J1 acylase, a glutaryl-7-aminocephalosporanic acid acylase from *Bacillus laterosporus* J1, is a member of the α/β-hydrolase fold superfamily

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Abstract J1 acylase, a glutaryl-7-aminocephalosporanic acid acylase (GCA) isolated from *Bacillus laterosporus* J1, has been conventionally grouped as the only member of class V GCA, although its amino acid sequence shares less than 10% identity with members of other classes of GCA. Instead, it shows higher sequence similarities with *Rhodococcus* sp. strain MB1 cocaine esterase (RhCoE) and *Acetobacter turidans* α-amino acid ester hydrolase (AtAEH), members of the αβ-hydrolase fold superfamily. Homology modeling and secondary structure prediction indicate that the N-terminal region of J1 acylase has an αβ-hydrolase folding pattern. The catalytic triads in RhCoE and AtAEH were identified in J1 acylase as S125, D264 and H309. Mutations to alanine at these positions were found to completely inactivate the enzyme. These results suggest that J1 acylase is a member of the αβ-hydrolase fold superfamily with a serine-histidine-aspartate catalytic triad.

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1. Introduction

Glutaryl-7-aminocephalosporanic acid acylase (GCA) is a group of enzymes that hydrolyze the linkage between C6 of the β-lactam ring and the glutaryl side-chain of glutaryl-7-aminocephalosporanic acid (GL-7-ACA) [1]. These enzymes are industrially important and are exploited in the synthesis of 7-aminocephalosporanic acid (7-ACA), the starting material for the production of semi-synthetic cephalosporin antibiotics. Five classes of GCA have been identified [2]. Members from class I to IV are all heterodimeric in nature, having a smaller α-subunit (16–28 kDa) and a larger β-subunit (40–61 kDa) arisen from a common precursor peptide. Structurally and functionally, they belong to the N-terminal nucleophile hydrolase superfamily [3] and are closely related with the penicillin acylases in the structural classification of protein (SCOP) database (http://scop.mrc.lmb.cam.ac.uk/scop/) [4]. In contrast, class V GCA, with J1 acylase isolated from *Bacillus laterosporus* J1 [5] as the only member, is remarkably different. J1 acylase is monomeric with a single polypeptide chain of 70 kDa [5]. Its amino acid sequence shares only 5–7% identity with those of other classes of GCAs. Limited information is available on its structural and catalytic properties; nevertheless, it is generally believed that J1 acylase has a minor role in antibiotic industry because of its relatively low hydrolytic activity on GL-7-ACA [6].

Amino acid sequence comparisons show that J1 acylase has the highest degree of identity with two recently characterized αβ-hydrolases, namely cocaine esterase (CoCE) of *Rhodococcus* sp. strain MB1 (RhCoE) [7] and α-amino acid ester hydrolase (AEH) of *Acetobacter turidans* (AtAEH) [8]. The αβ-hydrolase fold superfamily is one of the largest protein superfamilies with more than 30 families listed in the SCOP database. These enzymes have diverse amino acid sequences and substrate specificities [9]. Nevertheless, their N-terminal regions all contain a canonical β-α-β arrangement folded into an eight-stranded β-sheet surrounded by α-helices on both sides. Another distinguishable feature of αβ-hydrolase is the presence of the highly conserved catalytic motif, G-X-S-X-G (where X can be any small amino acid); although minor variations, for example, an insertion of Y after the catalytic S, can be observed in some members. In the present report, evidence suggesting the structural classification of J1 acylase as an αβ-hydrolase is presented. Computer modeling of J1 acylase was carried out using the structure of RhCoE as template. With the amino acid sequence alignment and structural model, the catalytically important amino acid residues, including the catalytic triad and the oxyanion-hole residues, were identified in J1 acylase and their roles were confirmed by site-directed mutagenesis.

