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Open Reading Frame 176 in the Photosynthesis Gene Cluster of *Rhodobacter capsulatus* Encodes *idi*, a Gene for Isopentenyl Diphosphate Isomerase

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Isopentenyl diphosphate (IPP) isomerase catalyzes an essential activation step in the isoprenoid biosynthetic pathway. A database search based on probes from the highly conserved regions in three eukaryotic IPP isomerases revealed substantial similarity with ORF176 in the photosynthesis gene cluster in *Rhodobacter capsulatus*. The open reading frame was cloned into an *Escherichia coli* expression vector. The encoded 20-kDa protein, which was purified in two steps by ion exchange and hydrophobic interaction chromatography, catalyzed the interconversion of IPP and dimethylallyl diphosphate. Thus, the photosynthesis gene cluster encodes all of the enzymes required to incorporate IPP into the ultimate carotenoid and bacteriochlorophyll metabolites in *R. capsulatus*. More recent searches uncovered additional putative open reading frames for IPP isomerase in seed-bearing plants (*Oryza sativa*, *Arabadopsis thaliana*, and *Clarkia breweri*), a worm (*Caenorhabiditis elegans*), and another eubacterium (*Escherichia coli*). The *R. capsulatus* enzyme is the smallest of the IPP isomerases to be identified thus far and may consist mostly of a fundamental catalytic core for the enzyme.

Isoprenoid metabolism, with more than 23,000 known products, is the most chemically diverse biosynthetic pathway in nature. Isoprenoids are found in all organisms (25) and comprise several classes of essential compounds, including sterols (18), carotenoids (12), dolichols (16), ubiquinones (4), and prenylated proteins (8). All of these metabolites are derived from linear isoprenoid diphosphates synthesized from the isomeric five-carbon precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (20). In eukarya and archaea, in which the pathway is well established, IPP is synthesized from three molecules of acetyl coenzyme A by the classical mevalonate pathway (18). However, in some bacteria, IPP is synthesized by a nonclassical route that is not yet fully characterized (21). Beyond IPP, the pathway for constructing polyisoprenoid chains is similar for organisms in all three major kingdoms.

Isopentenyl diphosphate:dimethylallyl diphosphate isomerase (IPP isomerase; EC 5.3.3.2) catalyzes a crucial activation step in the isoprenoid pathway by converting IPP to its highly electrophilic isomer, DMAPP. These two metabolites are the initial substrates for prenyltransferases that synthesize polyisoprenoid chains, as illustrated in Fig. 1. A gene encoding IPP isomerase, *IDI1*, was first isolated from *Saccharomyces cerevisiae* (2). Subsequently, we identified *IDI1* in *Schizosaccharomyces pombe* by plasmid shuffle-mediated complementation of the disrupted yeast gene (13). Xuan et al. (29) found a human cDNA sequence in a phorbol-induced library with an open reading frame (ORF) encoding a protein with a high degree of similarity to both fungal IPP isomerases (13). All three proteins contained conserved regions surrounding two residues, corresponding to cysteine 139 and glutamate 207 in the *S. cerevisiae* enzyme, that were shown to be essential for catalysis by a combination of affinity labeling and site-directed mutagenesis experiments (27). By searching databases with full-length protein sequences and a shorter peptide based on the region surrounding the conserved cysteine residue, we found a putative eukaryotic IPP isomerase encoded in a previously unidentified ORF from the worm *Caenorhabditis elegans* and a putative bacterial isomerase in an unidentified ORF in the photosynthesis gene cluster of *Rhodobacter capsulatus*.

The ORF for IPP isomerase in *R. capsulatus*, a purple nonsulfur bacterium able to grow photosynthetically under anoxygenic conditions, is the first eubacterial IPP isomerase gene to be identified and is located in the photosynthesis gene cluster along with other enzymes required for the biosynthesis of bacteriochlorophyll and carotenoids. The chlorophylls are part of the light-harvesting apparatus, while carotenoids offer protection against reactive species generated during photosynthesis. Both classes of metabolites contain hydrocarbon chains derived from geranylgeranyl diphosphate (GGPP). Previously, genes for all of the isoprenoid enzymes needed to synthesize bacteriochlorophyll and carotenoids from IPP and DMAPP had been identified in the photosynthesis gene cluster. We also report biochemical experiments which establish that ORF176 in the gene cluster encodes IPP isomerase.

