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RESEARCH PAPER

Characterization of ELP-fused ω-Transaminase and Its Application for the Biosynthesis of β-Amino Acid

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Abstract Optically pure amines, β-amino acids and γ-amino acids are the valuable precursors to produce biologically active compounds. The ω-TAs are the class of enzymes which are widely used to produce such compounds. In this work (*S*)-ω-transaminase from the thermophilic eubacterium *Sphaerobacter thermophilus* (St-TA) was fused with Elastin-like polypeptides (ELPs) through the cloning process and expressed in *E. coli* cells. The characterization of this fusion complex was performed with respect to thermostability and effect of DMSO. Where in case of St-TA-ELP-$V_{60,}$ major difference in the transition temperature (T_t) was observed, wherein a T_t of 38 and 70ºC was observed at the increasing concentration of DMSO from 5 to 25% (v/v). Interestingly, these fusion proteins the activity was preserved even after the aggregation of fusion complex at T_t. The substrate specificity and product inhibition analysis showed that ω-TA-ELPs had comparable results as that of wild type ω-TA. Moreover, the fused ω-TA could be efficiently reused for up to 20 batches of transamination reaction. Furthermore, the applicability of the fusion protein for the production of a sitagliptin precursor (*R*)-3-amino-4-(2,4,5-triflurophenyl) butanoic acid (3-ATfBA) was evaluated, wherein 3-ATfBA was synthesized with good conversion (65%).

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Keywords: (*S*)-ω-transaminase, elastin-like polypeptides, chiral amines, bio-catalysis, thermostability.

1. Introduction

Chiral amines and amino acids are essential building blocks in the synthesis of a broad range of biologically active compounds, pharmaceuticals and agrochemicals [1-7]. Moreover, chiral amino acids are consistently used in bimolecular structure and functional studies. The chemical synthesis of chiral amines is labor intensive, requiring harsh reaction conditions and use of toxic intermediates. The use of toxic metals also warrants a purification step, thus further complicating the synthetic systems and the cost thereof [4]. The ω-TAs are the class of enzymes which are widely used to produce such compounds for example an enantioselective synthesis of β-amino acids has been reported by employing various enzymes such as amino-acylase [8], β-aminopeptidase [9], phenylalanine amino-mutase [10], and ω-transaminase (ω-TA) [11-13]. The advances in the protein engineering techniques have consistently improved the substrate scope of these biocatalysts. Among the various biosynthetic routes for the production of chiral β-amino acids, ω-TAs are significantly used due to its broad substrate specificity and high enantioselectivity [14-19]. For instance, (*S*)-ω-transaminase from the thermophilic eubacterium *Sphaerobacter thermophilus* (St-TA) [20] has been successfully used in the biosynthesis of optically pure β- and γ-amino acids (*ee* >99%) *via* asymmetric synthesis [21-24].

Elastin-like polypeptides (ELPs) are the synthetic, genetically encodable polypeptides that respond to reaction temperature, ionic strength and pH [25]. ELPs are composed of the repeating pentapeptide sequence VPGXG, where the

residue (X) can be any naturally occurring amino acid except proline [26]. ELPs undergo an inverse phase transition that is analogous to the lower critical solution temperature which could be used as an alternative to purify proteins [25,27]. ELPs are highly soluble in aqueous solution below a critical transition temperature (T_t); however, at temperatures above its T_t, ELPs undergoes a phase transition to insoluble form, leading to aggregation of the polypeptide [28]. Economic feasibility of the transaminases-catalyzed reactions is firmly related to efficient utilization of ω-TA in the reaction [29]. The fusion of ω-TA with ELPs can meet the requirements of improving enzymatic activity and their reuse [29]. In this work, the St-TA was fused to an ELP through genetic engineering of the enzyme. This fused ω-TA-ELP complex was purified by using reverse phase transition method. This fusion complex was compared with wild type St-TA with respect to substrate specificity, substrate inhibition and reusability of fusion protein. Furthermore, to broaden the scope of ω-TA-ELP complex, this fusion protein was employed for the synthesis of valuable β-amino acid named as (*R*)-3-amino-4-(2,4,5-triflurophenyl) butanoic acid (3-ATfBA). this 3-ATfBA is used as a precursor for the synthesis of valuable antidiabetic drugs such as sitagliptin, retagliptin and evogliptin [30,31]. 3-ATfBA can be synthesized by enantioselective amination of 3-oxo-4-(2,4,5-triflurophenyl) butanoic acid. In our previous studies, we have reported the synthesis of aromatic (*S*)-β-amino acids, including 3-ATfBA, from their corresponding β-keto esters by employing the cascade of lipase and ω-TA [32]. Here, we attempted to utilize an ELP-fused St-TA for the biocatalytic synthesis of 3-ATfBA. The results of the present studies demonstrate the applications of the ELP-fused ω-TA for the production of industrially valuable β-amino acid, 3-ATfBA [30,31].

