Unusual Mechanism for an Aminomutase Rearrangement: Retention of Configuration at the Migration Termini†

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ABSTRACT: The phenylalanine aminomutase from *Taxus* catalyzes the vicinal exchange of the amino group and the *pro*-3*S* hydrogen of (2*S*)-α-phenylalanine to make (3*R*)-*β*-phenylalanine. While the migration of the amino group from C2 of the substrate to C3 of the product is already known to proceed intramolecularly with retention of configuration, the stereochemistry of the hydrogen transfer remained unknown, until now. The chemical shifts of the prochiral hydrogens of authentic (3*R*)-*β*-phenylalanine were established by ^{1}H NMR, and the configuration of each hydrogen was assigned by ^{2}H NMR analysis of a racemic mixture of [2,3-$^2H_2$]-(2*S*,3*R*)- and (2*R*,3*S*)-*β*-phenylalanines synthesized via *syn* addition of deuterium gas with palladium catalyst to stereospecifically reduce the double bond of an *N*-acetyl enamine. After the aminomutase was incubated with [3,3-$^2H_2$]-(2*S*)-α-phenylalanine, the derived deuterium-labeled *β*-diastereoisomer product, derivatized as the *N*-acetyl methyl ester, was analyzed by ^{2}H NMR, which revealed that the mutase shuttles the *pro*-3*S* hydrogen to C2 of the *β*-isomer product (designated 2*S*,3*R*) with retention of configuration. Retention of configuration at both reaction termini is unique among all aminomutase mechanisms examined so far. Furthermore, the dynamics of the C_β−H bond of the substrate were measured in a competitive experiment with deuterium-labeled substrate to calculate a primary kinetic isotope effect on V_{max}/K_M of 2.0 ± 0.2, indicating that C−H bond cleavage is likely rate limiting. Isotope exchange data indicate that the migratory deuterium of [$^2H_8$]-(2*S*)-α-phenylalanine, at saturation, dynamically exchanges up to 75%, with protons from the solvent during the reaction after the first 10% of product is formed. The calculated equilibrium constant of 1.1 indicates that the *β*-isomer was slightly favored relative to the α-isomer at 30 °C.

Amino acid aminomutases typically catalyze the isomerization of α-amino acids to *β*-amino acids, which are usually found on the biosynthetic pathways of biologically active natural products such as blasticidin S (*1*), enediyne C-1027 (*2*), viomycin (*3*), and Taxol (*4*). Of the seven aminomutases identified, the stereochemical course of the lysine-2,3- (*5*, *6*), tyrosine-2,3- (*7*, *8*), arginine-2,3- (*1*), and *β*-lysine-5,6- (*9*) aminomutase reaction has been evaluated (Table 1). The vicinal transfer of the amino group of each substrate was shown to invert the configuration at the receiving carbon, replacing one of the diastereotopic hydrogens formerly at this position. Of these isomerases, the stereoselectivity of the reciprocal hydrogen transfer is known for only the arginine-2,3- and lysine-2,3-aminomutase reactions, wherein the configuration at the receiving prochiral carbon is inverted.

The mechanisms of the α-ornithine-4,5- (*10*), *β*-lysine-5,6-, and α-lysine-2,3-aminomutases involve radical intermediates, and each employs adenosylcobalamin or *S*-adenosylmethionine and pyridoxal phosphate as cofactors. The stereochemistry of the arginine-2,3-isomerase reaction was assessed by feeding stereospecifically ^{2}H-labeled α-arginine to *Streptomyces* (*1*), and consequently, the cofactor dependency of this enzyme could not be assessed in this in vivo experiment. Nevertheless, this catalyst likely involves homolysis and free radical coupling processes that initiate the removal of a relatively nonacidic alkyl hydrogen ($pK_a \geq 45$) at C_β from the arginine substrate, which is chemically and structurally similar to the α-ornithine and α-lysine substrates of radical-mediated isomerases, as described above. Moreover, α-arginine lacks electronic stabilization by an adjacent aromatic or allylic group that would increase the acidity of the hydrogen at the adjacent C_β in a heterolytic reaction. In contrast to the radical-initiated mechanisms described, the tyrosine aminomutase (TAM)[1] from *Bacillus* requires only ATP as a cosubstrate, while the functionally similar orthologue from *Streptomyces* requires no cofactors; both apparently proceed through heterolytic, ionic mechanisms to form (3*S*)-*β*-tyrosine (*7*, *8*).

Phenylalanine aminomutase (PAM) (*11*, *12*) is deemed similar in mechanism to the TAM from *Streptomyces* and catalyzes the transformation of α-phenylalanine to *β*-phe-

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[1] Abbreviations: ASG, alanine−serine−glycine sequence motif; $E1_{cb}$, first-order elimination reaction that proceeds via a carbanion intermediate; EtOAc, ethyl acetate; GC, gas chromatography; MIO, 3,5-dihydro-5-methylidene-4*H*-imidazol-4-one; EI-MS, electron impact-mass spectrometry; PAL, phenylalanine ammonia lyase; PAM, phenylalanine aminomutase; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TAM, tyrosine aminomutase.

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Table 1: Stereochemistry and Cofactor Dependency of Aminomutases

Aminomutase Source (ref)	Substrate/ Cofactors	Product and *Stereochemistry at carbon accepting NH_3 (H)*
Lysine 2,3- *Clostridium* (*5, 6*)	(2*S*)-α-lysine/AdoMet, PLP	(3*S*)-β-lysine *inverted (inverted)*
Arginine2,3- *Streptomyces* (1)	(2*S*)-α-arginine	(3*S*)-β-arginine *inverted (inverted)*
Tyrosine 2,3- *Bacillus* (*7*)	(2*S*)-α-tyrosine/ ATP	(3*S*)-β-tyrosine *inverted (unknown)*
Tyrosine 2,3- *Streptomyces* (*8*)	no cofactors	*unknown (unknown)*
Lysine 5,6- *Clostridium* (*9*)	(3*S*)-β-lysine	(3*S*,5*S*)-diaminohexanoate *inverted (unknown)*
Ornithine 4,5- *Clostridium* (*10*)	*(2R)*-α-ornithine AdoCbl, PLP	(2*R*,4*S*)-diaminopentanoate *unknown (unknown)*
Leucine 2,3- *Andrographis* (*25*)	(2*S*)-α-leucine	(3*S*)-β-leucine *unknown (unknown)*

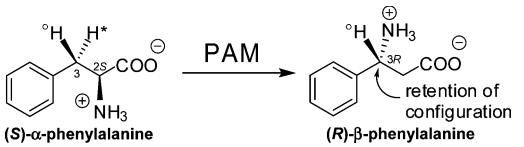


FIGURE 1: Isomerization of (2*S*)-α-phenylalanine to (3*R*)-*β*-phenylalanine by PAM. The amino group replaces the *pro*-3*S* hydrogen (designated H*) with overall retention of configuration at C3.

