J. Microbiol. Biotechnol. (2006), **16**(9), 1434–1440

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Stabilization of Quinonoid Intermediate E-Q by Glu32 of D-Amino Acid Transaminase

RO, HYEON-SU[1,2], CHE-OK JEON[2], HAK-SUNG KIM[3], AND MOON-HEE SUNG[4]*

[1]*Department of Microbiology and Research Institute of Life Science,* [2]*Environmental Biotechnology National Core Research Center, Gyeongsang National University, Chinju 660-701, Korea*
[3]*Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejon 305-701, Korea*
[4]*Department of Bio- and Nanochemistry, Kookmin University, Seoul 136-702, Korea*

Received: April 4, 2006
Accepted: May 12, 2006

Abstract The stable anchorage of pyridoxal 5'-phosphate (PLP) in the active site of D-amino acid transaminase (D-AT) is crucial for the enzyme catalysis. The three-dimensional structure of D-AT revealed that Glu32 is one of the active site groups that may play a role in PLP binding. To prove the role of Glu32 in PLP stability, we firstly checked the rate of the potential rate-limiting step. The kinetic analysis showed that the rate of the α-deprotonation step reduced to 26-folds in E32A mutant enzyme. Spectral analyses of the reaction of D-AT with D-serine revealed that the E32A mutant enzyme failed to stabilize the key enzyme-substrate intermediate, namely a quinonoid intermediate (E-Q). Finally, analysis of circular dichroism (CD) on the wild-type and E32A mutant enzymes showed that the optical activity of PLP in the enzyme active site was lost by the removal of the carboxylic group, proving that Glu32 is indeed involved in the cofactor anchorage. The results suggested that the electrostatic interaction network through the groups from PLP, Glu32, His47, and Arg50, which was observed from the three-dimensional structure of the enzyme, plays a crucial role in the stable anchorage of the cofactor to give necessary torsion to the plane of the cofactor-substrate complex.

Key words: Transaminase, quinonoid, cofactor, pyridoxal, D-amino acid

D-Amino acid transaminase (D-AT, EC 2.6.1.21) is a pyridoxal 5'-phosphate (PLP) dependent enzyme and catalyzes the following transamination reaction between various D-amino acids and α-keto acids, where E-PLP is the PLP-bound enzyme and E-PMP is the pyridoxamine 5'-phosphate (PMP)-form D-AT [22]. Both the half reactions have been thought to proceed with the formation of a key quinonoid intermediate (E-Q) (Fig. 1A).

E-PLP+D-amino $acid_1$=E-PMP+α-keto $acid_1$
E-PMP+α-keto $acid_2$=E-PLP+D-amino $acid_2$

D-AT plays a crucial role in providing D-amino acids for bacterial cell wall biosynthesis [12]. Because the requirement of D-amino acids is unique in the bacterial cell wall system, D-AT has been studied as a good target for antibiotic drug discovery. Additionally, D-AT has been one of the target enzymes to produce D-amino acids from L-glutamate by a coupled reaction with glutamate racemase [1]. D-Amino acids are important building blocks for bioactive peptides [8, 9]. Other PLP-dependent enzymes such as tyrosine phenol-lyase and typtophan indole-lyase have also been sought for their industrial use [10, 11].

Stabilization of the enzyme-substrate complex and stable anchorage of cofactor are crucial for enzyme activity [15]. Numerous studies have indicated that the stable binding of PLP in the active site of all PLP-dependent enzymes is critical not only to the confinement of the cofactor itself within the active site but also to the faithful coordination of the enzyme catalysis [2, 4, 6, 18, 20, 21]. For example, the cofactor (PLP) in the active site of the tryptophan synthase β2 is tightly held by hydrogen bonds formed between the phosphate group of the cofactor and the Gly232-Gly233-Gly234-Ser235 loop and His86 [6, 16]. The substitution of His86 to leucine led to partial loss of the cofactor and shift of the optimal pH from 7.5 to 8.8 [18]. Similarly L-aspartate aminotransferase, which is one of the most well known PLP-dependent enzymes, also holds its cofactor

*Corresponding author
Phone: 82-2-910-4808/5098; Fax: 82-2-910-4415;
E-mail: smoonhee@kookmin.ac.kr

through hydrogen bonds between the phosphate group and Tyr70, Arg266, Thr109, and Gly110 [7].