2. Materials and methods

2.1. Fold recognition and homology modeling

The amino acid sequence of J1 acylase, with the first 27 amino acid residues corresponding to the signal peptide omitted, was submitted to the 3D-PSSM server (http://www.sbg.bio.ic.ac.uk/~3dpssm/index2.html) [10] for secondary structure prediction, fold recognition and
zyme expression was induced by 1 mM isopropyl-
D-thiogalactopyranoside (1 mM IPTG) as described in [19,20], with slight modifications. Equal vol-
umes (125 µl) of enzyme and substrate (GL-7-ACA) were allowed to react in a Thermo-Mixer Eppendorf) at 37°C for a certain period of time before the reaction was terminated by the addition of 1 ml mixture of 20% acetic acid and 0.1 M sodium hydroxide. Then, 0.25 ml of 0.5% 4-(dimethylamino)-benzaldehyde in methanol (w/v) was added for color development. The absorbance at 410 nm, which reflects the amount of the product 7-ACA formed, was measured. For the determination of kinetic parameters, the final enzyme concentration was kept at 25 µg/ml with the substrate concentration increasing from 0.1 to 5 mM. In the study on the pH dependence, three different buffers were used, namely, sodium phosphate buffer (100 mM) for pH 5.5–7.5; Tris-C1 buffer (100 mM) for pH 7.5–8.5; and sodium bicarbonate buffer (100 mM) for pH 8.5–10.0. Buffer controls were performed. The substrate was found to be stable and did not contribute any background under all the experiments conditions employed. Protein concentra-
tion was determined by the method of Bradford using bovine serum albumin as the standard.

2.4. Site-directed mutagenesis
Single amino acid mutations were introduced into J1 acylase by site-
directed mutagenesis using pRSETKan-J1 as the template. The results from the site mutagenic primers used for generating the various mutants were as follows, with the mutated codons underlined: Y57A: 5′-tttgaaacgtggacagattcataa-3′; Y57F: 5′-tttgaaacgtttcatacagatcataa-3′; S125A: 5′-acaatgacctagctatgcctat-3′; Y176A: 5′-atgggcctatctggagtgaatcataa-3′; Y126F: 5′-atgggctattcttttagttgtcataa-3′; W173A: 5′-cgctattttctagctttgcagcctaa-3′; D264A: 5′-aaggagtsgcagctttgcagcctaa-3′; and H308A: 5′-gacttcgctattgcagcctaa-3′. All the antisense primers were exactly complementary. The mutated fragments were filled in with Platinum Taq DNA Polymerase High Fidelity (Invitrogen) and were cloned into pRSETKan. Mutations were confirmed by nucleotide sequencing. All mutants were expressed and purified with the same method as described above for the wild-type enzyme.

3. Results and discussion
3.1. Amino acid sequence and structural analysis
J1 acylase is an enzyme isolated from B. laterosporus J1 which hydrolyzes GL-7-ACA to glutaric acid and 7-ACA. Although its amino acid sequence (only 5–7% identity with other GCAs) and overall protein structure (monomeric instead of heterodimeric) are quite different from other GCAs, J1 acylase has long been classified as a class V GCA. Such assignment is based entirely on its catalytic activity, but not structural characteristics. Recently, it was reported that J1 acylase has higher amino acid sequence similarity to several α/β-hydrolases including RhCoE and AEHs [7,8]; however, a detailed study on the folding and 3D structure of J1 acylase is still lacking. In this study, we provide evidence showing that J1 acylase should be classified as an α/β-hydrolase.

Results from the 3D-PSMM server show that the folding pattern of J1 acylase is closely related to those of dipeptidases, ser-
ne proteases and hydrolases. The secondary structures of J1 acylase predicted by using the different computer programs, including PSI-Pred in the 3D-PSMM server, JPred and SOPMA, are similar to each other and show a β-α-β arrangement in the N-terminal region (Q1-A164) (Fig. 1). The J1 acylase structure exhibits significant similarity with the secondary structures of α/β-hydrolases with >95% similarity. Among α/β-hydrolases, RhCoE shows the highest hit (E-value = 4.89e-18, Pdb 1ju3 [21]), followed by AEHs from A. turidens (E-value = 2.7e-3, Pdb 1nxs [22]) and Xanthomonas citri (E-value = 2.7e-3; Pdb 1mpx [23]) and also a X-prolyl dipeptidyl aminopeptidase (PepX) from Lactococcus lactis (E-value = 2.7e-3, Pdb 1lns [24]).

