2-oxoadipate (2-OA) as an amino acceptor to yield AAA, is suggested to be involved in lysine biosynthesis. Matsuda & Ogur (1969) isolated and characterized glutamate:2-OA aminotransferases 1 and 2 from Saccharomyces cerevisiae and reported, based on the catalytic properties, that enzymes 2 and 1 were biosynthetic and catabolic enzymes, respectively, in lysine metabolism. However, to date, the corresponding genes have not been identified in S. cerevisiae or other organisms. To elucidate the whole pathway of lysine biosynthesis in T. thermophilus and the evolutionary relationships between lysine biosynthesis and other metabolic pathways, including leucine biosynthesis, arginine biosynthesis and the TCA cycle, we attempted to clone the gene from the micro-organism and analyse its function.

In this paper, we describe the cloning of a gene (lysN) encoding a mammalian kynurenine/AAAAT homologue, which is presumably involved in lysine biosynthesis in T. thermophilus HB27. We also report the kinetic properties of LysN, which shows AAAAT activity and branched-chain amino acid aminotransferase activity.

**Abbreviations:** AAA, α-aminoadipate; AAAAT, α-aminoadipate aminotransferase; BcAT, branched-chain amino acid aminotransferase; 2-OA, 2-oxoadipate; 2-OIC, 2-oxoisocaproate; 2-OIV, 2-oxoisovalerate; 2-OMV, 2-oxo-3-methylvalerate.

The GenBank accession number for the sequence reported in this paper is AB097117.
METHODS

Strains, media and chemicals. The extreme thermophile *T. thermophilus* HB27 was cultivated as described previously (Kobashi et al., 1999; Koyoma et al., 1986; Tanaka et al., 1981). *Escherichia coli* DH5α was used for DNA manipulation, and *E. coli* BL21-Codon-Plus (DE3)-RII. [F- *ompT* *hsdS* (rK Mg) *dcm* Tet* gal* *λ* (DE3) enda* Hte* (argU ilv Ile* *Wam Cml*)] (Strategene) was used as the host for gene expression. The medium 2 × YT (Sambrook et al., 2001), was generally used for cultivation of *E. coli* cells.

All the chemicals were purchased from Sigma or from Kanto Chemicals. NAD+–dependent glutamate dehydrogenase was purchased from Toyobo. Enzymes for DNA manipulation were purchased from Takara Shuzo. Oligonucleotide primers used in this study are listed in Table 1. DNA manipulation was performed according to the methods of Sambrook & Russell (2001).

Molecular cloning and sequencing. Based on amino acid sequence alignments among DR1588 (NP_295311), ST1411 (NP_147018) and PH0207 (NP_142204), all of which possess significant identity in amino acid sequence to Kat2, oligonucleotides AAA1 and AAA2 were designed and used as degenerate primers for PCR to clone a DNA fragment containing a Kat2 homologue of *T. thermophilus* HB27. An amplified 666 bp fragment was blunted, phosphorylated and ligated into the EcoRI site of pBluescript II KS(−) (Stratagene). The resulting plasmid was named pKSAAA101. Based on the information on the partial nucleotide sequence of the cloned fragment, oligonucleotides AT-N3 and AT-C3 were designed and used as primers for inverse PCR (Sambrook et al., 2001). For the inverse PCR, a BglII-digested genome fragment of approximately 4.5 kb, which was positive in Southern hybridization using the 666 bp fragment as the probe, was purified by agarose gel electrophoresis, self-ligated and used as the template. The amplified DNA fragment of approximately 41 kb was then sequenced to assign both translational start and stop codons and AT-C3 were designed and used as primers for inverse PCR with the oligonucleotides HTK-1 and HTK-2 as primers, and inserted into pUC18/ A3AT at the XbaI site to yield pUCATKmR. Disruption of the chromosomal copy of *lysN* was performed by using pUCATKmR as described previously (Hidaka et al., 1999; Koyoma et al., 1986), and confirmed by PCRs using the oligonucleotides A3AT-1/AAA3 and A3AT-1/A3AT-5 as primers.