MATERIALS AND METHODS

Materials. [1^{14}C]IPP was purchased from American Radiolabeled Chemicals, Inc. [α-^{32}P]dATP was obtained from Amersham. Nalidixic acid was from Sigma. DE52 ion exchange resin was from Whatman. All restriction endonucleases and T7 DNA polymerase were purchased from New England Biolabs. T4 DNA ligase was from Boehringer Mannheim. *R. capsulatus* genomic DNA was provided by J. E. Hearst (Lawrence Berkeley Laboratories, Berkeley, Calif.). Oligonucleotide primers and linkers were synthesized by Bob Schackmann, Utah Regional Cancer Center, Protein/DNA Core Facility.

General procedures. Minipreparations of plasmid DNA for restriction analysis were obtained by the boiling method as described by Sambrook et al. (22). Large-scale plasmid preparations (>100 μg) were performed with the purification kit from Qiagen. DNA fragments were purified on agarose gels (IBI) with a Geneclean II kit from Bio101. Restriction digests, ligations, and *Escherichia coli* transformations were conducted as described by Sambrook et al. (22). The transfer of *R. capsulatus* genomic DNA to nitrocellulose and Southern hybridizations were performed as described by Sambrook et al. (22). The probe was ORF176, labeled by random priming with [α-^{32}P]dATP by using the Rad Prime DNA Labeling System (Bethesda Research Laboratories).

IPP isomerase was assayed by the acid lability procedure (23). Radioactivity was measured in CytoScint scintillation fluid (ICN) with a Packard Tri-Carb 2300TR liquid scintillation analyzer. Ion exchange and hydrophobic interaction chromatographies were conducted at 4°C with a Pharmacia FPLC system. So-

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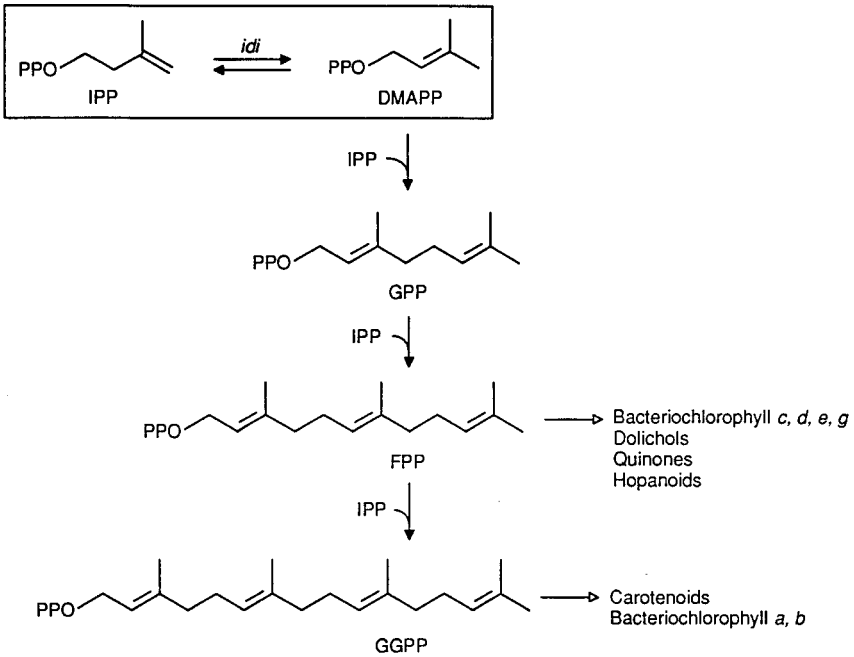

FIG. 1. Early steps in bacterial isoprenoid biosynthesis. The interconversion of IPP and DMAPP, catalyzed by IPP isomerase, is shown in the box.

dium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with the discontinuous buffer system of Laemmli (15), and the gels were stained with Coomassie brilliant blue R (Sigma). Protein concentrations were determined by the method of Bradford (7) with bovine serum albumin as a standard.