Sequence analyses by PSI-BLAST also show that J1 acylase is closely related to the α/β-hydrolase fold enzymes (Fig. 2). It exhibits a sequence identity of 34.4% with RhCoE (E-value = 1e-115), 26.7% with Xanthomonas citri α-amino acid ester hydrolase (XAEH) (E-value = 8e-99) and 25.5% with AraEH (E-value = 6e-95). In addition, J1 acylase also shows a high degree of similarity with a putative S15 peptidease from Solibacter usitatus (SuPep; 33.7% identity, E-value = 1e-136). The α/β-hydrolase fold enzymes employ a catalytic triad of nucleophile-histidine-acid in their reaction mechanism. For example, in RhCoE and AraEH, the catalytic triad is the

potential modeling templates retrieval. JPred (http://www.comp-
ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_software.html) [12] were also used for secondary structure prediction. J1 acylase homol-
ogues were searched for using PSI-BLAST (http://www.ncbi.nlm.nih.
gov/BLAST/) [13] and multiple sequence alignments were constructed by CLUSTAL W version 1.82 (http://www.ebi.ac.uk/clus
talw) [14] with default gap penalty. For homology modeling, the preliminary sequence alignments between J1 acylase and potential templates were constructed by FUGUE (http://www.cryst.bioc.cam.ac.uk/~fugue/) [15] followed by manual optimization using Swiss-PdbViewer [16]. Alignment gaps were adjusted after considering secondary structure con

2.2. Expression and purification
The J1 acylase gene was amplified, using the plasmid pACY11-3 (International Patent Organism Depository, Tsukuba, Japan) as tem
plate, by the sense primer 5′-tgagctagtatcagatagaaagaa-3′ and the anti-sense primer 5′-aaggatctttcattgctaa-3′, with a Nhel and a BamHI restriction site added to the 5′ and 3′ ends, respectively. The 1.9 kb fragment obtained which contains the coding region for the sig
nal peptide was purified and ligated to the Nhel/BamHI-opened pRSETKan [18], a modified version of pRSET-A (Invitrogen), to generate the construct pRSETKan-J1. An 1 ml aliquot of an overnight culture of Escherichia coli strain BL21 (DE3) E. coli transformed with pRSETKan-J1 was inoculated into 200 ml of 2x LB broth containing 50 µg/ml of kanamycin. After a 24-h incubation at 37°C (250 rpm), en
zyme expression was induced by 1 mM isopropyl-β-D-thiogalactopyra
noside for 5 h under the same conditions. Cells were collected by centrifugation at 5000 rpm for 20 min and resuspended in 50 mM so
dium phosphate buffer, 2 mM EDTA, pH 7.5. All the subsequent steps were carried out at 4°C. The resuspended cells were lysed by sonica
tion and centrifuged at 13000 rpm for 30 min to remove the insoluble de
bris. The supernatant was then applied to a frac
tage column (25 x 80 mm; EMD Bioscience) equilibrated with the re-suspen
sion buffer. After washing with the same buffer, the bound proteins were eluted with an increasing gradient of 0–0.2 M sodium chloride. Fractions showing acylase activity were pooled, concentrated and dia
lyzed against 0.8 M ammonium sulfate in 50 mM sodium phosphate buffer, pH 7.0. The dialyzed sample was loaded onto a phenyl-Sephar
ose CL-4B column (25 x 23 mm; Pharmacia) equilibrated with the dialysis buffer. Fractions were pooled and concentrated with a decreasing gradient of 0.8–0.4 M ammonium sulfate in buffer. The active fractions were con
centrated and dialyzed against 20 mM Tris-C1 buffer, 0.15 M sodium chloride, pH 8.0, before being loaded onto another Hi
Load 16/60 Superdex 200 gel-filtration column (Amersham Bioscience) operated by the AKTA Purifier System (Amersham Bioscience) with a flow rate of 1.5 ml/min. The active fractions were concentrated and dia
lyzed against 20 mM Tris-C1 buffer, pH 8.0, before for further analyses. The purity of the recombinant J1 acylase was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), with stacking and running gels of 5% and 12% polyacryl
amide, respectively.