2. Materials and Methods

2.1. Chemicals

β-ketoesters, β-amino acids, GITC (2, 3, 4, 6-Tetra-O-acetyl-β-D-glucopyranosyl iso-thiocyanate), lipase (Catalog No. L1754), PLP (Pyridoxal 5′-phosphate hydrate), dimethyl sulfoxide (DMSO), amino donors and amino acceptors were obtained from Sigma-Aldrich [Sigma-Aldrich Korea, Seoul]. Isopropyl-β-D-thiogalactopyranoside (IPTG) was obtained from EMD chemicals (San Diego, USA)

2.2. Gene construction for the ω-TA and ELP fusion protein

In the present case, ELP V_{60} and V_{120} represent the 60 and 120 repetitive units of VPGXG respectively [33]. The elongation was performed as reported by wang *et al.* [34] to synthesize the pQE_mod_4: V_{60} or V_{120}. Next, these desired ELPs (V_{60} or V_{120}) were fused in pQE_mod_3: ω-TA using following procedure: First, the gene encoding ω-TA was cloned into *Sac*I-*Kpn*I restriction sites of pQE_80L vector [35] by using PCR ω-TA-F (5′-ATTAGGA TC CAGCTCTGGCTCCCGTC-3′) and ω-TA-R (5′-ATTAGG TACCGCCTCTCGCATCTT GC-3′) primers. Next, we synthesized an oligomer 'mod3' (5′-**GGTACC**AGCGGC GTTGGC GTCCTGAGACCCACTGGTCACGGTCTC GG TCCCGGTTAATAA**CTGCAG**-3′) and (5′-**CTG CAG**TTATTAACCGGGACCGAGACCGTGACCAGTG GGTCTCAGGACGCCA ACGCCGCT**GGTACC**-3′) containing the *Bsa*I restriction sites. This oligomer was cloned in *Kpn*I site and *Pst*I restriction sites of pQE_ω-TA vector system (pQE_mod3: ω-TA). The ELP tags, from pQE_mod_4: V_{60} or V_{120} were prepared by digestion with *Bbs*I and *Bsa*I, and further ligated with *Bsa*I digested pQE_mod3: ω-TA (Fig. 1).

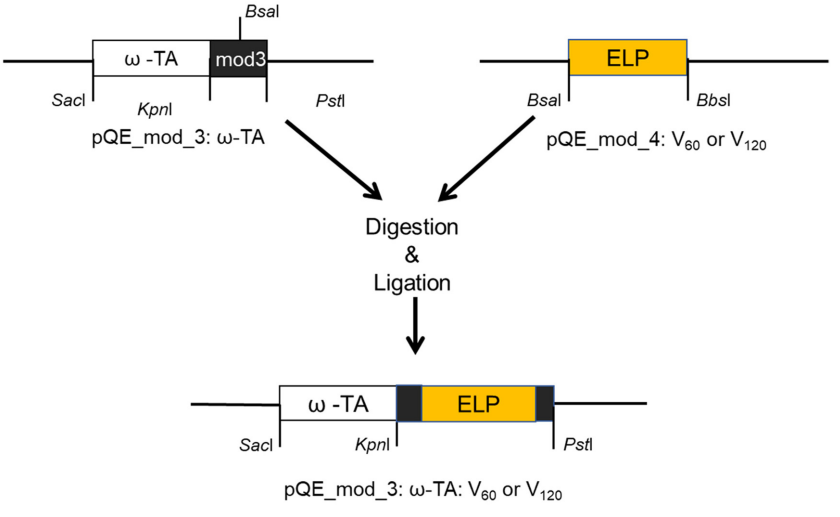

Fig. 1. Gene construction of the ω-TA-ELP (V_{60} and V_{120}) fusion proteins.

2.3. Expression and purification of recombinant St-TA-ELPs

The ω-TA and ELPs were fused as mentioned above and the fusion proteins ω-TA-ELPs (ω-TA-ELP-V_{60} and ω-TA-ELP-V_{120}) were expressed in *E. coli.* Next the *E. coli* was harvested by centrifugation at 4ºC and resuspended in Tris-HCl buffer (100 mM) [34]. The cells were lysed by ultrasonic disruption in the ice bath, and the lysate was heated up to 42ºC for 10 min and centrifuged at 10,000 g at 42ºC for 10 min to remove the soluble proteins (supernatant). The precipitate (including cell debris and aggregated ω-TA-ELPs) was resuspended in Tris-HCl buffer 1 mL and centrifuged at 10,000 g at 4ºC for 10 min to remove the other proteins. The resulting supernatant solution containing ω-TA-ELPs was reheated at 42ºC for 10 min followed by centrifugation at 42ºC for 10 min. The purification process was repeated three times. The enzyme activity was assayed at 42ºC. Protein concentration was measured by the Bradford method [36] using Bovine Serum Albumin as a standard.

2.4. Enzyme assay

Amino donor specificity of ω-TA-ELPs was examined in a reaction containing 100 mM Tris–HCl buffer (pH 8.0), 15 mM amino donor, 5 mM pyruvate, 0.1 mM Pyridoxal 5′-phosphate (PLP) and 0.23 mg/mL Enzyme at 25°C for 30 min. Total volume of the reaction was 200 µL. The amino acceptor activity of ω-TA-ELPs was determined by performing a 200 µL enzyme reaction containing 100 mM Tris–HCl buffer (pH 8.0), 15 mM amino acceptor, 5 mM (*S*)-α-Methyl benzylamine ((*S*)-α-MBA), 0.1 mM PLP and 0.23 mg/mL enzyme at 25°C. Enzyme reactions were stopped after 30 min by adding 10 µL perchloric acid (50% v/v), and centrifuged at 13,000 rpm for 15 min. The supernatant was analyzed by HPLC. One unit of enzyme activity was defined as the amount of enzyme that depleted 1 µmol pyruvate/ (*S*)-α-MBA in 1 min.