X-ray crystallographic data on D-AT revealed that the cofactor is tightly held by electrostatic interactions with several active site residues including Glu177, Lys145, Arg50, Thr241, Thr205, etc [20]. In addition, experimental data hinted that the amino acid residues in the β strand III region of D-AT might be involved in constituting the active site, because mutations at the residues in this region led to a marked decrease in the enzyme activity [17]. The three-dimensional structure showed that the side chains of Tyr31, Glu32, Val33, and Lys35 in the strand faces the active site. Specifically, the carboxyl group of Glu32 interacts with the guanidinium moiety of Arg50, which forms a hydrogen bond with the phosphate group of the cofactor, and with the imidazole group of His47 (Fig. 1B). The ε-amino group of the active site lysine (Lys145), which catalyzes the crucial deprotonation reaction at the α-carbon of the substrate, is located within the space formed by the side chains of Arg50, Glu32, and Tyr31 (Fig. 1B).

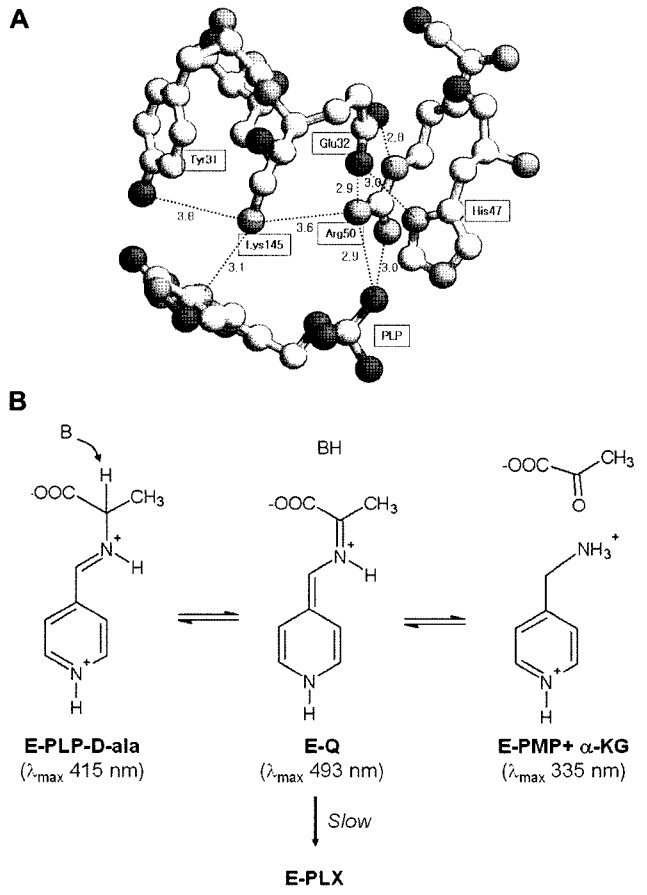

Fig. 1. Reaction intermediates of D-AT with D-alanine (**A**) and spatial arrangement of the active site groups (**B**).
The active site structure was prepared with selected residues from the reported structure in the Protein Data Bank (PDB ID: 2DAA) using Rasmol. Numbers represent the distance in angstrom.

Previous study demonstrated that a mutation on Glu32 induced loss of the Schiff base proton at high pH and consequently resulted in loss of 99% of its activity [17]. This and X-ray crystallographic data led us to speculate the involvement of Glu32 in the catalytic process by stabilizing the cofactor through a series of electrostatic interactions.

MATERIALS AND METHODS

Plasmids, Bacterial Strain, and Culture Conditions

Escherichia coli XL1 Blue was used as the host strain. Cells were grown in LB medium (1% tryptone, 0.5% yeast extract, and 1% NaCl) containing 1 mM IPTG and 100 μg/ml ampicillin at 37°C. Restriction enzymes were obtained from NEB (U.S.A.). T4 DNA ligase was from Takara (Japan). DNA polymerase used for the site-directed mutagenesis was Vent polymerase from NEB. Oligonucleotides were synthesized at Bioneer (Korea). All other chemicals were of analytical grade.

Cloning of D-AT from *Bacillus* sp. YM1

D-AT from *Bacillus* sp. YM1 was PCR-amplified using a set of synthetic primers, N-terminal primer 5'-GCC ATG GGA TAC ACT TTA TGG AA-3', and C-terminal primer 5'-CCT GCA AAG CTT ATT ATA TAT GAA GCG GTT TTG-3'. The restriction sites *Nco*I and *Hind*III, were introduced into the N- and C-terminal primers, respectively. The resulting PCR fragment was cloned into the *Nco*I/*Hind*III sites of an overexpression vector, pTrc99A (AP Biotech, Uppsala, Sweden). To generate Glu32Ala (E32A) mutant D-AT, a site-directed mutagenesis was carried out by the megaprimer method using a two-stage polymerase chain reaction [17].

