Effect of Site-directed Mutagenesis of Conserved Aspartate and Arginine Residues upon Farnesyl Diphosphate Synthase Activity*

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Alison Joly and Peter A. Edwards
From the Departments of Biological Chemistry and Medicine, University of California, Los Angeles, California 90024

All polyprenyl synthases catalyze the condensation of the allylic substrate, isopentenyl diphosphate, with a specific homoallylic diphosphate substrate. Polyprenyl synthases from *Homo sapiens*, *Rattus rattus*, Escherichia coli, Saccharomyces cerevisiae, Neurospora crassa, and Erwinia herbicola contain two conserved “aspartate-rich domains” (Asby, M. N., and Edwards, P. A. (1992) J. Biol. Chem. 267, 4128-4136). In order to determine the importance of these domains in catalysis, the conserved aspartates or arginines in domains I and II of rat farnesyl diphosphate synthase were individually mutated to glutamate or lysine, respectively. The putative “active site” arginine (Brems, D. N., Breunger, E., and Rilling, H. C. (1981) Biochemistry 20, 3711-3718) was mutated to lysine. Each mutant enzyme was overexpressed in *E. coli* and purified to apparent homogeneity. Detailed kinetic analyses of the wild type and mutant enzymes indicated that mutagenesis of Asp104, Asp107, Arg113, Arg118, and Asp243 resulted in a decreased \( V_{\text{max}} \) of approximately 1000-fold compared to wild type. However, no significant change in the \( K_{\text{m}} \) values for either the isopentenyl diphosphate or geranyl diphosphate substrate were observed. The results strongly suggest that these amino acids, and to a lesser extent Asp243, are involved in either the condensation of isopentenyl diphosphate and geranyl diphosphate to form farnesyl diphosphate and/or the release of the farnesyl diphosphate product from farnesyl diphosphate synthase. The conservation of these amino acid residues in different enzymes from several species suggests that these domains play a similar role in other polyprenyl synthases.

Farnesyl diphosphate (FPP) synthase (EC 2.5.1.1) is a polyprenyltransferase which is a regulated enzyme of cholesterol biosynthesis (1). FPP synthase catalyzes the sequential 1'-4 condensation of the 5-carbon isoprenoid compounds isopentenyl diphosphate (IPP) and dimethylallyl diphosphate to form the 10-carbon geranyl diphosphate (GPP). GPP is then condensed with another IPP molecule to form the 15-carbon product, FPP (2). FPP is utilized within the mammalian cell for several biosynthetic pathways namely in the synthesis of squalene and sterols, dolichols, ubiquinone, prenylated proteins, and the isoprenoid side chain of heme (3). FPP synthase is a member of a large family of enzymes collectively termed the prenyltransferases. This class of enzyme can be subdivided into three groups according to the type of reactions which they perform. Certain prenyltransferases, of which FPP synthase is one (1, 4), catalyze the irreversible 1'-4 condensation of the allylic diphosphate isoprene unit, IPP, to a homoallylic isoprene primer, such as GPP. This type of condensation is often termed “head to tail.” Other enzymes of this type include hexaprenyl diphosphate synthase (5) and geranylgeranyl diphosphate synthase (6, 7). However, prenyltransferases such as squalene synthase (8, 9) and phytoene synthase (crtB) (10, 11) catalyze the “head to head” condensation of two allylic diphosphate substrates to form a diphosphate intermediate followed by a rearrangement to give the final product. A third group of prenyltransferases are involved in the transfer of isoprenoid diphosphate substrates to several nonterpenoid acceptor molecules; examples include tRNA-dimethylallyl transferase (MOD5), p-hydroxybenzoate-polyprenyltransferase, adenosine monophosphate-dimethyl allyl transferase (Ti-Bo542, TiT37, TiAch5) (see Ref. 5 and references cited therein), and the recently discovered protein:prenyltransferases (12).

