Product Variability. The chain length of the synthesized polyprenyl pyrophosphate is dramatically affected by the concentration of Mg^{2+} in the medium.[6] At 0.1 m*M* Mg^{2+}, C_{25}, C_{30}, and C_{35} compounds are produced with C_{25} predominating; at 0.5 m*M* Mg^{2+}, C_{25}, C_{30}, C_{35}, and C_{40} compounds are produced with C_{35} and C_{40} predominating; and at 20 m*M* Mg^{2+}, C_{40} and C_{45} are produced without accumulation of shorter compounds.

Physiological Significance. Since *M. luteus* produces menaquinones having prenyl chains of C_{30}, C_{35}, C_{40}, and C_{45} with a predominance of C_{40}, the role of this enzyme is probably to provide the isoprenoid precursors for these menaquinones, though it is uncertain whether or not this enzyme preparation is a mixture of more than one enzyme protein. Geranyl pyrophosphate, which is needed as a substrate to prime the chain elongation by nonaprenylpyrophosphate synthetase, will be provided by geranylpyrophosphate synthetase which has also been isolated from this bacteria.[5]

[6] H. Fujii, H. Sagami, T. Koyama, K. Ogura, S. Seto, T. Baba, and C. M. Allen, *Biochem. Biophys. Res. Commun.* **96,** 1648 (1980).

[26] Enzymatic Synthesis of Phytoene

By BENJAMIN L. JONES and JOHN W. PORTER[1]

Enzyme systems in the chromoplasts of tomato fruits[1a] and in extracts of spinach leaves[2] convert isopentenyl pyrophosphate to phytoene by the reactions shown in Fig. 1. The enzyme system from tomato fruits (which has been studied more extensively than the spinach system) behaves as a complex on BioGel filtration.[3] However, during subsequent DEAE-cellulose chromatography phytoene-synthesizing activity is lost but activities for the formation of acid-labile compounds are retained.[1a,4] Subsequent purification steps result in the separation of activities for isopentenylpy-

[1] Deceased, June 27, 1984.

[1a] F. B. Jungalwala and J. W. Porter, *Arch. Biochem. Biophys.* **119,** 209 (1967).

[2] C. Subbarayan, S. Kushwaha, G. Suzue, and J. W. Porter, *Arch. Biochem. Biophys.* **137,** 547 (1970).

[3] B. Maudinas, M. L. Bucholtz, C. Papastephanou, S. S. Katiyar, A. V. Briedis, and J. W. Porter, *Arch. Biochem. Biophys.* **180,** 354 (1977).

[4] M. A. Islam, S. A. Lyrene, E. M. Miller, Jr., and J. W. Porter, *J. Biol. Chem.* **252,** 1523 (1977).

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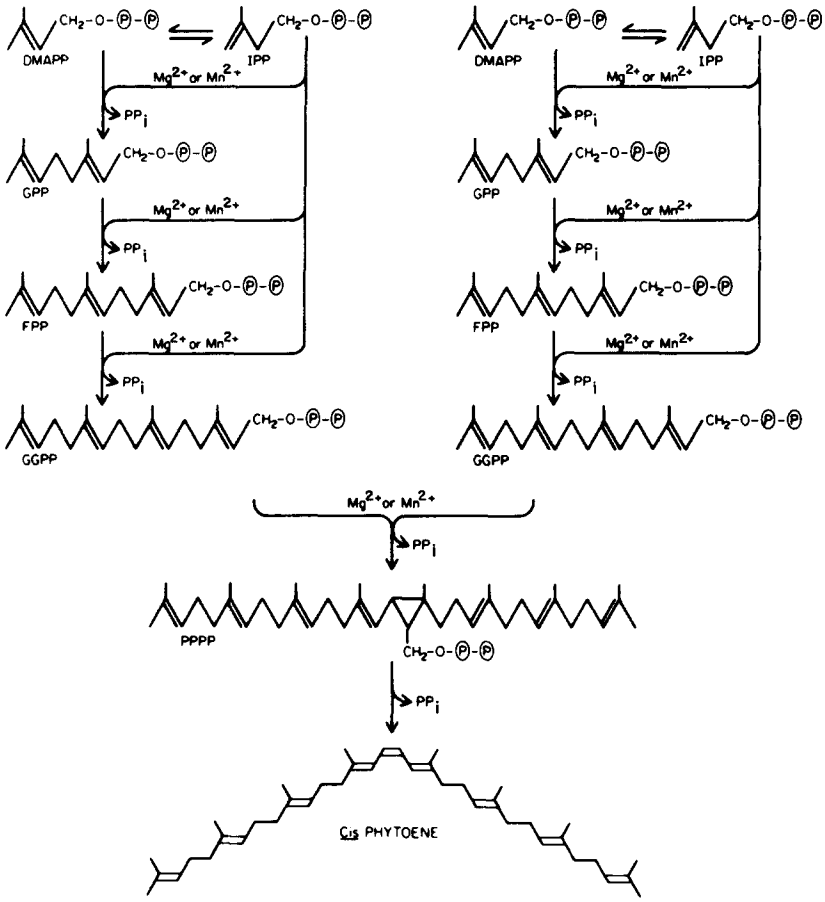

