X-ray Crystallographic Analysis of 6-Aminohexanoate-Dimer Hydrolase

MOLECULAR BASIS FOR THE BIRTH OF A NYLON OLIGOMER-DEGRADING ENZYME

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6-Aminohexanoate-dimer hydrolase (EII), responsible for the degradation of nylon-6 industry by-products, and its analogous enzyme (EII’) that has only ~0.5% of the specific activity toward the 6-aminohexanoate-linear dimer, are encoded on plasmid pOAD2 of Arthrobacter sp. (formerly Flavobacterium sp.) KI72. Here, we report the three-dimensional structure of Hyb-24 (a hybrid between the EII and EII’ proteins; EII’-level activity) by x-ray crystallography at 1.8 Å resolution and refined to an R-factor and R-free of 18.5 and 20.3%, respectively. The fold adopted by the 392-amino acid polypeptide generated a two-domain structure that is similar to the folds of the penicillin-recognizing family of serine-reactive hydrolases, especially to those of D-alanyl-D-alanine-carboxypeptidase from Streptomyces and carboxylesterase from Burkholderia. Enzyme assay using purified enzymes revealed that EII and Hyb-24 possess hydrolytic activity for carboxyl esters with short acyl chains but no detectable activity for D-alanyl-D-alanine. In addition, on the basis of the spatial location and role of amino acid residues constituting the active sites of the nylon oligomer hydrolase, carboxylesterase, D-alanyl-D-alanine-peptidase, and β-lactamases, we conclude that the nylon oligomer hydrolase utilizes nucleophilic Ser112 as a common active site both for nylon oligomer-hydrolytic and esterolytic activities. However, it requires at least two additional amino acid residues (Asp181 and Asn266) specific for nylon oligomer-hydrolytic activity. Here, we propose that amino acid replacements in the catalytic cleft of a preexisting esterase with the β-lactamase fold resulted in the evolution of the nylon oligomer hydrolase.

Microorganisms are believed to be highly adaptable toward environmental conditions. This can be elucidated from the observations that microorganisms capable of degrading unnatural synthetic compounds can be isolated relatively easily. Unnatural synthetic compounds include various chemicals such as endocrine disrupters and toxic compounds, which have unfavorable effects on living cells. A suitable system to enhance the biodegradability of these compounds is important from an environmental point of view. We have been studying the degradation of a by-product of nylon-6 manufacture (i.e. 6-aminohexanoate oligomers (namely nylon oligomers)) (1), by Flavobacterium sp. KI72 as a model for studying the adaptation of microorganisms toward unnatural compounds (1, 2). Three enzymes, 6-aminohexanoate-cyclic dimer hydrolase (3), 6-aminohexanoate-dimer hydrolase (EII) (4), and endo-type 6-aminohexanoate-oligomer hydrolase (5), encoded on the plasmid pOAD2 (45,519 bp) (6) in strain KI72, were found to be responsible for the degradation of the nylon oligomers. It was also established that the EII-analogous protein (EII’) is located on a different part of the pOAD2 (7, 8). EII’ has 88% homology to EII (7) but has very low catalytic activity (2% of EII activity) toward the 6-aminohexanoate-linear dimer (Ald), suggesting that EII has evolved by gene duplication followed by base substitutions from its ancestral gene (8).

Enzyme assay using the purified enzyme revealed that EII is active on 6-aminohexanoate-linear oligomers from the dimer to theicosamer. It is more active on 6-aminohexanoyl-8-aminooctanoate (Ahx-Aoc) and 6-aminohexanoyl-aniline (Ahx-Ani) than Ald but is barely active on 4-aminobutyryl-6-aminooxanoate-Ahx or 8-aminooctanoyl-6-aminooxanoate (Aoc-Ahx) (1, 9). In addition, this enzyme has no detectable activity on the 6-aminohexanoate-cyclic dimer (substrate for 6-aminohexanoate-cyclic dimer hydrolase enzyme) (1, 3), 6-aminohexanoate-oligomer hydrolases (degree of polymerization >3, substrates for the endo-type 6-aminohexanoate-oligomer hydrolase enzyme) (1, 5), or more than 60 kinds of various peptides tested such as i-aminois-l-aminole (1, 4). Thus, the EII enzyme specifically recognizes amide compounds containing 6-aminohexanoate as the N-terminal residue in the substrate, but the recognition of the C-terminal residue in the substrate is not stringent.