Comparison of the amino acid sequences of a number of prenyltransferases (5) revealed the presence of three conserved regions. Two of these regions have been referred to as domains I and II and are characterized by an aspartate-rich motif which is flanked by other conserved residues (see Fig. 1). The third region of homology is similar to an active site peptide proposed from the studies of Brems et al. (13) on avian FPP synthase. Since all prenyltransferases and synthases share the common feature of binding at least one prenyl diphosphate substrate, it was hypothesized that the conserved domains I and II might be involved in substrate binding (5). In order to begin testing this hypothesis a previous study was performed (14) in which two of the conserved aspartate residues in domain II of rat FPP synthase were separately changed to glutamate residues. The results of this analysis suggested that the first aspartate in domain II was involved in the catalytic reactions of the enzyme (14).

We report here an extension of the initial study in which we have created point mutants of rat FPP synthase in both conserved domains I and II. In addition, we mutated the rat FPP synthase arginine at position 192 to a lysine residue in order to examine the effect(s) of a conservative mutation within the proposed active size peptide (13).

The wild type and mutant proteins were expressed in *Escherichia coli*, purified by ion exchange chromatography and the kinetic parameters were determined. The results of this study imply that several of the conserved residues of domains I and II play an essential role in the catalytic reactions of FPP synthase since the mutations described greatly reduced the enzyme’s specific activity.

EXPERIMENTAL PROCEDURES

Materials—[1-14C]IPP (52.7Ci/mmol) was purchased from Amersham Corp. Geranyl diphosphate was the generous gift of Dr. H. C. Rilling (University of Utah). \( \alpha\)-3SS-dATP of sequencing grade was purchased from DuPont NEN. DE52 ion-exchange cellulose powder was obtained...
Analysis of Proteins—Proteins were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using the discontinuous buffer system of Laemmli (16). Following electrophoresis the gels were either stained with Coomassie Brilliant Blue R or were transferred to Amer sham Hybond ECL nitrocellulose membranes for 16 h at 20 V in transfer buffer (20 mM Tris, 150 mM glycine, 20% [v/v] methanol). Following transfer the membrane was air-dried, rinsed with distilled water, soaked in 15% hydrogen peroxide for 15 min, and blocked with Tris-buffered saline (TBS), pH 7.6, containing 0.5% Tween 20 and 5% nonfat dry milk for 1 h. The membrane was washed to remove excess milk, incubated with affinity purified anti-FPP synthase antibodies (see below) in TBS, 0.5% Tween 20 for 2 h, washed three times with TBS, 0.3% Tween 20 for 10 min each, and incubated with the secondary antibody, anti-rabbit IgG coupled to horseradish peroxidase in TBS, 0.3% Tween 20 for 1 h. The membrane was finally washed with TBS, 0.3% Tween 20 (3 x 5 min washes) and TBS, 0.1% Tween 20 (5 x 5-min washes). The membrane was then soaked in the Amersham ECL detection reagents (11, v/v) for 1 min and exposed to x-ray film for 1-6 s. Anti-FPP synthase antiserum for Western analysis was generated by injection of rabbits with purified recombinant wild type FPP synthase. The antiserum was purified by passing it over an affinity column consisting of purified wild type FPP synthase coupled to Tresyl-activated agarose (Schleicher & Schuell) according to the manufacturers instructions. Purified anti-FPP synthase antibodies were eluted from the affinity column with 100 mM glycine, pH 2.0, and the identity confirmed by Western analysis. Protein estimations were determined by the method of Bradford (17) using bovine serum albumin (Sigma) as a reference protein.

