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Significance of Asn-77 and Trp-78 in the Catalytic Function of Undecaprenyl Diphosphate Synthase of *Micrococcus luteus* B-P 26[1]

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The primary structures of *cis*-prenyltransferases are completely different from those of *trans*-prenyltransferases. To obtain information about amino acid residues relating to catalytic function, random mutation of the undecaprenyl diphosphate synthase gene of *Micrococcus luteus* B-P 26 was carried out to construct a mutated gene library using an error-prone polymerase chain reaction. From the library, the mutants showing poor enzymatic activity were selected by the colony autoradiography method. Among 31 negative clones selected from 3,000 mutants, two clones were found to contain only one amino acid substitution at either Asn-77 or Trp-78. To determine the functional roles of these interesting residues, we prepared six mutated enzymes with substitutions at residues Asn-77 or Trp-78 by site-directed mutagenesis. Substitution of Asn-77 with Ala, Asp, or Gln resulted in a dramatic decrease in catalytic activity, but the K_m values for both allylic and homoallylic substrates of these mutant enzymes were comparable to those of the wild-type. On the other hand, three Trp-78 mutants, W78I, W78R, and W78D, showed 5–20-fold increased K_m values for farnesyl diphosphate but not for *Z*-geranylgeranyl diphosphate. However, these mutants showed moderate levels of enzymatic activity and comparable K_m values for isopentenyl diphosphate to that of the wild-type. These results suggest that the Asn-Trp motif is involved in the binding of farnesyl diphosphate and enzymatic catalysis.

Key words: isoprenoid, prenyltransferase, undecaprenyl diphosphate synthase, site-directed mutagenesis, substrate recognition.

Isoprenoids are the most chemically diverse family of naturally occurring compounds, and are widely distributed among archaea, bacteria, and eukarya. Over 23,000 isoprenoid compounds have been characterized, and most of them are essential components of the cellular machinery of all organisms serving as visual pigments, reproductive hormones, defensive agents, constituents of membranes, components of signal transduction, and so on (*1*). Isoprenoids are all produced biochemically from linear prenyl diphosphates, which are synthesized by sequential condensations of isopentenyl diphosphate (IPP) with allylic prenyl diphosphates. These condensation reactions are catalyzed by a family of prenyltransferases (so-called prenyl diphosphate synthases) that can be classified into two major subgroups (*cis* and *trans*-type) according to the geometry of the products. These enzymes are extremely interesting from an enzymological viewpoint in that the reactions are regulated so as to proceed consecutively and terminate precisely at definite chain lengths depending on the specificities of the individual enzymes.

During the past decade, the structural genes for many kinds of prenyltransferases that catalyze *trans*-prenyl chain elongation have been cloned and characterized (*2*). Comparison of the deduced amino acid sequences of these enzymes has revealed the presence of seven highly conserved regions, including two characteristic aspartate-rich DDXXD motifs, which have been shown to be essential for catalytic function in several site-directed mutagenesis studies of farnesyl diphosphate (FPP) synthases (*3–5*). Tarshis *et al.* (*6*) have determined the crystal structure of avian FPP synthase to 2.6 Å resolution, which is the first three-dimensional structure for any prenyltransferases. In the crystal structure of the FPP synthase, most of the conserved regions are found in a large central cavity, which is presumed to be the catalytic site. X-ray crystallography (*7*) and site-directed mutagenesis (*8–12*) have uncovered the precise catalytic mechanism of FPP synthase.