Sequence analysis. Sequence searches were performed with the Genetics Computer Group (GCG) package provided by the University of Wisconsin. Several databases were searched, including SWISS-PROT 30.0, PIR 43.0, the coding sequence translations from GenBank 87.0, EMBL 41.0, and the database of expressed sequence tags. Updated versions of these databases were also accessed at the National Center for Biotechnology Information with the BLAST network service. Searches were performed with the BLAST, FASTA, and TFASTA database search programs as well as the Intelligenetics PC Gene software (CD-ROM release 9.0, June 1993; SWISS-PROT 25, EMBL 34, and UgenBank 76-34). Pairwise sequence alignments were accomplished by using the BESTFIT and GAP programs provided in the GCG package. Multiple sequence alignments were initially based upon the pairwise sequence alignments and subsequently optimized by hand.

Strains and plasmids. The strains and plasmids used in this study are listed in Table 1. Growth conditions for the strains have been described previously (13).

Expression and purification of *R. capsulatus* IPP isomerase. *Nde*I and *Sal*I sites were introduced into pRH2, a pBluescript derivative containing ORF176, by mutagenesis (14) with the Muta-Gene Phagemid in vitro mutagenesis kit, version 2 (Bio-Rad), and the following primers: 5′-CCGCCGCAATGGACCTG<u>CAT ATG</u>GCCGAGGAGATGATC-3′ and 5′-CCGGTGACGGCC**TGA**<u>GTCGAC</u>G CCCGGGGCGCGCTTGCG-3′. The start and stop codons of ORF176 are in boldface, and the new restriction sites are underlined. Restriction with *Nde*I and *Sal*I gave a 0.5-kb DNA fragment containing ORF176, which was subcloned into the *E. coli* expression vector pARC306N. The resulting plasmid, pFMH14, was used to transform *E. coli* JM101. Protein synthesis was induced with nalidixic acid as previously described (13).

All steps in the purification of protein were done at 4°C. Cell paste from

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description[a]	Reference or source
E. coli		
DH5α	Host for cloning vectors	Life Technologies
JM101	Host for protein expression	30
S. cerevisiae FH1	Diploid; heterozygous for *idi1*::*leu2*; allows determination of ability to complement disrupted yeast IPP isomerase gene	17
Plasmids		
pFL205	pBR325 with the 6.5-kb *Bam*HI fragment corresponding to bp 18791–25244 of 46-kb photosynthesis gene cluster (EMBL accession no. Z11165)	J. E. Hearst
pBS(KS$^-$)	pBluescript cloning vector; Ap^r	Stratagene
pRH1	pBS(KS$^-$) with the 6.3-kb *Bam*HI-*Hin*dIII fragment of pFL205	This work
pRH2	pRH1 with a 2.8-kb *Sal*I deletion	This work
pRH3	pRH2 with *Nde*I and *Sal*I sites introduced by mutagenesis	This work
pARC306N	*E. coli* expression vector; Ap^r; *rec-7* hybrid promoter	13
pFMH14	pARC306N with the 532-bp *Nde*I-*Sal*I fragment of pRH3	This work
pRH4	pRH2 with a *Sal*I site introduced by mutagenesis	This work
pBSF19	pBSII (SK$^+$) with the *S. pombe* 0.9-kb cDNA fragment encoding IPP isomerase	13
pRH5	pBSF19 with the 532-bp *Nco*I-*Sal*I fragment of pRH4	This work
pRH6	pRH5 with a 348-bp *Nco*I-*Eco*RI deletion	This work
pYES2.0	Yeast expression-*E. coli* shuttle vector; Ap^r; *URA3*; *GAL1* promoter	Invitrogen
pFMH15	pYES2.0 with the 547-bp *Eco*RI-*Xho*I fragment of pRH6	This work

[a] Ap, ampicillin.