Recombinant DNA Reagents—T4 DNA ligase, polynucleotide kinase, and restriction enzymes used in the construction of plasmids were obtained from Life Technologies Inc. Preparation of plasmid DNA, restriction enzymes used in the construction of plasmids were obtained from Life Technologies Inc. Preparations of plasmid DNA, restriction enzyme digests, and agarose gel electrophoresis were performed by standard methods as described by Maniatis et al. (18). Sequence analysis of plasmid was performed by the dyeoxy chain termination method of Sanger et al. (19) using the Sequenase Version 2.0 sequencing kit from U. S. Biochemical Corp. Specific oligonucleotide internal primers were designed to obtain sequence throughout the FPP synthase gene.

Construction of the Reading Frame Cassette and the Prokaryotic Expression Vector for FPP Synthase cDNA—The strategy used in the construction of the reading frame cassette for FPP synthase is explained in previous publications (14, 20).

Site-directed Mutagenesis of FPP Synthase—DNA mutants for rat FPP synthase were generated by the oligonucleotide-directed in vitro mutagenesis system from Amer sham Corp. Single strand wild type FPP synthase, used as a template in the mutagenesis reaction, was generated by the VCS-M13 interference resistant helper phage (Stratagene) infection of XL1-Blue cells which contained the wild type FPP synthase gene inserted into the Bluescript vector. The resulting sense single strand DNA template was isolated and purified by standard methods (18) and sequenced to confirm identity. The synthetic anti-sense oligonucleotides designed to produce the desired point mutations (with the changes to the wild type sequence underlined and in bold type) were as follows:

| Site-directed mutants for rat FPP synthase | 5'-AAAGATGTCAGTGACCTGCGATCTGAG-3' |
|------------------------------------------|---------------------------------------|
| Asp<sup>188</sup>-Glu                    | 5'-AAAGATGTGACCTGCGATCTGAG-3'        |
| Asp<sup>189</sup>-Glu                    | 5'-AAAGATGTGACCTGCGATCTGAG-3'        |
| Asp<sup>233</sup>-Glu                    | 5'-AAAGATGTGACCTGCGATCTGAG-3'        |
| Asp<sup>234</sup>-Glu                    | 5'-AAAGATGTGACCTGCGATCTGAG-3'        |
| Arg<sup>112</sup>-Lys                    | 5'-AAAGATGTGACCTGCGATCTGAG-3'        |
| Arg<sup>113</sup>-Lys                    | 5'-AAAGATGTGACCTGCGATCTGAG-3'        |
| Arg<sup>116</sup>-Lys                    | 5'-AAAGATGTGACCTGCGATCTGAG-3'        |
| Arg<sup>117</sup>-Lys                    | 5'-AAAGATGTGACCTGCGATCTGAG-3'        |

FIG. 1. The alignment of the highly conserved amino acid residues of domains I and II and the active site peptide of prenyltransferase enzymes. The optimum amino acid alignment between domains I and II and the active site peptide of farnesyl diphosphate synthase (FPPS), hexaprenyl diphosphate synthase (HPPS), and geranylgeranyl diphosphate synthase (GGPPS) from several different species is shown. E. coli FPPS contains two extra amino acid residues in the middle of domain I. The numbers above the rat FPP synthase sequence reflect the residue number according to the rat sequence (1,26).
Following mutagenesis, transformants were screened by dioxygenase sequencing to confirm the mutation. The mutant cDNA was excised from the Bluescript vector with two restriction enzymes, NdeI and Sall. This produced a 1.07-kilobase pair fragment which was ligated into the expression vector pARC360N and used to transform JM101 cells.