Enzyme Purification

Cells were suspended in 30 mM Tris-HCl (pH 7.3) containing 0.01% β-mercaptoethanol and 20 mM PLP and then broken by sonication. After centrifugation, the supernatant was heated at 50°C for 30 min and centrifuged to remove aggregated proteins. The supernatant was applied to an Resource Q anion-exchange column (Pharmacia), which had been equilibrated with 30 mM Tris-HCl (pH 7.3). Proteins were eluted with a linear gradient from 0 to 1 M NaCl. Fractions showing D-AT activity were collected and concentrated by ultrafiltration. The concentrated protein solution was loaded on Phenyl superose column (Pharmacia), which had been equilibrated with 30 mM Tris-HCl (pH 7.3) containing 25% ammonium sulfate. Proteins were eluted with a linear gradient of 25%-0% ammonium sulfate. Fractions showing D-AT activity were collected and dialyzed against 30 mM Tris-HCl (pH 7.3) containing 20 mM PLP, 0.01% β-mercaptoethanol, 0.2 mM EDTA,

10 μM phenylmethylsulfonylfluoride (PMSF), and 5 μM *p*-toluenesulfonyl-L-phenylalanine chloromethylketone (TPCK). All column procedures were carried out by an FPLC system (Pharmacia).

CD and UV/Vis Spectrophotometric Analyses, and Enzyme Activity Assay

Absorption spectra were obtained at 25°C by using a spectrophotometer (Shimadzu UV2100, Japan). Circular dichroism spectra were taken using a Jasco spectropolarimeter (Japan). All experiments were conducted in 100 mM HEPES buffer (pH 7.5), unless otherwise noted. A continuous-coupled assay system using lactate dehydrogenase (LDH) was employed to determine the enzyme activity. The reaction mixture contained 100 mM Tris-HCl (pH 8.5), 0.5 mM NADH, 10 mg/ml LDH, 100 mM D-alanine, and 100 mM α-ketoglutarate (α-KG). The reaction was initiated by addition of enzyme solution. The decrease in the absorbance at 340 nm was measured at 50°C, and the enzyme activity was calculated with a molar extinction coefficient of 6,220 M^{-1} cm^{-1} for NADH.

Analysis of Data

The half reaction of D-AT with D-amino acid can be expressed as:

$$\text{E-PLP+D-AA} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \text{E-PLP-D-A} \overset{k_2}{\rightleftharpoons} \text{E-PMP-}\alpha\text{-KA}$$

The dissociation constant (K_d) of the enzyme for D-amino acid was calculated using the following equations.

$$[E]_{total}=[\text{E-PLP}]+[\text{E-PLP-D-AA}]+[\text{E-PMP-}\alpha\text{-KA}] \quad (1)$$

$$K_d=[\text{E-PLP}][\text{D-AA}]/[\text{E-PLP-D-AA}]=k_{-1}/k_{+1} \quad (2)$$

$$[\text{E-PMP-}\alpha\text{-KA}]=k_2\,[E]_{total}/\{(k_2+1)+K_d/[\text{D-AA}]\} \quad (3)$$

$[E]_{total}$, [E-PLP], [E-PLP-D-AA], [E-PMP-α-KA], and [D-AA] are concentrations of total enzyme, the PLP form enzyme, the PLP-D-amino acid complex, the PMP-α-keto acid complex, and D-amino acid, respectively. k_{-1}, k_{+1}, and k_2 are rate constants. Since [E-PMP-α-KA] absorbs at 335 nm, the dissociation constant (K_d) can be calculated from the fitting of Eq. (4) to the absorbance at 335 nm vs. concentration of D-amino acid.