On the other hand, *cis*-prenyltransferases catalyze *cis*-prenyl chain elongation to produce prenyl diphosphates with *E,Z*-mixed stereochemistry. In bacteria, the sequential *cis*-addition of IPP onto FPP as an allylic primer to give undecaprenyl diphosphate (UPP, C_{55}), which is an essential intermediate in the biosynthesis of cell wall peptidoglycans,

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Abbreviations: FPP, farnesyl diphosphate; *Z*-GGPP, (*Z,E,E*)-geranylgeranyl diphosphate; IPP, isopentenyl diphosphate; IPTG, isopropyl-β-D-thiogalactopyranoside; UPP, undecaprenyl diphosphate.

is catalyzed by UPP synthase. This enzyme has been partially purified and characterized from *Micrococcus luteus* B-P 26 (*13*), *Bacillus subtilis* (*14*), *Escherichia coli* (*15*), and *Lactobacillus plantarum* (*16*). It is associated with the periplasmic membrane so loosely that it is easily solubilized, but requires a phospholipid or detergent such as Triton X-100 for enzymatic activity. Molecular cloning of the gene for the UPP synthase of *M. luteus* B-P 26 has recently been carried out (*17*). Surprisingly, the primary structure of the protein encoded by this gene is totally different from those of *trans*-prenyltransferases. Homologous genes for the bacterial enzyme were identified and isolated shortly afterward from *E. coli*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* (*18*). Dehydrodolichyl diphosphate synthase, which is responsible for *cis*-prenyl chain elongation in eucaryotic cells, catalyzes a much longer chain elongation than does the bacterial enzyme. This enzyme has been solubilized and characterized from the membrane fractions of rat liver (*19*) and *Saccharomyces carlsbergensis* (*20*). Recently, the *Saccharomyces cerevisiae RER2* gene has been identified to encode the dehydrodolichyl diphosphate synthase responsible for the biosynthesis of dolichol in yeast (*21*).

However, little is known about the molecular mechanism of enzymatic *cis*-prenyl chain elongation. In order to obtain information about the amino acid residues related to catalytic function, we introduced random mutations on the *M. luteus* B-P 26 UPP synthase gene using an error-prone polymerase chain reaction (PCR), and screened clones that showed poor enzymatic activity by the colony autoradiography method. Thirty-one negative clones were isolated from 3,000 mutants, and their gene sequences were determined. Two mutants at Asn-77 or Trp-78, which were found to contain only one amino acid substitution, were selected for further analysis. To better understand the functional roles of Asn-77 and Trp-78 during catalysis, site-directed mutagenesis of the two residues was carried out. This paper describes the elucidation of Asn-77 and Trp-78 as being important for the catalytic function and for the binding of the allylic substrate, respectively.

EXPERIMENTAL PROCEDURES

Materials and General Procedures—[1-^{14}C]IPP (1.95 TBq/mol) was purchased from Amersham. Non-labeled IPP, FPP, and (*Z,E,E*)-geranylgeranyl diphosphate (*Z*-GGPP) were synthesized according to the procedure of Davisson *et al.* (*22*). Precoated reversed phase thin layer chromatography (TLC) plates, LKC-18, were purchased from Whatman. Restriction enzymes and other DNA-modifying enzymes were from Takara Shuzo and New England Biolabs. Potato acid phosphatase was a product of Sigma. Chromosomal DNA of *M. luteus* B-P 26 was isolated according to the method described by Saito and Miura (*23*). *E. coli* K12 strain JM109 or *E. coli* B strain BL21 (DE3) was used as the host for expression of the target gene. Restriction enzyme digestions, transformations, and other standard molecular biology techniques were carried out as described by Sambrook *et al.* (*24*). Bacteria were cultured in LB or M9YG medium (*25*). All other chemicals were of analytical grade.