**Purification of Recombinant FPP Synthase**—A stationary phase culture (1 ml) was inoculated into 500 ml of M9+CAGM media and grown at 37 °C with aeration until the A600 nm was 0.5–0.6 (typically 6–8 h). The bacterial cells were harvested by centrifugation (4,000 rpm, 15 min). The resulting paste was resuspended in 5 ml of buffer A (10 mM potassium phosphate, pH 7.0, 10 mM 2-mercaptoethanol, 1 mM EDTA). The cells were disrupted by three cycles of sonication for 30 s each with 1 min cooling on ice in between. Undisrupted cells and cell membranes were separated from the soluble material by centrifugation (15,000 × g, 30 min, 4 °C) and the total protein content and FPP synthase activity of the extract were determined. The protein precipitating between 30 and 65% ammonium sulfate was collected, dissolved in 2 ml of buffer A, and dialyzed against two 2-liter changes of FPLC buffer. The dialyzed material was centrifuged (15,000 × g, 10 min, 4 °C) and the total protein concentration estimated before diluting to a final protein concentration of approximately 10 mg/ml. The sample was pumped onto a 2.5-cm DE52 column (at a flow rate of 6–8 ml/min) and the absorbance at 280 nm of the eluate had returned to baseline (typically 300 ml). The bound protein was eluted with 200 ml of buffer B (90 mM potassium phosphate, pH 7.0, 10 mM 2-mercaptoethanol, 1 mM EDTA), and precipitated by the addition of ammonium sulfate to a final concentration of 70%. The protein was dissolved in 2 ml of FPLC buffer (50 mM Tris-HCl, pH 7.0, 1 mM EDTA, 1 mM dithiothreitol, 0.01 M NaCl) and dialyzed for 18 h at 4 °C against two 2-liter changes of FPLC buffer. The dialyzed DE52 purified material was chromatographed on a Mono Q 5/5 HR ion-exchange column with an FPLC system (Pharmacia LKB Biotechnology Inc.). The column was equilibrated with FPLC buffer at a flow rate of 1 ml/min and the sample was applied to the column via a 2-ml injection loop. Following sample application the column was washed with 25 ml of FPLC buffer followed by a 75-ml linear gradient from 0.01 to 1 x NaCl in the same buffer. Fractions (1 ml) were collected and assayed for FPP synthase activity.

**In Vitro Assay of Farnesyl Diphosphate Synthase**—FPP synthase was assayed essentially as described by Holloway and Popjak (21) as reported in previous publications (14, 22). Routine assays contained (in a final volume of 50 µl) 40 µM [1-14C]IPP (4 Ci/mol), 20 µM GPP, 1 mM dithiothreitol, 1 mM MgCl2, 10 mM HEPES buffer, pH 7.2. Assays were carried out at 37 °C for 10 min and were terminated by the addition of 50 µl of stop mixture (2.5 x HCl in 70% ethanol, 10 µg/ml farnesol). Assay products were acid hydrolyzed for 30 min at 37 °C (23). The samples were neutralized by the addition of 50 µl of 10% NaOH and then extracted with 150 µl of hexane. 75 µl of each hexane extract were counted in 5 ml of scintillation fluid for 10 min/sample.

For kinetic studies with purified enzymes the amount of [1-14C]IPP and GPP substrates were varied as indicated in the legend to Fig. 3. 1.1 ng of wild type, 1.5 ng of Asp284-Glu, 9.8 µg of Arg113-Lys, 2.5 µg of Asp284-Glu, 2.5 µg of Asp101-Glu, 5 µg of Arg113-Lys, or 5 µg of Arg113-Lys FPP synthase proteins were assayed. [1-14C]IPP of specific activity 4 or 50.7 Ci/mol was used dependent upon the specific activity of the various mutant proteins. Assays were carried out in quadruplet or triplicate and the variation between the multiple values was less than 10%. Each analysis was performed on at least three
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Fig. 3. Lineweaver-Burk plots for wild type and mutant FPP synthase. Double reciprocal plots of $1/v$ (pmol min$^{-1}$ mg$^{-1}$) against $1/v$ varied substrate (pmol) at different fixed concentrations of the second substrate are shown. A shows varied concentrations (pmol) of IPP at the following fixed concentrations of geranyl diphosphate: a, 1 μm; b, 2 μm; c, 5 μm; d, 10 μm. Each point is the average of triplicate or quadruplicate determinations. B shows varied concentrations (pmol) of GPP at the following fixed concentrations of isopentenyl diphosphate: a, 1 μm; b, 2 μm; c, 5 μm; d, 10 μm. Each point is the average of triplicate or quadruplicate determinations.

separate occasions with essentially similar results. In all cases less than 10% of the substrate was converted into product. A separate control assay which contained no GPP was performed for each assay utilizing a different concentration of [1-14C]IPP.