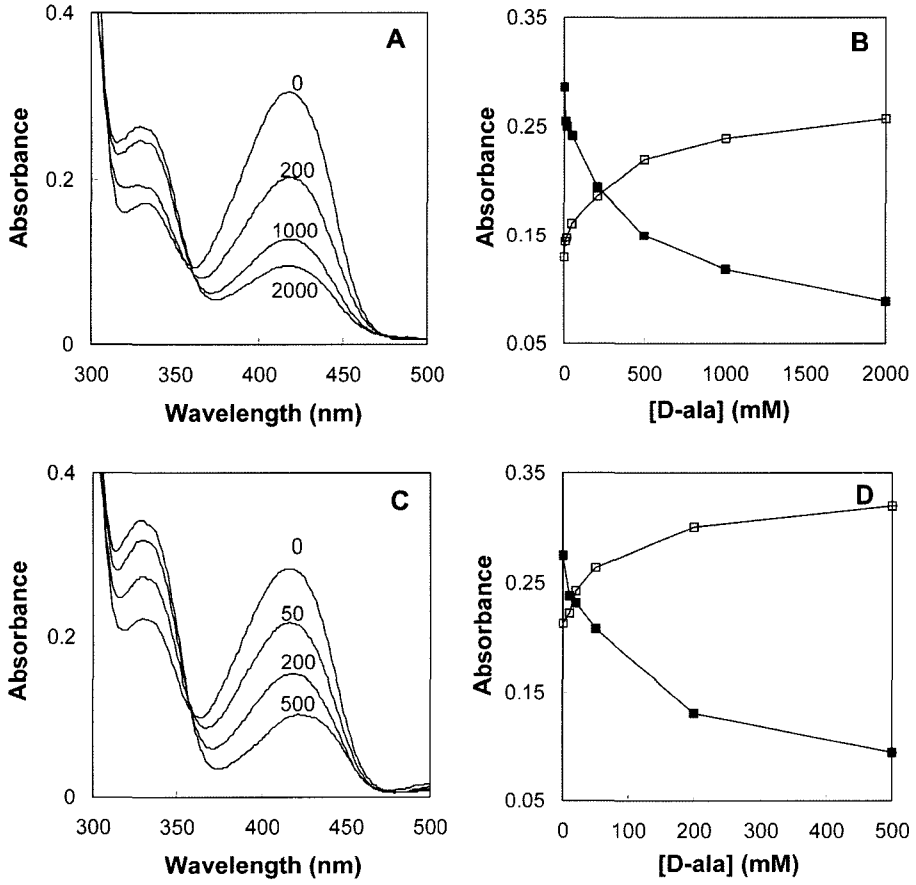

Fig. 2. Absorption spectra of the wild-type (**A**) and E32A (**C**) enzymes in the presence of various concentrations of D-alanine.
The protein concentration was 1 mg/ml. The spectra were obtained 3 min after addition of D-alanine at 25°C. Numbers represent the concentration of added D-alanine in μM. The absorbance vs. D-alanine concentration plots for the wild-type (**B**) and the E32A (**D**) enzymes were drawn using the absorbance measured at 335 nm (□) and 415 nm (■) with the increasing concentrations of D-alanine.

$$A_{335\,nm}=C_1/(C_2+K_d/[D\text{-}AA]) \quad (4)$$

C_1 and C_2 are constants corresponding to $k_2[E]_{total}$ and (k_2+1) in Eq. (3), respectively. The relationship between the substrate dissociation (K_d) and the Michaelis constants (K_m) can be expressed as Eq. (7).