JM101/pFMH14 was suspended in 10 ml of 10 mM potassium phosphate–10 mM β-mercaptoethanol (BME)–1 mM phenylmethylsulfonyl fluoride, pH 7.0, and disrupted by sonication. The resulting homogenate was centrifuged at 23,700 × *g* to remove cellular debris. The supernatant was then loaded on a DE52 cellulose column (1.5 by 30 cm) previously equilibrated with 10 mM potassium phosphate–10 mM BME, pH 7.0 (buffer A). The column was eluted at 1.5 ml/min with a linear gradient of buffer A to 10% buffer B [10 mM potassium phosphate, 2 M $(NH_4)_2SO_4$, 10 mM BME (pH 7.0)] over 60 ml. Active fractions were collected and combined. $(NH_4)_2SO_4$ (2.0 M)–10 mM potassium phosphate–10 mM BME, pH 7.0, was added to the combined fractions with stirring on ice to a final concentration of 1.0 M $(NH_4)_2SO_4$. The solution was then loaded onto an HR10/10 phenyl-Superose column (Pharmacia) preequilibrated with 1.0 M $(NH_4)_2SO_4$–10 mM potassium phosphate–10 mM BME, pH 7.0 (50% buffer A plus 50% buffer B). The column was eluted with a linear gradient to 100% buffer A containing 10 mM potassium phosphate and 10 mM BME, pH 7.0, over 30 ml. Fractions were assayed for IPP isomerase activity and protein content after each purification step.

Product analysis by proton NMR. A reaction mixture containing 10 mM potassium phosphate, 10 mM BME, 5 mM $MgCl_2$, 7 mM IPP, and 0.6 μM *R. capsulatus* IPP isomerase was allowed to incubate for 6 h at 37°C. Water was removed from the reaction mixture on a Speed Vac. The residue was resuspended in 99.9% D_2O, and a ^1H nuclear magnetic resonance (NMR) spectrum was acquired.

Complementation of disrupted yeast *IDI1*. The yeast expression plasmid containing ORF176, pFMH15, was used to transform the heterozygous diploid yeast strain FH1 (17) to uracil prototrophy by the modified lithium acetate procedure of Elbe (10). Transformants were sporulated and dissected as previously described (13). Spores that were both uracil and leucine prototrophs were identified by replica plating. Haploid spores were identified by mating type analysis as previously described (13).

RESULTS

Database searches and sequence alignments. Database searches were conducted with the complete amino acid sequence for IPP isomerase from *Schizosaccharomyces pombe* (13), *S. cerevisiae* (2), and *Homo sapiens* (29), as well as the amino acid sequence surrounding the essential Cys-139 in the active site of *S. cerevisiae* IPP isomerase (27). The last probe was instrumental for identifying the ORF for IPP isomerase in the *R. capsulatus* photosynthesis gene cluster. Nucleic acid searches with the isomerase gene from *Clarkia breweri* (5) yielded putative IPP isomerases from two other plant sources, *Arabadopsis thaliana* and *Oryza sativa*, in the database of expressed sequence tags. The searches yielded a total of five putative IPP isomerases, including homologs in seed-bearing plants (*O. sativa*, *A. thaliana*, and *C. breweri*), a worm (*C. elegans*), and a eubacterium (*R. capsulatus*). A more recent search uncovered a newly added entry for *E. coli* ORF182 at 64 to 65 min in the K-12 genome. The ORFs in *C. elegans*, *E. coli*, and *R. capsulatus* had not previously been assigned. Pairwise sequence alignments of the IPP isomerases for which the entire amino acid sequences were available were conducted to identify conserved regions. These results are listed in the form of a multiple sequence alignment in Fig. 2. The pairwise alignments indicated a high degree of identity (typically 50%) among the eukaryotic IPP isomerases. However, the identity between the two bacterial enzymes was only 32% and not substantially different from their identities with the eukaryotic IPP isomerases. The percentage of conserved amino acids increases substantially in a core region, defined by the residues from I74 to W255 in the *S. cerevisiae* protein, that encompasses the essential active-site cysteine and glutamate residues. The length of this core region ranges from 152 amino acids in *R. capsulatus* to 181 in *S. cerevisiae*.