2.5. Analytical conditions

The quantitative analysis of β-amino acid was performed using HPLC with a Crownpak CR column (Daicel Co., Japan) at 210 nm, with an elution of PCA solution (pH 1.5; 0.6 mL/min) as previously reported [32]. Quantitative chiral analysis of all substrates was performed using a C_{18} Symmetry column (Waters, MA) with a Waters HPLC system at 254 nm after the derivatization of sample with GITC. Separation of each enantiomer was achieved through an isocratic elution with a mixture of 50% methanol and 50% water (0.1% TFA) at a flow rate of 1.0 mL/min. Acetophenone was quantified by HPLC with a C_{18} column (Agilent) at 254 nm using the mobile phase comprising of methanol (0.1% TFA)/ water (0.1% TFA) (45:55. v/v) at 1.0 mL/min. The injection volume was 20 µL.

3. Results and Discussion

3.1. SDS PAGE analysis of recombinant St-TA-ELPs

The purity of ω-TA-ELPs was analyzed by SDS-PAGE where a prominent protein bands were clearly observed (Fig. 2). In SDS-PAGE analysis the wild type ω-TA showed a band of ~47.7 kDa (Fig. 2A Lane 3). ω-TA-V_{60} and ω-TA-V_{120} obtained during the purification cycles are shown in Fig. 2B. ω-TA-V_{60} and ω-TA-V_{120} showed prominent bands at ~74.5 kDa and ~99.1 kDa, respectively (Fig. 2B Lane 4 and 9, respectively), implying the homogencity of the purified protein complexes.

3.2. Analysis of the transition temperature (T_t) of ω-TA-ELPs

The transition temperature (T_t) is defined as the temperature

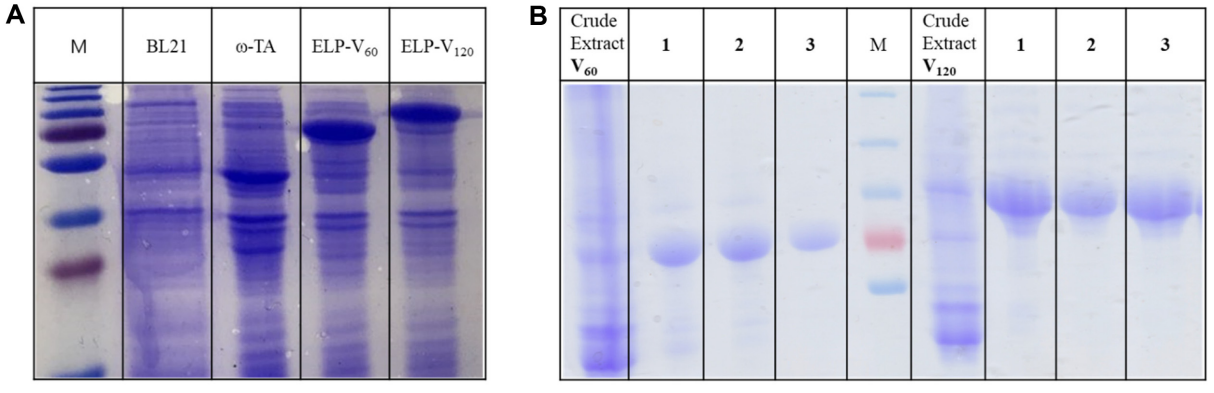

Fig. 2. (A) Analysis of SDS-PAGE for expression of (A) ELP-V_{60} and ELP-V_{120}; where, from left side lane 1 represents the markers, lane 2 is BL21 cells, lane 3 shows wild type St-TA whereas lane 4 and 5 shows ELP-V_{60} and ELP-V_{120} respectively; (B) ω-TA-ELP-V_{60} and ω-TA-ELP-V_{120}; where, from left side lane 1 represents the crude extract of ω-TA-ELP-V_{60}, lane 1 to 3 shows ω-TA-ELP-V_{60} first to third purification cycles respectively, lane 4 represents the markers, BL21 cells, lane 3 shows wild type ω-TA whereas lane 4 and 5 shows ELP-V_{60} and ELP-V_{120} respectively.

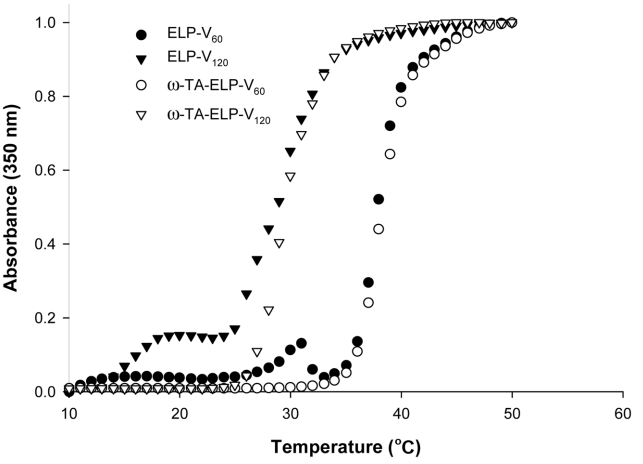

Fig. 3. T_t analysis of ELPs with varying lengths (V_{60} and V_{120}) and ω-TA-ELPs (V_{60} and V_{120}): ELP-V_{60} (Dark down triangle), ELP-V_{120} (Dark circle), ω-TA-ELP-V_{60} (Empty down triangle) and ω-TA-ELPs-V_{120} (Empty circle). Solutions were heated at rate of 1°C/min with constant monitoring of the absorbance at 350 nm.