$$K_m=(k_{-1}+k_2)/k_{+1} \quad (5)$$

$$K_d=k_{-1}/k_{+1} \quad (6)$$

$$K_m=K_d+k_2/k_{+1} \quad (7)$$

RESULTS AND DISCUSSION

Kinetic Analysis of the Role of Glu32

Reaction of D-AT with D-alanine yielded an intense peak at 335 nm with concomitant decrease in 415 nm (Fig. 2). The 335 nm peak is attributed to E-PMP-pyruvate and the 415 nm peak is attributed to E-PLP-D-alanine [3, 19]. The decrease of the 415 nm peak with concomitant increase of the 335 nm peak indicates conversion of E-PLP+D-alanine to E-PMP-pyruvate. By measuring the increase of the 335 nm peak and the decrease of the 415 nm peak by increasing D-alanine concentration, we were able to determine the binding constant (K_d) of D-AT for D-alanine by fitting of Figs. 2B and 2D using Eq. (4). The apparent K_d value of the wild-type enzyme was 300 μM whereas that of the E32A mutant enzyme was 30 μM. The 10 times higher binding affinity of the E32A mutant enzyme for substrate indicates that the carboxylic group of Glu32 may hinder the access of substrate to the active site. These values were much less than those of the Michaelis constants (K_m) for D-alanine, which we had previously determined as 2.1 mM (WT) and 0.1 mM (E32A) [17], indicating a rapid conversion of the E-PLP-D-AA complex to E-PMP-α-KA. The values of k_{-1} and k_2 with respect to k_{+1} could be calculated from Eqs. (6) and (7) using predetermined K_d and K_m values and were summarized in Table 1. Under the assumption that the rate constants k_{+1} for the wild-type and E32A mutant enzymes were identical, which might not reflect the real case, the turnover rate of E-PLP-D-ala (k_2) for the E32A mutant enzyme was 3.8% of that of the wild-type enzyme. This was comparable to the experimental value that we had previously measured as 1% of the wild-type activity. The large reduction of activity of E32A, in spite of increased substrate accessibility, suggests that the carboxyl group of Glu32 is important for enzyme catalysis. One possible explanation of this result is that the hydrogen bond network formed among PLP, Arg50, Glu32, and Tyr31 may play a role in stabilizing the cofactor PLP, so that each reaction intermediate step converts to another step with minimal activation energy requirement. In relationship with this interpretation, the rate constant k_2 of the E32A mutant enzyme, which may indicate the rate for the rate-limiting α-deprotonation step (k_2), was 26 times slower than that of the wild-type enzyme.

Table 1. Kinetic constants derived from the absorbance vs. D-alanine concentration plot.

	k_{-1}	k_2	K_d (mM)	K_m (mM)
WT (A)	0.3 k_{+1}	1.8 k_{+1}	0.3	2.1
E32A (B)	0.03 k_{+1}	0.07 k_{+1}	0.03	0.1
(A)/(B)	10	26	10	21

Glu32 Stabilizes the Reaction Intermediate E-Q

To investigate the role of Glu32 in the stabilization of cofactor, we examined the stability of a crucial quinonoid intermediate (E-Q). E-Q is a reaction intermediate generated after deprotonation of the α-carbon at the E-PLP-D-Ala

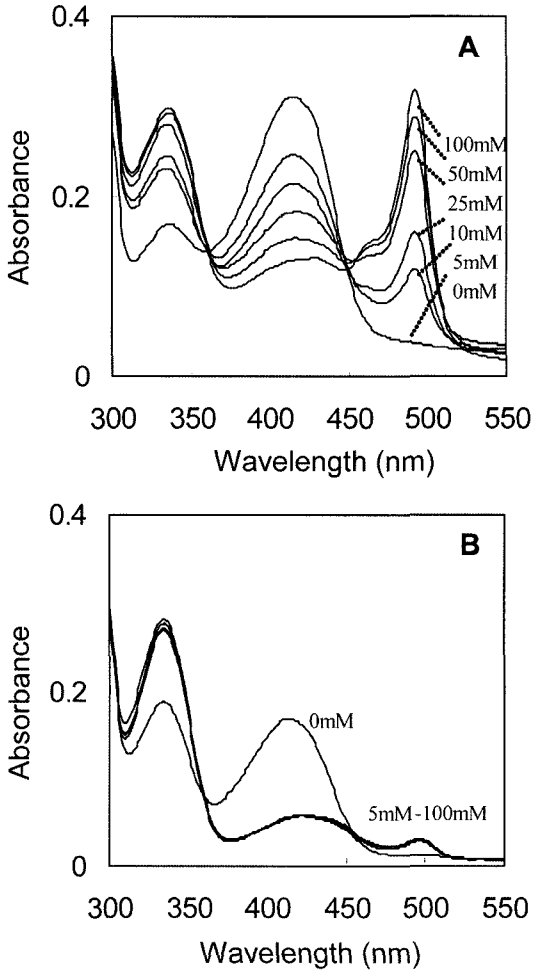

Fig. 3. Absorption spectra of the wild-type (**A**) and E32A (**B**) mutant enzymes in the presence of various concentrations of D-serine.

The protein concentration was 1 mg/ml. The spectra were obtained immediately after addition of D-serine at 25°C.

complex (Fig. 1A). D-Serine is a pseudosubstrate of D-AT with which the enzyme accumulates E-Q intermediate as one of the major forms along with E-PMP. The reaction of D-AT with D-serine yielded a strong absorption peak at 493 nm, which is attributed to E-Q (Fig. 3) [13, 14]. For the wild-type enzyme, the 493 nm peak increased with increasing concentration of D-serine and reached saturation at 100 mM D-serine (Fig. 3A). The E32A mutant enzyme produced a much less amount of the quinonoid intermediate and exhibited saturation at a low concentration of D-serine (Fig. 3B). This reflects the low K_d value of the E32A mutant enzyme for substrate, as described above.