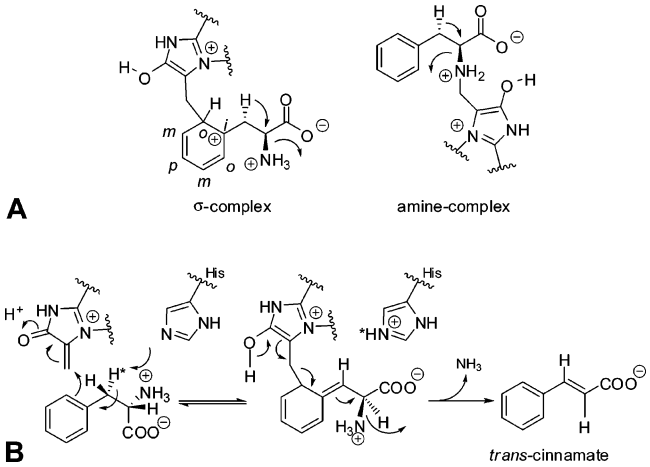


FIGURE 2: Two separate, hypothetical MIO intermediates for the ammonia lyase reaction. The MIO is attacked by the aromatic ring electrons, and the pK_a of the *β*-hydrogens is lowered for easy removal to quench the carbocation at the *ipso* carbon (*σ*-complex). Alternatively, the MIO is attacked by the amino group to facilitate Hoffmann-type elimination of the NH_3/H^+ (amine complex). The *ipso* (*i*), *ortho* (*o*), *meta* (*m*), and *para* (*p*) positions are indicated (panel A). The literature-supported mechanism of the phenylalanine ammonia lyase (PAL) via MIO-aryl ring coupling chemistry. After removal of the *pro*-3*S* hydrogen putatively by a His residue, ammonia is lost in the final, irreversible step (panel B).

nylalanine, without cofactors (*11*). The product of the PAM reaction is an obligatory biosynthetic precursor of the phenylisoserine side chain of the antimitotic pharmaceutical Taxol (*13*). Previous investigations showed that the PAM reaction modifies the phenylalanine substrate by removing the *pro*-3*S* hydrogen which is subsequently replaced by the amino group of the substrate with retention of configuration at C3 of the (3*R*)-*β*-regioisomer (*11*) (Figure 1). This retention of stereochemistry is uncommon compared to the described radical-mediated aminomutase mechanisms, and in particular, it is opposite that of the 3*S* product made in the TAM reaction. Despite this apparent divergence in stereochemistry between the aryl amino acid aminomutases, the lack of cofactor dependency and similarity (30% amino acid identity) of the amino acid sequences of PAM and TAM (from *Streptomyces*) reveal that these enzymes belong to the same family, which includes several ammonia lyases (*14*−*16*). The lyases share a characteristic active site motif, alanine−serine−glycine (ASG), that autocatalytically rearranges to form a functional 3,5-dihydro-5-methylidene-4*H*-imidazol-4-one (MIO) prosthesis acting as a Lewis acid to promote the elimination of NH_3 and H^+ from the arylalanine substrate (*8*). The MIO moiety purportedly couples with the substrate by nucleophilic attack of the *ortho* carbon of the aromatic ring or by the amino group (Figure 2A). The mode of MIO−substrate coupling via the aryl ring in the PAL reaction (and other aryl amino acid lyase reactions) has been widely studied and is supported empirically; thus, we incorporate this step into the proposed mechanism. The PAL-mediated elimination process begins with removal of the *pro*-3*S* hydrogen likely by the ^{137}His residue of PAL from *Rhodosporidium toruloides* (*17*), followed by $E1_{cb}$-type displacement of ammonia from the substrate to form *trans*-cinnamate (*15*, *17*) (Figure 2B). In terms of reaction chemistry and substrate specificity, PAL provides a practical model upon which to base the PAM and TAM reactions; therefore, similar MIO involvement is proposed for the aminomutase mechanisms where the NH_3 and H^+ swap places and rebound to the phenylpropanoid to make the corresponding *β*-amino acid product (*11*, *12*, *18*).

On the surface, the net vicinal rearrangement reactions of all the 2,3-aminomutases appear to be similar; however, there is obvious diversity among the α,*β*-isomerase mechanisms. Thus, understanding the complete stereochemistry of the aminomutase reactions can help to identify the determinants that sort these enzymes into coherent subclasses. In this study, the cryptic stereochemistry of the PAM reaction involving the hydrogen transfer from C3 to C2 of the phenylpropanoid substrate is defined. A deuterium-label tracer method is described for the measurement of the overall kinetic isotope effect of the C_β−H bond cleavage. The exchange of the transient hydrogen with protons from an alternative source was also investigated in deuterium-labeling studies, and the equilibrium constant of the PAM reaction

was measured to assess the difference in free energy between the reactant and product.

EXPERIMENTAL PROCEDURES

General. A Varian Inova-300 instrument was used to acquire ^{13}C and ^{1}H NMR spectra, and proton-decoupled ^{2}H NMR experiments were performed on a Varian Unity Plus 500 magnet at 76.77 MHz. A gas chromatograph (model 6890N; Agilent, Palo Alto, CA) coupled to a mass selective detector (model 5973 inert; Agilent) was used for analysis of derivatized products isolated from enzyme assays that were loaded onto a 5HS GC column (0.25 mm inner diameter × 30 m, 0.25 μm film thickness) mounted in the GC oven. The EI-MS conditions were set with an ion scan mode from 100 to 300 atomic mass units at 70 eV ionization voltage. The GC conditions were as follows: the column temperature was programmed from 70 °C (3 min hold) to 320 °C at 10 °C/min and then a 3 min hold at 320 °C, splitless injection was selected, and helium was used as the carrier gas (1.2 mL/min).

Chemicals and Reagents. Deuterium gas, [ring-2,3,3-$^{2}H_8$]- and [3,3-$^{2}H_2$]-(2*S*)-α-phenylalanines, and D_2O (each at >98% isotope enrichment) were purchased from Cambridge Isotope Laboratories (Andover, MA), and ethyl benzoylacetate (90%), [$^{2}H_8$]ethyl acetate (>98% isotope enrichment), and all other reagents were purchased from Sigma/Aldrich (St. Louis, MO), unless otherwise noted, and used without further purification.

Heterologous Expression and Partial Purification of PAM. For the following procedures minimal growth media [1 L contained 12.8 g of $Na_2HPO_4 \cdot 7H_2O$, 3 g of KH_2PO_4, 0.5 g of NaCl, 1.0 g of NH_4Cl, 2 mL of 1 M $MgSO_4$, 100 μL of 1 M $CaCl_2$, 10 mL of 100× Basal Medium Eagle vitamins solution (Sigma/Aldrich), and 20 mL of 20% D-glucose solution] containing ampicillin (100 μg/mL) were employed at 25 °C to propagate the bacterial cells expressing the *pam* clone, described below.

