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Overproduction, in *Escherichia coli,* of Soluble Taxadiene Synthase, a Key Enzyme in the Taxol Biosynthetic Pathway

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Taxadiene synthase catalyzes the conversion of the universal precursor of diterpenoids, geranylgeranyl diphosphate, to taxadiene, a key intermediate in Taxol (paclitaxel) biosynthesis. The gene encoding taxadiene synthase was cloned recently. Here we report a method for the heterologous overexpression of cDNA encoding taxadiene synthase in *Escherichia coli* using a thioredoxin fusion expression system, which increases the solubility of expressed protein. Taxadiene synthase cDNA was amplified by polymerase chain reaction and then subcloned into pET3d and pET32a(+) to form pET3dTX and pET32TX, respectively. The expressed taxadiene synthase from *E. coli* BL21(DE3)/pET3dTX was present completely as inclusion bodies. The transformant *E. coli* BL21(DE3)/pET32TX produced a thioredoxin fusion taxadiene synthase (15–20% of total soluble protein) when induced with isopropyl β-D-thiogalactopyranoside at low temperature (20°C). The recombinant enzyme was purified by a single step with a His-binding metal affinity column. The maximal production attained was 13 mg of purified, active fusion protein per 500 ml culture of *E. coli* BL21(DE3)/pET32TX. The purified recombinant taxadiene synthase fusion protein was similar to native protein in steady-state kinetic parameters and mobility on sodium sulfate–polyacrylamide gel electrophoresis. The protein purified from *E. coli* BL21(DE3)/pET3dTX had the expected N-terminal (AQLSFNA) sequence.

Key Words: **diterpenes; Taxol; diterpene cyclases; taxadiene synthase.**

The diterpenoid Taxol (paclitaxel) first isolated from bark of Pacific Yew (*Taxus brevifolia*) (1) is one of the most powerful antitumor drugs so far discovered. It shows excellent activity against a range of cancers including breast and ovarian (2,3). A crisis in the supply of Taxol had at one time threatened its widespread clinical use. The total synthesis of Taxol has been achieved by several elegant routes (4,5), but the yields are too low to be commercially viable. Today, although semisynthesis (6) of Taxol and its analog taxotere (docetaxel) is regarded as a viable interim measure, the continued supply of Taxol in the future could be augmented by biological methods of production via genetic engineering of *Taxus* species. To develop an improved biological process, we must first understand the biosynthetic pathway to Taxol, the enzymes catalyzing the sequence of reactions, especially the rate-limiting step, and the genes encoding these proteins. To study these features, it will be necessary to develop technologies that allow for the routine, high-level expression of the biosynthetic proteins using hosts such as *Escherichia coli.* Ideally, this should be followed by a simple purification technique to allow biochemical, kinetic, and structural studies of the enzymes. Several diterpene cyclases have been expressed in *E. coli,* but either the expression level was very low (7,8) or the expressed protein was present in inclusion bodies (9–11). This paper describes a system for overexpression in *E. coli* of a Taxol biosynthetic enzyme, taxadiene synthase, in soluble form.

The biosynthesis of Taxol is presumed to involve the initial cyclization of geranylgeranyl diphosphate (GGPP[2]) to a transient verticillyl cation intermediate, with transfer of C11 α-proton to C7 to initiate transan-

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[2] Abbreviations used: DTT, D,L-dithiothreitol; GC–MS, capillary gas chromatography–mass spectrometry; GGPP, geranylgeranyl diphosphate; IPTG, isopropyl β-D-thiogalactopyranoside; PMSF, phenylmethanesulfonyl fluoride; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; Tris, tris(hydroxymethyl) aminomethane.

