Structure and Catalytic Mechanism of Yeast 4-Amino-4-deoxychorismate Lyase*

Received for publication, April 24, 2013, and in revised form, June 29, 2013 Published, JBC Papers in Press, July 1, 2013, DOI 10.1074/jbc.M113.480335

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Background: *Saccharomyces cerevisiae* Abz2 is a pyridoxal 5′-phosphate-dependent lyase that converts 4-amino-4-deoxychorismate (ADC) to para-aminobenzoate and pyruvate. To investigate the catalytic mechanism, we determined the 1.9 Å resolution crystal structure of Abz2 complexed with PLP, representing the first eukaryotic ADC lyase structure. Unlike *Escherichia coli* ADC lyase, whose dimerization is critical to the formation of the active site, the overall structure of Abz2 displays as a monomer of two domains. At the interdomain cleft, a molecule of cofactor PLP forms a Schiff base with residue Lys-251. Computational simulations defined a basic clamp to orientate the substrate ADC in a proper pose, which was validated by site-directed mutageneses combined with enzymatic activity assays. Altogether, we propose a putative catalytic mechanism of a unique class of monomeric ADC lyases led by yeast Abz2.

Folate and coenzyme Q (ubiquinone or CoQ)2 are very important cofactors during biological processes. Folate and its derivatives play key roles in one-carbon transfer reactions, which are involved in the biosynthesis of a wide variety of essential compounds such as methionine, purines, and thymidylate (1). Folate deficiency in humans can cause genomic instability by misincorporation of uracil into the DNA (2) and is associated with many physical disorders such as cardiovascular disease, impaired cognitive performance, and Alzheimer’s disease (3). As an aromatic precursor of the benzoquinone ring of CoQ, PABA is synthesized in *Escherichia coli* step by step via three steps; in the first step chorismate is converted to 2-amino-2-deoxyisochorismate (23). The biosynthesis pathway of PABA has been identified in a few species of bacteria, microbial eukaryotes, and plants. However, both folate and CoQ can utilize a common precursor PABA in vivo (11–17). In the folate synthesis pathway, PABA and 7,8-dihydro-6-hydroxymethylpterin pyrophosphate are coupled to produce 7,8-dihydroprotoberine, which is subsequently gluta- mylated to generate 7,8-dihydrofolate and ultimately reduced to yield tetrahydrofolate. The CoQ biosynthesis pathway, mostly based on the findings of Tran and Clarke (18) in yeast, usually starts with the formation of a hydroxybenzoic acid head group and a lipophilic polyisoprenoid tail (5). Alternatively, PABA could enter the CoQ biosynthesis pathway (15, 16) as a competitor of *para*-hydroxybenzoate, which has long been recognized as an aromatic precursor of the benzoquinone ring of CoQ.

The biosynthesis pathway of PABA has been identified in a few species of bacteria, microbial eukaryotes, and plants (12–14, 19–21). In most cases, PABA is synthesized from chorismate via two steps (Fig. 1): first, ADC is synthesized from chorismate and glutamine; and second, ADC is converted to PABA (22). As an exception, the PABA biosynthesis pathway in *Bacillus subtilis* contains three steps; in the first step chorismate is converted to 2-amino-2-deoxyisorhoccumarate (23). PABA is synthesized in *Escherichia coli* step by step via three enzymes encoded by genes designated *pabA*, *pabB*, and *pabC*, respectively (12, 22). The first step involves two reactions: PabA acts as a glutamine amidotransferase to provide the amino group to PabB, which has a synthase activity for the replacement of the hydroxyl group at the C4 position of chorismate by

*This work was supported by Ministry of Science and Technology of China Project 2012CB911000 and National Natural Science Foundation of China Grant 31170695.

The atomic coordinates and structure factors (code 4K6N) have been deposited in the Protein Data Bank (http://wwpdb.org/).