By using a cohesive-end PCR method (*19*), *pam* cDNA was subcloned from expression vector pET1981His (*11*) into pET14b to place the poly-His tag at the C-terminus (designated pET14-1981His). Preliminary purification of small-scale preparations of PAM protein expressed from pET1981His (*11*) and from pET14b vector in *Escherichia coli* BL21(DE3) indicated that the N-terminal-tagged protein bound to the Ni-affinity column matrix 8-fold better than the C-terminal-tagged protein (<10% relative binding). The oligonucleotide primers for this subcloning procedure have been described (*11*). *E. coli* BL21(DE3) cells transformed with pET14-1981His were grown for 16 h in 50 mL of minimal growth medium, and these bacteria were used to inoculate 6 L of the same broth supplemented with 100 μg/mL ampicillin. The cells were grown at 37 °C until OD_{600} = 0.6, overexpression was induced by the addition of 1 mM isopropyl D-thiogalactopyranoside, and the cultures were grown at 18 °C for 18 h. The remaining steps were conducted at 4 °C, unless otherwise noted. The cells were harvested by centrifugation at 5000*g* (20 min), diluted in 100 mL of resuspension buffer (50 mM potassium phosphate, pH 8.0), and lysed by brief sonication [five 20 s bursts at 50% power (Misonix Sonicator, Farmingdale, NY)], followed by removal of cellular debris by centrifugation at 15000*g* (30 min). Residual membrane debris was removed by centrifugation at 45000*g* (2 h). The clarified crude supernatant was partially purified by anion-exchange (primarily to remove small molecules) and HIS-Select (Sigma/Aldrich) Ni-affinity gel chromatographies (PAM eluted in 150−200 mM imidazole). Fractions containing active soluble PAM (~76 kDa) were combined (10 mL total) and loaded into a size-selective centrifugal filtration unit (Centriprep centrifugal filter units, 30000 MWCO; Millipore, Billerica, MA). The protein solution was concentrated to 3 mL and diluted several cycles until the imidazole concentration was <1 μM so that the diazole would not confound the efficiency of our in situ amino acid derivatization chemistry (see section below) nor contaminate the GC column used routinely in these analyses. The quantity (~50 μg/mL) and purity (~70%) of concentrated enzyme was assessed by SDS−PAGE and Coomassie Blue staining using Kodak 1D image analysis software (version 3.6.3) to integrate the relative intensities of the scanned protein bands.

General PAM Assay. (2*S*)-α-Phenylalanine (~500 μM) was added to 1 mL of 50 mM phosphate buffer (pH 8.0) containing partially purified PAM (~1 μg) and incubated at 30 °C for 2 h, and the production of (3*R*)-β-phenylalanine was evaluated. The assay buffer was basified to pH ≥10 with dropwise addition of 0.1 M sodium hydroxide and treated with acetic anhydride (2 × 300 μL for 20 min between each addition), and the reaction was quenched by acidifying to pH 2 with 1 M HCl. The *N*-acetyl amino acids were extracted with ethyl acetate (3 × 1 mL), the organic fractions were combined, and the carboxylic acids were methyl esterified by diazomethane treatment. The solvent was removed in vacuo, the derivatized amino acid mixture was dissolved in 300 μL of ethyl acetate, and 1 μL of this solution was analyzed by coupled GC/EI-MS.

Synthesis of N-Acetyl [2,3-$^{2}H_2$]-(2S,3R)- and (2R,3S)-β-Phenylalanine Methyl Ester Racemate. The following synthesis procedure is an adaptation of an established method (*20*). To a stirred solution of ethyl benzoylacetate (57 mmol, 11 mL) dissolved in methanol was added concentrated sulfuric acid solution (0.5 mL). After 20 h, the solvent was evaporated, and the resulting oil was partitioned between dichloromethane and water. The organic layer was collected and evaporated to yield methyl benzoylacetate and used in the next step without further purification. The oil was dissolved in methanol (150 mL), excess ammonium acetate (20 g) was added, and the solution was refluxed for 5 h. The reaction was cooled, and the resulting crystals were removed by vacuum filtration. The solvent was evaporated to yield the desired methyl (*Z*)-3-amino-3-phenylacrylate enamine as an oil, which was purified over silica gel (9:1 hexanes/ethyl acetate, v/v). The solvent was evaporated, and the enamine was obtained in 90% yield based on the ethyl benzoylacetate (9.0 g, 51 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.70 (OCH_3), 4.98 (HC═C−), 7.48−7.60 (phenyl-H). GC/EI-MS: *m*/*z* 177 (M^+), 146 ($M^+ - OCH_3$).

To the purified enamine (11.3 mmol, 2 mL) in dry tetrahydrofuran (50 mL) was added freshly distilled pyridine (10 mL), and the solution was cooled to 0 °C. Freshly distilled acetyl chloride (84.8 mmol, 7.5 equiv, 6 mL) was added dropwise, and the reaction was warmed to room temperature and stirred overnight. The reaction progress was monitored by thin-layer chromatography, and additional

acetyl chloride was added dropwise to complete the reaction. The sample was quenched by dilution with brine (50 mL) and extracted with chloroform (3 × 25 mL). The solvent was evaporated and the *N*-acetyl enamine methyl ester was purified by silica gel (85:15 hexanes/ethyl acetate, v/v). The solvent was evaporated, and the desired methyl (*Z*)-3-acetamido-3-phenylacrylate was obtained quantitatively in 95% yield (2.3 g, 11 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.14 (s, $CH_3C{=}O$), 3.70 (s, OCH_3), 4.98 (s, C═CH), 7.48−7.60 (phenyl-H). GC/EI-MS: *m*/*z* 219 (M^+), 177 ($M^+ - CH_3$-OH), 160 [$M^+ - CO_2CH_3$ or $M^+ - HNC(O)CH_3$].

To the (*Z*)-acrylate (6.0 mmol, 1.3 g) dissolved in methanol (30 mL) in a Parr flask was added 10% (w/w) Pd/C (25 mg). The chamber was pressurized with deuterium gas at 30 psi to stereospecifically reduce the substrate by *syn* addition. The reaction was shaken for 24 h and then filtered through Celite overlaying a short (∼2 cm) column of silica to remove the catalyst. The methanol was evaporated to provide a racemic mixture of dideuterio *N*-acetyl-(2*S*,3*R*)- and (2*R*,3*S*)-β-phenylalanine methyl esters (>98% yield, 1.2 g). Proton-decoupled ^{2}H NMR (79.77 MHz, ethyl acetate): δ 2.82 (C_αDH), 5.41 (C_βDN). GC/EI-MS: *m*/*z* 223 (M^+), 180 [$M^+ - CH_3C(O)$, 100%], 149 [$M^+ - HC(D)C(O)OCH_3$), 20%]. This compound was used to evaluate the stereochemistry of the hydrogen migration from C3 to C2 of the phenylpropanoid substrate in the PAM reaction. Since authentic β-phenylalanine deuterium-labeled at C2 was not available, the synthetically derived ^{2}H-labeled compound, described above, was also used to assess the extent of D for H exchange at C2 under the same basic and acidic conditions employed to derivatize the α- and β-phenylalanines to their *N*-acetyl methyl esters. Approximately 500 μM dideuterio *N*-acetyl amino acid ester was suspended in 1 mL of H_2O which was adjusted to pH 11 (0.1 M NaOH) in a 10 mL glass screw-capped tube and shaken vigorously (Vortex) for 1 min. The solution was then acidified (pH 2) with dropwise addition of 1 M HCl and shaken for 1 min. The suspension was extracted with ethyl acetate (300 μL), and the organic fraction was dried by passage through a column a sodium sulfate powder. A 1 μL aliquot of the eluant was analyzed by GC-EI/MS, and the diagnostic ion fragments revealed that the isotopic abundance of the analyte after base/acid treatment was identical to that of the starting material, which is at 98% deuterium isotope enrichment.