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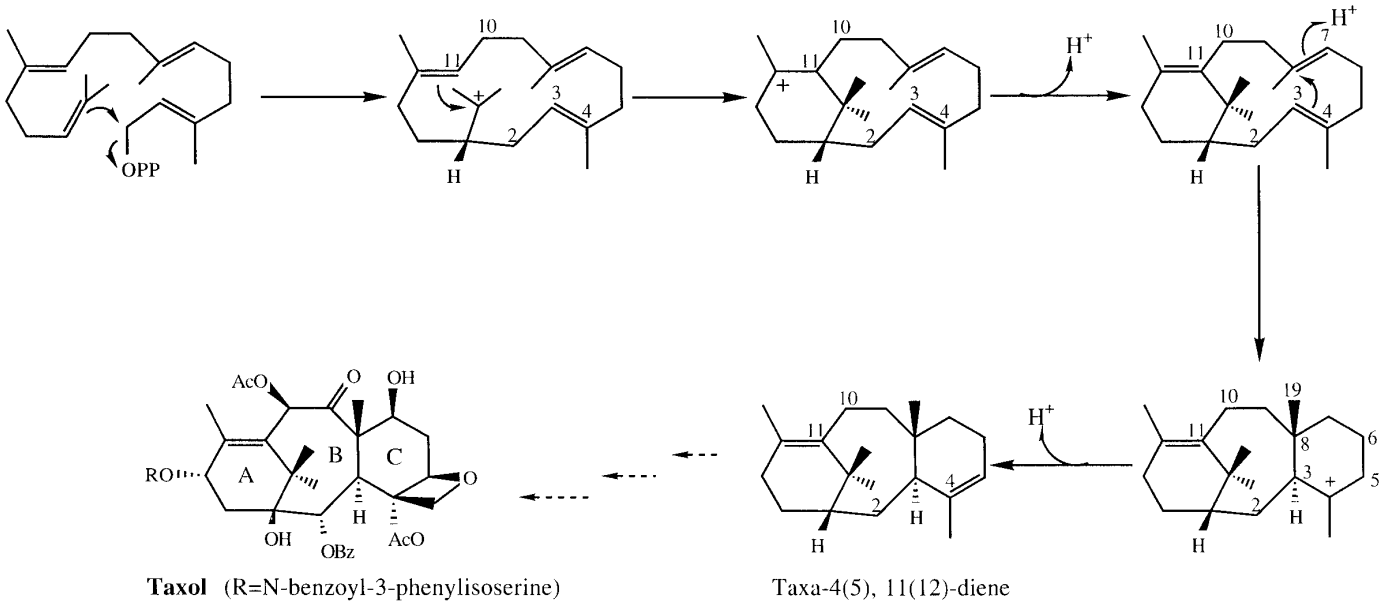

FIG. 1. Cyclization of GGPP to taxadiene.

nular B/C-ring closure to the taxenyl cation, followed by deprotonation at C5 to form taxa-4(5), 11(12)-diene. This is then processed by extensive oxidative modification and elaboration of the side chain (12) (Fig. 1).

The cyclization enzyme, Taxadiene synthase, was characterized (13) and partially purified from Pacific Yew (*T. brevifolia*) (14). Based on consensus sequences of related monoterpene, sesquiterpene, and diterpene cyclases, degenerate primers were designated to amplify sequences from a cDNA library of Pacific Yew (*T. brevifolia*) stem. Two of these primers amplified a 83-base-pair fragment showing similarity to other cyclases and were used to screen the cDNA library. A full-length cDNA clone, pTB42.1, was isolated recently (7). The cDNA insert comprised 2568 bases and encodes a protein of molecular weight 98,303. Analysis of the translated sequence shows that taxadiene synthase has 32% identity and 55% similarity to the monoterpene cyclase, (−)-limonene synthase from spearmint (*Mentha spicata*) (15), 31% identity and 54% similarity to the sesquiterpene cyclase, vetispiradiene synthase from *Hyoscyamus muticus* (16), 30% identity and 54% similarity to *epi*-aristolochene synthase from tobacco (*Nicotiana tabacum*) (17), 30% identity and 55% similarity to cadinene synthase from cotton (*Gossypium arboreum*) (18); 31% identity and 56% similarity to the diterpene cyclase, casbene synthase from castor bean (*Ricinus communis.* L) (19), 33% identity and 56% similarity to kaurene synthase A from *Arabidopsis thaliana* (9) and *Zea maize* (20), 30% identity and 55% similarity to kaurene synthase B from pumpkin (*Cucurbita maxima* L.) (10), and 45% identity and 67% similarity to (−)-abietadiene synthase from grand fir (*Abies grandis*) (8). All of these proteins are of plant origin and catalyze intramolecular cyclizations of a prenyl diphosphate substrate to a specific cyclic hydrocarbon product.