2.3. Enzyme kinetics
The activity of J1 acylase was determined by a spectrophotometric method as described in [19,20], with slight modifications. Equal vol
umes (125 µl) of enzyme and substrate (GL-7-ACA) were allowed to react in a Thermo-Mixer Eppendorf) at 37°C for a certain period of time before the reaction was terminated by the addition of 1 ml mixture of 20% acetic acid and 0.1 M sodium hydroxide. Then, 0.25 ml of 0.5% 4-(dimethylamino)-benzaldehyde in methanol (w/v) was added for color development. The absorbance at 410 nm, which reflects the amount of the product 7-ACA formed, was measured. For the determination of kinetic parameters, the final enzyme concentration was kept at 25 µg/ml with the substrate concentration increasing from 0.1 to 5 mM. In the study on the pH dependence, three different buffers were used, namely, sodium phosphate buffer (100 mM) for pH 5.5–7.5; Tris-C1 buffer (100 mM) for pH 7.5–8.5; and sodium bicarbonate buffer (100 mM) for pH 8.5–10.0. Buffer controls were performed. The
classical serine–histidine–aspartate combination. Alignment of the sequences reveals that the catalytic triad residues of RhCocE and AtAEH can also be found in J1 acylase as S125, D264 and H309 (Fig. 2). The putative catalytic S125 is located within the highly conserved G-L-S-Y-M-A motif (G123-A128). The presence of this consensus motif allows Fig. 1. Predicted secondary structure of J1 acylase. The secondary structures were predicted by three different methods (PSI-Pred, JPred and SOPMA) with the first 27 amino acid residues corresponding to the signal peptide omitted. C = random coil, H = α-helix, E = β-strand, and T = β-turn.

Fig. 2. Multiple sequence alignments of J1 acylase (J1) with CocE of Rhodococcus sp. strain MB1 (RhCocE), AEH of A. turbidans (AtAEH) and S15 peptidase of S. usitatus (SuPep) using CLUSTAL W version 1.82. The sequences of RhCocE and AtAEH were derived from the corresponding PDB files. The consensus catalytic motifs are underlined while the catalytic triads and oxyanion-hole residues are highlighted in gray and black, respectively.
the easy identification of the catalytic serine. In α/β-hydrolases, this motif is bent sharply to form the ‘nucleophile elbow’ such that the catalytic serine is at the turn and the following residue, using its backbone NH, forms part of the oxyanion-hole [9]. The tyrosine insertion after the catalytic serine is a characteristic of the PepX family in the α/β-hydrolase superfamily [25]. In J1 acylase, the last residue in this consensus motif is an alanine, but not glycine which exists in most α/β-hydrolases. Such replacement has also been observed in Lactobacillus rhamnosus PepX [26] and Agrobacterium radiobacter AD1 epoxide hydrolase [27]. Conserved glycine residues are commonly found near catalytically active residues to allow the productive interaction of the substrate with the active site residues [9,28]. The replacement by an alanine may thus increase the steric constraint in the nucleophile elbow because of the reduced rotation freedom of the C–N and C–C bonds. In addition to the consensus motif surrounding the catalytic S125, the sequences flanking D264 (G-G-W-Y-D) and H309 (G-P-W-X-H) in J1 acylase are also highly conserved. The sequence alignments show that the oxyanion-hole residues (Y44 and W166) in RhCocE [21,29] also exist at corresponding positions in J1 acylase (Y57 and W173).

The X-ray structure of RhCocE (Pdb 1ju3) [21] was chosen as the template for homology modeling of J1 acylase because the two enzymes share the highest degree of similarity in both primary and secondary structures. The resultant 3D-model of
J1 acylase has an overall PROCHECK G-factor of −0.1, indicating that the backbone architecture is acceptable with a root-mean-square deviation of 0.03 Å from that of the template. Over 98% of the residues were found in the most allowed region in the Ramachandran plot. However, the putative catalytic nucleophile S125 was found in the disallowed region, an observation which is not uncommon among z/β-hydrolases, with torsion angles of $\phi = 64.9^\circ$ and $\psi = −119^\circ$. The overall secondary structure of the 3D-model of J1 acylase in general agrees with those predicted by PSI-Pred and JPred.