Phenotypic analysis of *lysN* mutant. The *lysN* knockout mutant of *T. thermophilus* was cultured in 1 ml TM medium overnight. TM medium contains 0.8% polypeptone, 0.4% Bacto Yeast Extract, 0.2% NaCl, 0.4 mM CaCl2, and 0.4 mM MgCl2. After centrifugation of the culture at 20 000 g for 1 min, the precipitate was washed with minimal medium (MP medium; Kobashi et al., 1999; Tanaka et al., 1981) four times and resuspended in 1 ml MP medium. A 10 μl volume of the resuspended culture was added to 5 ml MP medium and incubated at 70 °C. Growth of the cells was analysed by monitoring the OD600 of the cell culture at appropriate intervals.

Purification of recombinant *LysN* from *E. coli*. *E. coli* BL21-Codon-Plus (DE3)-RII cells harbouring pETLysN7 were cultured in 800 ml of 2 × YT medium containing 50 μg kanamycin ml−1 and 30 μg chloramphenicol ml−1. When the culture had reached an OD600 of 0.5, IPTG (final concentration, 0.1 mM) was added. The culture was continued at 25 °C for an additional 12 h after the induction. *E. coli* pETLysN7 cells (wet weight, 4.5 g) collected from the 800 ml culture were suspended in 27 ml buffer I (20 mM potassium phosphate buffer, pH 6.5, 0.5 mM EDTA) and disrupted by sonication. The supernatant prepared by centrifugation at 40 000 g for 20 min was heated at 80 °C for 20 min, and denatured proteins from *E. coli* cells were removed by centrifugation at 40 000 g for the resulting plasmid carrying the correct sequence was named pETLysN7 and used for expression of the *lysN* gene. The nucleotide sequence was determined by the method of Sanger et al. (1977).

Disruption of *lysN* in *T. thermophilus* HB27. To introduce an XbaI site in the *lysN* gene, PCRs were performed using two pairs of oligonucleotides as primers, A3AT-1/A3AT-2 and A3AT-3/A3AT-4. After appropriate treatment with restriction endonucleases, the two amplified fragments were inserted into pUC18 to generate pUC18/ A3AT. The 1.1 kb DNA fragment encoding the thermostable kanamycin resistance gene (Hoseki et al., 1999) was amplified as the XbaI fragment by PCR, using the oligonucleotides HTK-1 and HTK-2 as primers, and inserted into pUC18/A3AT at the XbaI site to yield pUCATKmR. Disruption of the chromosomal copy of *lysN* was performed by using pUCATKmR as described previously (Hidaka et al., 1999; Koyoma et al., 1986), and confirmed by PCRs using the oligonucleotides A3AT-1/AAA3 and A3AT-1/A3AT-5 as primers.