A certain spatial coordination of the active site groups has been known to be required for the stabilization of the quinonoid intermediate [5]. For example, the crystal structure of aspartate aminotransferase with *erythro*-β-hydroxy-L-aspartate, which corresponds to D-serine for D-AT, revealed that a hydrogen bond network between the β-hydroxyl group of substrate and the enzyme groups (Tyr70, Lys258) stabilizes E-Q [5]. The lowered accumulation of E-Q for the E32A mutant enzyme suggests that Glu32 is critical in the stabilization of E-Q, presumably a cofactor anchoring role through the PLP-Arg50-Glu32-Tyr31 network. It is notable that the interpretation is correlated well with the reduced activity of the E32A mutant enzyme, since the stability of E-Q is important to enhance the rate of the α-deprotonation step.

Cofactor Stability is Also Required for the Substrate-Induced Inactivation

To confirm the cofactor anchoring role of Glu32 as described above, we investigated the change of E-Q concentration with a longer period of incubation. E-Q, after an initial rapid accumulation, has been known to slowly convert into an irreversible E-PLX, resulting in eventual inactivation of D-AT (Fig. 1A) [13]. The E-Q concentration (493 nm band) reached its maximum immediately after mixing of D-AT with D-serine (Fig. 4A). Further incubation of the reaction mixture eventually decreased the 493 nm peak. More than half the amount of E-Q disappeared 30 min after the initial accumulation for the wild-type D-AT enzyme, while it took as much as 2 h for the E32A mutant enzyme. The disappearance of E-Q was directly related to the inactivation of the enzyme, as shown in Fig. 4B. Coincidently with the E-Q disappearance, more than half the amount of the wild-type enzyme was inactivated after 30 min incubation with D-serine, while 80% of the activity of the E32A mutant enzyme was still retained even after 1 h incubation. It indicates that the accumulation of E-Q is a prerequisite for the substrate-induced enzyme inactivation, and the removal of the carboxylic group of Glu32 causes catalytic incompetence to the enzyme to maintain its activity, though negligible, against substrate-induced inactivation.

CD Spectra Prove Effect of Glu32 on Cofactor Stability

Finally, we examined circular dichroic (CD) spectra for the wild-type and E32A enzymes. D-AT has been known to show a strong negative CD signal at 415 nm owing to the optically active arrangement of PLP through the interaction of active site groups [22]. Unfolded enzyme loses the optical activity and thereby the CD signal even in the presence of PLP. As shown in Fig. 5, the wild-type D-AT showed a strong 415 nm signal, whereas the E32A enzyme exhibited only a weak negative peak. This indicates that the removal of the carboxylic group relaxes the optically active enzyme environment into an optically inactive one, suggesting the cofactor anchoring role of Glu32.

Maintaining a torsional stress to the plane of the PLP-substrate complex is important for the PLP-mediated enzyme catalysis. The tight anchorage of the cofactor in the enzyme active site is, therefore, crucial for D-AT activity. This study provides evidence that an active site residue, Glu32, functions as such a role to generate and stabilize

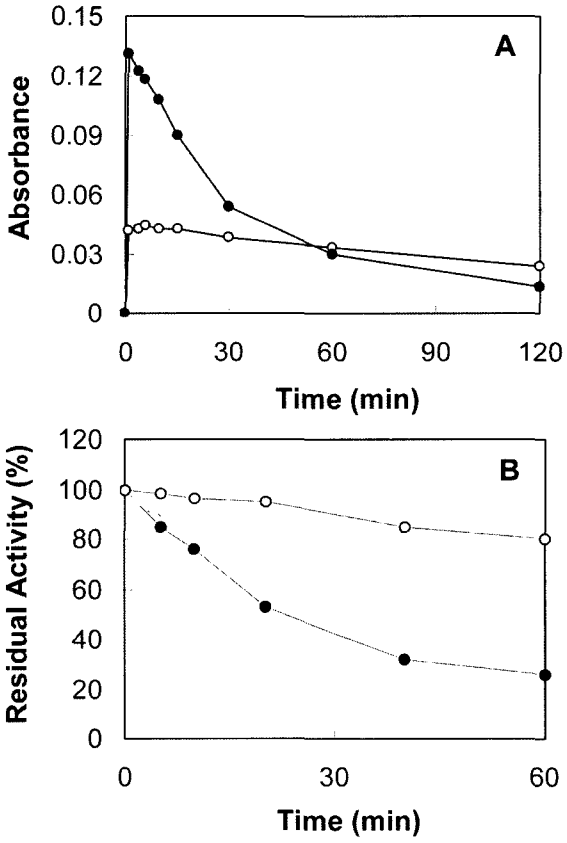

Fig. 4. The time course of changes in absorbance at 493 nm (**A**) and in the residual activity (**B**) of the wild type (●) and the E32A (○) enzymes.