Measurement of the Equilibrium Constant. To assess the equilibrium between product and substrate in the PAM reaction at 30 °C, α-phenylalanine at 100 μM was added to 10 mL of PAM (at ∼10 μg·mL^{-1} instead of 1 μg·mL^{-1} used for routine assays) in 50 mM phosphate buffer (pH 8.0). Aliquots (1 mL) were withdrawn from the reaction at various time points between 0.25 and 50 h. The reaction in each aliquot was stopped by addition of 0.1 M sodium hydroxide, and the amino acids were derivatized and analyzed by GC/EI-MS, as described above. The relative amounts of product and reactant at equilibrium were determined by linear regression analysis of the area of the base peak ion of the derivatized α- or β-phenylalanines (*m*/*z* 162 and 178, respectively). Peak area was converted to concentration of product (or substrate) by solving the corresponding linear equation, derived by plotting the area of the base peak ion (produced by the corresponding authentic standard) against concentration ranging from 0 to 30 μM, at 5 μM intervals.

Evaluation of the Stereochemistry of the Hydrogen Migration in the PAM Reaction. [3,3-$^2H_2$]-(2*S*)-α-Phenylalanine (5 mM) was incubated with PAM (∼50 μg) in each of two 1 mL samples (10 μmol total substrate) in phosphate buffer (pH 8.0) at 30 °C. The reaction progress and deuterium distribution in the product were tracked by GC/EI-MS analysis at regular intervals after the start of the reaction by removing aliquots (10 μL) from each sample, and the amino acids were derivatized as described earlier. Under these assay conditions, where 50-fold more protein was used than in a routine assay, the reaction was halfway to equilibrium (described above) at 6 h. Thus, the reaction was terminated at 10 h by basification to pH 10 with dropwise addition of 0.1 M sodium hydroxide solution, and the isotopomeric amino acids were derivatized as before, except that 20 μmol of acetic acid was used. An estimated 830 μg of product (5 μmol, ∼50 mol % conversion of substrate to product) was made, which was sufficient for analysis by ^{2}H NMR. The biosynthesized dideuterio-β-phenylalanine isotopomer was present at ∼30% (∼330 μg, 1.5 μmol) compared to the monodeuterio-β-isotopomer, as judged by GC/EI-MS analysis. Diagnostic fragment ions were found at *m*/*z* 179 [$PhC_\alpha D(NH)C_\beta H_2CO_2CH_3^+$, 100%], 180 [$PhC_\alpha D(NH)C_\beta$-$DHCO_2CH_3^+$, 28%, corrected for ^{13}C contributions from *m*/*z* 179 ion], and 149 [PhC(D)═$NHAc^+$, 20%]. The dried mixture of derivatized deuterium-labeled α- and β-amino acids extracted from the aqueous assay solution was dissolved in 300 μL of ethyl acetate and analyzed by proton-decoupled ^{2}H NMR, with overnight scanning. The chemical shifts corresponding to the deuterium of the labeled β-phenylalanine in the sample were compared to those of the authentic *N*-acetyl [2,3-$^2H_2$]-(2*S*,3*R*)- and (2*R*,3*S*)-β-phenylalanine methyl ester racemate dissolved in ethyl acetate to assess the stereochemistry at C2 of the biosynthetically derived product. For reference, the [3,3-$^2H_2$]-(2*S*)-α-phenylalanine substrate (5 mM) was isolated from 1 mL of assay buffer (50 mM phosphate buffer, pH 8.0) without PAM after 12 h at 30 °C. The substrate was derivatized to its *N*-acetyl methyl ester by described methods, dissolved in 300 μL of ethyl acetate, and analyzed by ^{2}H NMR.

Assessment of the Kinetic Isotope Effect. PAM and an equal mixture of [ring,2,3,3-$^2H_8$]- and unlabeled (2*S*)-α-phenylalanines (500 μM of each) were incubated for 3 h until ∼0.2% conversion of α- to β-phenylalanine (2.3 nmol) was observed under standard assay conditions. The amino acids were converted to their *N*-acetyl methyl esters as described previously, and the relative amounts of product derived from labeled and unlabeled substrate were compared. The isotopomers generated diagnostic base peak fragment ions in the mass spectrometer at *m*/*z* 178 (unlabeled product) and at *m*/*z* 185 and 186 (D_7- and D_8-labeled product, respectively). Since the deuterium originating at C3 in the labeled substrate is partially lost during its transfer to C2 in the isomerization reaction, the ratio of the peak area for ion *m*/*z* 178 to the combined areas of ions *m*/*z* 185 and 186 was used to calculate the kinetic isotope effect on V_{max}/K_M in this competitive assay (*21*).

Assessment of Hydrogen Exchange during the PAM Reaction. PAM (∼1 μg/mL) was incubated with [ring,2,3,3-$^2H_8$]-(2*S*)-α-phenylalanine (250 μM) in 12 mL of 50 mM phosphate buffer (pH 8.0) at 30 °C, and a 1 mL aliquot of

sample was removed at various time intervals up to 50 h. The substrate remained at saturation through 50 h (<10 mol % product made) to prevent dynamic equilibrium during the time course of the experiment and thus minimized contributions from the reverse reaction.

A complementary experiment was conducted where the water (H_2O) of a 50 mM phosphate buffer solution (2 mL, pH 8.0) was removed in vacuo, the remaining phosphate salts were redissolved in D_2O (2 mL), and the deuterated solvent was evaporated. The sample was again diluted with 2 mL of D_2O, and to this volume was added PAM (~1 μg) solvated by 100 μL of protio-labeled buffer and 100 μL of a 5 mM solution (in D_2O) of unlabeled α-phenylalanine; this reaction was incubated for 17 h. The isolated β-phenylalanine isotopomers were converted to *N*-acetyl methyl esters as described previously and subjected to mass spectrometric analysis to assess the deuterium content.

RESULTS

In a previous study, recombinant PAM was expressed in *E. coli* cells grown in LB medium, and several steps where needed to separate PAM from the abundant α-phenylalanine present in this rich broth (*11*). This exogenous α-amino acid was also circumstantially converted to β-phenylalanine in vivo during the overexpression of the *pam* gene in the transformed bacterial cells. Thus as an alternative, the cells were grown in minimal growth medium, and the levels of these amino acids were reduced significantly in the enzyme preparations isolated in the present study.

His-tagged PAM of modest purity (~70%) obtained after Ni-affinity chromatography was only observed for protein preparations from large-scale (6 L) bacteria cell cultures expressing *pam*. In these instances, several batches of protein extract were serially loaded on and eluted from the affinity matrix column over several hours. The relative purity of PAM eluting from the column diminished over time, and this reduction in quality was attributed to either fouling of the Nickel resin or proteolysis of the target protein during short-term storage between column loading. To acquire enough PAM to conduct the experiments described herein, all fractions from the Ni column containing at least half the activity as the most active fraction were combined. The imidazole eluant was removed by size-selective filtration to yield a solution of PAM at the described purity, and this enzyme preparation was judged adequate for the purposes of this investigation.