Table 1. Primers

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA1</td>
<td>5′-AAAGGT(G/C)TTCTCT(G/C)GACGAG-3′</td>
</tr>
<tr>
<td>AAA2</td>
<td>5′-CTCCATCCA(G/C)AGGAACAT(G/C)CC-3′</td>
</tr>
<tr>
<td>AAA3</td>
<td>5′-CTCCATCCAGACGAGCAATGCCC-3′</td>
</tr>
<tr>
<td>AT-N3</td>
<td>5′-CGCCAGGCTTTTTCCAGTCAGACCAGCCGCGGGCACCCTGAGGAAGCG-3′</td>
</tr>
<tr>
<td>AT-C3</td>
<td>5′-GACGGGATACAAATTTTCAACAGAGGTGCTGCAACAGATGCTGGTTCGAGCAG-3′</td>
</tr>
<tr>
<td>A3AT-1</td>
<td>5′-CCCCCAATTACCAAGGGTCTTCCTGAGCAGGGG-3′</td>
</tr>
<tr>
<td>A3AT-2</td>
<td>5′-GAGGCTCGGGATGTCATAGACTCCCGGAAGTA-3′</td>
</tr>
<tr>
<td>A3AT-3</td>
<td>5′-TACCTGGGGAGGTCTAGATCCCGAGGCCTC-3′</td>
</tr>
<tr>
<td>A3AT-4</td>
<td>5′-CCACAGAGGAGCATCCGGCCCTTGGGCCCCTTG-3′</td>
</tr>
<tr>
<td>A3AT-5</td>
<td>5′-GCAATGGCCGGGCTTGGTAGGCGGA-3′</td>
</tr>
<tr>
<td>lysN-N</td>
<td>5′-GGGGTCTAGACATATGAAAGCGCTAAGCTG-3′</td>
</tr>
<tr>
<td>lysN-C</td>
<td>5′-GGGGCAATTCTTAGACCCAAGCGCAGAAGCC-3′</td>
</tr>
<tr>
<td>HTK1</td>
<td>5′-CCCTCTAGACGGTGAGCGGGAATGATGTA-3′</td>
</tr>
<tr>
<td>HTK2</td>
<td>5′-AAATCTAGACGTAACCAATGATTAACA-3′</td>
</tr>
</tbody>
</table>
20 min. Supernatant fractions were applied to an anion-exchange column (DE-52; Whatman), pre-equilibrated with buffer I and eluted with buffer I containing 0.1 M NaCl. After the addition of ammonium sulfate to a final concentration of 65% saturation to active fractions, the resultant precipitate was collected by centrifugation at 40,000 g for 30 min. The precipitated proteins were solubilized with buffer I and applied onto a Hi-load 26/60 Superdex 200 prep-grade column (Amersham-Pharmacia Biotech) equilibrated with buffer I containing 0.2 M NaCl to yield the purified preparation. Protein concentration was determined by the method of Bradford (1976) using a protein assay kit (Nippon Bio-Rad).

**RESULTS**

**Strategy for cloning the T. thermophilus gene encoding AAAAT**

We have previously reported that *T. thermophilus* synthesizes lysine through AAA. To determine the whole lysine biosynthetic pathway, we attempted to clone the gene that encodes the enzyme AAAAT, which catalyses the transamination reaction of 2-OA to produce AAA in lysine biosynthesis of *T. thermophilus*. However, a corresponding gene has not been identified even in fungi or yeast, although the lower eukaryotes are known to synthesize lysine through AAA. To determine the whole lysine biosynthesis through AAA carry a gene encoding a protein that shows considerable identity to Kat2 in amino acid sequence: YGL202W (32%) from *S. cerevisiae*, DR1588 (31%) from *Deinococcus radiodurans*, PH0207 (31%) from *Pyrococcus horikoshii*, ST1411 (32%) from *Sulfolobus tokodaii* and APE0169 (30%) from *Aeropyrum pernix*. We therefore decided to clone a *Kat2* homologue from *T. thermophilus*. Determination of the nucleotide sequence of the cloned full-length *Kat2* homologue revealed that the gene encodes a protein composed of 397 amino acid residues with a predicted molecular mass of 43,845 Da. The *Kat2* homologue showed significant identity in amino acid sequence to DR1588 (44%), PH0207 (35%), ST1411 (37%) and APE0169 (30%).

![Fig. 1. Growth curves of the wild-type T. thermophilus HB27 (○) and the *lysN*-deficient mutant (△) in a minimal (MP) medium, and of the *lysN*-deficient mutant in minimal MP medium supplemented with 0.1 mM 2-OA (■), AAA (●) or lysine (○).](image)

**Disruption of Kat2 homologue in T. thermophilus**

We next investigated the role of the *Kat2* homologue in *T. thermophilus*. For this purpose, we constructed a mutant of *T. thermophilus* with disruption in the chromosomal *Kat2* homologue as described in Methods. The *Kat2*-homologue disruptant, TM1001, required longer lag time for growth in the shift-down experiments from rich medium to minimal MP medium: the lag time was about 20 h for the mutant and 10 h for the wild-type (Fig. 1). The extended lag phase was significantly reversed by addition of 0.1 mM lysine or AAA, but not affected by addition of 0.1 mM 2-OA. Furthermore, the growth rate of the mutant (doubling time, 190 min) was found to be lower than that of the wild-type strain (84 min) in minimal medium. The doubling
Expression and purification of LysN

To characterize the lysN gene product, we expressed the gene in E. coli and obtained a purified preparation of LysN by using a T7 promoter expression system. LysN was accumulated as insoluble inclusion bodies when the E. coli cells carrying pETLysN7 were cultured in the presence of 1 mM IPTG at 37°C. Hereafter, we refer to this gene as lysN.