RESULTS AND DISCUSSION

The alignment of the conserved amino acid residues in domains I and II and the active site peptide of prenyltransferases from several different species is summarized in Fig. 1. All of the enzymes share the common feature of binding both allylic and homoallylic diphosphate substrates.

In order to test the hypothesis that the conserved amino acids might play a role in enzymatic activity we systematically mutated several of the conserved residues. The mutations were designed so that the charge of the residue in question remained constant; thus, an aspartate was changed to a glutamate and an arginine was changed to a lysine. The residues mutated in domain I were the aspartate at positions 103, 104, and 107 and the arginines at positions 112 and 113 according to the numbering of the rat FPP synthase protein. In domain II aspartate 244 was changed to a glutamate and in the active site peptide the arginine at position 192 was changed to a lysine.

Following oligonucleotide site-directed mutagenesis of the wild type gene each plasmid was sequenced to confirm that the desired mutation was present. Next, either the wild type or mutated gene fragments were inserted into the bacterial expression vector pARC360N which was then used to transform E. coli strain JM101. The wild type or mutant enzymes were expressed in E. coli after growth on minimal media and purified as described under “Experimental Procedures.” Table I summarizes the purification of the wild type and mutant FPP synthase enzymes as monitored by the specific activity at each step in the purification. Fig. 2A shows a Coomassie Brilliant Blue-stained SDS-PAGE analysis of the purification steps using the expression of the wild type protein as an example. As can be seen from Fig. 2A, JM101 which had been transformed with pARC360N containing the wild type insert (lane 2) showed the presence of a protein band which migrated with an apparent molecular mass of 39 kDa. This protein was not present in the soluble extract obtained from
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B

WILD TYPE

D104E

R113K

D244E

D107E

R192K

D103E

R112K

Fig. 3.—continued

JM101 which had been transformed with the expression vector alone (lane 1). An apparent subunit molecular mass of rat liver FPP synthase of 39 kDa as seen here is consistent with previous studies (14) and with the predicted molecular mass as calculated from the cDNA sequence (24, 26, 27). Lanes 2-6 of Fig. 2A show the three-step purification of the expressed wild type enzyme while lanes 7-12 show the expressed mutant proteins obtained after the final step of purification. The purified proteins appear homogeneous. Immunoblot analysis of an identical gel with affinity purified anti-FPP synthase antibodies confirmed the identity of the homogeneous band (Fig. 2B).

The purified proteins were used in multiple in vitro enzyme assays to determine the $K_m$ values for the IPP and GPP substrates and the $V_{max}$ values for both the wild type and mutant FPP synthase proteins. Fig. 3A shows the double reciprocal plots of the initial velocities versus varied substrate concentration with IPP being the varied substrate at constant GPP concentrations. These data were used to determine the $K_m$(IPP) values summarized in Table II. Fig. 3B shows the double reciprocal plots of initial velocities versus varied substrate concentration with GPP being the varied substrate at constant IPP concentrations. These data were used to determine the $K_m$(GPP) values summarized in Table II. In order to determine the $V_{max}$ values of the enzymes which are also summarized in Table II, the intercept on the $y$ axis of the Lineweaver-Burk plots were replotted against the reciprocal of the constant substrate concentration. These straight line plots (data not shown) intersected the $y$ axis at a value corresponding to the reciprocal of the $V_{max}$ value (28, 29).