Complete Stereochemistry of the PAM Reaction. The ^{1}H NMR chemical shift resonances of the diagnostic protons of unlabeled *N*-acetyl (3*R*)-β-phenylalanine methyl ester (~25 μM in [$^2H_8$]ethyl acetate) were observed at δ 5.43 (doublet of doublets, not shown) for $C_\beta HN$ and at δ 2.92 (doublet of doublets) and δ 2.82 (doublet of doublets) for the $C_\alpha H_2$ protons. This sample also contained unlabeled *N*-acetyl (2*S*)-α-phenylalanine methyl ester (~50 μM), and its chemical shift resonances for the benzylic protons [$C_\beta H_2$, at δ 3.15 (doublet of doublets) and δ 3.08 (doublet of doublets)] were well resolved from the signals of β-phenylalanine (Figure 3, profile 1). This peak resolution indicated that the signals for both compounds could be measured simultaneously by ^{2}H NMR, precluding the need to chromatographically separate the ^{2}H-labeled α- (substrate) and

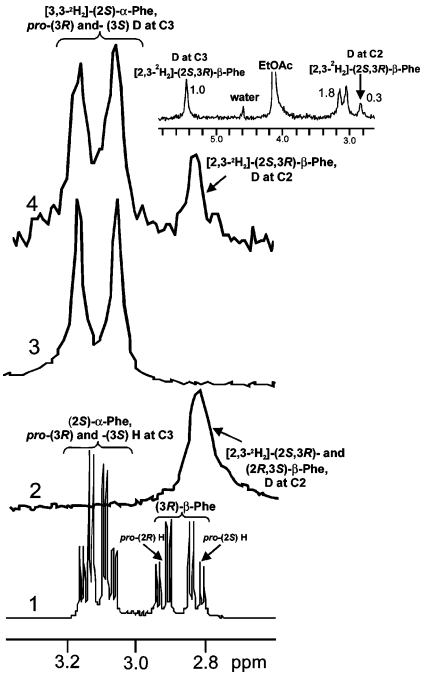


FIGURE 3: NMR analysis of α- and β-phenylalanine derivatives. A partial ^{1}H NMR (500 MHz, [$^2H_8$]ethyl acetate) spectrum of a mixture of authentic unlabeled (2*S*)-α-phenylalanine (at ~50 μM) and unlabeled (3*R*)-β-phenylalanine (at ~25 μM) as their *N*-acetyl methyl ester analogues reveals a pair of doublet of doublets for the C3 hydrogens of the α-isomer at δ 3.04−3.20 and a pair of doublet of doublets for the C2 hydrogens of the β-isomer at δ 2.79−2.96 (profile 1). Partial proton-decoupled ^{2}H NMR (76.77 MHz, EtOAc) spectra of labeled α-phenylalanine and/or biosynthetic β-phenylalanine were analyzed as their *N*-acetyl methyl esters. Authentic *N*-acetyl-[2,3-$^2H_2$]-(2*S*,3*R*)- and (2*R*,3*S*)-β-phenylalanine methyl ester racemates displayed a signal for C2 at δ 2.82 for the enantiomers (profile 2), authentic [3,3-$^2H_2$]-(2*S*)-α-phenylalanine isolated from assay buffer in which no enzyme was added reveals deuterium resonances for the prochiral deuteriums at δ 3.09 and 3.15 (profile 3), and derivatized [3,3-$^2H_2$]-(2*S*)-α-phenylalanine substrate and biosynthetic β-phenylalanine, isolated from an assay to assess the stereochemistry of the mutase reaction, reveal resonance signals at δ 2.82 for the *vicinal* dideuterio product and at δ 3.08 and 3.15 for the unreacted *geminal* dideuterio substrate (profile 4). The relative peak integrals of the ^{2}H chemical shift signals at C2 (δ 2.82) and C3 (δ 5.42) of the biosynthetic β-phenylalanine and at C3 (δ 3.08 and 3.15) of the unreacted [3,3-$^2H_2$]-(2*S*)-α-phenylalanine that correspond to the partial spectrum shown in profile 4 are listed as numerical values next to the corresponding peaks (inset).

β- (biosynthetic product) amino acids in the biosynthetic assay. The relative configuration of the prochiral hydrogens at C2 of β-phenylalanine were assigned by employing the racemate of *N*-acetyl [2,3-$^2H_2$]-(2*S*,3*R*)- and (2*R*,3*S*)-β-phenylalanine methyl ester that was synthesized by stereospecific reduction (palladium/D_2 gas) of an *N*-acetyl *trans*-enamine methyl ester (Figure 4); the (2*S*)- and (2*R*)-deuteriums in the enantiomeric pair are spectroscopically equivalent. Proton-decoupled ^{2}H NMR analysis of the synthetic dideuterio product, dissolved in ethyl acetate, revealed chemical shift resonances at δ 5.42 (C_βDN, singlet) and at δ 2.82 (C_αDH, singlet); the latter signal corresponds to the deuterium at the 2*S* and 2*R* positions of the enantiomeric mixture (Figure 3, profile 2).

A ^{2}H NMR spectrum of the 3,3-$^2H_2$-labeled substrate was acquired for referencing the chemical shift signals of the unused substrate in the enzyme assay to examine the

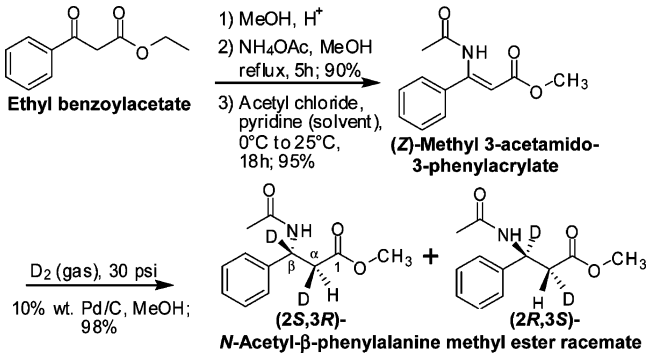


FIGURE 4: Synthesis of racemic *N*-acetyl [2,3-$^2H_2$]-(2*S*,3*R*)- and (2*R*,3*S*)-β-phenylalanine methyl ester. Abbreviations: MeOH, methanol; NH_4OAc, ammonium acetate; D_2, deuterium gas; Pd/C, palladium catalyst on carbon. The percentage yield for each step is given.

stereochemistry (described below). The labeled α-amino acid was incubated for 10 h in assay buffer without PAM and processed like the amino acids in the catalytic assays. The derivatized amino acid showed deuterium chemical shift resonances at δ 3.15 and 3.09 for the diastereotopic deuteriums (Figure 3, profile 3) in the proton-decoupled ^2H NMR spectrum; the chemical shifts were identical to those of the unlabeled isotopomer that was analyzed by ^1H NMR (Figure 3, profile 1). Incidentally, D−D spin couplings are about 40 times smaller than H−H spin couplings; consequently, the resolution of peak splitting caused by this coupling is not observed in ^2H NMR, and the deuterium signals appear as singlets.

^1H NMR analysis of unlabeled (2*S*)-α- and (3*R*)-β-phenylalanines, each at 200 μM in perdeuterio ethyl acetate, revealed that the relative chemical shifts of the protons at C3 of the α-isomer and those at C2 of the β-isomer were resolved; therefore, chromatographic separation of substrate and product was unnecessary prior to NMR analysis of the biosynthesis assay. Analogous ^2H NMR analysis of the ^2H-labeled β-phenylalanine isotopomer isolated after [3,3-$^2H_2$]-(2*S*)-α-phenylalanine (5 mM) was incubated for 10 h with 50-fold more PAM enzyme than used in general assays (see Experimental Procedures) revealed chemical shifts at δ 5.43 ($NC_\beta D$) and at δ 2.82 ($HC_\alpha D$) (Figure 3, profile 4), which were identical to those of the authentic deuterated racemic compound. These NMR data, coupled with the known, exclusive 3*R* stereochemistry of the biosynthetically derived [^2H]-β-phenylalanine product (*22*), establish the biosynthetic product as the 2*S*,3*R* enantiomer.