Catalytic properties of LysN

For an initial evaluation of the substrate specificity of LysN, we determined specific activities for several compounds in the crude extract prepared by heat treatment of the E. coli cell lysate and successive centrifugation steps. As expected, T. thermophilus LysN showed transamination of 2-OA to AAA with a specific activity of 6788 U (mg protein)^−1 (Table 2). When similar analysis was done in the reverse reaction at the same pH with AAA and 2-oxoglutarate as the amino donor and the amino acceptor, respectively, only negligible activity was detected. From this analysis, we concluded that LysN was a biosynthetic enzyme for lysine biosynthesis. We next analysed the transamination reaction with several 2-oxoacids as the amino acceptor by using glutamate as an amino donor; we found that LysN recognized all these compounds as a substrate.

Kinetic parameters of LysN

Since, as shown above, LysN recognized branched 2-oxoacids as well as 2-OA as a substrate, we performed kinetic analysis of the purified enzyme using 2-OA, 2-OIC, 2-OIV or 2-OMV, which are intermediates of lysine, leucine, valine or isoleucine biosynthesis, respectively, as an amino acceptor, and glutamate as an amino donor. Double reciprocal plots indicated that the reaction catalysed by LysN proceeded through a ping-pong bi-bi mechanism (data not shown), similar to results obtained with other aminotransferases. Kinetic parameters indicate that although LysN recognizes 2-OA most efficiently, the enzyme can also utilize other compounds involved in biosynthesis of branched-chain amino acids with comparable efficiency (Table 3).

DISCUSSION

The gene encoding the fourth enzyme in the lysine biosynthesis pathway, which catalyses the conversion of 2-OA to AAA, has not previously been identified in any organism

Table 2. Specific activities of LysN for several 2-oxoacids

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Metabolism</th>
<th>Specific activity [U (mg protein)^−1]^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-OA (1 mM)</td>
<td>Lysine</td>
<td>6788 ± 120</td>
</tr>
<tr>
<td>2-OIC (1 mM)</td>
<td>Leucine</td>
<td>5079 ± 305</td>
</tr>
<tr>
<td>2-OIV (1 mM)</td>
<td>Valine</td>
<td>1102 ± 81</td>
</tr>
<tr>
<td>2-OMV (10 mM)</td>
<td>Isoleucine</td>
<td>561 ± 64</td>
</tr>
<tr>
<td>Pyruvate (10 mM)</td>
<td>Alanine</td>
<td>568 ± 32</td>
</tr>
<tr>
<td>Oxoacetate (10 mM)</td>
<td>Aspartate</td>
<td>661 ± 50</td>
</tr>
<tr>
<td>Phenylpyruvate (1 mM)</td>
<td>Phenylalanine</td>
<td>513 ± 119</td>
</tr>
</tbody>
</table>

*Heat-treated lysate of E. coli without the recombinant plasmid had no activity.