Our interests are focused on the structural and functional relationships of diterpene cyclases and the enzymatic synthesis of diterpenes, such as Taxadiene, casbene (21), and *ent*-kaurene (22). We now describe a system for high-level expression, and a single-step purification, of taxadiene synthase in *E. coli.*

MATERIALS AND METHODS

Materials and general procedures. [1-^{3}H]GGPP (1 mCi/ml) was purchased from American Radiolabelled Chemicals (St. Louis, MO). Radioactivity was measured in vials containing 5 ml premixed scintillation solution (Econofluor, New England Nuclear, Boston, MA) in a Beckman LS5801 liquid scintillation spectrometer. Oligonucleotides were synthesized by the phosphoramidite method (23) by the Biotech Lab at Texas A&M University. Proteins were analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) with the discontinuous buffer of Laemmli (24). The gels were stained with Coomassive brilliant blue R. Protein concentration was determined by the method of Bradford (25) using a Bio-Rad assay kit with bovine serum albumin as standard.

Recombinant DNA methods and reagents. Enzymes used in plasmid constructions were obtained from New England Biolabs. Restriction enzyme digestion, plasmid minipreps, DNA ligation, and other DNA manipulations were carried out by standard procedures as described by Maniatis *et al.* (26). DNA sequencing of

cDNA subclones was performed on an ABI 373 DNA sequencing system using the Dye Terminator cycle sequencing kit (Perkin–Elmer, Branchburg, NJ) employing either M13 universal primers or specific oligonucleotides. Polymerase chain reactions (PCR) were conducted with the *Pfu* polymerase from Stratagene (San Diego, CA) using a GeneE thermal cycler (Techne Ltd., Cambridge, UK).

Strains, media, and transformation. *E. coli* strains JM109, JM109(DE3), BL21(DE3), BL21(DE3)/PlysS, and AD494 are from Novagen (Madison, WI). *E. coli* JM109 was used for all plasmid manipulations. *E. coli* strain BL21 (DE3) was used for expression of recombinant taxadiene synthase. DNA transformation was carried out with a Bio-Rad *E. coli* Pulser transformation apparatus. The transformed cells were grown on LB medium containing ampicillin (100 μg/ml).

Construction of expression vector for taxadiene synthase cDNA. Plasmid pTB42.1 described previously (14) containing the *T. brevifolia* Taxadiene synthase coding sequence in pBluescript SK(+) was used as template for PCR. The forward primer is a 31-mer oligonucleotide containing an *Nco*I restriction site (italic) and the first nine codons of the taxadiene synthase gene, including the start codon (bold) [5′-CCGCC***ATG***GCTCAGCTCT-CATTTAATGCAGC-3′]. The reverse primer was a 36-mer containing a *Bam*HI restriction site (italic); the complementary sequence corresponding to the stop codon TCA was changed to TAA (bold) and the last eight codons of Taxadiene synthase [5′-GGATC*GAATTC*TTATACT-TGAATTGGATCAATATAA-3′]. A 100-μl PCR mixture containing 10 μl 10× buffer [10 μl containing of 200 mM Tris–HCl, pH 8.2, 100 mM KCl, 60 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$, 1% Triton X-100, and 100 ng/ml nuclease-free BSA], 8 μl of 2.5 mM dNTPs, 5 μl of 2.5 mM $MgCl_2$, 50 pmol each forward and reverse primer, 0.5 units *Pfu* polymerase, and sterile H_2O was carried out under standard conditions (94°C, 1 min; 55°C, 1 min; 72°C, 6 min; and an additional 10 min extension at 72°C). The 2.6-kb PCR product was gel purified and subcloned into pBluescript SK(−). The *Nco*I and *Bam*HI fragments were then subcloned into the corresponding sites of pET3d and pET32a(+) from Novagen to form pET3dTX and pET32TX. The constructs pET3dTX and pET32TX were transformed into *E. coli* strain BL21(DE3) according to an electroporation transformation procedure (Bio-Rad). The DNA sequence integrity was confirmed by DNA sequencing as described above.