The absorbance at 493 nm was measured while incubating the enzyme (0.5 mg/ml) with 100 mM D-serine at 25°C. The residual activity was measured by addition of the incubated enzyme solution from (A) in the reaction mixture containing 100 mM Tris-HCl (pH 8.5), 0.5 mM NADH, 10 mg/ml LDH, 100 mM D-alanine, and 100 mM α-ketoglutarate (α-KG).

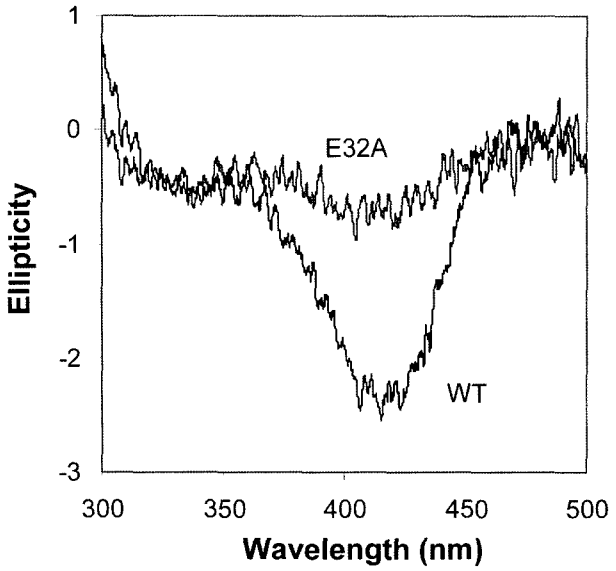

Fig. 5. Circular dichroism spectra of the wild-type and E32A enzymes.

the key intermediate E-Q complex, presumably through an electrostatic interaction network with neighboring residues (His47, Arg50) and the cofactor PLP.

Acknowledgments

This work was supported by grants from the KOSEF to the Environmental Biotechnology National Core Research Center (Grant R15-2003-012-02002-0) and the 2006 research fund of Kookmin University in Korea.