FIG. 1. The enzymatic reactions effected by the enzyme system that converts isopentenyl pyrophosphate to phytoene. The following abbreviations are used: IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; and PPPP, prephytoene pyrophosphate. [Reproduced with permission from J. W. Porter, S. L. Spurgeon, and N. Sathyamoorthy, Biosynthesis of Carotenoids, *in* "Isopentenoids in Plants" (W. D. Nes, G. Fuller, and L.-S. Tsai, eds.), p. 161. Marcel Dekker, New York, 1984.]

rophosphate isomerase and prenyltransferase.[5] Other purification steps result in the separation of the enzymes which convert geranylgeranyl pyrophosphate to phytoene from the isomerase and prenyltransferase.[6]

[5] S. L. Spurgeon, N. Sathyamoorthy, and J. W. Porter, *Arch. Biochem. Biophys.* **230,** 446 (1984).

[6] B. L. Jones and J. W. Porter, unpublished data (1983).

The separation and properties of the enzyme system for phytoene synthesis that exists in tomatoes are the concern of this chapter.

Assay Method—Tomato Enzymes

General Procedure. The enzymatic synthesis of phytoene is determined by assay for the incorporation of radioactivity of [1-^{14}C]isopentenyl pyrophosphate or [1-^{3}H]geranylgeranyl pyrophosphate into this product. The biosynthesized phytoene is extracted from the incubation mixture with petroleum ether and then separated from other compounds by high-pressure liquid chromatography (HPLC) or alumina chromatography. The incorporation of radioactivity into phytoene is confirmed by coelution with authentic phytoene on HPLC and alumina column chromatography.

Tomato System

Reagents

Borate buffer, 500 m*M*, pH 8.2
$MgCl_2$, 375 m*M*
$MnCl_2$, 100 m*M*
Dithiothreitol, 100 m*M*
ATP, 8 m*M*
$NADP^+$, 40 m*M*
Tween 80, 100 mg/ml
[1-^{14}C]Isopentenyl pyrophosphate, 0.2 m*M* or
[1-^{3}H]Geranylgeranyl pyrophosphate, 0.5 m*M*

Unlabeled isopentenyl pyrophosphate is synthesized chemically,[7,8] and purified by HPLC on an anion exchange column with a linear gradient of 0.5 to 4% ammonium bicarbonate. Substrate isopentenyl pyrophosphate (6000 dpm/nmol) is prepared by diluting the labeled compound, purchased from Amersham, with the synthetic compound. [1-^{3}H]Geranylgeraniol is prepared from geranylgeraniol by oxidation with manganese dioxide,[9] and reduction with ^{3}H-labeled sodium borohydride.[10] Phosphorylation of both labeled and unlabeled geranylgeraniol was by the same procedure as for the preparation of isopentenyl pyrophosphate.[7,8] Purification of labeled and unlabeled geranylgeranyl pyrophosphate is carried out on a silica gel column with a convex gradient from 12 : 1,

[7] R. H. Cornforth and G. Popják, this series, Vol. 15, p. 359.
[8] Y. Gafni and I. Shechter, *Anal. Biochem.* **92,** 248 (1979).
[9] L. J. Altman, R. C. Kowerski, and D. R. Laungani, *J. Am. Chem. Soc.* **100,** 6174 (1978).
[10] W. Rudiger, J. Benz, and C. Guthoff, *Eur. J. Biochem.* **109,** 193 (1980).

n-propanol : ammonium hydroxide to 6 : 3 : 1, *n*-propanol : ammonium hydroxide : water.[8] Substrate geranylgeranyl pyrophosphate (30,000 dpm/nmol) is prepared by diluting the labeled compound with unlabeled compound.