Enzyme assay for taxadiene synthase. The assay of taxadiene synthase was based on the previously described procedure (7) with the following modification. Enzyme extract was added to 0.5 ml buffer (50 mM Tris–HCl, pH 8.0, 5 mM $MgCl_2$, 1 mM PMSF, 1 mM DTT, 10% glycerol) and 15 μM [1-^{3}H]GGPP (1.1×10^6 dpm). The mixture was incubated at 32°C for 1 h. The reaction was terminated by addition of 50 μl EDTA (0.5 M, pH 8.0) and the olefinic product was extracted with two 1-ml portions of hexanes. After vortexing, followed by centrifugation, the supernatant was passed through a 3-cm silica gel column. The mini-column was washed with 1 ml hexane, and the collected solution was reduced to 200 μl by flushing with nitrogen and used for radioactivity measurement. One unit of taxadiene synthase corresponds to the formation of 1 nmol of taxadiene per minute. The identity of the recombinant enzymatic reaction product was verified by TLC and MS by comparison with authentic taxa-4(5), 11(12)-diene (27).

Kinetic experiments were carried out in a total volume of 500 μl containing assay buffer and [1-^{3}H]GGPP at 0.5, 1, 1.5, 2.0, 2.5, 3.0, 6.0, 10, and 15 μM. The assay mixtures were prewarmed for 5 min at 32°C before the reaction was initiated by adding Taxadiene synthase. After 30 min, the reactions were stopped by adding 50 μl 0.5 M EDTA at pH 8.0 and then overlaying with 1 ml hexane. The samples were vortex mixed, and the hexane extracts were analyzed as described above. For calculation of the kinetic parameters, the average values obtained from duplicate experiments were used. All determinations were made in the linear region of the progress curve where <20% of substrate was consumed. A blank containing buffer and [1-^{3}H]GGPP was run for each different concentration of GGPP. K_{cat} and k_m were initially estimated from Lineweaver–Burk double-reciprocal plots and calculated by direct fitting of data to the Michaelis–Menten equation using standard nonlinear least-squares methods.

Expression and purification of taxadiene synthase in E. coli. In a typical preparation, a single clone of *E. coli* BL21(DE3)/pET32TX was used to inoculate 50 ml of LB medium containing 100 μg ampicillin/ml in a 150-ml flask and the culture was incubated overnight at 37°C at 200 rpm. Ten milliliters of the overnight culture was inoculated into 500 ml of the same medium and the culture was grown at 37°C for ca. 4 h at 200 rpm until the OD (550 nm) reached 0.6–1.0. IPTG was then added to a final concentration of 0.1 mM and the culture was incubated a further 8–10 h at 20°C. For assay of taxadiene synthase activity, 1.5-ml aliquots of the cultures were subsequently removed. The cells were collected by centrifugation for 2 min in a microcentrifuge, and the pellet was resuspended in 100 μl lysis buffer (100 mM Tris–HCl, pH 8.0, 1 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 10% glycerol), vortexed, and sonicated four times for 10 s each time. The sonicated solution was centrifuged for 10 min at 4°C in a microcentrifuge at 15,000*g*, and the supernatant was used for taxadiene synthase activity determinations. After 8 h induction by IPTG, the taxadiene synthase activity had reached maximum (data not shown).

Purification of recombinant taxadiene synthase was performed according to the procedure described in the Talon metal affinity resin manual (Clontech, CA). The purification data were based on a 500-ml culture. Protein concentrations were determined by the method of Bradford (23) using the Bio-Rad protein assay kit and bovine serum albumin as standard. SDS–PAGE gel (10%) was carried out according to the modified procedure described by Laemmli (22). After electrophoresis, gels were stained with Coomassie brilliant blue.