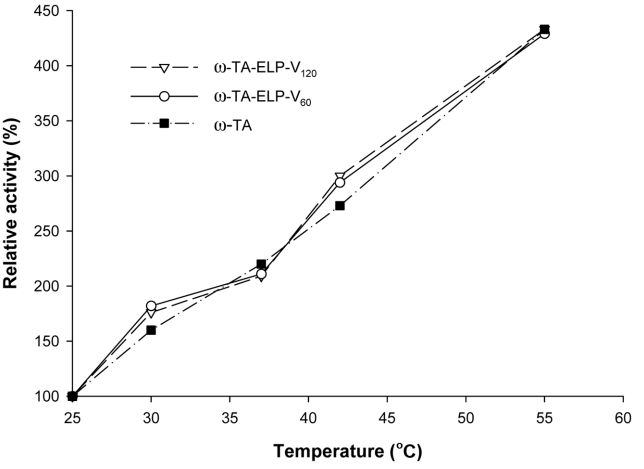

Fig. 4. Effect of temperature on ω-TA-ELPs (V_{60} and V_{120}) fusion proteins. Reaction condition: 10 mM (*S*)-α-MBA, 10 mM pyruvate, 0.115 mg/mL Enzyme, 100 mM potassium phosphate buffer, pH 8.0, total volume 200 μL, reaction time 30 min.

at which the turbidity change reaches half the maximum value [37,38]. The T_t of the ELP fusion proteins was measured at 350 nm by varying temperature using a UV-VIS spectrophotometer. ELP solutions were heated at a rate of 1°C/min with constant monitoring of the absorbance. Fig. 3 demonstrates the behavior of ELPs (V_{60} and V_{120}) and ω-TA-ELPs (ω-TA-ELP-V_{60} and ω-TA-ELP-V_{120}) with respect to the changing temperature. T_t of ω-TA-ELPV_{120} and ω-TA-ELPV_{60} was found to be 30°C and 38°C, respectively. In response to temperature generally, a broad and flat curve will observe in case of ELP fusion proteins in comparison to natural ELPs (Sharp curve). Interestingly, in this case of ω-TA-ELP the sharp and similar pattern of response to temperature was observed. Thus, these results suggested that both the modified ELP-fusion proteins showed similar temperature behavior as that of natural ELPs (Fig. 3).

3.3. Effect of temperature on the fusion of ELPs with ω-TA

After successful purification of ω-TA-ELPs, further the thermostability of these fusion proteins was investigated with respect to different temperature conditions ranging from 0-50°C and compared with the activity of wild type ω-TA. The results suggested that both the fusion complexes start showing 100% relative activity at 25°C, which was considerably similar to the wild type ω-TA. Recent advances in the structural details of ω-TAs have established a fact that the active form of ω-TAs is a homodimer [4]. Thus, it was estimated that the fusion of ω-TA with ELPs would negatively affect the activity of the ω-TA, plausibly this may be due to the disruption of the homo-dimeric structure. Interestingly, no significant difference in activity was observed after aggregation of fusion proteins in comparison with wild type ω-TA. This result suggested that the dimeric form of the ω-TA was preserved well (Fig. 4).

3.4. Effect of DMSO on transition temperature (T_t)

Generally, in case of ω-TA catalyzed reaction various organic co-solvents are often used to increase the solubility of substrates [5,6]. Hence, the effect of co-solvent on ω-TA-ELPs was evaluated and compared with the non-fused ELP proteins (V_{60} and V_{120}). In this case, the DMSO was used as a co-solvent with increasing concentration ranging from the 0-25%. It was observed that, the thermostability of both the ω-TA-ELPs linearly increased with the increasing concentration up to 25% of DMSO. In case of the ω-TA-ELP-$V_{60,}$ major difference in the T_t was observed, wherein a T_t of 38 and 70°C was observed at the increasing concentration of DMSO from 5 to 25% (v/v). It is worth emphasizing that 10% DMSO increased the melting temperature of both the ω-TA-ELPs by 8°C (Fig. 5).

3.5. Substrate specificity of St-TA-ELPs

Following the optimization of reaction parameters, we decided to investigate the substrate specificity of the ω-TA-ELPs by using different amino donors as substrates (Table 1), which included amines (A_1 to A_6), β-amino acids (B_1 to B_5) and γ-amino acids (C_1 to C_3). The results were compared with the activity of wild type ω-TA, wherein the activity of substrate A_3 and C_1 was considered as 100% for amines and amino acid substrates respectively. Using amine compounds as substrates, the activity of ω-TA-ELP-V_{60} was better for A_5. Similarly, both the fused proteins showed nearly two times higher activity compared to wild-