Random Mutagenesis of the UPP Synthase Gene—The introduction of random mutations in the structural gene for UPP synthase of *M. luteus* B-P 26 was carried out using an error-prone PCR according to the method by Cadwell and Joyce (*26*). The sense and antisense primers, 5′-GAGGTTGACATGTTTCCAATTAA-3′ and 5′-CATGATTTAAAGCTTATAATCCACC-3′ (newly created restriction sites are underlined), were designed to create an *Afl*III site overlapping the starting codon, ATG, and a *Hin*dIII site downstream of the stop codon, TAA, respectively. PCR was performed in a final volume of 100 μl containing 10 mM Tris-HCl buffer, pH 8.3, 50 mM KCl, 7 mM $MgCl_2$, 0.2 mM dATP, 1.0 mM dCTP, 0.2 mM dGTP, 1.0 mM dTTP, 1.0 μM of amplification primer pairs, 500 ng of *M. luteus* B-P 26 genomic DNA, and 5 units of *Taq* polymerase (Takara). The protocol used was 30 cycles of 1 min at 94°C, 1 min at 45°C, and 1 min at 72°C. The PCR products were digested with *Afl*III and *Hin*dIII, and then were ligated into pTrc 99A (Pharmacia Biotech), which was digested with *Nco*I and *Hin*dIII. *E. coli* JM109 cells were transformed with these plasmids to construct a mutagenic gene library of *M. luteus* B-P 26 UPP synthase.

Colony Autoradiography Screening of the Mutagenic Gene Library of M. luteus B-P 26 UPP Synthase—The screening of mutants expressing poor UPP synthase activity was carried out essentially according to the colony autoradiography method as described in our previous work (*17*). An appropriate amount of *E. coli* cells carrying the mutagenic UPP synthase gene library was inoculated on a nylon membrane (132-mm diameter, Pall) laid on an LB-agar plate containing 60 μg/ml ampicillin, and the filter was incubated at 37°C for 12 h. A replica filter was then made by filter-to-filter contact. The replica filter was incubated for another 6 h on a fresh LB-agar plate containing 60 μg/ml ampicillin and 57 μg/ml isopropyl-β-D-thiogalactopyranoside (IPTG), and then floated on TE buffer (10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA) to remove nutrient broth absorbed in the filter. The incubation mixture for the direct assay of the replica filter contained, in a final volume of 0.6 ml, 100 mM Tris-HCl buffer, pH 7.5, 0.5 mM $MgCl_2$, 5 μM FPP, and 0.46 μM [1-^{14}C]IPP (1.95 TBq/mol). After incubation with the assay mixture at 37°C for 6 h, the replica filter was exposed on a Fuji imaging plate at room temperature for 1 day, and then the distributions of radioactivity on the filter were analyzed with a Fuji BAS 1000 Mac bioimaging analyzer. Negative colonies that showed no spot in the autoradiogram of the replica filter were isolated from the master plate for further analysis.

DNA Sequence Analysis—All DNA sequences were determined by the dideoxy chain termination method (*27*) with a DNA sequencer (LI-COR model 4200). Computer analysis and comparison of DNA sequences were performed using GENETYX genetic information processing software (Software Development).

Site-Directed Mutagenesis of UPP Synthase—To uncover the functional roles of amino acid residues Asn-77 and Trp-78, site-directed mutagenesis was performed using the procedure of Kunkel *et al.* (*28*). The single-stranded wild-type UPP synthase gene, used as a template in the mutagenesis reaction, was prepared by M13KO7 helper phage infection of *E. coli* CJ236 (Takara) cells containing pMluUEX (*17*). The mutagenic oligonucleotides designed to produce the desired point mutations were as follows:

N77A, 5′-CATCTTTAGGTCGCGACCAAGCTTCAGTTG-

3′; N77D, 5′-CATCTTTAGGTCGCGACCAATCTTCAGTTG-3′; N77Q, 5′-CATCTTTAGGTCGCGACCA*TTG*TTCAGTTG-3′; W78I, 5′-CATCTTTAGGTCGCGA*AAAT*ATTTTCAGTTG-3′; W78R, 5′-CATCTTTAGGTCGCGACCG*A*TTTTCAGTTG-3′; W78D, 5′-CATCTTTAGGTCGCGA*GT*CATTTTCAGTTG-3′ (mismatched bases are in italics, newly created *Nru*I restriction sites are underlined). After mutagenesis, all the mutations were confirmed by DNA sequencing.