Incubation System

The incubation system for the biosynthesis of phytoene, which is a slight modification of previous systems,[1a,11] contains the following: $MnCl_2$, 1 μmol; $MgCl_2$, 7.5 μmol; dithiothreitol, 5 μmol; $NADP^+$, 2 μmol; ATP, 0.4 μmol; Tween 80, 5 mg; borate buffer, pH 8.2, 50 μmol; enzyme protein, 0.5 to 2 mg, and either [^{14}C]isopentenyl pyrophosphate, 20 μmol and 120,000 dpm or [1-^{3}H]geranylgeranyl pyrophosphate, 25 nmol and 750,000 dpm, in a final volume of 0.5 ml. Incubation of this mixture under nitrogen is carried out for 2 hr at 22°.

Isolation of Phytoene

The enzyme reaction is stopped by the addition of four volumes of absolute ethanol and nonpolar compounds are then extracted with two 4.0-ml aliquots each of silica gel purified petroleum ether (bp 40 to 60°). The extract is dried over anhydrous sodium sulfate, concentrated by a stream of nitrogen, and then chromatographed on alumina with increasing concentrations of diethyl ether in petroleum ether.[1a]

More routinely, the extract is concentrated under a stream of nitrogen to near dryness, resuspended in acetonitrile : tetrahydrofuran, 1 : 1, 66%, and methanol : water, 3 : 1, 34%, and analyzed on a C_{18} reverse phase column by HPLC with the same solvent mixture.[12] Phytoene is detected by absorbance at 280 nm. Eluate fractions are collected and aliquots are taken for liquid scintillation counting for radioactivity.

Enzyme Activity

Total enzyme activity is expressed as nanomoles of substrate incorporated into phytoene under the described assay conditions.

Assay for Isopentenyl Pyrophosphate Isomerase

General Procedure. The conversion of isopentenyl pyrophosphate to dimethylallyl pyrophosphate is catalyzed by isomerase. Isopentenyl pyro-

[11] D. V. Shah, D. H. Feldbruegge, A. R. Houser, and J. W. Porter, *Arch. Biochem. Biophys.* **127,** 124 (1968).

[12] P. Beyer, K. Kreuz, and H. Klenig, *Planta* **150,** 435 (1980).

phosphate is stable to acid while dimethylallyl pyrophosphate is not, and the latter is converted to the alcohol form in the presence of acid. This alcohol is extracted with petroleum ether, and an aliquot is taken for liquid scintillation counting for radioactivity.

Reagents

TES buffer, 1 *M*, pH 7.5
$MgCl_2$, 373 m*M*
$MnCl_2$, 100 m*M*
Dithiothreitol, 100 m*M*
Hydrochloric acid, 10.0 *N*
[1-^{14}C]Isopentenyl pyrophosphate, 0.2 m*M*

Incubation System

The incubation system, modified from a previous report,[5] for the assay of isomerase contains $MnCl_2$, 1 μmol; $MgCl_2$, 7.5 μmol; dithiothreitol, 5 μmol; TES buffer, pH 7.5, 100 μmol; [1-^{14}C]isopentenyl pyrophosphate, 20 nmol and 120,000 dpm, and enzyme protein, 0.02 to 0.5 mg in a final volume of 0.5 ml. Incubation is carried out at 37° for 30 min.

Assay of Product

The reaction is terminated by the addition of four volumes of absolute ethanol. The product is hydrolyzed by the addition of 100 μl of 10.0 *N* hydrochloric acid and incubation at 37° for 20 min. Acid-labile product is extracted with 4 ml of petroleum ether (bp 40 to 60°). A 1-ml aliquot of the extract is taken for liquid scintillation counting for radioactivity.

Activity

Total enzyme activity is expressed as nanomoles of substrate converted to acid-labile product.