Expression of *R. capsulatus idi* and purification of IPP isomerase. Plasmid pFMH14 was constructed to see if ORF176 directed synthesis of a protein with IPP isomerase activity. Starting with pFL205, containing a 6.5-kb *R. capsulatus* DNA fragment (see Table 1), a 6.3-kb *Bam*HI-*Hin*dIII fragment was isolated and subcloned into pBS(KS$^-$) to create pRH1. The *R. capsulatus* DNA contained in pRH1 was then trimmed to 3.5 kb by restriction with *Sal*I. Separation of the resulting 6.4-kb and 2.8-kb fragments by agarose gel electrophoresis, purification of the 6.4-kb fragment by Geneclean, and recircularization yielded pRH2. Site-directed mutagenesis by the procedure of Kunkel (14) was then used to introduce an *Nde*I site at the start codon and a *Sal*I site just downstream from the stop codon in ORF176 to create pRH3. The 0.5-kb *Nde*I-*Sal*I fragment containing ORF176 was cloned into *E. coli* expression vector pARC306N to form pFMH14, which was used to transform *E. coli* JM101. Cultures of JM101/pFMH14 in supplemented M9 minimal medium (28) were grown at 37°C, and plasmid-directed protein synthesis was induced by addition of nalidixic acid. *R. capsulatus* IPP isomerase was purified from cell extracts in two steps by ion-exchange chromatography on DE52-cellulose and hydrophobic interaction chromatography on phenyl-Superose following procedures similar to those for the yeast protein (28). The purification is summarized in Table 2. This procedure gave an enzyme that was greater than 95% pure, as judged by SDS-PAGE (Fig. 3). The specific activity for purified *R. capsulatus* IPP isomerase was approximately 100-fold lower than that of the *S. cerevisiae* enzyme (28).

Product analysis. The reaction catalyzed by purified ORF176 gene product was analyzed by incubating 7 mM IPP with catalytic amounts of the protein at pH 7.0 in buffer containing $MgCl_2$. After 6 h at 37°C, water was removed from the reaction mixture at reduced pressure, and the residue was dissolved in 99.9% D_2O. A ^1H NMR spectrum of a sample of IPP treated in the same manner except for addition of protein gave resonances typical of those reported for IPP (9). After incubation with ORF176 protein, a ^1H NMR spectrum that had additional resonances centered at 1.77, 4.70, and 5.46 ppm, in accord with those previously reported for DMAPP (9), was acquired. The ratio of IPP to DMAPP in the reaction mixture was calculated by integration of the resonances for the methylene protons in IPP and DMAPP to give a 2.4:1 ratio for DMAPP to IPP. This is in good agreement with the reported value of 2.2:1 for this equilibrium (26).

Complementation of disrupted yeast *IDI1*. The yeast expression plasmid pFMH15 was constructed to determine if ORF176 would complement the disrupted IPP isomerase gene, *IDI1*, in *S. cerevisiae* to give a viable yeast strain. Plasmid pRH4, containing a *Sal*I site introduced just after the stop codon of ORF176 but without an *Nde*I site at the start codon, was isolated during the site-directed mutagenesis experiment described for pRH3. pBSF19, a pBluescript derivative containing the ORF for *S. pombe* IPP isomerase, has a unique *Nco*I site within the 0.9-kb *S. pombe* cDNA insert. An *Nco*I-*Sal*I digestion removed a 0.6-kb fragment containing the 3′ region of the *S. pombe* cDNA insert in pBSF19. By using a unique *Nco*I site at the start codon, a 0.5-kb *Nco*I-*Sal*I fragment containing ORF176 was isolated from pRH4 and subcloned into the pBSF19 fragment to form pRH5. pRH5 was then digested with *Nco*I and *Eco*RI to remove the remaining *S. pombe* DNA, and a synthetic linker,

5′ AATTCGACGTCC
GCTGCAGGGTAC 5′

was used to close the incompatible *Eco*RI-*Nco*I ends to form pRH6. ORF176 was removed from pRH6 as a 0.5-kb *Eco*RI-*Xho*I fragment and ligated into the yeast expression vector pYES2.0 to produce pFMH15.

Diploid *S. cerevisiae* strain FH1, an *idi1*::*leu2* heterozygote, was transformed to uracil prototrophy with pFMH15. Following sporulation of isolated transformants, asci were dissected,