Knowledge of the three-dimensional structures of the EII and EII’ enzymes allows us to study the catalytic mechanism and the evolution of these enzymes in comparison with proteins having the analogous three-dimensional structures. The EII enzyme was purified to homogeneity on SDS-PAGE. However, according to the light scattering-diffraction pattern, the three-dimensional structure of the enzyme was still heterogeneous, and the purified enzyme gave no crystal formation under any conditions.

2 Strain KI72 was previously identified as Flavobacterium sp., since the strain is judged to be Gram-negative by the ordinary Gram staining method and produces a yellow pigment typical of Flavobacterium sp. However, we reinvestigated the phylogenetic relationship of strain KI72 on the basis of the sequences of 16 S rDNA and concluded that the strain should be classified as Arthrobacter sp. (K. Yasuhira, A. Ohara, I. Kawamoto, M. Takeo, and S. Negoro, unpublished results).

3 The abbreviations used are: EII, 6-aminohexanoate-dimer hydrolase; EII’, a protein with 88% homology to EII encoded on plasmid pOAD2; Ald, 6-aminohexanoate-linear dimer; Ahx, 6-aminohexanoate; Hyb-24, an EII/EII’ hybrid protein; Aoc, 8-aminooctanoate; Ani, aniline; MES, 4-morpholineethanesulfonic acid; DD, d-Ala-d-Ala; EstB, carboxylesterase from Burkholderia: r.m.s., root mean square; PBP, penicillin-binding protein.
Three-dimensional Structure of Nylon Oligomer Hydrolase

experimental procedures

Construction of a Hybrid Plasmid Expressing the Hyb-24 Protein

To construct plasmid pHY3, which expresses high levels of the EII’-type protein (Hyb-24), the 1,344-bp DNA fragment containing the nylB gene was initially amplified by PCR using *Ex Taq* DNA polymerase (Takara Co.). Two primers, NYL3 (5’-GCCGAGGCCCAGGCTACTCGATCTC-3’) and NYL2 (5’-CCACCGCTGTAGCGACGTGCAGGATCCA-3’), which annealed to 81 bp upstream and 31 bp downstream of the *nylB* gene, respectively, were used for the PCR. The amplified fragment was ligated with pT7Blue-T-Vector (Novagen), and plasmid pTA1 was obtained. The 1,441-bp EcoRI-HindIII fragment obtained from pTA1 was ligated with pKP1500 (10), which had been digested with EcoRI and HindIII, and plasmid pT7Blue was obtained. To fuse the His-tagged region to the N terminus of the EII’ gene, respectively, were used for the PCR. The plasmid fragment was ligated with pT7Blue-T-Vector (Novagen), and plasmid pTA1 was obtained. The 1,441-bp EcoRI-HindIII fragment obtained from pTA1 was ligated with pKP1500 (10), which had been digested with EcoRI and HindIII, and plasmid pT7Blue was obtained. To fuse the His-tagged region to the N terminus of the EII’ gene from the expression vector pQE-80L (Qiagen). The His-tagged enzymes were purified by conventional methods.

Enzyme Assay

To measure the Ald-hydrolytic activity, enzyme reactions were carried out in 20 mM phosphate buffer (pH 7.0) containing 10 mM Ald at 30 °C, and the reaction product, Alx, was analyzed by reverse phase high pressure liquid chromatography (8). To measure the esterolytic activity, enzyme reactions were carried out in 20 mM phosphate buffer (pH 6.5) containing 10 mM tributyrin. The reaction product was analyzed by reverse phase HPLC. To measure the hydrolytic activity of the purified enzymes, enzyme reactions were carried out in 20 mM phosphate buffer (pH 6.5) containing 10 mM tributyrin. The reaction product was analyzed by reverse phase HPLC.
FIGURE 2. Multiple three-dimensional alignment of Hyb-24, DD-peptidase (DDA; Protein Data Bank code 3PTE) from Streptomyces sp. (18), EstB carboxylesterase (Protein Data Bank code 1CIB) from Burkholderia gladioli (20), class C β-lactamases (Protein Data Bank code 1GCE) from Enterobacter cloacae (33), and class A β-lactamase (Protein Data Bank code 1BTL) from E. coli (26). Helices and β-strands of Hyb-24 shown in Fig. 3A are illustrated at the top with green cylinders (α-helix), green shaded cylinders (310-helix), and orange arrows (β-strands). Multiple three-dimensional alignment was carried out using the method of secondary structure matching (48), and the secondary structures are shown.
activity, reactions were carried out in 50 mM phosphate buffer (pH 7.0) containing 0.2 mM p-nitrophenylacetate and p-nitrophenylbutyrate, and release of p-nitrophenol was monitored by absorbance at 400 nm (ε = 6,710 M⁻¹ cm⁻¹).