Table 3. Kinetic parameters of LysN

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Biosynthesis</th>
<th>K_M (µM)</th>
<th>k_cat (s^-1)</th>
<th>k_cat/K_M (s^-1 M^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-OA</td>
<td>Lysine</td>
<td>24±2 ± 1±3</td>
<td>22±3 ± 0±4</td>
<td>(9±2±0±5) × 10^3</td>
</tr>
<tr>
<td>2-OIC</td>
<td>Leucine</td>
<td>28±8 ± 3±4</td>
<td>17±8 ± 0±6</td>
<td>(6±2±0±8) × 10^3</td>
</tr>
<tr>
<td>2-OIV</td>
<td>Valine</td>
<td>13±3 ± 3±6</td>
<td>2±1 ± 0±1</td>
<td>(1±6±0±4) × 10^3</td>
</tr>
<tr>
<td>2-OMV</td>
<td>Isoleucine</td>
<td>148±3 ± 15±4</td>
<td>3±5 ± 0±1</td>
<td>(2±4±0±3) × 10^4</td>
</tr>
</tbody>
</table>
known to synthesize lysine through AAA. In this study, we cloned the Kat2 homologue, lysN, and the gene product possessed AAAAT activity. Unexpectedly, the lysN mutant of T. thermophilus, TM1001, did not show a lysine-auxotrophic phenotype. However, the mutant required a longer lag time and showed slower growth in minimal MP medium, which was significantly reversed by exogenous addition of either 0.1 mM lysine or AAA. These results indicate that LysN plays a primary role in the conversion of 2-OA to AAA, which is the fourth reaction in lysine biosynthesis by T. thermophilus. When the evolutionary relationship of LysN to other aminotransferases was phylogenetically analysed, LysN was found to be outgrouped with aspartate aminotransferases but grouped with putative aminotransferases from organisms that have been suggested to synthesize lysine through the AAA pathway (Fig. 2). Therefore, this phylogenetic analysis suggests that all the putative aminotransferases related to LysN are involved in lysine biosynthesis in these micro-organisms.

Our previous studies suggested that T. thermophilus lysine biosynthesis has evolutionary relationships with leucine biosynthesis, arginine biosynthesis and the TCA cycle (Miyazaki et al., 2003; Nishida et al., 1999). The fourth reaction in the lysine biosynthesis pathway, the conversion of 2-OA to AAA, is thought to proceed in a way similar to that of the related reaction catalysed by branched-chain amino acid aminotransferase (BcAT) in leucine, valine and isoleucine biosyntheses, which are the conversion of 2-OIC to leucine, 2-OIV to valine and 2-OMV to isoleucine, respectively (Fig. 3). The fourth reaction in the lysine biosynthesis pathway is also similar to this reaction, i.e. the conversion of 2-oxoglutarate to glutamate catalysed by aspartate aminotransferase, which connects the TCA cycle and arginine biosynthesis. As shown in Tables 2 and 3, LysN exhibits activity for branched-chain amino acids and aspartate. Therefore, these results support our previous assumption that lysine biosynthesis has functional and evolutionary relationships with branched-chain amino acid biosynthesis, arginine biosynthesis and the TCA cycle.

The fact that lysN-knockout did not confer the complete lysine (AAA)-auxotrophic phenotype suggests the presence of another enzyme(s) capable of compensating for lysN deficiency of the mutant. BcAT may be a possible candidate to compensate the loss of LysN because BcAT is known to have broad substrate specificity (Hall et al., 1993; Yvon et al., 2000). Alternatively, an aromatic aminotransferase may be involved in the process. YGL202W, a Kat2 homologue in S. cerevisiae, has another name, ARO8. ARO8 encodes aromatic aminotransferase I involved in the last step of the reaction in phenylalanine and tyrosine biosyntheses, which transaminates amino groups of glutamate to phenylpyruvate and p-hydroxyphenylpyruvate. Interestingly, Aro8p was found to have the ability to convert 2-OA to AAA (Urrestarazu et al., 1998). In that study, the authors reported that the action of the aromatic aminotransferase I accounted for half of the glutamate : 2-OA aminotransferase activity in a cell-free extract of S. cerevisiae when the aminotransferase activity in the wild-type and an