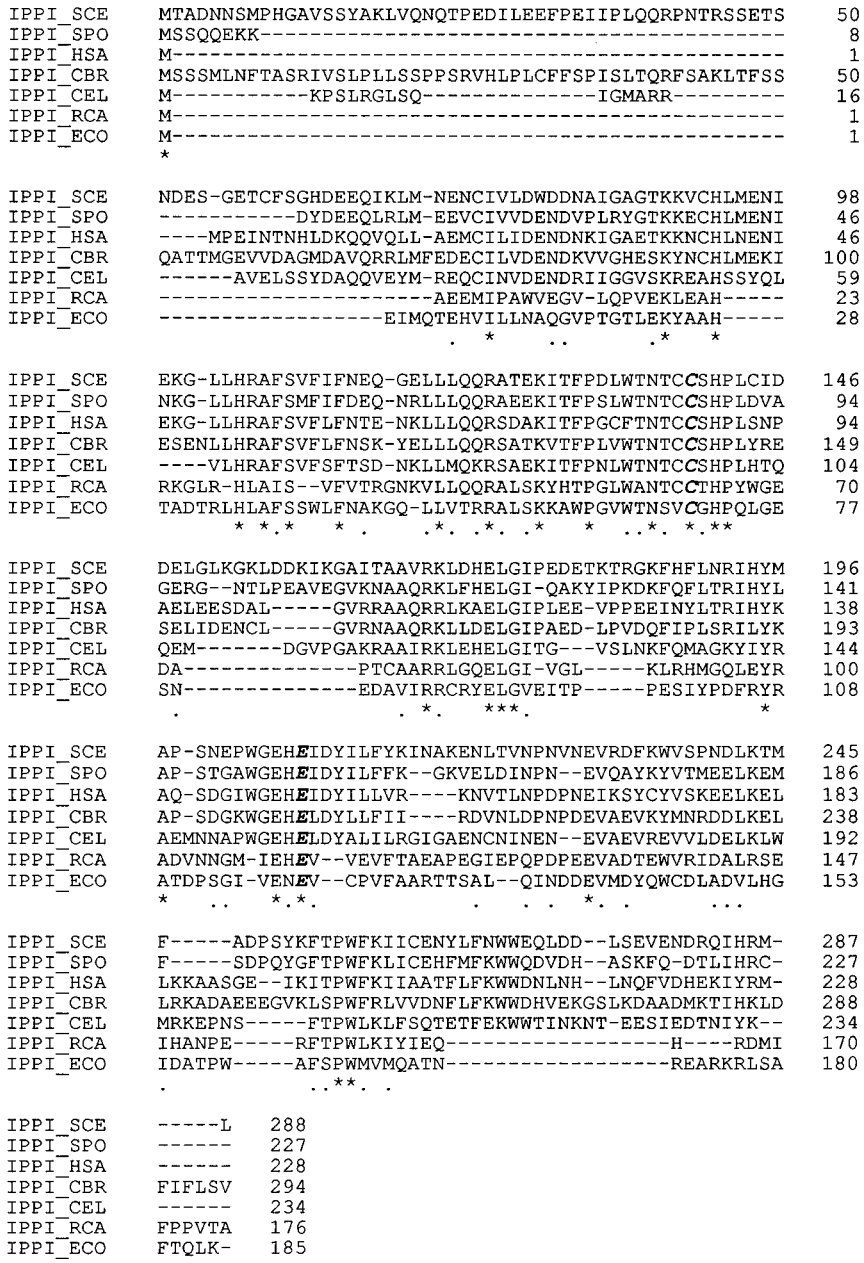

FIG. 2. Sequence alignments for IPP isomerases from *S. cerevisiae* (IPPI-SCE), *S. pombe* (IPPI-SPO), *H. sapiens* (IPPI-HSA), *C. breweri* (IPPI-CBR), *C. elegans* (IPPI-CEL), *R. capsulatus* (IPPI-RCA), and *E. coli* (IPPI-ECO). *, identity; •, similarity. The conserved active-site residues, identified in *S. cerevisiae* IPP isomerase as C139 and E207 (27), are shown in boldface italic.

and haploid spores able to grow without uracil and leucine were identified by replica plating. Thus, *R. capsulatus* IPP isomerase, encoded by ORF176, had sufficient activity to complement disrupted yeast *IDI1*.

TABLE 2. Purification of recombinant *R. capsulatus* IPP isomerase

Purification step	Total activity (μmol/min)	Sp act (μmol/min·mg)	Yield (%)	Purification factor[a]
Cell supernatant	7.7	0.03	100	1
DE52	6.4	0.09	83	2.7
Phenyl-Superose	3.4	0.14	44	4.2

[a] Fold increase in specific activity.