Enzyme activity for Ald and d-alanyl-d-alanine (D-Ala-D-Ala) were measured qualitatively by TLC. 75 μl of the purified enzyme (EII, 0.1 and 1.5 mg/ml; Hyb-24, 1.5 mg/ml) was mixed with an equal volume of 20 mM Ald or 20 mM D-Ala-D-Ala. After the reactions were carried out at 30 °C, 25-μl aliquots were sequentially sampled, and the reactions were stopped by heating in boiling water for 3 min. Then the reaction mixtures (1 μl) were spotted onto a silica gel plate. The samples were developed by solvent mixture (1-propanol/water/ethyl acetate/ammonia = 24:12:4:1.3), and then degradation products were detected by spraying with 0.2% ninhydrin solution (in butanol saturated with water).

**SDS-PAGE and Nucleotide Sequencing**

The concentrations of samples were adjusted to A₅₉₀ = 10, 20 μl of the sample was applied to SDS-polyacrylamide gel, and electrophoresis was carried out by conventional methods (16). Nucleotide sequences were determined by the dideoxy method (16) using a Thermo Sequenase fluorescent labeled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Biosciences).

**RESULTS AND DISCUSSION**

**Overall Structure of Hyb-24 Protein**

The Hyb-24 enzyme consists of a single polypeptide chain of 392 amino acid residues (Fig. 2) (6, 8). The overall structure of the molecule is divided into two domains, the α and α/β domains (Fig. 3A). The α domain contains eleven helices (H1, H2, H5–H13) and three antiparallel β-strands (β₁, β₃, and β₅). The central helix (H5) is surrounded by six helices (H8 and H11–H15). The α/β domain consists of a central eight-stranded antiparallel β-sheet (β₁–β₆ and β₈–β₁₃) flanked by one long carboxyl-terminal helix (H18) and three other helices (H3, H4, and H16) on one face and two helices (H14 and H15) on the opposite face. In the electron density map, 415 water molecules were assigned and included in the final refined model.

**Structural and Functional Comparison with Other Proteins in the Protein Data Bank**

A homology search based on the Hyb-24 structure was carried out using the DALI program (17). Although the sequence identity between Hyb-24 and the proteins in the penicillin-recognizing family of serine-reactive hydrolases is low, ranging from 10 to 19% (Fig. 2 and TABLE ONE), their overall structures were very similar (Fig. 3). DD-peptidase (18, 19) and carboxylesterase (EstB) (20) possessed the highest and second highest Z-scores, respectively. In addition, class A β-lactamases (21–30) with relatively high Z-scores have been thoroughly studied in this family. Accordingly, the following experiments and discussions focus mainly on a comparison between the nylon oligomer hydrolases (EII, Hyb-24), DD-peptidase, carboxylesterase, and class A β-lactamase.

**Comparison of Overall Structures**

**DD-peptidase**—The structurally superimposable regions between Hyb-24 and DD-peptidase comprise 277 amino acid residues, and the r.m.s. deviations of the superimposed Cα atoms was calculated to be 2.8 Å. Major structural differences observed in Hyb-24 are as follows (Fig. 3B). (i) The 61 residues from the N terminus, including H1, H2, and β₁, are present in Hyb-24. (ii) Helix H9 including Gly₁₈₁ in Hyb-24 is divided into two helices (H6 and H7) in DD-peptidase. DD-peptidase has no corresponding residues at the position of Gly¹₈¹ of Hyb-24, although it is located in the vicinity of the H7 of DD-peptidase. As described below, however, replacement of Gly¹₈¹ in Hyb-24 by Asp highly increases the nylon oligomer-degrading activity, suggesting that Asp¹₈¹ can be a substrate binding site in the nylon oligomer-degrading enzyme (Fig. 2) (15, 31). (iii) The regions, including β₆–β₁₁–H₁₁ in DD-peptidase, are absent in Hyb-24.