Preparation and Purification of Mutated UPP Synthase—E. coli BL21 (DE3) was transformed with each of the mutant plasmids, pN77A, pN77D, pN77Q, pW78I, pW78R, pW78D, and cultured according to the methods described previously (*17*). The cells were harvested and disrupted by sonication in TE buffer. The protein fraction precipitated from the 100,000 ×*g* supernatant by 30–60% saturation with ammonium sulfate was dissolved in TE buffer containing ammonium sulfate at 30% saturation and chromatographed on a Phenyl Sepharose HP column (Pharmacia Biotech) with elution of a decreasing linear gradient of ammonium sulfate from 30 to 0% saturation. The UPP synthase fractions were desalted on a HiTrap desalting column (Pharmacia Biotech), applied to a Mono Q HR 5/5 column (Pharmacia Biotech), and eluted with a stepwise gradient of 0–0.5 M NaCl in TE buffer. The fractions containing the mutated proteins were analyzed for purity by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie Brilliant Blue staining, and the fractions that showed greater than 90% purity were used for further characterization. Protein concentrations were measured by the method of Bradford (*29*) with bovine serum albumin (BSA) as a standard.

UPP Synthase Assay and Product Analysis—The enzyme activity was measured by determining the amounts of [1-^{14}C]IPP incorporated into butanol-extractable polyprenyl diphosphate. A standard assay mixture contained, in a final volume of 0.2 ml, 100 mM Tris-HCl buffer, pH 7.5, 0.5 mM $MgCl_2$, 10 μM FPP or *Z*-GGPP, 10 μM [1-^{14}C]IPP (37 MBq/mol), 0.2% (w/v) Triton X-100, and a suitable amount of enzyme solution. After incubation at 37°C for 15 min, the reaction products were immediately extracted with 1-butanol saturated with water, and the radioactivity in the butanol extract was measured with an Aloka LSC-1000 liquid scintillation counter.

For kinetic studies, the concentration of either the allylic substrate FPP/*Z*-GGPP or homoallylic substrate [1-^{14}C]IPP was varied, while the other was kept constant at 200 μM. The calculation of kinetic parameters was performed using EnzymeKinetics software version 1.5 (Trinity Software).

The radioactive prenyl diphosphate products in the reaction mixture were hydrolyzed to the corresponding alcohols with potato acid phosphatase according to our method reported previously (*30*). The alcohols were extracted with pentane and analyzed by reversed phase TLC plate with a solvent system of acetone/water (19:1). The positions of authentic standards were visualized with iodine vapor, and the distribution of radioactivity was detected. The TLC plates were exposed on a Fuji imaging plate at room temperature for 1 day, and then analyzed with a Fuji BAS 1000 Mac bioimage analyzer.

RESULTS

Random PCR Mutagenesis of the UPP Synthase Gene and Colony Autoradiography Screening—A random mutagenesis strategy using an error-prone PCR was used to introduce mutations on the entire UPP synthase gene of *M. luteus* B-P 26. The UPP synthase gene was amplified by the mutagenic PCR (*26*), and a plasmid library containing mutated UPP synthase genes was constructed.

To screen for mutants showing poor UPP synthase activity, we applied a colony autoradiography method as described previously (*17*). Before screening, we confirmed the background level of adsorption of radioactive IPP to *E. coli* cells or the membrane filter as well as the effect of the intrinsic UPP synthase activity of the host cells. In this screening system, if a clone carrying a mutated UPP synthase gene expresses poor UPP synthase activity, no radioactive spot is seen at the corresponding position in the autoradiogram of the replica filter. Approximately 3,000 transformants of *E. coli* cells harboring mutated UPP synthase genes were screened by this method. As a result, we obtained 31 negative clones that showed no spot in the autoradiogram of the replica filter. These clones were then isolated from the master plate and their UPP synthase activity in cell-free homogenates of the clones was examined using FPP or *Z*-GGPP as an allylic primer with [1-^{14}C]IPP. All mutants showed less than 20% relative activity of the wild-type. Then, the 31 mutants were analyzed for their UPP synthase gene sequences. Although the conditions employed for PCR mutagenesis were milder than those described by Cadwell and Joyce (*26*), 18 mutants (58% of all mutants) were found to contain heavy gene disruptions within the UPP synthase gene cassette, including 3 mutants with frame shifts, 7 mutants with introduced stop codons, and 8 mutants with more than ten amino acid substitutions. Table I lists the amino acid changes of the other 13 mutants (42% of all mutants). Two mutants with changes at Asn-77 or Trp-78 (N77S or W78R), which were found to contain only one amino acid substitution, were selected for further analysis.