Three domains can be distinguished in the J1 acylase model (Fig. 3A). Domain 1 (Q1-A164 and W252-A386) consists of a $\beta$-sheet surrounded on one side by two $\alpha$-helices and on the other side by four $\alpha$-helices. The folding topology of Domain 1 is a typical canonical $\alpha/\beta$-hydrolase (Fig. 3B) with the second strand ($\beta_2$) being antiparallel to the others. The catalytic triad and oxyanion-hole residue Y57 are all located within Domain 1 at positions typical in other $\alpha/\beta$-hydrolases. As predicted by Swiss-PdbViewer, their side chains protrude towards the bottom of the active site and are in close proximity to allow hydrogen bond formation (Fig. 4). The catalytic triad S125, H309 and D264 are linked by two hydrogen bonds: one between S125-OG and H309-NE2 (3.06 Å) and the other between H309-ND2 and D264-OD1 (2.53 Å). Domain 2 (M165-T251) is a cap insertion between strands $\beta_6$ and $\beta_7$ of Domain 1 (Fig. 3A). It is a helix-rich region and is common in $\alpha/\beta$-hydrolases for the regulation of substrate binding and activity [9]. Domain 3 (R387-E607) is a C-terminal extension of Domain 1 (Fig. 3A). Interestingly, the sequence and structure of Domain 3 are uncommon in other $\alpha/\beta$-hydrolases or PepX, except RhCocE and AEHs. Actually, the sequence identity of this region among the four enzymes (J1 acylase, RhCocE, AtAEH and SuPep) shown in Fig. 2 is the highest when compared with the other two domains. An insertion within this domain is present in AtAEH (M554-G563) but not in J1 acylase, RhCocE or SuPep (Fig. 2). Such an insertion can also be found at the corresponding position in XcAEH (E525-G535) and is known to be involved in the tetramerization of the molecule [23]. On the other hand, RhCocE, the closest homologue of J1 acylase, exists as a monomer [7]. Thus, the exact role of Domain 3 in these enzymes remains to be identified.

![Fig. 4. The catalytic triad and oxyanion-hole residues in J1 acylase.](image)

3.2. Enzyme expression, purification and kinetics

J1 acylase was successfully expressed in E. coli strain BL21(DE3)pLysS. The recombinant J1 acylase accounts for ~10% of the total soluble protein (Fig. 5). After three chromatographic steps, the J1 acylase preparation was homogeneous, as demonstrated by a single band on SDS-PAGE (Fig. 5). Using the present expression and purification systems, over 20 mg of purified J1 acylase could be obtained per liter of bacterial culture. The recombinant enzyme could hydrolyze GL-7-ACA with $k_{\text{cat}}$ and $K_M$ values of 7.54 ± 0.64 s$^{-1}$ and 0.19 ± 0.01 mM, respectively (Table 1). Substrate specificity study shows that J1 acylase could also act on glutaryl-7-aminodesacetoxycephalosporanic acid (GL-7-ADCA) with $k_{\text{cat}}$ and $K_M$ values of 7.04 ± 0.46 s$^{-1}$ and 0.33 ± 0.04 mM, respectively. However, it shows no detectable activity on cephalaxin (p-phenylglycinyl-7-ADCA), cephalorixin (p-hydroxy-phenylglycinyl-7-ADCA), ampicillin (p-phenylglycinyl-6-aminopenicillanic acid), amoxicillin (p-hydroxy-phenylglycinyl-6-aminopenicillanic acid) and glutaryl-6-aminopenicillanic acid at concentrations up to 25 mM. These antibiotics are readily hydrolyzed by other AEHs which fail to act on GL-7-ACA or GL-7-ADCA [8].

The pH dependence of J1 acylase kinetic parameters towards GL-7-ACA is shown in Fig. 6. J1 acylase has the highest $k_{\text{cat}}$ at pH 8.0–8.5 (Fig. 6A). At higher pH, activity decreases such that at pH $\geq$ 10, a complete loss of activity was observed. On the other hand, the binding between the substrate and the enzyme appears to be most efficient at pH 6.5–7.0; at higher pH, the $K_M$ value increases sharply (Fig. 6B).

![Fig. 5. SDS-PAGE of J1 acylase at different stages of purification.](image)
Table 1
Kinetic parameters of J1 acylase and its mutants towards the hydrolysis of GL-7-ACA

<table>
<thead>
<tr>
<th></th>
<th>[k_{cat} \text{ (s}^{-1}) ]</th>
<th>[K_M \text{ (mM)}]</th>
<th>[k_{cat}/K_M \text{ (s}^{-1}\text{mM}^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>7.54 ± 0.64</td>
<td>0.19 ± 0.01</td>
<td>39.5 ± 4.49</td>
</tr>
<tr>
<td>S125A</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>D264A</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H309A</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Y57A</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Y57F</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Y126A</td>
<td>7.16 ± 1.02</td>
<td>1.69 ± 0.37</td>
<td>4.31 ± 0.43</td>
</tr>
<tr>
<td>Y126F</td>
<td>6.05 ± 0.08</td>
<td>0.15 ± 0.02</td>
<td>39.5 ± 4.15</td>
</tr>
<tr>
<td>W173A</td>
<td>0.23 ± 0.03</td>
<td>1.20 ± 0.21</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Wild-type</td>
<td>7.54 ± 0.64</td>
<td>0.19 ± 0.01</td>
<td>39.5 ± 4.49</td>
</tr>
</tbody>
</table>

Enzyme activities were determined in 100 mM Tris–Cl, pH 8.0, with 0.1–5 mM GL-7-ACA as substrate. NA: no activity. Data represent the average ± S.D. obtained from three independent preparations of enzyme.