**Class A β-lactamase**—Comparison between the amino acid sequences revealed only 11% of strict identity between the Hyb-24 enzyme and class A β-lactamases (TABLE ONE). Although the total number of amino acids and number of helices and β-strands are quite different between the Hyb-24 and class A β-lactamase, their three-dimensional structures are similar (Fig. 3D). The structurally superimposable regions comprise 201 amino acids, and the r.m.s. deviation of the superimposed Cα atoms was 3.3 Å. Major differences observed in Hyb-24 are summarized as follows (Fig. 3D): (i) the presence of N-terminal 65 residues including H1, H2, β₁, and a part of H3; (ii) the presence of a large insertion including H9–H10, including Gly³¹⁸¹; (iii) insertion of 52 residues between H9–H10 between positions 129 and 130 in β-lactamase; (iv) the absence of the Ω loop, which contains the class A β-lactamase-specific “Glu-X-Glu-Leu-Asn” motif; (v) alteration of the sequence in the “KTG box” (Lys-Ser/Thr-Gly) located at β₁ in β-lactamase to Gly-Ile-Gly.

Other proteins in the penicillin-recognizing family of serine-reactive hydrolases exhibited similar folds with some variations in the width of the central β-sheet, class C β-lactamase (nine strands) (33–37) and class D β-lactamase (seven strands) (38, 39), although detailed comparisons were not carried out.

**Comparison of Substrate Specificity**

To examine the activity of the EII and Hyb-24 proteins on substrates recognized by penicillin-recognizing enzymes, we assayed the activity on d-Ala-D-Ala (DD-peptidase activity) (Fig. 4), and p-nitrophenylacetate (C2-ester) and p-nitrophenylbutyrate (C4-ester) (carboxylesterase activity) (TABLE TWO). TLC analyses demonstrated that no product (D-Ala) was obtained from D-Ala-D-Ala, even after continuing the reaction; therefore, reactions were carried out in 50 mM phosphate buffer (pH 7.0) containing 0.2 mM p-nitrophenylacetate and p-nitrophenylbutyrate, and release of p-nitrophenol was monitored by absorbance at 400 nm (ε = 6,710 M⁻¹ cm⁻¹).
tion for 48 h using 0.75 mg/ml EII or Hyb-24 enzymes. Product (Ahx) was detected from Ald after only a 15-min reaction time using the wild-type EII enzyme (0.05 mg/ml). This suggests that the DD-peptidase activity in EII is less than 0.05% of the Ald-hydrolytic activity. Moreover, enzyme assay using purified EII demonstrated that the enzyme has no significant level of β-lactamase activity toward ampicillin or cephalothin (data not shown).

In contrast, the EII and Hyb-24 enzymes possessed hydrolytic activity toward the C2-ester (6.4 μmol/min (unit)/mg of protein (EII); 4.8 units/mg of protein (Hyb-24)) and lower activity toward the C4-ester (22–54% of the values toward the C2-ester) (TABLE TWO). Moreover, E. coli clones harboring the EII gene (nylB) or Hyb-24 gene (nylB24) produced clear halos on the LB-Tributyrin plate, suggesting that these enzymes hydrolyze the ester linkages in the glyceryl tributyrate (data not shown). Since EstB esterase hydrolyzes C4–C6 fatty acid esters (20), we have concluded that the EII and Hyb-24 enzymes are structurally and functionally related to EstB esterase.
Active Site Amino Acid Residues

From the three-dimensional structure and functional analyses of the Hyb-24 protein, several amino acid residues in the cleft between the α and α/β domains were identified as the active sites.

Ser<sup>12</sup>

The wild-type EII polypeptide includes 26 Ser residues. Previously, we found that EII activity was inhibited by the specific binding of diisopropylfluorophosphate to Ser<sup>12</sup> (40). Moreover, site-directed
mutagenesis of Ser112 to Ala caused a drastic decrease in the enzyme activity to undetectable levels (40), suggesting the possible involvement of Ser112 in the catalysis. Moreover, the sequence motif common to the EII and EII/H11032 proteins (i.e., Ser112-Val-Ser-Lys115, is located at the beginning of the 5 helix. This motif is located at the structurally equivalent position in the penicillin-recognizing family of serine-reactive hydro-lases (Fig. 5). From these results, we concluded that Ser112 acts as a nucleophile in the catalysis.