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2 The abbreviations used are: CoQ, coenzyme Q; PLP, pyridoxal 5′-phosphate; ADC, 4-amino-4-deoxychorismate; PABA, *para*-aminobenzoate; SeMet, selenomethionine; EcPabC, *E. coli* PabC; LpPabC, *L. pneumophila* PabC; MIPabC, *M. iotii* PabC; RMSD, root mean square deviation.
the amino group. PabA and PabB normally work together to produce ADC, thus collectively termed ADC synthase. When sufficient ammonia is supplied, PabB can execute the synthase activity in the absence of PabA in vitro (22). In the second step, PabC serves as a PLP-dependent ADC lyase catalyzing the release of pyruvate and the aromatization of the ADC ring to give PABA. In contrast to E. coli, genetic and genomic data or biochemical analyses have demonstrated that ADC synthases encoded by Streptomyces griseus and Plasmodium spp. in addition to several types of fungi and plants are enzymes of two fused domains that are homologous to PabA and PabB, respectively (13, 20, 24–26). For instance, Saccharomyces cerevisiae ABZ1/YMR289w encodes a bifunctional ADC synthase of two domains (26), whereas ABZ2/YMR829w encodes an ADC lyase (EC 4.1.3.38) (17).

To date, four crystal structures of prokaryotic ADC lyases have been deposited in the Protein Data Bank, including E. coli PabC (EcPabC, Protein Data Bank code 1ET0) (27), Pseudomonas aeruginosa PabC (Protein Data Bank code 2Y4R) (28), Thermus thermophilus PabC (Protein Data Bank code 2ZGI) (29), and L. pneumophila PabC (LPpPabC, Protein Data Bank code 3LUL). All of these display a similar dimeric overall fold, but with differences in the active pockets. In addition, a protein of unknown function from Mesorhizobium loti (Protein Data Bank code 3QQM) has been annotated as a putative amino acid aminotransferase. However, comparisons of both the overall structure and active sites indicated that it is most likely an ADC lyase.

Nevertheless, the structure of eukaryotic ADC lyase has not been determined. Here, we present the crystal structure and active sites indicated that it is most likely an ADC lyase.

EXPERIMENTAL PROCEDURES

Overexpression and Purification of Abz2 and Mutants—The ABZ2 gene was amplified from purified genomic DNA extracted from S. cerevisiae S288C. The coding sequence was cloned into a PET28a-derived expression vector with an N-terminal His6 tag and overexpressed in E. coli BL21 (DE3) strain (Novagen) at 37 °C using LB culture medium (10 g of NaCl, 10 g of Bacto Tryptone, and 5 g of yeast extract per liter) containing 30 μg/ml kanamycin. When the Abz2-expressing cells were grown to an A600nm of 0.8, expression of the recombinant proteins was induced with 0.2 mM isopropyl β-D-1-thiogalactopyranoside, and cell growth was continued for another 20 h at 16 °C before harvesting. The cells were collected and resuspended in the lysis buffer (100 mM NaCl, 20 mM Tris-Cl, pH 8.0). After 30 min of sonication and centrifugation at 12,000 × g for 30 min, the supernatant containing the soluble target protein was collected and loaded onto a nickel-chelating column (GE Healthcare) equilibrated with the binding buffer (100 mM NaCl, 20 mM Tris-Cl, pH 8.0). The target protein was eluted with 300 mM imidazole and further loaded onto a HiLoad 16/60 Superdex™ 200 column (GE Healthcare) pre-equilibrated with 100 mM NaCl, 20 mM Tris-Cl, pH 8.0. Fractions containing the target protein were pooled and concentrated to 10 mg/ml for crystallization. Protein samples for enzymatic activity assays were collected at a lower concentration (1 mg/ml). Protein purity was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the protein sample was stored at −80 °C.

Selenomethionine (SeMet)-labeled Abz2 protein was expressed in E. coli strain B834 (DE3) (Novagen). A culture of transformed cells was inoculated into LB medium and incubated at 37 °C overnight. The cells were harvested when the A600nm reached 0.2 and were then washed twice in the M9 medium. The cells were then cultured in SeMet medium (M9 medium with 25 mg/liter l-SeMet and the other essential amino acids at 50 mg/liter) to A600nm of 0.6–0.8. The remaining steps of protein expression, purification, and storage were the same as those for the native protein.

Site-directed mutagenesis was performed by using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) with the plasmid encoding the wild-type Abz2 as the template. The mutant proteins were expressed, purified, and stored in the same manner as the wild-type protein.

Crystallization, Data Collection, and Processing—Both native and SeMet-substituted crystals of native Abz2 were grown at 16 °C using the hanging drop vapor diffusion technique, with the initial condition of mixing 1 μl of 10 mg/ml protein sample with an equal volume of the reservoir solution (20% (v/v) polyethylene glycol monomethyl ether 5,000, 0.1 M Bis-Tris, pH 6.2). Typically, crystals appeared in 1–2 days and reached the maximum size in 1 week. The SeMet derivative crystals were grown under the same conditions. The crystals were transferred to cryoprotectant (reservoir solution supplemented with
25% glycerol) and flash-frozen with liquid nitrogen. Both the native and SeMet derivative data for single crystals were collected at 100 K in a liquid nitrogen stream using Beamline 17U. The native and SeMet derivative data for single crystals were collected at 100 K in a liquid nitrogen stream using Beamline 17U.