Southern analysis. A Southern analysis of *R. capsulatus* genomic DNA digested with *Cla*I, *Pst*I, *Bam*HI, *Aat*II, and *Apa*I is shown in Fig. 4. The 0.5-kb *Eco*RI-*Xho*I fragment from pFMH15, containing ORF176, was ^{32}P labeled and used as a probe. All of the fragments that contain ORF176, as predicted by a restriction analysis of the *R. capsulatus* photosynthesis gene cluster sequence, hybridized to the probe. The predicted fragment sizes are 5,921 bp, *Cla*I; 2,384 bp, *Pst*I; 6,453 bp, *Bam*HI; 3,224 bp, *Aat*II; and 4,941 bp, *Apa*I. However, bands presumably other than those containing ORF176 were also seen. For example, an additional 4-kb band from the *Cla*I digestion, an additional 8-kb band from the *Bam*HI digestion, and additional bands of 4.9, 3.0, and 1.9 kb from the *Pst*I digestion were detected.

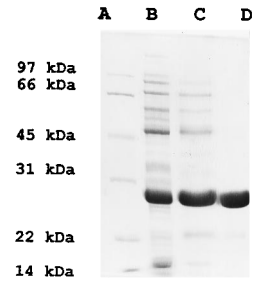

FIG. 3. SDS-PAGE gel of samples from each step in the purification of recombinant *R. capsulatus* IPP isomerase. Lanes: A, molecular weight standards; B, supernatant from the cell extract; C, after chromatography on DE52; D, combined fractions after chromatography on phenyl-Superose.

DISCUSSION

A database search with protein and DNA probes based on conserved regions from *S. cerevisiae*, *S. pombe*, and *H. sapiens* IPP isomerases surrounding the known essential active-site cysteine and glutamate residues uncovered complete or partial sequences for six putative enzymes, three in seed-bearing plants (*O. sativa*, *A. thaliana*, and *C. breweri*), one in a worm (*C. elegans*), and two in eubacteria (*R. capsulatus* and *E. coli*). The sequences in *R. capsulatus* and *E. coli* are the first to be assigned to a bacterial IPP isomerase.

The encoded proteins vary in size from 20 kDa for the *R. capsulatus* enzyme to 34 kDa for IPP isomerase from *C. breweri*. Each protein contains a conserved core region that corresponds to amino acids from I74 to W255 in the *S. cerevisiae* enzyme. The core constitutes virtually the entire protein in bacteria and is flanked by amino- and carboxy-terminal extensions in the eukaryotic isomerases. The highest region of similarity within the core is a 38-residue sequence corresponding to H104 to P142 in the yeast protein. This region contains the essential, highly conserved active-site cysteine at position 139 in *S. cerevisiae*. The affinity labeling experiments that originally uncovered C139 also labeled C138. Street et al. (27) subsequently showed that the latter cysteine is not essential by site-directed mutagenesis. It is interesting that this residue is conserved in all but the *E. coli* protein.

The function of the protein encoded by ORF176 was established biochemically. Recombinant protein was synthesized in *E. coli* and purified to homogeneity. The enzyme catalyzed the isomerization of IPP to DMAPP. Although *R. capsulatus* IPP isomerase was somewhat more stable than its yeast counterpart, its specific activity was approximately 100-fold less.

The *R. capsulatus* photosynthesis gene cluster encodes enzymes required for the biosynthesis of light-harvesting bacteriochlorophylls and photoprotective carotenoids. With the discovery that ORF176 encodes IPP isomerase, all of the isoprenoid genes required for the synthesis of these metabolites from IPP have been identified in the cluster. ORF176 is located in an operon that contains *bchE*, *bchJ*, *bchG*, *bchP*, and the unidentified ORF ORF428 (1). The *bch* genes encode enzymes required for bacteriochlorophyll biosynthesis—*bchE*, Mg protoporphyrin IX monomethyl ester oxidative cyclase subunit; *bchJ*, 4-vinyl reductase; *bchG*, geranylgeranyl bacteriochlorophyll synthase; and *bchP*, geranylgeranyl bacteriochlorophyll reductase (1). The last two enzymes catalyze reactions with isoprenoid substrates. Geranylgeranyl diphosphate synthase, the chain elongation enzyme that synthesizes GGPP from IPP and DMAPP, is encoded by the *crtE* gene, located on another operon in the photosynthesis gene cluster (1). There is no obvious explanation at this time for why ORF176 is located in the operon with the *bch* genes.