Prenyltransferase

General Procedure. The condensation of isopentenyl pyrophosphate with dimethylallyl pyrophosphate to form geranyl pyrophosphate and subsequent condensation with isopentenyl pyrophosphate to form farnesyl pyrophosphate and geranylgeranyl pyrophosphate is catalyzed by prenyl transferase. The reaction is assayed by the incorporation of [1-^{14}C]isopentenyl pyrophosphate into acid-labile products.

Reagents

Potassium phosphate buffer, 0.1 *M*, pH 7.0
Tween 80, 50 mg/ml
$MgCl_2$, 375 m*M*
$MnCl_2$, 100 m*M*
Iodoacetamide, 25 m*M*
Dimethylallyl pyrophosphate, 1.3 m*M*
[1-^{14}C]Isopentenyl pyrophosphate, 0.2 m*M*
Hydrochloric acid, 10.0 *M*

Incubation System

The assay for prenyltransferase activity is essentially the same as reported previously.[5] This assay consists of two incubations: the first to destroy isomerase, and the second for prenyltransferase activity. The assay system contains the following: $MnCl_2$, 1 μmol; $MgCl_2$, 7.5 μmol; Tween 80, 5 mg; iodoacetamide, 0.5 μmol; potassium phosphate, pH 7.0, 10 μmol; and enzyme protein, 0.02 to 0.5 mg. The incubation is for 30 min at room temperature. At the end of the incubation, [1-^{14}C]isopentenyl pyrophosphate, 20 nmol and 120,000 cpm, and dimethylallyl pyrophosphate, 33 nmol, are added and the volume of the incubation mixture is adjusted to 0.5 ml. Blank assays are handled in the same manner except that dimethylallyl pyrophosphate is omitted. Incubation is carried out for one hour at 37°.

Acid Hydrolysis

The reaction is terminated and the products are extracted and assayed for radioactivity as described for isomerase. Activity is expressed as nmoles [1-^{14}C]isopentenyl pyrophosphate incorporated into product.

Enzymatic Hydrolysis for Product Analysis

Reagents. The reagents are the same as described above for prenyltransferase with the addition of the following:

EDTA, pH 7.0, 250 m*M*
Ammonium hydroxide, 10.0 *M*
Sodium acetate buffer, pH 4.7, 1 *M*
Triton X-100, 100 mg/ml
Potato acid phosphatase in 10 m*M* sodium acetate, pH 7.5, 2 mg protein/ml (Sigma)
Sodium hydroxide, 6 *N*

The prenyltransferase reaction is stopped by the addition of 25 μmol of EDTA and 1 mmol of ammonium hydroxide. The incubation mixture is then passed through a C_{18} Sep-pak that has been preconditioned according to the manufacturer's instructions. The Sep-pak is washed with 4 ml of water, and the reaction products are eluted with two 2-ml aliquots of methanol. The methanol is blown off under a stream of nitrogen to dryness and the residue is resuspended in 10 m*M* Tris, pH 8.5, to a suitable volume (less than 1 ml). Polyprenyl pyrophosphates are then hydrolyzed with acid phosphatase.[13] The incubation mixture contains sodium acetate buffer, pH 4.7, 0.1 mmol; Triton X-100, 3 mg; acid phosphatase, 3.8 units; an aliquot of the products to be hydrolyzed, and methanol to 60% in a volume of 2.8 ml. The mixture is incubated at 37° overnight, stopped with 0.6 mmol sodium hydroxide, and extracted three times, each with 4 ml of hexane. The extracts are pooled, carrier farnesol, geraniol, and geranylgeraniol are added, and the mixture is blown to dryness under a stream of nitrogen. The sample is resuspended in tetrahydrofuran : acetonitrile, 1 : 1, 60%, and methanol : water, 2 : 1, 40%, and analyzed by HPLC on a C_{18} reverse phase column in the same solvent mixture.[12] Terpenyl alcohols are detected by absorbance at 218 nm or by a change in refractive index. Fractions are collected and aliquots are taken for liquid scintillation counting for radioactivity.

Product analysis of the alcohols may also be performed on reverse phase thin layer chromatography (TLC) plates.[5] Silica gel plates are impregnated with paraffin oil by dipping the plates in a solution of 5% paraffin oil in petroleum ether. The developing system is methanol : water (80 : 20). Geraniol, farnesol, and geranylgeraniol are spotted as standards. The positions of compounds are determined by iodine vapors and radioactive products are determined by cutting or scraping the plate into scintillation vials, adding cocktail and counting for radioactivity.