### RESULTS AND DISCUSSION

**Overall Structure**—Each asymmetric unit of the crystal contains one molecule of Abz2 (Fig. 2A). The largest total interface area between any two molecules in the crystal is 517 Å², which is too small to form a stable dimer. In fact, Abz2 also exists as a monomer in solution with an approximate mass of 44 kDa as determined by gel filtration chromatography. We applied the full-length Abz2 for crystallization; however, the segment of residues Met-1–Asn-22 could not be traced in the electron density map. Electrophoresis assay of the crystals confirmed that the protein had not been degraded during the crystallization process, indicating the high flexibility of this segment. The overall structure of Abz2 (Pro-23–Tyr-374) is composed of an N-terminal domain (Domain I, Pro-23–Ser-229) and a C-terminal domain (Domain II, Thr-230–Tyr-374) (Fig. 2A). Domain I comprises eight α-helices (α1–α8) and six β-strands (β1–β6) (Fig. 2B). An open β-sheets structure is formed by β-strands (β1, β5, and β6) that arrange in an antiparallel manner. Structural analysis demonstrated that apart from this β-sheet structure and two α-helices (α4 and α5), the remaining texture of Domain I displays as a unique fold referred to as the auxiliary subdomain, which is absent from all previous PabB structures (Fig. 2A). Three α-helices (α1–α3), combined with three short β-strands (β2–β4) that also arrange in an antiparallel manner, assemble the fold that link β1 to α4. Domain II consists of three α-helices (α9–α11) and eight β-strands (β7–β14). Three pairs of β-hairpins (β8/β9, β10/β11, and β13/β14) combined with strands β7 and β12 are sandwiched by two solvent-exposed α-helices (α10 and α11) on both sides. The PLP cofactor that is covalently bound to Lys-251 fits well in the large cleft between the two domains (Fig. 2A).

**Structural and Biochemical Analysis of Yeast Abz2**

Abz2-PLP and Se-Abz2

| Data collection | Abz2-PLP | Se-Abz2 |
|----------------|----------|---------|
| Space group    | C222₁    | C2      |
| μ, b, c (Å)    | 90.97, 113.89, 86.00 | 90.69, 114.04, 85.78 |
| Resolution range (Å) | 50.00–1.90 (1.97–1.90) | 50.00–2.20 (2.28–2.20) |
| Unique reflections | 35,258 (3,487) | 43,953 (4,338) |
| Completeness (%) | 99.9 (100.0) | 99.3 (98.7) |
| | <Rmerge>(%) | 20.4 (20.4) | 22.1 (22.3) |
| | <Rmerge>(%) | 20.4 (20.4) | 22.1 (22.3) |
| | Average redundancy | 6.2 (6.2) | 6.9 (6.8) |
| Structure refinement | | |
| Resolution range (Å) | 36.79–1.90 | 36.79–1.90 |
| Rmerge/(%) | 17.4/20.2 | 17.4/20.2 |
| Number of protein atoms | 2815 | 2815 |
| Number of water atoms | 260 | 260 |
| RMSD bond lengths (Å) | 0.10 | 0.10 |
| RMSD bond angles (°) | 1.259 | 1.259 |
| Mean B factors (Å²) | 31.7 | 31.7 |
| Ramachandran plot (residues, %) | | |
| Most favored (%) | 96.6 | 96.6 |
| Additional allowed (%) | 3.4 | 3.4 |
| Outliers (%) | 0 | 0 |
| Protein Data Bank entry | 4K6N | 4K6N |

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**TABLE 1**

Crystal parameters, data collection, and structure refinement

The data values in parentheses refer to statistics in the highest bin.

| Abz2-PLP | Se-Abz2 |
|----------|---------|
| Completeness (%) | 99.9 (100.0) | 99.3 (98.7) |
| Outliers (%) | 0 | 0 |
| Average redundancy | 6.2 (6.2) | 6.9 (6.8) |
| Resolution range (Å) | 50.00–1.90 (1.97–1.90) | 50.00–2.20 (2.28–2.20) |
| Unique reflections | 35,258 (3,487) | 43,953 (4,338) |
| Completeness (%) | 99.9 (100.0) | 99.3 (98.7) |
| | <Rmerge>(%) | 20.4 (20.4) | 22.1 (22.3) |
| | Average redundancy | 6.2 (6.2) | 6.9 (6.8) |