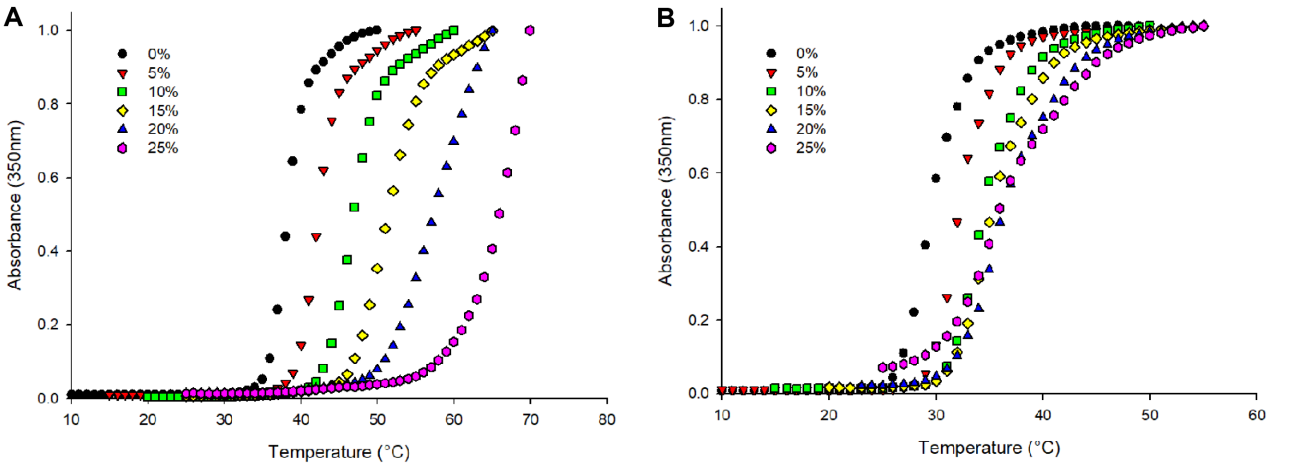

Fig. 5. Effect of DMSO on T_t of A) ELPs (V_{60} and V_{120}); and B) ω-TA-ELPs (V_{60} and V_{120}). 0% (Dark circle), 5% (Dark down triangle), 10% (Dark square), 15% (Dark Diamond), 20% (Dark up triangle), 25% (Dark hexagonal). Solutions were heated at a rate of 1°C/min with constant monitoring of the absorbance at 350 nm.

Table 1. List of substrates used

Sr. No	Substrates	Abbreviation	Sr. No.	Substrate	Abbreviation
1	Isopropyl amine	A_1	12	4-amino-4-phenylbutanoic acid	C_1
2	Benzylamine	A_2	13	4-amino-4-(4-fluorophenyl) butanoic acid	C_2
3	α-methyl benzylamine	A_3	14	4-amino-4-(*p*-tolyl) butanoic acid	C_3
4	4-fluoro α-methyl benzylamine	A_4	15	Acetone	D_1
5	α-ethyl benzylamine	A_5	16	Pyruvic acid	D_2
6	4-phenylbutan-2-amine	A_6	17	α-Keto butyric acid	D_3
7	3-amino-3-phenylpropanoic acid	B_1	18	methyl 2-oxopropanoate	D_4
8	3-amino-3-(4-methoxyphenyl) propanoic acid	B_2	19	3,3-dimethyl-2-oxobutanoic acid	D_5
9	3-amino-3-(3, 4-dimethoxyphenyl) propanoic acid	B_3	20	2-oxohexanoic acid	D_6
10	3-amino-4-phenylbutanoic acid	B_4	21	2-oxopentanedioic acid	D_7
11	3-Amino-3-benzo [1,3] dioxol-5-yl-propionic acid	B_5			

type enzyme in the case of substrate A_1. In the case of substrate A_6, the activity of ω-TA-ELP-V_{60} was approx. four times compared to wild-type ω-TA. In case of amino acid substrates, and wild type enzyme exhibited lower activity towards B_1 compared to the activity of fused-enzymes and the wild-type enzyme was comparable towards most of the other amino acid substrates (Fig. 6).

After testing the activity of ω-TA-ELPs towards various amino donors as substrates, we further checked the activity towards different amino acceptors (carbonyl compounds; D_1 to D_7) which are important counterpart of transamination reaction. In this case, the activity against compound D_2 was considered as 100% and all results were further compared with wild type St-TA. The results suggested that except ω-TA-ELP-V_{60} all other ω-TAs (wild type ω-TA and fused) had no or negligible activity against compound D_5. Whereas, all the enzymes displayed the highest activity towards D_4 substrate (Fig. 7). Moreover, both the fusion proteins showed similar activity in comparison with wild type ω-TA towards substrates D_3 and D_6 (Fig. 7). It has been reported that the ELP tags do not affect the activity of ω-TAs. For instance, Gao *et al*. [27], have reported that the (*R*)-ω-TA fused with ELP exhibited similar specific activity compared to that of its wild-type counterpart [27].