REFERENCES

1. Bae, H. S., S.-P. Hong, S.-G. Lee, M.-S. Kwak, N. Esaki, and M.-H. Sung. 2002. Application of a thermostable glutamate racemase from *Bacillus* sp. SK-1 for the production of D-phenylalanine in a multi-enzyme system. *J. Mol. Catal. B-Enzym.* **17:** 223–233
2. Burkhard, P., G. S. Rao, E. Hohenester, K. D. Schnackerz, P. F. Cook, and J. N. Jansonius. 1998. Three-dimensional structure of O-acetylserine sulfhydrylase from *Salmonella typhimurium*. *J. Mol. Biol.* **283:** 121–133.
3. Futaki, S., H. Ueno, A. Martinez del Pozo, M. A. Pospischil, J. M. Manning, D. Ringe, B. Stoddard, K. Tanizawa, T. Yoshimura, and K. Soda. 1990. Substitution of glutamine for lysine at the pyridoxal phosphate binding site of bacterial D-amino acid transaminase. *J. Biol. Chem.* **265:** 22306–22312.
4. Gallagher, D. T., G. L. Gilliland, G. Xiao, J. Zondlo, K. E. Fisher, D. Chinchilla, and E. Eisenstein. 1998. Structure and control of pyridoxal phosphate dependent allosteric threonine deaminase. *Structure* **6:** 465–475.
5. Hayashi, H. and H. Kagamiyama. 1995. Reaction of aspartate aminotransferase with L-*erythro*-3-hydroxyaspartate: Involvement of Tyr70 in stabilization of the catalytic intermediate. *Biochemistry* **34:** 9413–9423.
6. Hyde, C. C., S. A. Ahmed, E. A. Padlan, E. W. Miles, and D. R. Davies. 1988. Three-dimensional structure of the tryptophan synthase α2β2 multienzyme complex from *Salmonella typhimurium*. *J. Biol. Chem.* **263:** 17857–17871.
7. Jensen, R. A. and W. Gu. 1996. Evolutionary recruitment of biochemically specialized subdivisions of family I within the protein superfamily of aminotransferases. *J. Bacteriol.* **178:** 2161–2171.
8. Jung, B. R., Y. Lee, Y. Lim, and J. H. Ahn. 2005. Structure prediction of the peptide synthesized with the nonribosomal peptide synthetase gene from *Bradyrhizobium japonicum*. *J. Microbiol. Biotechnol.* **15:** 656–659.
9. Jung, H. J., K. S. Choi, and D. G. Lee. 2005. Synergistic killing effect of synthetic peptide P20 and cefotaxime on methicillin-resistant nosocomial isolates of *Staphylococcus aureus*. *J. Microbiol. Biotechnol.* **15:** 1039–1046.
10. Kim, J. H., J. J. Song, B. G. Kim, M. H. Sung, and S. C. Lee. 2004. Enhanced stability of tyrosine phenol-lyase from *Symbiobacterium toebii* by DNA shuffling. *J. Microbiol. Biotechnol.* **14:** 153–157.
11. Lee, S. G. and M. H. Sung. 1999. Development of an enzymatic system for the production of dopamine from catechol, pyruvate, and ammonia. *Enz. Microb. Technol.* **25:** 298–302.
12. Lee, S. G., S. P. Hong, J. J. Song, S. J. Kim, M. S. Kwak, and M. H. Sung. 2006. Functional and structural characterization of thermostable D-amino acid aminotransferases from *Geobacillus* spp. *Appl. Environ. Microbiol.* **72:** 1588–1594.
13. Martinez del Pozo, A., M. A. Pospischill, H. Ueno, J. M. Manning, K. Tanizawa, K. Nishimura, K. Soda, D. Ringe, B. Stoddard, and G. A. Petsko. 1989. Effects of D-serine on bacterial D-amino acid aminotransferase: Accumulation of an intermediate and inactivation of the enzyme. *Biochemistry* **28:** 8798–8803.
14. Morino, Y. and S. Tanase. 1985. Quasisubstrates and irreversible inhibitors of aspartate aminotransferase, pp. 251–265. *In* Christen, P. and D. E. Metzler (eds.), *Transaminases*. John Wiley and Sons, New York, U.S.A.
15. Park, H., K. S. Lee, S. M. Park, K. W. Lee, A. Y. Kim, and Y. M. Chi. 2005. Relationship between enhancement of electrostriction and decrease of activation energy in porcine pancreatic lipase catalysis. *J. Microbiol. Biotechnol.* **15:** 587–594.
16. Rhee, S., K. D. Parris, C. C. Hyde, S. A. Ahmed, E. W. Miles, and D. R. Davies. 1997. Crystal structures of a mutant (β-K87T) tryptophan synthase α2β2 complex with ligands bound to the active sites of the α- and β-subunits reveal ligand-induced conformational changes. *Biochemistry* **36:** 7664–7680.
17. Ro, H. S., S. P. Hong, T. Yoshimura, N. Esaki, K. Soda, H. S. Kim, and M. H. Sung. 1996. Site-directed mutagenesis of the amino acid residues in β-strand III of D-amino acid aminotransferase. *FEBS Lett.* **398:** 141–145.

18. Ro, H. S. and E. W. Miles. 1999. Structure and function of the tryptophan synthase α2β2 complex: Roles of β subunit histidine 86. *J. Biol. Chem.* **274:** 36439–36445.
19. Soper, T. S. and J. M. Manning. 1985. Enzyme-activated inhibitors of pyridoxal-phosphate enzymes, pp. 266–285. *In* Christen, P. and D. E. Metzler (eds.), *Transaminases*. John Wiley and Sons, New York, U.S.A.
20. Sugio, S., G. A. Petsko, J. M. Manning, K. Soda, and D. Ringe. 1995. Crystal structure of a D-amino acid aminotransferase: How the protein controls stereoselectivity. *Biochemistry* **34:** 9661–9669.
21. Toney, M. D., E. Hohenester, J. W. Keller, and J. N. Jansonius. 1995. Structural and mechanistic analysis of two refined crystal structures of the pyridoxal phosphate-dependent enzyme dialkylglycine decarboxylase. *J. Mol. Biol.* **245:** 151–179.
22. Yonaha, K., H. Misono, T. Yamamoto, and K. Soda. 1975. D-Amino acid aminotransferase of *Bacillus sphaericus*. Enzymologic and spectrometric properties. *J. Biol. Chem.* **250:** 6983–6989.