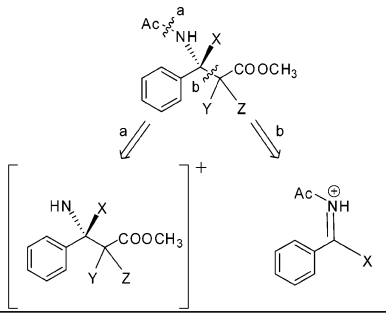
Analysis of the ^2H NMR spectrum of the derivatized biosynthetic product (Figure 3, profile 4) showed that when the integral of the peak area for the resonance signal at δ 5.43 ($C_\beta D$) was set to the expected value of 1.0 deuterium, the relative abundance of deuterium at C2 was a fraction (∼0.30) of its expected value of 1.0 (Figure 3, inset). This lower deuterium peak area at C2 supports the level of hydrogen exchange observed in the isotope replacement assays, described later. The isotopic ratio was confirmed by GC/EI-MS analysis of the product, and after correction for ^{13}C natural abundance contributions, the relative diagnostic ion abundances were at 100% (*m*/*z* 179, D_1 species) and 28% (*m*/*z* 180, D_2 species) (Table 2A).

The reaction progress of the enzyme assay was monitored by GC/EI-MS for this investigation, and the reaction was stopped when it was estimated that sufficient [2,3-^2H]-β-phenylalanine was available for ^2H NMR detection. These conditions were met when equilibrium was reached at a substrate to product ratio near 50:50, which was determined by comparing the relative peak areas of the deuterium signals in the ^2H NMR spectrum of the α- and β-phenylalanine derivatives isolated from the enzyme assay (Figure 3, inset). A peak area of 1.8 corresponded to the chemical shift signals of the two diastereotopic deuteriums at C3 of the remaining 3,3-$^2H_2$-labeled substrate. The peak area (set to 1.0) of the deuterium signal at C3 ($C_\beta DN$) of the biosynthetic β-amino acid indicates an approximate substrate to product ratio of 47:53, given that a theoretical 50:50 mixture of ^2H-labeled α- and β-phenylalanines would correspond to a 2.0:1.0 area ratio for the observed signals, respectively.

Notably, if the D for H exchange observed in the biosynthetic product resulted from the chemical derivatization of the amino acids, then racemization at C_α of β-phenylalanine would have been highly anticipated; yet, this was not observed in the ^2H NMR spectrum of the biosynthesized [^2H]-β-phenylalanine derivative.

Assessment of the Equilibrium Constant. The PAM enzyme catalyzes a reversible transformation of α-phenylalanine to β-phenylalanine, with the β-isomer in ∼10% excess at equilibrium (K_{eq} = 1.1 at 30 °C). This corresponds to a small free energy change ($\Delta G° = -RT \ln K_{eq}$, where R =

Table 2: Diagnostic Ions Resulting from Electron Impact-Mass Spectrometry Fragmentation[a]



(2*S*)-α-Phe Substrate incubated with PAM	Ions (*m*/*z*) observed by EI-MS analysis	
A)	*m*/*z* 179 (D_1): X,Y,Z=H *m*/*z* 180 (D_2): X,Z=D, Y=H	m/z 149 (D_1): X=D
B) unlabeled	*m*/*z* 178 (D_0): X,Z,Y=H	m/z 148 (D_0): X=H
C) [$^2H_8$]-	*m*/*z* 185 (D_7): X,Z=D, Y=H, ring D_5 *m*/*z* 186 (D_8): X,Y,Z=D, ring D_5	m/z 154 (D_6): X=D, ring D_5
D) unlabeled (in D_2O)	*m*/*z* 178 (D_0): X,Y,Z=H *m*/*z* 179 (D_1): X,Y=H, Z=D	m/z 148 (D_0): X=H

[a] Listed are mass spectral fragments after bond fissure at sites "a" and "b", yielding diagnostic ions of the corresponding *N*-acetyl-β-phenylalanine methyl esters.

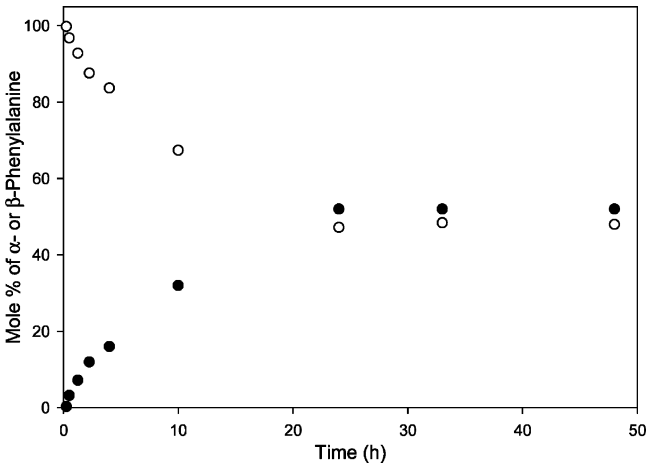


FIGURE 5: Evaluation of the equilibrium of the phenylalanine aminomutase reaction. The relative mol % of (2*S*)-α-phenylalanine (open circles, ○) and (3*R*)-*β*-phenylalanine (filled circles, ●) is plotted relative to time. The assay contained PAM (~10 *μ*g/mL) and 100 *μ*M α-phenylalanine at 30 °C. The standard error for each point was ±1%.

1.987 cal·K^{-1}·mol^{-1} and $T = 298$ K) of approximately −0.05 kcal·mol^{-1}, suggesting that the forward and reverse reactions are nearly equally favored; *β*-phenylalanine comprises 53 ± 1% of the amino acid mixture at equilibrium (Figure 5).

Rate-Determining Step on the Isomerization Pathway. An equal mixture of unlabeled and perdeuterio-(2*S*)-α-phenylalanines (at saturation) was incubated for 3 h with PAM under typical assay conditions, and the reaction was processed for analysis as described previously. The resulting biosynthetically derived *β*-phenylalanine isotopomers (~0.7 mol % relative to substrate) were comprised of 66 mol % of D_0 product (Table 2B) and 34 mol % of a mixture of D_7 and D_8 product, both derived from labeled substrate (Table 2C). This suggests a primary isotope effect on V_{max}/K_M of approximately 2.0 ± 0.2 for the removal of the *pro*-3*S* hydrogen, which is likely a rate-determining step of the catalytic cycle. No D_1-labeled *β*-phenylalanine was detected, indicating that intermolecular deuterium crossover from the [$^2H_8$]-α-phenylalanine to the D_0 *β*-isomer was below detection limits.