3.6. Substrate inhibition

The enzyme efficiency in the biocatalytic reactions is generally affected by the enzyme properties such as substrate inhibition [4-6]. In the case of ω-TAs, the catalytic efficiency is affected by the higher concentrations of amino acceptor (ketone substrates). Therefore, an experiment was performed to investigate the substrate inhibition by amino acceptor using different concentrations of pyruvate (0-200 mM) as model amino acceptor compound. (*S*)-α-MBA was used as amino donor in these reactions. The results were compared with the wild type ω-TA, where the highest activity (100%)

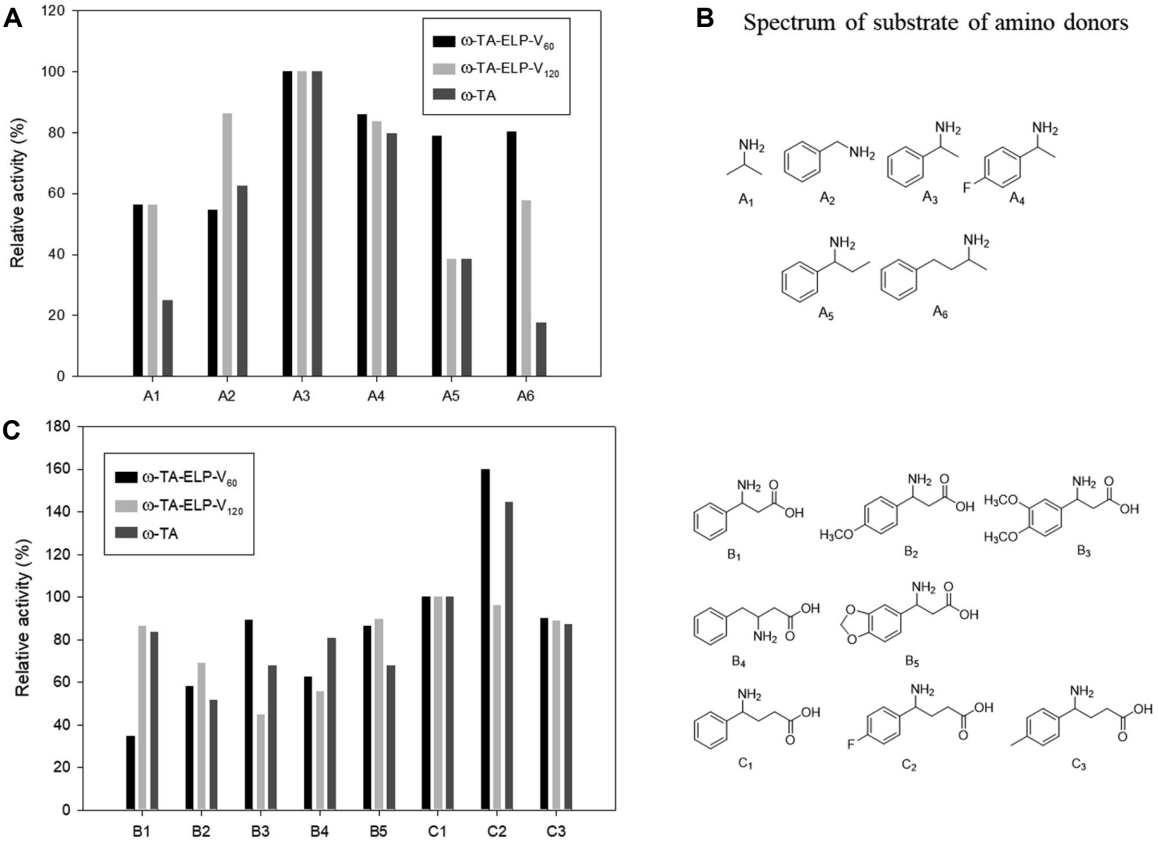

Fig. 6. Amino donor specificity of A) ω-TA-ELPV$_{60}$ and ω-TA-ELPV$_{120}$. Reaction conditions: 15 mM amino donor, 5 mM pyruvate, 0.23 mg/mL enzyme, 0.1 mM PLP, 100 mM potassium phosphate buffer, pH 8.0, 25°C, total volume 200 μL, 30 min.

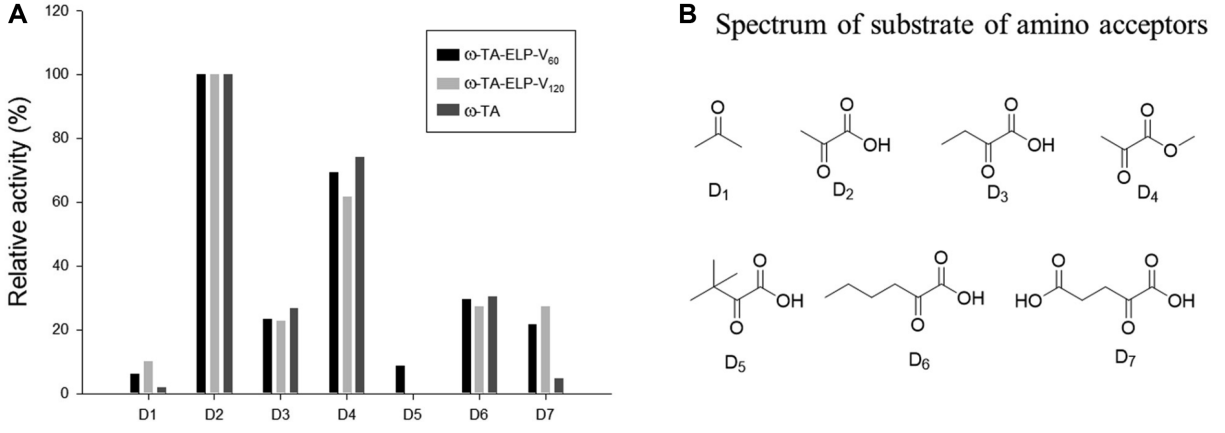

Fig. 7. Amino acceptor specificity of ω-TA-ELPs (V$_{60}$ and V$_{120}$). Reaction conditions: 15 mM amino acceptor, 5 mM (*S*)-α-MBA, 0.23 mg/mL enzyme, 0.1 mM PLP, 100 mM potassium phosphate buffer, pH 8.0, 25°C, total volume 200 μL, for 30 min.

was measured at 5 mM pyruvate concentration. However, further increase in the pyruvate concentration showed decrease in the activity of all TAs (including wild type ω-TA). The activity of all the enzymes decreased to ~45% at 50 mM and the lowest activity (~20%) was observed at 200 mM pyruvate concentration. Furthermore, both of the fused proteins also showed similar pattern of substrate inhibition by amino acceptor (Fig. 8A).