The pyrophosphate form of the product is analyzed by TLC.[14] The plates are silica gel H buffered with 0.1 M ammonium phosphate, pH 6.5, and they are activated for 20 min at 110° prior to use. The solvent system is chloroform : methanol : water (60 : 40 : 9). Standards of farnesyl pyrophosphate, geranyl pyrophosphate, and geranylgeranyl pyrophosphate are spotted. After development spots are detected by iodine vapor or by spraying for phosphate with Rosenburg's reagent.[15] Radioactive products are determined by scraping the gel into a scintillation vial, adding cocktail, and counting for radioactivity.

[13] H. Fujii, T. Koyama, and K. Ogura, *Biochim. Biophys. Acta* **712,** 716 (1982).
[14] H. Rilling, *J. Lipid Res.* **10,** 183 (1969).
[15] H. Rosenburg, *J. Chromatogr.* **2,** 487 (1959).

Preparation of Enzyme System

Partial Purification. All operations are carried out at 4° unless otherwise stated.

Preparation of Plastids. Tomato fruit plastids are prepared essentially as described previously.[1a] Tomatoes are cut in half, most of the seeds are removed, and the residual tomato sections are placed in a commercial Waring blender with a buffer consisting of 0.2 *M* Tris, pH 8.2, 1 m*M* EDTA, and 1 m*M* 2-mercaptoethanol in a ratio of 800 g tomato tissue to 800 ml of buffer. The mixture is homogenized by three 5-sec bursts of the blender. The slurry is filtered first through a double layer of cheesecloth and then a double layer of cheesecloth with a glass wool mat in it. This mixture is centrifuged at 13,000 *g* for 30 min, the supernatant is poured off, the centrifuge tube refilled with filtrate, and centrifuged again. When the filtrate is exhausted, the pellet is suspended in a minimal volume of 0.1 *M* potassium phosphate buffer, pH 6.5, and 5 m*M* dithiothreitol. The slurry is poured into 20 volumes of −20° acetone and stirred for 20 min at −20°. The acetone is decanted off, fresh acetone added, and the process repeated until the acetone is almost clear. The plastids are then extracted in an analogous fashion with diethyl ether. After extraction plastids are collected on Whatman #1 filter paper and washed with cold diethyl ether. The plastids are placed under vacuum over silica gel overnight, then collected and stored at −20°.

Extraction and Partial Purification of Enzymes of Tomato Fruits

Phytoene Synthetase. Plastids are extracted as described previously.[1a,3] Plastids, usually about 5 g, are extracted in buffer (0.1 *M* potassium phosphate, pH 7.0, 1% Tween 80, 2 m*M* dithiothreitol) at a ratio of 1 g of plastids to 20 ml of buffer. Homogenization is carried out in a tissue homogenizer with a Teflon pestle. The resulting crude extract is centrifuged at 12,000 *g* for 20 min and the resulting supernatant is fractionated with ammonium sulfate. The fraction precipitating at 20 to 60% saturation is collected by centrifugation as described above. The precipitate is dissolved in a minimum volume of 0.1 *M* potassium phosphate buffer, pH 7.0, containing 30% glycerol and 2 m*M* dithiothreitol. The crude enzyme fraction is applied to a column of BioGel A 1.5m (5 × 75 cm), equilibrated in 100 m*M* potassium phosphate, pH 7.0, 2 m*M* dithiothreitol and 30% glycerol. Isomerase, prenyltransferase, and phytoene synthetase activities coelute from this column. Fractions containing activity are pooled and stored at −20°. This preparation synthesizes phytoene from isopentenyl pyrophosphate.