Production of Site-Directed Mutagenesis of UPP Synthase—To examine the functional roles of Asn-77 and Trp-78, site-directed mutagenesis studies were carried out with *M. luteus* B-P 26 UPP synthase. The Asn-77 residue was changed to Ala, Asp, or Gln, to examine the significance of the carbamoyl group, and Trp-78 was replaced with Ile, Arg, or Asp, to evaluate the importance of the aromatic moiety.

To construct expression systems for these mutants, oligo-

TABLE I. **Amino acid changes in UPP synthase mutants with five or fewer substitutions.**

Mutant	The number of amino acid substitution	Amino acid changes
1	5	I26N/D29G/N77I/D121V/N181I
2	3	I26N/D118G/Q211L
3	3	K135N/L172V/E201D
4	3	M40T/F144L/L194S
5	2	N97Y/E202D
6	2	T134P/N147D
7	2	H23R/A72P
8	2	R203C/Q234H
9	2	K11T/N147D
10	2	A72N/I175V
11	2	A162T/F207I
12	1	N77S
13	1	W78R

TABLE II. **Kinetic parameters for the wild-type and mutant UPP synthases.**

Enzyme	IPP[a]		FPP		Z-GGPP	
	K_m (μM)	k_{cat}[b]×10³ (s^{-1})	K_m (μM)	k_{cat}[b]×10³ (s^{-1})	K_m (μM)	k_{cat}[b]×10³ (s^{-1})
Wild-Type	7.8±2.8	1850±200	8.3±1.3	1220±60	8.2±4.0	1770±250
N77A	12.0±0.9	1.64±0.1	14.7±3.2	1.25±0.2	10.2±0.9	2.61±0.1
N77D	15.0±3.0	4.73±0.1	13.9±7.4	2.72±0.4	8.0±1.7	4.15±0.1
N77Q	9.0±1.4	2.70±0.1	10.4±2.0	1.55±0.1	5.0±0.4	2.31±0.1
W78I	14.1±7.0	783±180	49.5±10.9	456±40	19.0±7.3	1370±160
W78R	16.5±2.0	803±50	120.7±30.1	434±40	15.0±2.0	899±60
W78D	13.1±2.0	397±30	155.0±23.0	151±3.0	8.0±2.1	300±20

[a]For the reaction with FPP. [b]k_{cat} was calculated from the V_{max} value.

nucleotide-mediated mutagenesis was done into plasmid pMluUEx, which carries the wild-type UPP synthase gene. After transformation of *E. coli* BL21 (DE3) with each of the expression plasmids, the UPP synthase mutant was overproduced and purified. All the mutated enzymes showed chromatographic properties similar to those of the wild-type UPP synthase during the purification procedures. SDS-PAGE analyses with Coomassie Brilliant Blue staining showed the purities of these mutants to be greater than 90%.