Additionally, we examined the activity of ELP-fused TAs in the presence of increasing concentration of (*S*)-α-MBA from 0 to 500 mM and compared with the activity of that of wild type ω-TA. In this case, pyruvate (15 mM) was used as an amino acceptor counterpart., Similarly to that of wild type ω-TA, the activity of ELP fused ω-TAs

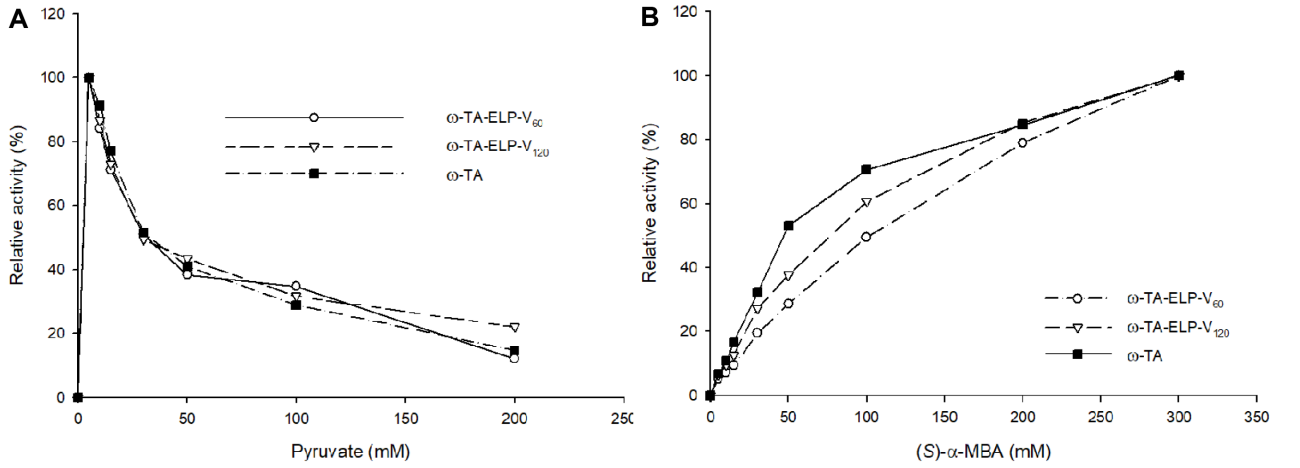

Fig. 8. Substrate inhibition of ω-TA-ELPs (V_{60} and V_{120}) by A) Amino acceptor; Reaction parameters:0-200 mM pyruvate, 15 mM (*S*)-α-MBA, 0.23 mg/mL enzyme, 0.1 mM PLP, 100 mM potassium phosphate buffer, pH 8.0, 25°C, total volume 200 µL, for 30 min; and B) Amino donor; Reaction conditions:0-300 mM (*S*)-α-MBA, 15 mM pyruvate, 0.23 mg/mL enzyme, 0.1 mM PLP, 100 mM potassium phosphate buffer, pH 8.0, 25°C, total volume 200 µL, for 30 min.

gradually increased to 100% at 300 mM substrate concentration (Fig. 8B).

3.7. Reusability of St-TA-ELPs

After successfully investigating the activity of ω-TA-ELPs against the various amino donors and amino acceptors and their substrate inhibition patterns, we observed that the fusion of ELPs with ω-TA show similar activity that of wild type ω-TA. Moreover, unique response of ELPs to the temperature changes can help in the recycling of the ω-TA. Hence, we further decided to examine the reusability of the fusion proteins. A series of twenty cycles was carried out with ω-TA-ELP-V_{120} and *rac*-α-MBA as amino donor and pyruvate as amino acceptor, wherein the enzyme was recovered from the previous reaction, purified and reused for the next cycle. Interestingly, >99% of enantiomeric excess of unreacted substrate (i.e. (*R*)-α-MBA) with 50% of conversion was measured for all the 20 cycles (Table 1). The initial concentration of the protein used was 4 mg/mL and the final concentration of 2.9 mg/mL was measured after 20 repetitive cycles of transamination. This result suggests that the reusability of ω-TA is feasible due to its fusion to ELP-V_{120}, which gives stability to ω-TA by possessing a unique response to the thermal behavior. Ideally, it is expected that the protein would lose the activity following its aggregation. Interestingly, the fusion complex of ω-TA and ELP was found to be active. This can be stated that fusion of ω-TA with ELP could stabilize the enzyme after the aggregation of the fusion complex. Furthermore, fusion with ELPs also helps in recyclizing the active form of the enzyme, which could efficiently complete the transamination reaction for 20 cycles. Generally, the ELP-fusion complexes have been reported for reusability of various enzymes such as α-Amylase [39], thioredoxin and chloramphenicol acetyltransferase [40] to name a few. Recently reported, the separately fused complexes of (*R*)-ω-TA and D-amino acid oxidase with ELP have been successfully immobilized on MnO_2 nanorods for recycling of the enzymes and used in the cascade reactions [36]. However, in the present study we have reported the methodology for recycling of the enzyme without using any additional support material.