The kinetic constants for the wild type rat FPP synthase given in Table II are in agreement with the previous analysis (14) and are similar to the values previously reported for the human, avian, and pig liver (23, 30-32), E. coli (33) and Saccharomyces cerevisiae (34) FPP synthases.

The results of the point mutations, summarized in Table II, indicate that the mutant enzymes fall into two groups, 1) those mutants with $K_m$(IPP), $K_m$(GPP), and $V_{max}$ values similar to the wild type enzyme namely Asp103-Glu, Asp244-Glu, and Arg192-Lys, and 2) those mutants with $K_m$(IPP) and $K_m$(GPP) values similar to the wild type enzyme but whose $V_{max}$ values are approximately 1000-fold lower than wild type, namely Asp104-Glu, Asp107-Glu, Arg112-Lys, and Arg113-Lys. The two notable exceptions to this generality are Asp244-Glu which caused a reduction in $V_{max}$ but an increase in the $K_m$(IPP) value (14) and the neighboring residue Asp244 which when mutated to Glu results in wild type $K_m$ values for both substrates and a 7-fold reduction in the $V_{max}$ value.

None of the mutations analyzed here significantly altered the $K_m$ values for either substrate. It is also clear that slight structural changes to several of these conserved residues drastically reduces the catalytic efficiency of the enzyme.
which is reflected in the reduced \( V_{\text{max}} \) values. The kinetic parameters can be expressed in terms of individual rate constants for

\[
E + \text{IPP} \rightarrow E-\text{IPP} \rightarrow E-\text{IPP+GPP} \rightarrow E+\text{FPP} + \text{EPP}
\]

where

\[
V_{\text{max}} = \frac{k_1 k_2 k_4 [E]_0}{k_1 + k_6}
\]

\[
K_{\text{mIPP}} = \frac{k_1 k_2}{k_5 (k_3 + k_4)}
\]

\[
K_{\text{mGPP}} = \frac{k_4 k_3}{k_6 (k_3 + k_4)}
\]

As discussed previously (14), FPP synthase obeys an ordered sequential mechanism for the synthesis of FPP from IPP and GPP (35, 36) for which there are several rate constants. The most likely interpretation for the reduced \( V_{\text{max}} \) value for the mutant Asp243-Glu was that once both substrates had bound, the rate of chemical conversion of GPP and IPP to FPP \((k_6)\) is reduced, or that the rate of release of FPP from the enzyme \((k_6)\) is much reduced (14). The decreased \( V_{\text{max}} \) in the mutant Asp243-Glu was most likely a result of a decrease in \( k_6 \) such that \( k_6 \) became rate-limiting (14). The product release step \((k_6)\) is rate-limiting in the wild type enzyme (35, 36). However, we previously concluded that the mutant Asp243-Glu was unlikely to decrease \( V_{\text{max}} \) as a result of a further decrease in \( k_6 \) since such a change would require compensatory changes in \( k_3 \) and \( k_4 \) that would in turn result in a reduced affinity for IPP and an increased affinity for the product FPP (14). Such a scenario appears unlikely.

Since several of the mutants analyzed here also exhibited a greatly reduced \( V_{\text{max}} \) value in the absence of changes in \( K_{\text{mIPP}} \) or \( K_{\text{mGPP}} \), we propose a similar interpretation, i.e. these mutations decrease \( k_6 \), the rate of the chemical step. Furthermore, since \( V_{\text{max}} / K_{\text{mIPP}} = k_1 \), when \( k_6 \ll k_5 \) (14), then the mutations, Asp104-Glu, Asp107-Glu, Arg112-Lys, and Arg113-Lys, that result in significant decreases in \( V_{\text{max}} \) but no change in \( K_{\text{mIPP}} \), will also have decreased values of \( k_1 \), the rate constant for addition of GPP to the enzyme.