In addition to the biosynthesis of carotenoids and bacteriochlorophylls, isoprenoids are required for respiration (ubiquinones) and cell wall biosynthesis (dolichols) in bacteria. Insertional inactivation of ORF176 was not lethal, nor was the gene product of ORF176 required for photosynthetic growth or carotenoid production (6). Similar experiments with *crtE*, the gene encoding GGPP synthase in the photosynthesis gene cluster, resulted in the loss of carotenoid production while allowing synthesis of prenylated bacteriochlorophylls (3). These observations strongly suggest that additional genes for IPP isomerase and a short-chain isoprenyl diphosphate synthase other than the GGPP synthase encoded by *crtE* are located outside the gene cluster. A Southern analysis of restricted genomic DNA with labeled ORF176 DNA as a probe suggests the possibility of two IPP isomerase genes. Intense bands were seen at positions expected for ORF176. In addition, a set of lighter bands indicates the presence of another genomic sequence with significant similarity to the ORF176 probe. The ability of the *crtE* mutant with a disabled GGPP synthase to support bacteriochlorophyll production is puzzling. Either another GGPP synthase gene is located outside the photosynthesis gene cluster, or perhaps farnesyl diphosphate serves as an alternative substrate for GGPP when *crtE* is disabled, to give functional bacteriochlorophylls.

The identification of IPP isomerase in *E. coli* was of interest because, as in *R. capsulatus*, the early steps of the isoprenoid pathway have not been established in this bacterium (21). The gene for IPP isomerase is located between 64 and 65 min on the *E. coli* genome and appears to be transcribed as an independent unit. Two promoter sequences, TTACAT and TATA AT, and a Shine-Dalgarno sequence, GUGAG, are located 10, 31, and 5 bp upstream, respectively, from the start codon, although their resemblance to *E. coli* consensus sequences is modest. These structures may be associated with the nominal levels of IPP isomerase activity seen in *E. coli* (24). A transcriptional termination hairpin, AACCCCGACAUUUGCCG GGGUU, is located 3 bp downstream from the stop codon. The gene for farnesyl diphosphate synthase, *ispA*, which en-

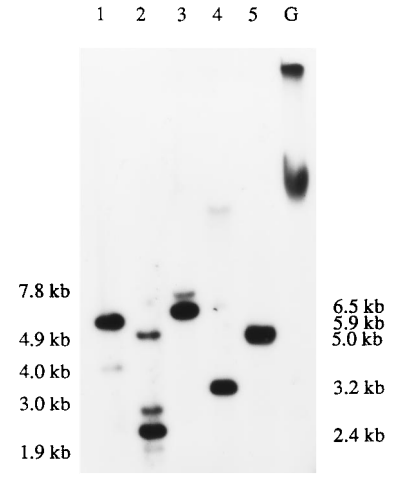

FIG. 4. Southern analysis of digested *R. capsulatus* genomic DNA. Lane 1, *Cla*I; lane 2, *Pst*I; lane 3, *Bam*HI; lane 4, *Aat*II; lane 5, *Apa*I; lane G, undigested genomic DNA. The five intense bands at 6.5, 5.9, 5.0, 3.2, and 2.4 kb correspond to those predicted to contain ORF176 and are labeled on the right. Bands labeled on the left, corresponding to lanes 1 to 3, presumably do not contain ORF176.

codes the next enzyme in the isoprenoid pathway, is located at 10 min on the *E. coli* genome and is transcribed independently.

In summary, genes for two bacterial and four eukaryotic IPP isomerases were identified during database searches with probes based on fungal and mammalian enzymes. The encoded bacterial proteins are smaller than their eukaryotic counterparts and appear to mostly consist of a ~20-kDa catalytic core. The *R. capsulatus* gene is located in the photosynthesis gene cluster along with all of the genes necessary to assimilate IPP into carotenoids and bacteriochlorophylls. Recombinant IPP isomerase from *R. capsulatus* was more stable than its eukaryotic counterparts but had a lower specific activity. Gene disruption experiments (6) and our Southern analysis of genomic DNA indicate that a second gene for IPP isomerase is located elsewhere in the *R. capsulatus* genome.

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