3.8. Synthesis of β-amino acid

The applicability of the fusion proteins was tested for the synthesis of β-amino acid 3-ATfBA. This 3-ATfBA is used as an important precursor for the production of various oral antidiabetic drugs such as sitagliptin and retagliptin [41]. In the synthesis of 3-ATfBA, the ω-TA-ELP-V_{120} was used in combination with lipase (*Candida rugosa*) (20 mg/mL) for the *in-situ* hydrolysis of ester form of the substrate. The ester form of β-keto acid was used due to instability of the keto intermediate which was a target substrate for the transamination reaction. This β-keto acid intermediate produced in the reaction was subsequently aminated by ω-TA-ELP-V_{120}. In this 20 mM reaction, initially 9 mM of product was obtained in 5 h, whereas, 13 mM of the β-amino acid (3-ATfBA) (65%) was produced after 21 h (Fig. 9).

4. Conclusions

Generally, the ELPs have been widely used to fuse with various proteins for their recycling [40]. In the present studies, (*S*)-ω-transaminase from the thermophilic eubacterium *Sphaerobacter thermophilus* was fused to ELPs

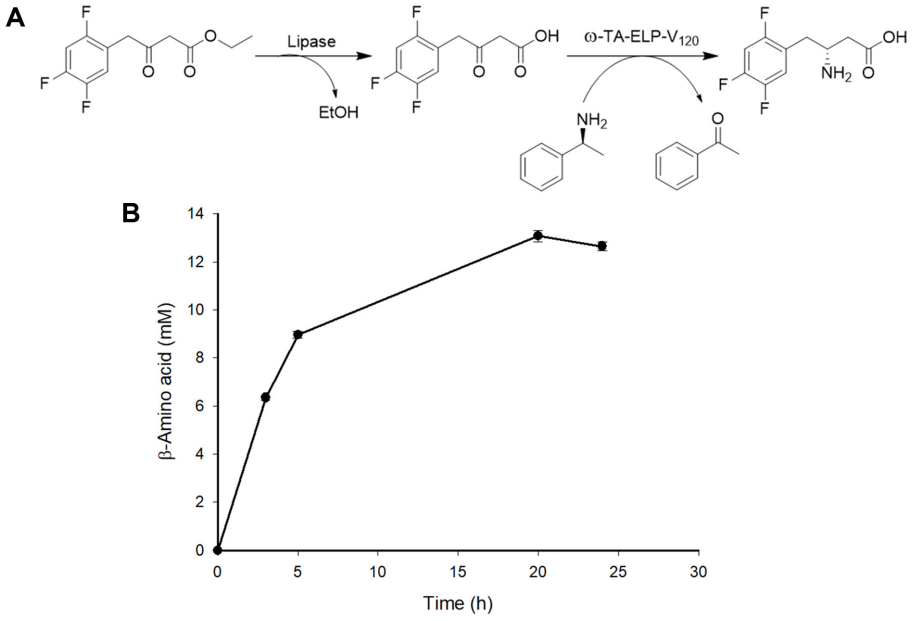

Fig. 9. (A) Schematic diagram for the synthesis of substituted β-amino acid (3-ATfBA) using (*S*)-α-MBA as amino donor by employing ω-TA-ELP-V_{120} in co-ordination with lipase. (B) Biocatalytic synthesis of substituted β-amino acid (3-ATfBA); Reaction condition: 20 mM substrate, 40 mM (*S*)-α-MBA, 1 mg/mL ω-TA-ELP-V_{120}, 0.5 mM PLP, 15 % (v/v) DMSO, 20 mg/mL lipase, 200 mM Tris-HCl Buffer, pH 8.0, 37°C.

(V_{60} and V_{120}) through the genetic engineering process and purified through reverse phase transition method. The active form of ω-TA was preserved after the fusion, due to the unique thermal response of ELPs. Furthermore, the activity of ω-TA-ELPs was investigated towards various amines, β-amino acids and γ-amino acids as a substrate, which suggested that ω-TA-ELPs exhibits similar activity with that of wild type ω-TA. Also, the substrate inhibition by amino donor as well as amino acceptor showed similar patterns for wild type ω-TA and its fused counterpart. The applicability of the fused protein system was evaluated for their reusability in the transamination of pyruvate as a model substrate. The fused protein ω-TA-ELPV_{120} was successfully used for 20 cycles, wherein 10 mM substrate (*rac*- α-MBA) was transformed to acetophenone with 50% conversion and excellent enantioselectivity (*ee* >99%) for (*S*)-α-MBA. Furthermore, this ω-TA-ELP-V_{120} was efficiently used for the production of Sitagliptin precursor 3-ATfBA with 65% conversion. The results of the present studies demonstrated that the fusion of ω-TA with ELPs helps to retain the active form of ω-TA and could be used as one of the efficient strategy for the reuse of ω-TAs for the production of various industrially important compounds.

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