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**High Performance Liquid Chromatography Analysis—ADC lyase activity** was determined using a coupled assay by reverse phase HPLC as previously described with minor modifications (17, 22). In this assay, recombinant *E. coli* PabB was applied to generate ADC, which is the substrate of Abz2. The assays were performed in a 50-μM system containing the buffer of 50 mM Tris-Cl buffer, pH 8.5, 100 mM (NH₄)₂SO₄, 5 mM MgCl₂, and 1 mM chorismic acid (Sigma). The reactions were initiated by the addition of 10 μM PabB, and the mixture was incubated at 25°C for 30 min followed by the removal of the protein by centrifuging with a 10-kDa cut-off filter. 20 μM wild-type Abz2 and mutants were added for further incubation at 30°C for 30 s. Meanwhile, reaction control lacking wild-type Abz2 or mutants was also performed. Then the reactions were terminated by mixing with 10 μl of 75% acetic acid each and then were centrifuged at 12,000 × g for 10 min. All supernatants were then subjected in volumes of 10 μl to RP-HPLC. Buffer A (0.5% acetic acid) and Buffer B (5% acetonitrile in 0.5% acetic acid) served as the mobile phase. Buffer A was used to equilibrate the C18 column (Zorbax, 300SB_C18, 4.6 × 250 mm; Agilent). The gradient was formed as follows: 100% Buffer A for 7 min followed by 100% Buffer B for 13 min and then 100% Buffer A for 30 min for re-equilibrating the column, at a flow rate of 1 ml/min. Also, ADC, chorismic acid, and PABA standards were quantified by HPLC analysis using a series concentrations ranging from 0.5 to 2 μM and 0.125 to 1 mM, respectively.

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**RESULTS AND DISCUSSION**

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**Structural and Biochemical Analysis of Yeast Abz2**

Abz2-PLP and Se-Abz2
Structural and Biochemical Analysis of Yeast Abz2

Superposition of Abz2 against all PabC structures yielded an overall root mean square deviation (RMSD) in the range of 1.7–2.3 Å over ~190 Ca atoms, implying that they share a quite similar core structure, although they adopt very low primary sequence identities. At a closer viewpoint on Domain II, we found that Abz2 and all PabC proteins adopt a quite similar pattern of twisted antiparallel β-sheet sandwiched by two α-helices. With regard to Domain I, despite the moiety defining the active cleft, it was well superimposed to the counterparts of PabC proteins, and the auxiliary subdomain makes Abz2 unique (Fig. 2, C and D). Further structural analysis of the auxiliary subdomain demonstrated that this fold is unnecessary for the formation of active sites, and no results were obtained when a DALI search was performed. However, it is probably required to maintain the integrity and stability of Abz2. In addition, a part of the dimeric interface of EcPabC is missing from Abz2 and M. loti PabC (Fig. 2, C and D).

The PLP Binding Site—A molecule of PLP cofactor is deeply buried in the cleft between Domains I and II (Fig. 3). PLP adopts the re-face specificity facing the protein side and is covalently linked to the catalytic residue Lys-251 by forming an internal aldime bond (Schiff base linkage), as previously observed in other prokaryotic PabC enzymes, which are classified into PLP-dependent fold type IV enzymes (27–29).

In addition to this covalent bond, PLP is further fixed by a network of hydrogen bonds and hydrophobic interactions. The phosphate group of PLP is stabilized by eight hydrogen bonds and acts as an anchor to fix the cofactor in the active site. The hydrogen bonds are partly donated by a cluster of main chain amide groups from Gly-328, Thr-329, Met-330, Asn-360, and Gly-361. Four well ordered water molecules (Wat-1–Wat-4) located in the active site also participate in the hydrogen bond network. Wat-1 is involved in fixing an oxygen atom of the phosphate group by positioning itself to the side chain of Asn-302. Wat-2 and Wat-3 mediate the hydrogen bonds that link two other oxygen atoms of the phosphate group to the side chain amino group of Arg-128 and the side chain hydroxyl group of Thr-30. The nitrogen atom of the Schiff base linkage accepts a hydrogen bond donated from Wat-4, which in turn accepts hydrogen bonds from the main chain carboxyl group of Ile-300 and Thr-301.