Cultures of *E. coli* BL21(DE3)/pET32TX were harvested at 8 h after IPTG induction by centrifugation for 10 min at 4000*g*. The pellet from 500 ml culture was resuspended in 20 ml lysis buffer containing 100 mM Tris–HCl, pH 8.0, 10% glycerol, 1 mM PMSF, 1 mM $MgCl_2$ and incubated for 30 min at 4°C with 75 μg/ml lysozyme. The cells were lysed by sonication at full power four times on ice while maintaining the temperature below 8°C. Unlysed cells and cell debris were removed by centrifugation at 12,000*g* for 15 min. The supernatant was mixed with Talon metal affinity resin, and the mixture was gently agitated at 4°C for 30 min and then centrifuged at 700*g* for 5 min. The supernatant was discarded and the resin washed four times with 30 ml of lysis buffer. The washed resin was then resuspended in lysis buffer and transferred to the gravity column. The column was washed first with 15 ml of lysis buffer. Subsequently, the taxadiene synthase was eluted with 20 ml lysis buffer containing 50 mM imidazole and 0.1 M NaCl. The eluted fractions were judged to be homogeneous by SDS–PAGE. The purified fusion taxadiene synthase was dialyzed overnight against 50 mM Tris–HCl, pH 8.0, 1 mM DDT, 10% glycerol and then stored at −70°C until used.

N-terminal sequencing. The inclusion bodies from 50 ml *E. coli* BL21(DE3)/pET3dTX were dissolved in 6 ml of 0.1 M Tris–HCl, pH 8.0, containing 8 M urea and allowed to stand at room temperature for 12 h. The solution was centrifuged at 37,000 rpm in a Beckman Ti 45 rotor and dialyzed extensively against two changes of 100 vol each of 0.1 M Tris–HCl, pH 8.0, containing 10% glycerol. The dialysate was centrifuged at 15,000 rpm for 5 min and then subjected to SDS–PAGE, and the gel was stained with Coomassie blue. The bands corresponding to taxadiene synthase were cut out and recovered using a Bio-Rad 422 Electro-Eluter. The recovered, pure taxadiene synthase (10 pmol) was subjected to automated Edman degradation in an ABI Model 477A protein sequencer at the Protein Technologies Laboratory, Department of Entomology, Texas A&M University.

GS–MS analysis of the taxadiene synthase product. For structure identification of the product from the taxadiene synthase reaction, large-scale incubations were conducted with GGPP (20 mg) in 50 mM Tris–HCl, pH 8.0, containing 5 mM $MgCl_2$, 1 mM PMSF, 10% glycerol, and 3 mg purified fusion protein in a total volume of 50 ml. After incubation for 15 h at 32°C, the reaction was extracted with 3 vol of hexane. The combined organic phases were dried with $MgSO_4$, filtered, and applied to a 5-cm silica gel column. The column was washed with 2–3 vol of hexane, and the collected hexane solution was concentrated *in vacuo.* The residue was chromatographed (TLC silica gel) in hexane, revealing a compound with an R_f value of 0.91, identical to that of authentic taxadiene. The mass spectrum of the enzymatically generated taxadiene showed *m/z* 272 [P^+]; 257 [P^+-15(CH_3)]; 229 [P^+-43(C_3H_7)]; 121, 122, 123 (C-ring fragment cluster); and 107 (*m/z* 122-15(CH_3)), a fragmentation pattern identical to that of synthetic taxadiene (27).

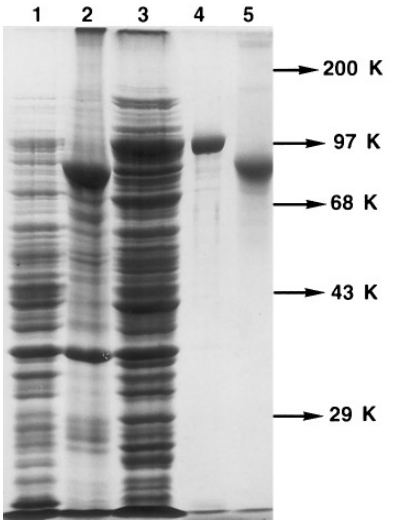

FIG. 2. SDS–PAGE analysis of recombinant taxadiene synthase: lane 1, total protein extracted from *E. coli* BL21(DE3)/pET32a(+) (15 μg); 2, the refolded protein from *E. coli* BL21(DE3)/pET3dTX (15 μg); 3, total soluble protein from *E. coli* BL21(DE3)/pET32TX (25 μg); 4, purified taxadiene synthase fusion protein (5 μg); 5, taxadiene synthase released from fusion protein by enterokinase (5 μg). Molecular marker positions are shown on the right.