\( K_{\text{mIPP}} \) can be simplified to \( k_4/k_3 \), when \( k_4 \ll k_3 \) (14). Thus the finding that \( K_{\text{mIPP}} \) does not change for the mutations Asp104-Glu, Asp107-Glu, Arg112-Lys, and Arg113-Lys (Table II) indicates that these mutations are unlikely to affect \( k_4 \), the dissociation of IPP from the \( E \)-GPP-IPP complex. The results are consistent with an important role for these four amino acids in \( k_4 \), the catalytic step that results in the formation of the \( E-\text{PP} \), FPP complex.

One of the mutants, Asp244-Glu, showed a \( V_{\text{max}} \) value that was only 7-fold lower than wild type (Table II). We hypothesize that one of the two rate constants for product formation/release were less affected by this mutation.

When either of the 2 conserved arginine residues in domain I (Arg112 and Arg113) were mutated to lysine residues there was approximately a 1000-fold reduction in \( V_{\text{max}} \) with little or no change in the \( K_m \) values for either substrate (Table II). Previous studies have reported the inactivation of pig liver FPP synthase by the arginine modifying agent phenylglyoxal (37). Bernard and Popjak (37) proposed that the biphasic nature of this observed inactivation was due to two important arginine residues per subunit that were both accessible to phenylglyoxal. These residues were postulated as being important in the binding of either the allylic or homoallylic substrates (37). In the current study, separate mutation of the 2 domain I arginine residues resulted in a relatively inactive enzyme, we conclude that these residues are important for the catalytic reactions of FPP synthase. Since mutation of either domain I arginine residues resulted in a 1000-fold decrease in \( V_{\text{max}} \) while mutation of the "active site" arginine (Arg112) resulted in only a 65% decrease in \( V_{\text{max}} \) (Table II), it appears possible that phenylglyoxal modification of the 2 domain I arginines was responsible for the observed enzyme inactivation in the original study by Bernard and Popjak (37).

The term "active site peptide" of prenyltransferases (summarized in Fig. 1) arose from an elegant series of experiments carried out in the laboratory of Billing (13, 38). These workers synthesized a radiolabeled photoactivatable analogue of IPP, \( \text{o-azidophenyl[1-3H]} \)ethyl pyrophosphate, which they incubated with FPP synthase purified from chicken liver. Following photoactivation a 30-amino acid CNBr fragment was found to contain 80% of the total radiolabel and upon Edman degradation of this peptide 16 of the 30 residues appeared to be modified (13). The two most extensively labeled being an arginine and an alanine (13). It was not known if this arginine was also modified by phenylglyoxal with the resulting loss in enzyme activity (37).

In order to determine the importance of this conserved active site arginine residue, FPP synthase containing a conservative mutation (Arg112-Lys) was generated and purified. The \( V_{\text{max}} \) and \( K_{\text{mIPP}} \) values of the Arg112-Lys FPP synthase mutant were approximately 65% of the wild type values, while the \( K_{\text{mIPP}} \) values remained unchanged (Table II). These apparent \( V_{\text{max}} \) and \( K_{\text{mIPP}} \) values are not significantly different from wild type values. These studies indicate that Arg112 does not play an important role in catalytic activity. Further studies to define a role for Arg112 might involve less conservative mutations, whereby the charge on the residue is altered to neutrality or negativity.

In conclusion, in the current report we tested the hypothesis that the conserved "aspartate-rich domains I and II" of prenyltransferases were involved in catalysis of the allylic and homoallylic substrates of these enzymes. This study demonstrates for the first time that a number of the conserved aspartate and arginine residues in rat FPP synthase domains I and II are critical for efficient enzyme catalysis, since conservative mutations result in a 1000-fold decrease in \( V_{\text{max}} \), but no change in the \( K_s \) for IPP and GPP. These studies, together with the previous report (14), indicate that domains I and II are most likely critically involved in either the chemical conversion of IPP and GPP to FPP and/or the release of the FPP product.
from the enzyme. Domains I and II in other polyprenyl synthases are likely to play a similar role.

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