An alternative procedure involves the preparation of the enzyme solution as described for isomerase and prenyltransferase. The majority of the enzyme activity which converts geranylgeranyl pyrophosphate to phytoene is separated from the isomerase and prenyltransferase by ammonium sulfate precipitation between 20 and 40% saturation. The phytoene-synthesizing activity which remains with isomerase and prenyltransferase is eluted from a DEAE-cellulose column by 0.25 *M* potassium phosphate.[6]

Prenyltransferase and Isomerase. This procedure is a modification of a previously published method.[5] Plastids are extracted as described for phytoene synthetase and the protein precipitating between 40 and 80% of saturation with ammonium sulfate is taken. The proteins are resuspended in a minimum volume of 0.1 *M* potassium phosphate buffer, pH 7.0, containing 30% glycerol and 2 m*M* dithiothreitol. The crude enzyme fraction is applied to a Sephadex G-25 column (2.5 × 37 cm) which has been equilibrated with 20 m*M* potassium phosphate, pH 7.0, containing 2 m*M* dithiothreitol. The fractions containing protein are collected and concentrated to less than 20 ml on a PM-10 membrane. The enzyme solution is made 30% with respect to glycerol and then stored at −20°. This is the last stage in which [1-^{14}C]isopentenyl pyrophosphate can be used as substrate for a phytoene synthetase assay without the addition of isomerase and prenyltransferase. The enzyme solution is loaded onto a DEAE-cellulose column (4 × 10 cm) which has been equilibrated with 20 m*M* potassium phosphate, pH 7.0, containing 2 m*M* dithiothreitol and 30% glycerol. The column is washed with the same buffer system containing 50 m*M* potassium phosphate, pH 7.0, then the same buffer system containing 100 m*M* potassium phosphate, pH 7.0. At this buffer concentration isomerase and prenyltransferase coelute.

The fractions containing isomerase and prenyltransferase activity are pooled and concentrated to about 2 to 4 ml on a PM-10 membrane. The enzyme mixture is loaded onto a Sephadex G-100 column (2.6 × 86 cm), equilibrated with 0.1 *M* potassium phosphate buffer, pH 7.0, and 2 m*M* dithiothreitol. The eluate fractions containing isomerase and those containing prenyltransferase activity are separately pooled, concentrated on a PM-10 membrane to about 2 ml, and then made 30% with glycerol. Each fraction is stored at −20° (see the table).

Preliminary results indicate that isomerase may be further purified by passage over hydroxylapatite. A suitable amount of enzyme is loaded onto a Sephadex G-25 column (1 × 45 cm) which has been equilibrated with 10 m*M* potassium phosphate, pH 7.0, 30% glycerol, and 2 m*M* dithiothreitol. The fractions containing protein are pooled and loaded onto a hydroxylapatite column (1.5 × 3 cm) which has been equilibrated with the same buffer. Isomerase elutes in the void volume. Fractions

PURIFICATION OF ISOPENTENYLPYROPHOSPHATE ISOMERASE OF TOMATO CHROMOPLASTS

	Volume (ml)	Protein (mg/ml)	Total protein (mg)	Total isomerase activity (nmol/hr)	Specific activity (nmol/hr/mg)	Yield	Fold purification
Whole homogenate	100.0	25.5	2550	9380	3.7	100	1.0
12,000 *g* Supernatant	82.0	16.6	1361	8315	6.1	89	1.6
40–80% ammonium sulfate precipitation after Sephadex G-25 filtration	31.0	6.4	198	3398	17.1	36	4.6
PM-10 concentrate after DEAE-cellulose chromatography	10.0	2.0	20	1946	96.4	21	26.1
PM-10 concentrate after Sephadex G-100 chromatography	2.8	0.73	2	546	276	6	74.6

containing isomerase activity are pooled, concentrated on a PM-10 membrane, and stored at −20°. Only one or two contaminating proteins are present with isomerase after this procedure is carried out. It should be noted that the G-100 purified isomerase is more stable to storage than the hydroxylapatite-purified enzyme.

Properties of Phytoene Synthetase

Cofactor and Substrate Requirements. Mn^{2+} and Mg^{2+} are required for the synthesis of phytoene from isopentenyl pyrophosphate; however, only Mg^{+} is required for the synthesis of phytoene from geranylgeranyl pyrophosphate.[11] Dithiothreitol is required for maximum phytoene synthetase activity.

Activators and Inhibitors. Dithiothreitol, as noted above, increases activity. In addition, ATP, although not a substrate, markedly stimulates phytoene synthesis.[16] The addition of $NADP^{+}$ stimulates activity up to 3-fold when isopentenyl pyrophosphate is the substrate,[3] but it has little effect when geranylgeranyl pyrophosphate is used.[11] Sulfhydryl reagents

[16] B. Maudinas, M. L. Bucholtz, C. Papastephanou, S. S. Katiyar, A. V. Briedis, and J. W. Porter, *Biochem. Biophys. Res. Commun.* **66,** 430 (1975).

such as *p*-hydroxymercuribenzoate, *N*-ethylmaleimide, and iodoacetamide[1a] markedly inhibit phytoene synthesis.