RESULTS

Expression of Taxadiene Synthase in E. coli

In an initial attempt to express taxadiene synthase in *E. coli,* a full-length taxadiene synthase cDNA was inserted into a commonly used expression plasmid, pET3d, which carries a T7 promoter. Significantly greater quantities of taxadiene synthase were detected in extracts of *E. coli* BL21(DE3)/pET3dTX (Fig. 2). Unfortunately, the expressed protein from *E. coli* BL21(DE3)/pET3dTX formed insoluble inclusion bodies. Varying the concentration of IPTG (0.05, 0.1, 0.3, 0.5, and 1.0 mM), temperature of induction (15, 20, 25,

and 37°C), and the host strain [*E. coli* JM109(DE3), BL21(DE3)/pLysS, AD494] all failed to increase the level of soluble taxadiene synthase expression. Assay of the supernatant obtained from *E. coli* pET3dTX/BL21 (DE3) with [1-^{3}H]GGPP did not show that any active taxadiene synthase had been produced. The taxadiene synthase from *E. coli* BL21(DE3)/pET3dTX could be refolded using 8 M urea, but no active protein was detected.

To overcome these problems, we turned to the use of an expression vector, pET32a(+), carrying a T7lac promoter, T7gene10, ribosome binding site and encoding a thioredoxin fusion protein (28), which was used to increase the solubility of expressed protein in *E. coli* (29,30). The constructed plasmid pET32TX was transformed into *E. coli* BL21(DE3). IPTG induction of the *E. coli* BL21(DE3)/pET32TX transformant again overexpressed taxadiene synthase exclusively in the form of inclusion bodies when the induction temperature was 37°C, but significantly soluble taxadiene synthase and enzyme activity were detected in extracts of *E. coli* BL21 (DE3)/pET32TX when the induction temperature was lowered to 20°C (Fig. 2).

Confirmation of the Recombinant Nucleotide Sequence

Various detergents and chaotropic agents were investigated for the ability to solubilize taxadiene synthase from *E. coli* BL21 (DE3)/pET3dTX. Sodium hydroxide (0.01 M) failed to solubilize taxadiene synthase, whereas both urea (8 M) and guanidinium hydrochloride (6 M) solubilized the inclusion-bound enzyme. In both cases, however, the refolded protein did not show any activity. To determine the N-terminal sequence of the recombinant protein, the refolded taxadiene synthase was separated by SDS–PAGE and the bands corresponding to taxadiene synthase were cut out and recovered using a Bio-Rad Model 422 Electro-Eluter. N-terminal sequencing of the recovered protein revealed a sequence of Ala-Gln-Leu-Ser-Phe-Asn-Ala, identical to that predicted from the corresponding cDNA sequence, minus the N-terminal Met.

Purification and Characterization of Recombinant Taxadiene Synthase

pET32a(+) encodes a fusion protein thioredoxin gene and a hepta-histidyl leader sequence, thus allowing simplification of the protein purification. The proteins produced from *E. coli* BL21(DE3)/pET32TX in the induced bacterial cultures were loaded onto a His-binding metal affinity column. Following elution from metal affinity resin, a single major band was observed on 10% SDS–PAGE (Fig. 2). The mobility of recombinant taxadiene synthase from pET3dTX upon SDS–PAGE corresponded to a M_r of 80,000, in close agreement with the value of 79,000 reported for the native protein (14).

TABLE 1

Purification of Recombinant Taxadiene Synthase from *E. coli* BL21(DE3)/pET32TX[a]

Step	Total protein (mg)	Total activity (nmol/h)	Specific activity (nmol/h/mg)	Recovery (%)
Crude extract	246	1304	5.3	100
Affinity column	12	978	81.5	75

[a] Data are based on protein from a 500-ml culture.

The fusion protein produced from *E. coli* BL21 (DE3)/pET32TX displays a mass of ca. 12,000 greater than estimated for the native protein.