Hydrogen Exchange at C2 of the PAM Reaction Product. Hydrogen exchange in the PAM reaction was tracked as a function of time to further evaluate cryptic aspects of the reaction mechanism. [ring,2,3,3-$^2H_8$]-(2*S*)-α-Phenylalanine, at saturation [500 *μ*M, $K_M \approx 45$ *μ*M (*11*)], was incubated with PAM in buffer dissolved in H_2O over a period of 50 h. After 0.25 h, 0.2 mol % (0.5 nmol) of the substrate had converted to *β*-phenylalanine that was comprised of ~57% D_7 isotopomer and ~43% D_8 isotopomer (Figure 6), as determined by GC/EI-MS analysis. The D_7 species contained the intact phenylpropanoid fragment ion (*m*/*z* 185), and a fragment ion derived from C2−C3 bond fissure at *m*/*z* 154 (a D_6 species) indicated that the deuterium resided at the phenyl (D_5) and benzylic (D_1) carbons; no D_5 species labeled in the ring only was observed (Table 2C). Thus, the H for D replacement was confirmed to have occurred at C2 and not at C3 nor in the aromatic ring. The slope of the time-dependent curve during the period from 0.25 to ~2 h estimated the exchange rate constant at 0.52 h^{-1} while the product abundance increased from 0.2 to 0.8 mol % relative to substrate (Figure 6). The H/D exchange rate decreased and finally reached equilibrium (~75% H for D replacement) at longer incubation times, while the product increased from 0.8 to 10 mol % relative to substrate between 2 and 50 h. GC/EI-MS analysis revealed that the substrate remained fully deuterium-labeled during the survey (data not shown).

The origin of the proton source in the exchange experiment was established through a reciprocal experiment conducted by incubating PAM with unlabeled α-phenylalanine in buffer with H_2O replaced by D_2O. After a 12 h incubation, the derivatized *β*-phenylalanine product isolated from this enzyme-catalyzed reaction was comprised of ~70% D_1 and ~30% D_0 isotopomers and confirmed the extent of hydrogen replacement observed in the previous experiment with unlabeled solvent (Table 2D). Evaluation of the *β*-amino acid isotopomers by GC/EI-MS fragmentation confirmed that the deuterium in the D_1 species was at C2. Furthermore, if the deuterium exchange described above resulted from the in situ chemical N-acetylation and methyl esterification steps used to derivatize the amino acids, then complete replacement of deuterium by hydrogen would have been anticipated, particularly for samples where the *β*-amino acid product was least abundant; yet, this was not observed.

DISCUSSION

Stereochemical Fate of the PAM Reaction. The PAM enzyme catalyzes the conversion of the [3,3-$^2H_2$]-α-phenylalanine substrate to a single (3*R*)-*β*-phenylalanine isomer (established by a GC method employing a chiral column and is described elsewhere (*22*); data not shown), which was judged by ^2H NMR and comparison to authentic stereospecifically deuterium-labeled *β*-isomer to have 2*S*,3*R* stereochemistry. Analysis of product stereochemistry indicated that, during the course of the reaction, retention of configuration at C2 and C3 occurs when the amino group formerly at the 2*S* position is replaced by the *pro*-3*S* deuterium, and reciprocally, the position formerly occupying the deuterium is replaced by the amino group. This mode of isomerization is unique among all of the aminomutases studied (Table 1).

To conform to the stereochemistry observed in the PAM-catalyzed product, the substrate may bind in the PAM active site with the carboxylate and phenyl ring in a *syn*-periplanar orientation (Figure 7). This arrangement positions the migrating NH_3 and H groups of the α-phenylalanine on the same side of the molecule, and if exchange and reattachment also occur from this side, then retention of configuration at both receiving carbons would result, as observed. The "cisoid" orientation proposed here is distinctive relative to the *anti*-periplanar docking orientation of the α-phenylalanine substrate in the mechanistically related PAL that makes *trans*-cinnamic acid (*23*) (cf. Figure 2B), and accordingly, this conformational difference in binding may likely contribute to the selectivity of the mutase and lyase reactions.

Although PAM (from a plant) and TAM (from bacteria) originate from disparate sources, these described aminomutases have 30% amino acid identity (Figure 8) and a signature active site motif (ASG), indicating that an MIO prosthetic group likely is formed and assists in catalysis. However, these arylalanine aminomutase mechanisms have distinct stereochemical differences at the carbon accepting the amino group.

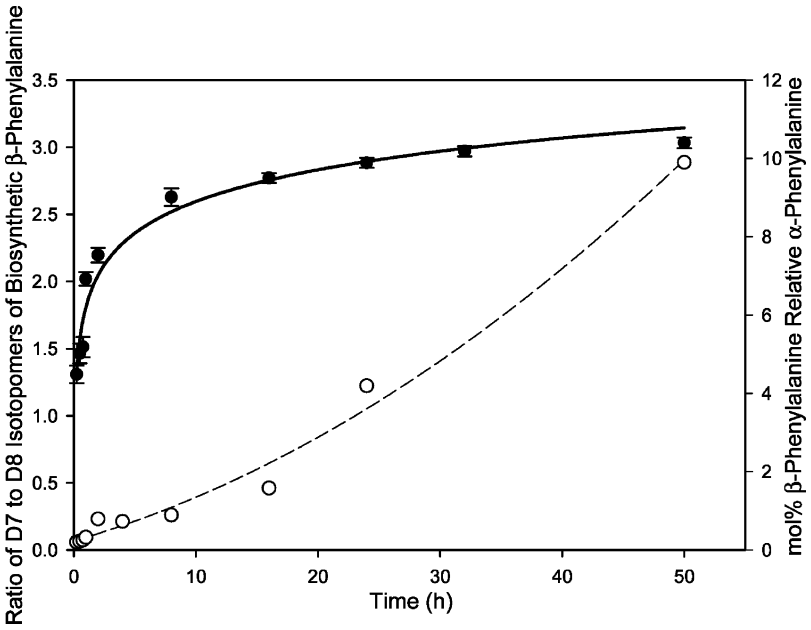


FIGURE 6: Hydrogen exchange between the substrate and bulk solvent observed in β-phenylalanine during PAM catalysis. Time-dependent hydrogen exchange is charted as a ratio of biosynthetic D_7 to D_8 ^{2}H-labeled β-phenylalanine products derived from the conversion of [ring,2,3,3-$^2H_8$]-(2*S*)-α-phenylalanine (at saturation) by PAM catalysis in H_2O at 30 °C and pH 8.0 (solid line, —; filled circles, ●). Also shown is the corresponding abundance of β-phenylalanine (as mol % relative to the [ring,2,3,3-$^2H_8$]-(2*S*)-α-phenylalanine substrate) over the same period; error bars not shown for clarity. Standard error ranged from 0.1% for low values to 0.01% for the highest value (dashed line, —; open circles, ○). The phenylalanines in these samples were N-acetylated and methyl esterified prior to quantification by GC/EI-MS analysis.

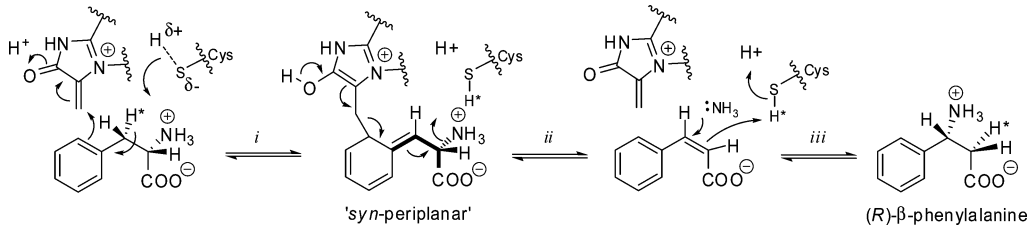


FIGURE 7: Proposed mechanism of PAM catalysis. Based on the evidence supporting the formation of a σ-complex in the presumed analogous PAL reaction mechanism, the PAM reaction is hypothesized to begin with the addition of the aromatic ring of the substrate to the electrophilic MIO moiety in the active site followed by removal of the *pro*-3*S* benzylic hydrogen (H*), possibly via ^{107}Cys (i). Collapse of the σ-complex is followed by ammonia group migration from C2 (ii) to C3 for stereoselective 1,4-Michael addition. Final transfer of the hydrogen purportedly from cysteine back to the phenylpropanoid proceeds with retention of configuration (iii) to yield the 2*S*,3*R* product. *syn*-Periplanar orientation (0° dihedral angle) is highlighted with bold, nonwedged lines of the proposed substrate intermediate to indicate the hypothetical docking conformation of the substrate.