Tyr215 and Lys115

The hydroxyl of the Ser112 probably forms an acyl intermediate with the substrate. The question is which residue acts as a general base. For class A β-lactamases, Lys73 in the “Ser-X-X-Lys motif” (24) or Glu166 in the Ω-loop (21, 23, 25, 28, 29) are candidates. To function as a general base, the pKₐ of Lys73 should be decreased. However, two estimates of the pKₐ values of Lys73 (i.e., pKₐ = 8.0 – 8.5 (24) and pKₐ > 10 (22)) lead to controversial conclusions that support or cast doubt on this hypo-

esis, respectively. In contrast, DD-peptidase, class C β-lactamase, and EstB esterase have no counterpart to Glu166 in the class A β-lactamase (Fig. 5). It is proposed that Tyr159 (DD-peptidase) (18), Tyr150 (class C β-lactamase) (37), and Tyr181 (EstB carboxylesterase) (20) are general bases and that Lys65 (DD-peptidase), Lys67 (class C β-lactamase), and Lys73 (EstB esterase) supply positive charges to stabilize the oxyanion (18, 20, 37). However, from the results of 13C NMR spectroscopy using [13C]Tyr-labeled class C β-lactamase of Citrobacter freundii is above 11, suggesting that Tyr150 does not directly participate in the activation of Ser64 as a general base (35). This suggests that positively charged Lys67 and Lys215 in the “KTG box” are involved in the reduction of the pKₐ of Tyr150 (35).

In Hyb-24, Nₛ of Lys115 and phenolic oxygen of Tyr215 are located 2.51 and 2.87 Å apart from the Oₛ of Ser112, respectively (Fig. 5A and C). These spatial locations are similar to those of DD-peptidase (Lys65/Tyr159) (Fig. 5B) (18), EstB carboxylesterase (Lys218/Tyr181) (Fig. 5C) (20),
and class C β-lactamase (Lys<sup>67</sup>/Tyr<sup>125</sup>) (33). In addition, Lys<sup>115</sup> in “KTG box” of class C β-lactamase was not conserved in Hyb-24 (Fig. 2).

From these considerations, Lys<sup>115</sup> and Tyr<sup>215</sup> are probably involved in maintaining the optimum electrostatic environment for the efficient catalytic activity in such a way that either one of these two residues functions as a general base or that both share the roles of promoting the acylation of Ser<sup>112</sup>.

Asp<sup>181</sup> and Asn<sup>266</sup>

We have found that of the 46 amino acid alterations that differed between the EII and EII’ proteins, two amino acid replacements in the EII’ protein (i.e. Gly to Asp (EII-type) at position 181 (G181D) and His to Asn (EII-type) at position 266 (H266N)) are sufficient to increase the Ald-hydrolytic activity back to the level of the parental EII enzyme. The other 44 amino acid alterations have no significant effect on the increase of the activity (41). Moreover, we confirmed that a single alteration in Hyb-24 from Gly<sup>181</sup> located at H9 to Asp increased the Ald-hydrolytic activity 11 times.<sup>4</sup> Therefore, it can be postulated that Asp<sup>181</sup> has a similar role to Glu<sup>166</sup> in class A β-lactamase (TEM-1). This structural alteration is apparently similar to that between the class A β-lactamase and penicillin-binding proteins (PBPs).

The class A β-lactamasases and PBPs react with β-lactams to form acyl enzymes. The stability of the PBP acyl enzymes results in the inhibition of transpeptidase function (42). However, the decylation of the β-lactamasases is extremely rapid, resulting in a high turnover of the β-lactam hydrolysis. This hydrolytic activity is due to the involvement of an acidic amino acid residue (Glu<sup>166</sup>, which is absent in PBP (43). Actually, it was reported that a single amino acid alteration from Phe<sup>450</sup> to Asp in EII resulted in the drastic decrease in the Ald-hydrolytic activity, especially in the Lys<sup>181</sup> mutant (42). The enzyme activity on p-nitrophenyl esters varied only in the range from 33 to 205% (p-nitrophenylacetate (C2)) and from 18 to 135% (p-nitrophenylbutyrate (C4)) among the mutant enzymes (TABLE TWO). Thus, the significant difference in the activity profiles raised a question as to how the EII enzyme discriminates between the esterolytic and nylon oligomer hydrolytic activity.