Steady-state kinetic parameters of the purified fusion protein were established for the substrate [1-^{3}H]-GGPP having a K_m of 3.1 μM, in close agreement with the value of 2.9 μM for native taxadiene synthase (14), and a maximal velocity of 0.9 $\pm$ 0.03 μmol min^{-1} mg^{-1}. The recombinant taxadiene synthase from a 500-ml culture was readily purified to homogeneity (>95%) in a single step (Table 1). The overall yield of the purification procedure was 80%, corresponding to 13 mg of purified taxadiene synthase from 500 ml of culture.

Hexane extracts of enzyme reaction mixtures were further purified by silica gel chromatography and used for GC–MC analysis. The major peak observed had the same retention time and mass fragmentation pattern as a synthetic sample of taxadiene (27).

DISCUSSION

The conversion of GGPP to taxadiene catalyzed by taxadiene synthase is the committed step in the biosynthesis of Taxol. The development of an expression system that produces a recombinant form of plant taxadiene synthase in the amounts described in this report will undoubtedly expedite progress toward elucidation of the molecular basis for catalysis of taxadiene formation and Taxol production. The single-step purification simplifies isolation of a stable homologous protein whose amino acid sequence matches that of the native plant protein.

Although the structural genes for several diterpene cyclases have been cloned and sequenced (7–10,19), heterologous expression of these genes was not successful due to very low expression levels (7,8) or to formation of inclusion bodies (9–11). In our first experiment, taxadiene synthase was first overexpressed using pET3d (Fig. 2), but the protein was found completely in inclusion bodies, even when the induction temperature, IPTG concentration, and host strains were varied. Taxadiene synthase itself may be toxic to the *E. coli* host, but taxadiene, which can be produced by taxadiene syn-

thase using endogenous GGPP as substrate, does not appear to be toxic to *E. coli* since taxadiene does not inhibit the growth of *E. coli* BL21(DE3), even when the *CrtE* gene (31) of *Erwina uredovora* encoding GGPP synthase was coexpressed in *E. coli* BL21(DE3) to increase the concentration of endogenous GGPP, in contrast to normal *E. coli* cells that only produce trace amounts of GGPP (9), with pET32TX (data not shown). We also tried to express the truncated taxadiene synthase (starting from the 138th N-terminal amino acid) using pET3d to determine whether a putative plastid transit peptide of 137 N-terminal amino acids affects the solubility, but the truncated taxadiene synthase was still located in inclusion bodies (data not shown). We are trying to express various truncated taxadiene synthases in *E. coli* with different start sites to investigate the native soluble form in plant cells.

When taxadiene synthase cDNA was inserted into pET32a(+), an expression vector containing the T7lac promoter and encoding a fusion protein thioredoxin, which dramatically increases the solubility of many proteins normally produced in an insoluble form in *E. coli*, about 70% of expressed taxadiene synthase appeared in soluble form following low-temperature (20°C) induction. Recently, we successfully used the thioredoxin expression system to overexpress casbene synthase (19), a cyclase which catalyzes the conversion of GGPP to the phytoalexin casbene (21) (Huang *et al.*, in preparation). Although the recombinant protein shows the same K_m for GGPP as that of native protein, a lower k_{cat} was observed, possibly due to the presence of the fusion protein. Although efforts were made to remove the thioredoxin N-terminal fusion protein by enterokinase, taxadiene synthase lost most activity and the yield was very low (data not shown).

Since diterpene biosynthesis is located exclusively within plastids (32,33), the initial translation product of taxadiene synthase should contain a targeting sequence to direct the protein to this organelle. The deduced amino acid sequence of taxadiene synthase indicates the presence of a putative plastid transit peptide of approximately 137 amino acids. It was not possible to predict a putative cleavage site, since there is no sequence closely resembling a consensus cleavage sequence for transit peptides. The amino terminus of the mature taxadiene synthase is blocked and its identity has not yet been determined (7). Thus, the transit peptide cleavage point is not yet known. Therefore, we decided to amplify the entire taxadiene synthase open reading frame from a full-length cDNA with the first putative methionine codon as the start codon.

In summary, we report for the first time the overexpression of plant taxadiene synthase. Recombinant protein, purified in one step on a His-binding affinity column, has physical and catalytic properties similar to those of the native plant protein.

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