Kinetic Analysis of UPP Synthase Mutants—Michaelis constants (K_m) for the allylic substrate FPP or *Z*-GGPP and the homoallylic substrate IPP and k_{cat} values were determined for all mutated enzymes as well as the wild-type, and the results are listed in Table II. All of the 6 mutated enzymes showed Michaelis constants for IPP comparable to that of the wild-type. Mutants with substitutions at Asn-77, N77A, N77D, and N77Q, showed K_m values for FPP and *Z*-GGPP comparable to those of the wild-type, but 400–1100-fold lower k_{cat} values. Hence, it was necessary to substantially increase both the enzyme concentrations and incubation times to obtain reliable values for the reaction rates for these less reactive mutated enzymes. On the other hand, substitution of the aromatic residue Trp-78 with Ile, Arg, or Asp resulted in marked increases in the K_m for FPP but slight decreases in k_{cat}. W78I showed a 5-fold higher K_m value for FPP, while the K_m values of W78R and W78D for FPP were 15–20-fold larger than that of the wild-type enzyme. These mutants, however, showed no significant changes in the K_m value for *Z*-GGPP compared with that of the wild-type.

Product Analysis—After enzymatic reaction of the mutant enzymes with [1-^{14}C]IPP and FPP or *Z*-GGPP as substrates, the radioactive prenyl diphosphate products were hydrolyzed to the corresponding alcohols. TLC analyses of the alcohols indicated that the three mutants at Trp-78, W78I, W78R, and W78D, catalyzed the formation of UPP as well as some intermediates having shorter prenyl chains, similar to the reaction catalyzed by the wild-type enzyme (data not shown). On the other hand, little production of the final product UPP was detected for the mutants with Asn-77 substitutions, N77A, N77D, and N77Q, which gave only small amounts of some intermediates with shorter prenyl chains when the amounts of the mutant enzyme in the reaction mixture was raised to 200 μg.

DISCUSSION

The primary structure of *M. luteus* B-P 26 UPP synthase is completely different from those of *trans*-prenyltransferases, which have been extensively studied by site-directed mutagenesis to define the roles of specific amino acids in catalysis and substrate binding (*3–5*, *8–12*) and X-ray crystallography (*6*, *7*). On the other hand, little is known about the molecular mechanism of enzymatic *cis*-prenyl chain elongation. In order to obtain information about amino acid residues important for catalysis, we constructed a mutated gene library of *M. luteus* B-P 26 UPP synthase by random PCR mutagenesis and carried out colony autoradiography screening (*17*). An essential element of this strategy is the efficient identification of mutants of interest among a large population of variants. Thirty-one mutants that showed poor UPP synthase activity were selected from 3,000 clones. Two of them, which were found to contain only one amino acid substitution at Asn-77 or Trp-78, were analyzed in more detail.

Six enzymes with mutations at Asn-77 and Trp-78 were prepared by oligonucleotide-mediated site-directed mutagenesis, and their kinetic parameters for allylic substrates FPP or *Z*-GGPP and homoallylic substrate IPP were determined. All mutants showed K_m values for IPP comparable to that of the wild-type enzyme, suggesting that neither Asn-77 nor Trp-78 is involved in the binding of IPP. Three mutants of Asn-77, N77A, N77D, and N77Q, showed dramatically decreased catalytic activities, with k_{cat} values 10^{-3}–10^{-2} that of the wild-type, but comparable K_m values for allylic substrates. This indicates that the Asn residue is critical for the catalytic function rather than for substrate binding. The Asn residue is so essential that it cannot be replaced by other residues, even Gln, which has a carbamoyl group with a longer side-chain, without a significant decrease in catalytic activity.

On the other hand, the mutants at Trp-78, W78I, W78R, and W78D, showed moderate levels of UPP synthase activity, although their k_{cat} values were 2–8-fold lower than that of the wild-type. Interestingly, these Trp-78 replacement mutants showed markedly increased K_m values for FPP. The K_m value for FPP of W78I was increased by 5-fold, while substitution with a charged amino acid, Arg or Asp, brought about a 15–20-fold increase compared with the wild-type. These results suggest that the Trp residue contributes to the binding of FPP by its hydrophobic nature with the prenyl chain moiety. Although *Z*-GGPP, which is the first intermediate in the enzymatic synthesis of UPP from the natural substrate FPP, can be accepted as a good substrate for UPP synthase, the K_m values of W78I and W78R for *Z*-GGPP were two times higher, there was no change with respect to W78D. *Z*-GGPP has an α-isoprene unit with a *cis*-configuration, while the configuration of the α-isoprene unit of FPP is *trans*. If the diphosphate moieties of FPP and *Z*-GGPP bind to the enzyme in the same confor-