FIGURE 2. Overall structure of yeast Abz2. A, Abz2 consists of two domains. The auxiliary subdomain of Domain I is colored green, and the rest of it is in cyan. Domain II is colored magenta. PLP covalently bound to Lys-251 is shown as sticks. B, topology diagram of Abz2. C, comparison of the overall structure between Abz2 and E. coli PabC (EcPabC, orange). The dimer interface missing from monomeric Abz2 and MIPabC is boxed. D, comparison of the overall structure between Abz2 and M. loti PabC (MIPabC, gray).
In addition, the pyridine ring of PLP is sandwiched between the main chain atoms of a tripeptide segment of Ser-299, Ile-300, and Thr-301 from the side of the si-face, and Leu-326 from the side of the re-face. Glu-297 forms an ion pair with N1, the protonated nitrogen atom of the pyridine ring of PLP, and is considered to strengthen the electron withdrawing effect of the pyridine ring of PLP as an electron sink (40, 41). PLP-O3’ is fixed by a hydrogen bond with NH1 of the side chain of Arg-255.

Remodeling of the Substrate Binding Site—To elucidate the catalytic mechanism, we attempted to determine the structure of Abz2 in complex with either the substrate or product. Because the substrate ADC is not commercially available, we tried crystallizing Abz2 in complex with the product molecules (PABA and/or pyruvate) by either cocrystallization or the soaking method but failed. Alternatively, we simulated a model of a complex of apo-form Abz2 and the external aldimine, which was formed by PLP and substrate ADC using the program HADDOCK. In the modeling process, we fixed the residues Arg-255, Thr-301, Glu-297, Arg-128, Thr-329, Met-330, and Gly-361 involved in PLP binding to ensure that the external aldimine intermediate lay in a relatively reasonable pocket. Among the three output clusters, a cluster of lowest energy with four members satisfied the best interaction restraints. The overall backbone RMSD of 0.3 ± 0.2 Å for the four members indicated that the model of Abz2-ADC is somewhat reliable.

In this model, PLP fits well in the cleft between Domains I and II (Fig. 4A). Upon the formation of an external aldimine, the amino group of the catalytic Lys-251 is hydrogen-bonded by the phosphate group of PLP and Thr-30, which might in turn

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**FIGURE 3.** The PLP-binding site. PLP (yellow) covalently bound to catalytic residue Lys-251 (magenta) is shown as bold sticks. The PLP-binding residues are shown as sticks. The polar interactions are indicated by dashed lines.

**FIGURE 4.** Simulation and validation of the substrate-binding model. A, a model of PLP (yellow)-ADC intermediate (green) bound to the active site of Abz2. The docked PLP-ADC intermediate and the main residues within 4 Å of PLP-ADC are shown as sticks. Hydrogen bonds between the active site and docked PLP-ADC are shown as black dashes. B, the relative activity of the wild-type Abz2 and mutants. C, multiple sequence alignment of Abz2 and homologs. The two highly conserved basic residues are indicated with red stars. The multisequence alignment was performed using programs ClustalW2 and ESPript. All sequences were downloaded from the NCBI database. The sequences are (NCBI accession numbers codes are in parentheses) S. cerevisiae Abz2 (NP_014016.1), Scheffersomyces stipitis CBS 6054 aminotransferase-like protein (XP_001382343.1), Fomitiporia mediterranea MF3/22 FOMMEDRAFT_27438 (EJD05274.1), Arabidopsis thaliana branched chain amino acid aminotransferase-like protein 3 (NP_200593.2), M. loti MAFF303099 mlr3007 (NP_104211.1), L. pneumophila str. Corby 4-amino-4-deoxychorismate lyase (YP_094631.1), and E. coli strain K-12 substrain MG1655 4-amino-4-deoxychorismate lyase (NP_415614.1).
decrease the free energy of the external aldimine (27). Also, polar groups of ADC make extensive interactions with a cluster of hydrophilic residues (Thr-30, Lys-180, and Arg-182). Accompanying the substrate binding, the side chains of residues Lys-180 and Arg-182 are slightly shifted toward the solvent, leading to a closed functional active site (Fig. 4A).