At the acylation step, as stated above, the hydroxyl of Ser<sup>112</sup> probably acts as a nucleophile and attacks the ester-carbonyl and amide-carbonyl of the substrates. The subsequent decylation step has to involve the attack of the acyl enzyme intermediate by a water molecule. Since EstB esterase has no counterparts to Glu<sup>166</sup> in class A β-lactamase, water molecules from the solvent are considered to function as a general base for the hydrolysis of the acyl intermediate (20). Since the spatial location of the residues of Ser<sup>112</sup>, Lys<sup>115</sup>, and Tyr<sup>215</sup> in the nylon oligomer hydrolyase (Hyb-24) is very similar to that of EstB esterase (Ser<sup>75</sup>, Lys<sup>78</sup>, and Tyr<sup>181</sup> in Fig. 5C) and since the esterolytic activity of EII is not so much affected by mutations at position 181, water molecules from the solvent are considered to function as a general base for the hydrolysis of the acyl intermediate. In contrast, the Ald-hydrolytic activity of EII is highly affected by substitutions at position 181, especially by substitutions to the basic amino acids (TABLE TWO). Moreover, the activity of the EII’-type enzyme is enhanced ~10-fold by the G181D substitution and ~200-fold by the G181D/H266N double substitutions (41). These results suggest that the nylon oligomer hydrolyase utilizes Ser<sup>112</sup>/Lys<sup>115</sup>/Tyr<sup>215</sup> as common active sites, both for Ald-hydrolytic and esterolytic activity, but requires at least two additional amino acid residues (Asp<sup>181</sup>/Asn<sup>266</sup>), specific for Ald-hydrolytic activity (Fig. 6).

**Evolutionary Implication**

We have previously proposed that the nylon oligomer hydrolyase (EII) evolved by gene duplication from the common antecedent of EII and cryptic EII’ proteins located on the same plasmid (8). However, the following two hypotheses have been proposed. (i) The EII enzyme is specified by an alternative open reading frame from a preexisting coding sequence that originally specified a 472-residue-long Arg-rich protein and a frameshift mutation in the ancestral gene, creating a gene responsible for nylon oligomer hydrolysis (45). (ii) There is a special mechanism for protecting a nonstop frame, namely a long stretch of sequence without chain-terminating base triplets, from mutations that generate the stop codons on the antisense strand, and such a mechanism enables the nonstop frame to evolve into a new functional gene (46).

Recently, through directed evolution from Hyb-24 using PCR random mutagenesis and selection for Ald-hydrolyase activity, we found that of seven clones that were enhanced in Ald-hydrolytic activity, three

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clones contained the G181D mutations in common. This suggests that the G181D mutation is preferentially selected during the evolution of the nylon oligomer hydrolase, when Ald-degradation has advantages in the selection.

Ser^112 and Lys^115 were conserved in all serine β-lactamases, and Tyr^215 was conserved in EstB esterase, DD-peptidase, class C β-lactamase, and 6-aminoheptaneoate-dimer hydrolase (EII and EII'), suggesting that these residues have been conserved during the evolution. In contrast, amino acid residues corresponding to Gly^181 in Hyb-24 have been diversified (Thr^123 (DD-peptidase), Val^142 (EstB esterase), Asp^127 (class C β-lactamase)). In the case of class A β-lactamase, no amino acid residue can be aligned to Gly^181 in Hyb-24. Similarly, amino acid residues corresponding to His^266 in Hyb-24 have also been diversified (Glu^220 (DD-peptidase), Gly^274 (EstB esterase), Pro^167 (class A β-lactamase)), and no amino acid residue can be aligned in class C β-lactamase (Fig. 1). However, because of the lack of structural similarities among the five enzymes, it has been impossible to do precise three-dimensional alignment at the regions 162–213 and 258–267 of Hyb-24. These results indicate that the G181D and H266N are amino acid alterations specific for the increase of nylon oligomer hydrolysis. Thus, the nylon oligomer-degrading enzyme (EII) is considered to have evolved from preexisting esterases with β-lactamase folds.

The structurally related proteins in the penicillin-recognizing family of serine-reactive hydrolases catalyze different distinct reactions (i.e. DD-transpeptidation, DD-peptide hydrolysis, β-lactam hydrolysis, and carboxyl ester hydrolysis). This illustrates how new enzyme functions evolve from a common ancestor while retaining the same basic fold. The present study suggests a strategy to create new enzymes active toward the nylon oligomer hydrolase, when Ald-degradation has advantages in the selection.