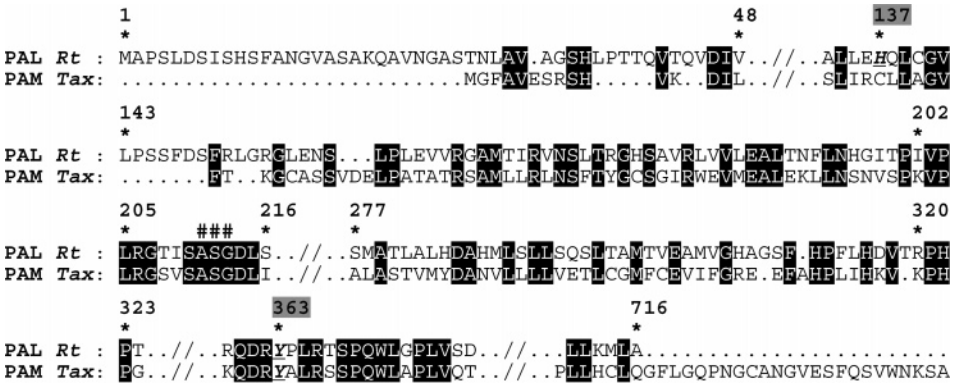


FIGURE 8: Alignment of partial sequences of the related PAM from *Taxus* (PAM *Tax*, GenBank accession AY582743) and PAL from *R. toruloides* (PAL *Rt*, EMBL accession X51513). Identical residues shared between the two sequences are highlighted in black boxes. To save space, portions of the sequences have been deleted (indicated by ..//..). A number and asterisk above a residue indicate the position of the residue relative to the PAL *Rt* sequence. Numbers 137 and 363, highlighted in gray, indicate proposed catalytic residues ^{137}His and ^{363}Tyr in the PAL *Rt*. The pound sign (#) above an amino acid designates residues comprising the signature ASG motif.

The β-amino acid product made by PAM is exclusively (3*R*)-β-phenylalanine, even after several hours at equilibrium, while, in contrast, TAM catalyzes the conversion of (2*S*)-α-tyrosine to (3*S*)-β-tyrosine, initially, and then produces the product racemate as the reaction reaches equilibrium. Therefore, the phenylalanine aminomutase likely binds its substrate

differently than TAM, and/or possibly the catalytic residues of PAM are positioned with an asymmetric bias that precludes racemization.

Moreover, the hydrogen/deuterium exchange observed in this investigation (discussed later) apparently does not epimerize the stereochemistry at C2 of the PAM-catalyzed product during the course of the reaction. The ^{2}H NMR spectrum of the ^{2}H-labeled product showed one signal (δ 2.82) for the deuterium at C2, which corresponded to a single diastereoisomer of the labeled biosynthetic (3*R*)-β-phenylalanine derivative.

Substrate/Product Distribution at Equilibrium. A general property considered of all amino acids is the greater strength of the $C_\beta-N$ bond over the $C_\alpha-N$ bond (*24*), as observed for the lysine-2,3-aminomutase reaction. The free energy ($\Delta G^\circ \approx -1.4$ kcal·mol^{-1}) was calculated to be considerably less than zero, and the forward reaction was assessed to be largely enthalpy-driven (*24*). However, in the PAM isomerization reaction, the product to substrate ratio of nearly 1.0 (cf. Figure 5) suggests that the equilibrium is not principally governed by the difference in C−N bond strength between the α- and β-isomers; apparently, the presence of the aromatic ring of β-phenylalanine minimizes the difference in $C_\alpha-N$ and $C_\beta-N$ bond energy.

Hydrogen Washout during Isomerization Chemistry. The mechanism of the PAM reaction is considered to proceed, in part, similar to that proposed for the reaction of the related ammonia lyases, as mentioned previously. By analogy, the ASG sequence of PAM from *Taxus* (35% sequence identity to PAL from *R. toruloides*; cf. Figure 8) is proposed to form a functional MIO that assists the isomerization reaction (*8*, *11*, *12*, *18*). Amino acid sequence comparison reveals that ^{107}Cys, instead of histidine, of the PAM sequence aligns with the proposed catalytic base (^{137}His) of PAL from *R. toruloides* (Figure 8); yet, this Cys residue could substitute as a general base in place of His to remove the *pro*-3*S* hydrogen in the PAM reaction. Alternatively, ^{322}Tyr of PAM that aligns with active site residue ^{363}Tyr of PAL (Figure 8) could also function as the general base that shuttles the hydrogen.

Deuterium-labeling studies incorporating GC/EI-MS analysis confirmed that as the *pro*-3*S* hydrogen of phenylalanine migrates to C_α of the product, it partially exchanges with protons from water (pH 8.0) during the isomerization reaction. The extent of deuterium exchange was measured by evaluating the level of deuterium retained in the β-amino acid product from $^2H_8$-labeled substrate incubated with PAM in H_2O solvent. While intramolecular transfer of deuterium back to the phenylpropanoid product (D-rebound) is evident, there is however significant loss (60%) of the migrating deuterium, 15 min after the start of the reaction, at which a 3:2 (mol/mol) ratio of D_7 to D_8 β-phenylalanine products is isolated at 0.2 mol %. Intriguingly, a transient deuterium exchange was detected within the first 2 h of the PAM reaction (Figure 6), and this exchange phenomenon likely involves several labile proton sources, including the substrate, PAM residues, the product, and solvent water. Thus far, the mechanism of this time-dependent H/D exchange remains phenomenological.

Conclusions. The results of the described isotopic labeling studies begin to define the boundaries of an acceptable mechanism for the PAM reaction. This investigation established that the amino group at C2 and the vicinal *pro*-3*S* hydrogen are interchanged by PAM with *retention* of configuration at *both* migration termini, which is an uncommon pathway for aminomutases. The removal of the hydrogen, which likely initiates the rearrangement, appears to be involved in the rate-determining step of catalysis, and this migratory hydrogen exchanges with protons from the solvent water. Isotopomeric experiments with perdeuterio-labeled substrate at saturation revealed that there is a significant, but fleeting time-dependent H for D replacement between substrate deuterium and H_2O during the reaction. The origins of this exchange process are, as yet, unexplainable, and the indication that solvent water can likely access the active site of PAM is patently different from the radical-forming lysine-2,3- and arginine-2,3-aminomutases, where the exchange of substrate hydrogens with those of solvent water is excluded.

Exploring the subtleties of the PAM mechanism is vital toward understanding how this enzyme, involved in Taxol biosynthesis, may direct product selectivity and retain ammonia after vicinal isomerization, compared to the homologous and mechanistically related PAL reaction that eliminates ammonia from the same substrate. Furthermore, as β-peptides, comprised of β-amino acids, continue to become more conventional in the development of synthetic mimics of natural protein antibiotics, PAM catalysis potentially provides an alternative, tractable method toward the production of novel β-aryl β-amino acid precursors for this application.

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