To validate the docking model, we subsequently performed a series of site-directed mutageneses in combination with activity assays. Arg-182 was proposed to stabilize the substrate via two hydrogen bonds with the carboxyl group (Fig. 4A). Enzymatic assays showed that mutation of R182A could lead to complete inactivation of the enzyme (Fig. 4B), implying that it is indispensable for substrate binding and/or catalysis. Moreover, multiple-sequence alignment demonstrated that Arg-182 is quite conserved except that in some species the counterpart residue is a lysine (Fig. 4C). In fact the mutant R182K retained ~30% of the activity (Fig. 4B). Moreover, residues Thr-30, Lys-180, and Asn-360 were also hypothesized to fix the substrate via hydrogen bonding.

FIGURE 5. A putative catalytic mechanism of ADC lyase. a, an internal aldimine formed between Lys-251 and PLP. b, a nucleophilic attack of the amino group of ADC towards the C4’ of PLP. c, an external aldimine formed between PLP and ADC. d, the Cα proton of ADC abstracted by Lys-251. e, the Cα anion stabilized by PLP. f, a quinonoid intermediate. g, the formation of the ketime.

FIGURE 6. Active site comparison of ADC lyases. Shown is a superposition of the active site of Abz2 against that of E. coli PabC (EcPabC, orange, A), L. pneumophila PabC (LpPabC, blue, B), and M. loti PabC (MnPabC, gray, C).
with various groups of ADC (Fig. 4A). The mutants of N360A and N360D were inactive, whereas mutants T30A and K180A retained partial activity (Fig. 4B), indicating that these residues could also participate in catalysis to some degree. In fact, fluorescence assays demonstrated that both mutants K180A and N360A had \( K_e \) values toward pyruvic acid of \(~2\)-fold compared with the wild-type Abz2, which further proved our docking model.

A Putative Catalytic Mechanism of Abz2—Based on the above findings, we propose a putative catalytic cycle of Abz2 (Fig. 5) following a previously hypothesized scheme (42). First, an internal aldimine is formed between the catalytic lysine (Lys-251) of Abz2 and the cofactor PLP through a Schiff base linkage (Fig. 5a). When approaching the substrate, the amino group of ADC triggers a nucleophilic attack toward the C4' of PLP (Fig. 5b). On dissociation of PLP from Lys-251, an external aldimine is formed between PLP and ADC (Fig. 5c), called an imine exchange. The \( \text{Co} \) proton of ADC is then abstracted by the noncovalently bonded Lys-251 (Fig. 5d), which is the only available basic active site lysine residue nearby (27). Meanwhile, PLP serves as an “electron sink” to stabilize the \( \text{Co} \) anion by delocalizing the negative charge through a much expanded network of conjugated \( \pi \)-bonds and the hydrogen bond between N1 of the PLP ring and Glu-297 (Fig. 5e) (39, 40). Afterward, the electron density of the PLP-stabilized \( \text{Co} \) anion intermediate improves to form a quinonoid intermediate (Fig. 5f), along with the release of a pyruvate and the aromatization of the six-membered ring of the substrate (Fig. 5g), also called formation of the ketimine. Finally, a molecule of PABA is released from PLP by Schiff base transfer to regenerate a starting Schiff base (Fig. 5a).

Structure-based Classification of ADC Lyases—As the first eukaryotic ADC lyase of known structure, Abz2 displays a monomer of two domains that form an active cleft. In contrast, the largest total interface areas in the dimeric EcPabC and LpPabC are 2127 and 2050 \( \text{Å}^2 \), respectively, indicating that both exist as stable dimers. PabC proteins have been classified into two groups depending upon whether the tyrosine fixing PLP-O3' is provided from the same subunit or the neighboring subunit in the dimer (28). Taking the structure of EcPabC for instance, the key residue Tyr-109' is from a loop of the neighboring subunit (Fig. 6A), whereas in LpPabC, the corresponding tyrosine (Tyr-144) is from the same subunit (Fig. 6B). As mentioned above, Arg-255 of Abz2 corresponds to Tyr-109' of EcPabC. Moreover, MIPabC shares a quite similar active site composition with the monomeric Abz2, including a superimposable arginine to fix PLP-O3' (Fig. 6C). In addition, MIPabC is most likely a monomer that does not contain a dimeric interface corresponding to that of EcPabC (Fig. 2D). Therefore, we could categorize the previous two groups of ADC lyase into one class, the members of which are dimers, whereas Abz2 leads another class of monomeric ADC lyases. Moreover, the two classes might be distinguished from each other by a tyrosine or an arginine to fix PLP.

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