mation, then their hydrocarbon tails must grow down the binding pocket for the allylic substrate in different ways. The hydrophobic tail of FPP might be accepted in the binding site, which contains Trp-78 whose aromatic ring forms a hydrophobic interaction with the prenyl chain of FPP. The reason that the K_m value for *Z*-GGPP was not affected very much by the replacements of Trp-78 may be explained if the hydrophobic prenyl tail of *Z*-GGPP becomes located at a different direction from the binding site containing Trp-78 in the hydrophobic pocket, which may well accomodate to the stretching of the *cis*-prenyl chain during the prenyl chain elongation up to C_{55}-length.

Taken together, these results suggest a hypothesis for the functional roles of Asn-77 and Trp-78 in enzymatic catalysis and the binding of allylic substrates (Fig. 1). Asn-77 seems to exert its hydrogen-bonding capability on the prenyloxyl moiety to facilitate the cleavage of the P-O bond, which forms a prenyl cation at the beginning of the catalytic reaction. Trp-78 may bind to the prenyl chain of FPP so as to hold the substrate molecule in the proper direction necessary for stereospecific condensation with IPP.

To date, several gene sequences for *cis*-prenyl chain elongating enzymes are available. Comparison of the deduced amino acid sequences of these enzymes shows the presence of five conserved regions (*18*). In Region III, the Asn residue corresponding to position of 77 in *M. luteus* B-P 26 UPP synthase is completely conserved and the next residue corresponding to the Trp-78 is also highly conserved as an aromatic residue Trp or Phe (Fig. 2). It is suggested that Region III, including the conserved Asn-Trp/Phe (NW/F) motif, plays an important role in the enzymatic catalysis and substrate binding for enzymatic *cis*-prenyl chain elongation. This motif is reminiscent of the Phe-Gln (FQ) motif of *trans*-prenyl chain elongating enzymes, which has been shown to be essential not only for the binding of allylic substrates but also for catalytic function (*31*). These motifs consisting of carbamoyl- and aromatic residues may be common and essential parts of the prenyl chain elongating machinery that triggers the consecutive condensation of IPP to produce polyprenyl chains stereospecifically. The *cis*- and *trans*-prenyltransferases are similar in that they both catalyze sequential condensations between IPP and allylic diphosphates with the concomitant release of inorganic pyrophosphate in the presence of magnesium ions (*32*). It is of particular interest to learn the similarities or differences in the molecular mechanisms of these enzymes. Although no sequence similarity was found, we postulate the presence of some common motifs playing similar functional roles during catalysis between *cis*- and *trans*-prenyltransferases. The conserved Phe-Gln (FQ) motif in region VI of