Fig. 6. pH dependence of (A) \(k_{cat}\), (B) \(K_M\), and (C) \(k_{cat}/K_M\) in the J1 acylase catalyzed hydrolysis of GL-7-ACA. The activity was determined with 0.1–5 mM GL-7-ACA in 100 mM sodium phosphate (pH 5.5–7.5), 100 mM Tris–Cl (pH 7.5–8.5), or 100 mM sodium bicarbonate (pH 8.5–9.5).

3.3. Site-directed mutagenesis

To confirm the importance of the putative catalytic triad (S125, D264 and H309) and oxyanion-hole residues (side chains of Y57 and W173, backbone NH of Y126) in J1 acylase, site-directed mutagenesis was employed. Each of these amino acids was mutated to alanine. The mutants were successfully expressed in E. coli strain BL21(DE3)pLysS and purified. The catalytic triad mutants S125A, D264A and H309A are completely inactive even at 25 mM GL-7-ACA (Table 1).

For the three conserved oxyanion-hole residues in J1 acylase, the effect of mutation is different. While Y57A is completely inactive, W173A is marginally active (\(k_{cat} = 0.23 ± 0.03 \text{s}^{-1}\)) and Y126A (\(k_{cat} = 7.16 ± 1.02 \text{s}^{-1}\)) is almost as active as the wild-type enzyme (Table 1). This is consistent with the observation that in \(\alpha/\beta\)-hydrolases, the residue immediately following the catalytic nucleophile (i.e., Y126 in J1 acylase) uses its backbone NH, not its side chain, to stabilize the oxyanion. The \(K_M\) values of mutants W173A and Y126A are increased 6.3- to 8.9-fold when compared with that of wild-type J1 acylase (Table 1). Alanine mutations of W166 in \(Rh\)CocE and Y206 in \(At\)AEH, the oxyanion-hole residues, have been reported to give similar results, with an 80- and 8.9-fold increase, respectively, in the \(K_M\) value [29,30]. In the crystal structure of \(Rh\)CocE, the W166 side-chain π-stacks and makes extensive van der Waals contacts with the benzoyl moiety of cocaine [29]. Thus, it is expected that a W166A mutation in \(Rh\)CocE would have a more deleterious effect on substrate binding than a W173A mutation in J1 acylase, as the substrate GL-7-ACA contains only an aliphatic acyl moiety.

To study the possible role of the side-chain OH groups in Y57 and Y126, they were also mutated to phenylalanine. Y57F is inactive, consistent with the 3D-model in which the hydroxyl group in Y57 forms a hydrogen bond with the side chain of W173 (Fig. 4). In contrast, Y126F retains ~80% of activity (\(k_{cat} = 6.05 ± 0.08 \text{s}^{-1}\)) and the \(K_M\) value (0.15 ± 0.02 mM) is also similar to that of the wild-type enzyme (Table 1). Such results suggest that the phenyl ring, not the hydroxyl group, in Y126 is more important for substrate binding. However, up to now, there is no structural evidence to support any interaction between the substrate and the corresponding tyrosine residue in \(Rh\)CocE and \(At\)AEH.

In summary, we have shown that J1 acylase possesses the following characteristics of \(\alpha/\beta\)-hydrolase: (1) the consensus catalytic motif G-L-S-Y-M-A; (2) the conserved catalytic triad (serine–histidine–aspartate); and (3) the canonical \(\alpha/\beta\)-hydrolase fold in the N-terminal region. Mutants of the catalytic triad and oxyanion-hole residues show similar catalytic behavior as their counterparts in \(Rh\)CocE and \(At\)AEH. Based on such structural and catalytic information, we propose that J1 acylase should be classified as a member of the \(\alpha/\beta\)-hydrolase fold superfamily.

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References