![Fig. 2. Phylogenetic tree of LysN and related aminotransferases, constructed by using neighbour-joining methods. The amino acid sequences were aligned by using CLUSTAL W (Thompson et al., 1994). Using the aligned data, a phylogenetic tree was constructed by the computer program MEGA (Kumer et al., 2001). Numbers on selected nodes indicate bootstrap values. The figure includes AAAAT and aspartate aminotransferases (AspAT) from T. thermophilus HB27, D. radiodurans (NP_295311, NP_294346), P. horikoshii (NP_142204, NP_143210), S. tokodaii (NP_377369, NP_377158), A. pernix (NP_147018, NP_148487), Rattus norvegicus (NP_058889), S. cerevisiae (NP_011313, NP_012816) and E. coli (NP_415448). AspAT, aspartate aminotransferase.](http://mic.sgmjournals.org)
The addition of AAA or lysine to the minimal MP medium indeed shortened lag phase for growth of the lysN-knockout mutant and improved its growth rate, but it did not completely reverse the extended lag and the slow growth. This observation may suggest some other roles of LysN that are required for the normal growth of the cells as suggested in the case of Aro8p (Urrestarazu et al., 1998; Zabriskie & Jackson, 2000). We would like to note that the expression of the lysN gene is not regulated by lysine in T. thermophilus cells (data not shown), which is in contrast to many other genes involved in amino acid biosynthesis. This might be evidence, though indirect, suggesting the possible involvement of LysN in several unrelated metabolic pathways. It is noteworthy that LysN recognizes 2-OIC more efficiently than oxaloacetate, as is the case for BcAT, which is involved in biosynthesis of branched-chain amino acids, although no significant identity in amino acid sequence has been found between LysN and BcAT (data not shown). Thus, since LysN has aminotransferase activity not only for AAA, but also for the branched-chain amino acids, there is the possibility that LysN might be involved at least in both lysine and branched-chain amino acid biosyntheses. There is a unique aminotransferase group, COG1167, in which a GntR-type DNA-binding HTH helix domain is fused at the N-terminus of type I aminotransferase. T. thermophilus AAAAT also belongs to the COG1167 group, but it lacks the N-terminal GntR-type HTH domain as found in normal aminotransferases. Therefore, T. thermophilus LysN does not function as a transcription regulator. However, it should be noted that the corresponding protein of Corynebacterium glutamicum, PdxR (cg0897), is involved in valine biosynthesis as well as pyridoxine biosynthesis (McHardy et al., 2003), which suggests a functional relationship between LysN and the MocR-type aminotransferase in that both the proteins play a putative role in branched-chain amino acid biosynthesis. In any case, such broad substrate specificity is a typical feature found in the enzymes involved in other reactions in lysine biosynthesis (Miyazaki et al., 2001, 2002, 2003; Wulandari et al., 2002). It should be noted that addition of branched-chain amino acids, isoleucine, leucine and valine, remarkably accelerated the growth of T. thermophilus cells in minimal medium and no difference was detected in growth between the wild-type and the lysN mutant (data not shown), suggesting that the amino group of the branched-chain amino acid might be, directly or indirectly, transferred to 2-OA to form AAA. Considering the similarity in amino acid sequence between LysN and YGL202W (ARO8), it might be possible to consider that LysN is involved in aromatic amino acid biosynthesis; however, this is unlikely because the activity of LysN for phenylpyruvate was quite low (Table 2) and addition of both phenylalanine and tyrosine had no effect on the growth of the T. thermophilus mutant lacking the lysN gene in the minimal medium (data not shown). To further elucidate the role of LysN in Thermus cells, detailed analysis of the nutrition required for the normal growth of the mutant will be necessary.

On the other hand, the Pyrococcus furiosus aminotransferase, PF0121, a possible orthologue of LysN and PH0207 of P. horikoshii, has been characterized biochemically and referred to as aromatic amino acid aminotransferase (Andreotti et al., 1995; Schut et al., 2003). Expression of PF0121 was upregulated in peptide-grown cells, suggesting its role in catabolism in the archaeon (Schut et al., 2003). On the other hand, we observed that the catabolic activity of LysN to convert AAA to 2-OA was negligible and addition of lysine had no effect on the expression profile of lysN in T. thermophilus (data not shown). These results could
suggest that LysN plays a role that is different from that of PF0121 in *P. furiosus*. Nevertheless, it is of interest to analyse the substrate specificity of PF0121 for these amino acids.

In this study, we have shown that LysN catalyses the fourth reaction of lysine biosynthesis through the AAA pathway. Detailed analysis of the mechanism used by LysN to recognize several compounds as substrates may provide a clue to elucidate the evolution of aminotransferases. For this purpose, it is necessary to determine the three-dimensional structure of LysN and the complexes it forms with substrate analogues.

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