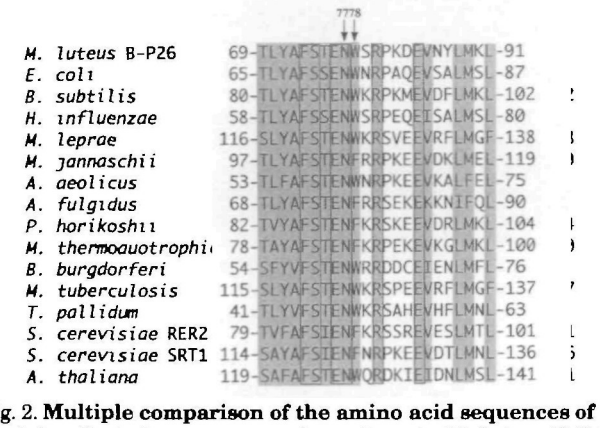

Fig. 2. **Multiple comparison of the amino acid sequences of 16 proteins that show sequence homology to *M. luteus* B-P 26 UPP synthase.** Only the residues corresponding to Region III are shown. The boxes show identical amino acid residues, and the borderless shading indicates similar residues. Sequences: *M. luteus* B-P 26 (GenBank AB004319), *E. coli* (SWISS-PROT Q47675), *B. subtilis* (SWISS-PROT Q31751), *H. influenzae* (SWISS-PROT P44938), *Mycobacterium leprae* (SWISS-PROT P38119), *Methanococcus jannaschii* (SWISS-PROT, Q58767), *Aquifex aeolicus* (SWISS-PROT O67291), *Archaeoglobus fulgidus* (SWISS-PROT O29049), *Pyrococcus horikoshii* (SWISS-PROT O59258), *Methanobacterium thermoauotrophicum* (SWISS-PROT O26334), *Borrelia burgdorferi* (SWISS-PROT O51146), *Mycobacterium tuberculosis* (SWISS-PROT O53434), *Treponema pallidum* (GenBank AE001235), *S. cerevisiae* RER2 (SWISS-PROT P35196), *S. cerevisiae* SRT1 (SWISS-PROT Q03175), *Arabidopsis thaliana* (GenBank AF162441).

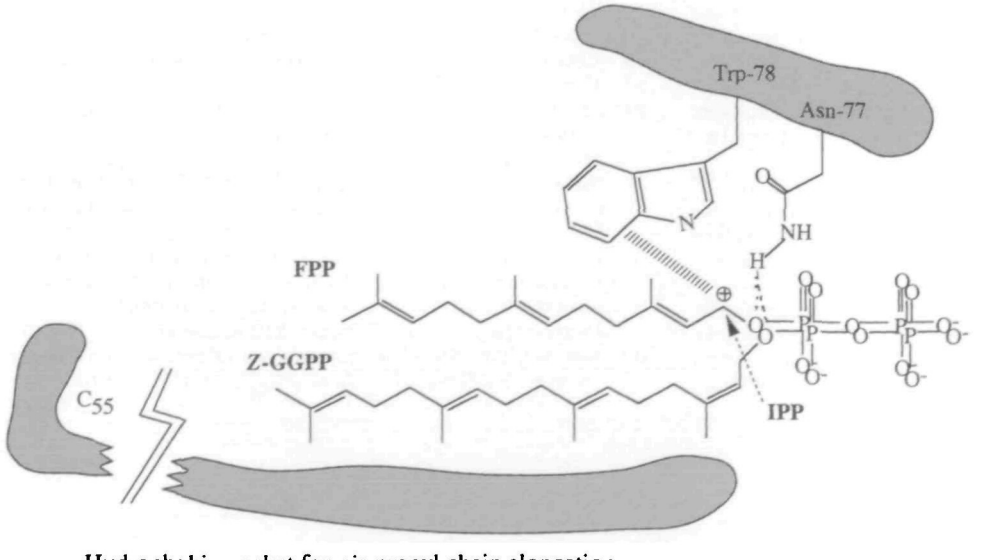

Fig. 1. **A hypothetical model for the functional role of the Asn-Trp (NW) motif of UPP synthase of *M. luteus* B-P 26.**

trans-prenyltransferases may be the counterpart of the Asn-Trp/Phe (NW/F) motif in *cis*-prenyltransferases, in that it is involved in the binding of allylic substrates and catalysis by exerting aromatic hydrophobicity and hydrogen bonding on the prenyloxyl moiety. Further studies on the structure-function relationship of *cis*-prenyl chain elongating enzymes are in progress involving site-directed mutagenesis as well as X-ray crystallographic analysis of *M. luteus* B-P 26 UPP synthase. These studies may accelerate the understanding of the molecular mechanism of enzymatic *cis*-prenyl chain elongation.

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