Substrate Affinity. The apparent K_m for isopentenyl pyrophosphate is 1.0×10^{-5} *M*.[3] Preliminary results indicate that the apparent K_m for geranylgeranyl pyrophosphate is about 2.6×10^{-5} *M*.[6]

Molecular Weight. As determined by gel filtration the phytoene synthetase complex has a molecular weight of about 200,000.[3]

Properties of Isomerase

Cofactor and Substrate Requirements. Only Mn^{2+} and Mg^{2+} are required for the conversion of [1-^{14}C]isopentenyl pyrophosphate to dimethylallyl pyrophosphate.[5] However, dithiothreitol or another thiol reagent is required for maximum enzyme activity.

Optimum pH. The enzyme has an optimum pH of 7.5 in TES buffer.[6]

Activators and Inhibitors. Dithiothreitol increases activity while sulfhydryl reagents such as iodoacetamide, inhibit the enzyme.[5] The enzyme is also inhibited by dimethylallyl and geranyl pyrophosphates and by pyrophosphate, but there appears to be little inhibition by farnesyl pyrophosphate.[5]

Substrate Affinity. The K_m for isopentenyl pyrophosphate is 5.7×10^{-6} *M*.[5]

Molecular Weight. As determined by gel filtration, the molecular weight of this enzyme is 34,000.[5]

Properties of Prenyl Transferase

Cofactor and Substrate Requirements. Mn^{2+} and Mg^{2+} are required for the synthesis of terpenyl pyrophosphates. [1-^{14}C]Isopentenyl pyrophosphate and a "starting" terpenyl pyrophosphate, usually dimethylallyl pyrophosphate, are required. However, geranyl pyrophosphate or farnesyl pyrophosphate may also be used in place of dimethylallyl pyrophosphate.[5]

Products of the Reaction. Analysis of the products of prenyltransferase indicate the production of farnesyl and geranylgeranyl pyrophosphates. Presumably, geranyl pyrophosphate is produced but it is not detected.

Optimum pH. The pH curve is rather broad with an optimum of about 7.0 in potassium phosphate buffer.[5]

Activators and Inhibitors. The enzyme activity is maximal in the presence of Tween 80,[5,6] but in the presence of $NADP^+$ and pyrophosphate, enzyme activity is inhibited.[5]

Substrate Affinity. The K_m for dimethylallyl pyrophosphate is 9.3×10^{-6} *M*, while the apparent K_m for isopentenyl pyrophosphate is 5.7×10^{-6} *M*.[6]

Molecular Weight. The molecular weight of the prenyl transferase, as determined by gel filtration, is 64,000.[5]

Acknowledgment

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[27] Carotene Mutants of *Phycomyces*

By ENRIQUE CERDÁ-OLMEDO

Color of *Phycomyces*

The young mycelia and sporangiophores of the Zygomycete fungus *Phycomyces* owe their yellow color to the accumulation of β-carotene. The same pigment is present throughout the life cycle (Fig. 1) but overshadowed, at times, by greenish, brown, or black melanins and sporopollenins. In fact in 1817 the fungus was misclassified as an alga, *Ulva nitens;* the definitive genus name, *Phycomyces,* meaning "algal fungus," is a reminder of this confusion.

Research has concentrated on the species *Phycomyces blakesleeanus*. Color and carotene content differ appreciably from one wild strain to another. Most of the physiological, biochemical, and genetic work has been carried out with strain NRRL1555, belonging to the (−) mating type. Strains largely isogenic with NRRL1555, but belonging to the (+) mating type, have been constructed by repeated backcrosses.[1] All future work should be concentrated on strains isogenic with NRRL1555 if possible. Otherwise the influence of variations in genetic background must be cautiously kept in mind.

Detection of Carotene Mutants

All available carotene mutants were originally detected by simple visual inspection of agar cultures. White, red, and deep-yellow mutants con-

[1] M. I. Álvarez and A. P. Eslava, *Genetics* **105,** 873 (1983).

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