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PARTIAL PURIFICATION AND PROPERTIES OF PRENYLTRANSFERASE FROM *PISUM SATIVUM**

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Key Word Index—*Pisum sativum*; Leguminosae; pea; prenyltransferase; enzyme purification; farnesyl pyrophosphate.

Abstract—Prenyltransferase (EC 2.5.1.1; assayed as farnesyl pyrophosphate synthetase) was purified 106-fold from an homogenate of 3-day-old seedlings of *Pisum sativum*. Some of the properties of the purified enzyme were determined and these differed in several significant respects from those reported for preparations from other sources, e.g. the apparent MW was 96000 ± 4000 and the preparation could be dissociated into two subunits of MW 45000 ± 3000. The total activity of the extractable enzyme went through a sharp maximum (in the range 1 to 28 days) 3 days after germination. Farnesyl pyrophosphate was formed in cell-free extracts of peas from either isopentenyl pyrophosphate alone, or this together with geranyl pyrophosphate (optimum yields 1.2 and 10% respectively). Use of [1-^{14}C]- and [4-^{14}C]-isopentenyl pyrophosphates as the sole substrates and degradation of the products showed that the crude extracts contained a pool of the biogenetic equivalent of 3,3-dimethylallyl pyrophosphate. No analogous pool of geranyl pyrophosphate could be detected.

INTRODUCTION

Prenyltransferase (EC 2.5.1.1; often designated as GPP† synthetase or FPP-synthetase) plays a fundamental role in terpene metabolism by coupling IPP with either DMAPP or with GPP to form GPP or FPP, respectively (see [1]). Further chain-lengthening of FPP to GGPP was not catalysed by the purified protein in the cases where this point was investigated. The enzyme has been highly (up to 600-fold) purified from liver [2, 3], yeast [4], castor bean [5] and cotton seed [6], and some preparations have been shown to comprise two subunits that exist in interconvertible forms [7, 8]. Detailed mechanistic studies have also been made [9]. However, the MWs and some kinetic properties of these various preparations differ appreciably. Prenyltransferase has also been partially (up to 20-fold) purified from pumpkin [10] and from a micro-organism [11], and cell-free extracts of the activity have been obtained from several higher plants [12–15].

We here record the extraction and partial purification of prenyltransferase from *Pisum sativum* L. cv Meteor (pea; Leguminosae). Others have prepared crude extracts from pea that converted MVA into FPP and other terpenoids, in very low yields [16, 17], or into diterpenes [18].

* Part 28 in the series "Terpene Biosynthesis". Reprints of this paper are not available. For Part 27 see Akhila, A. and Banthorpe, D. V. (1980) *Z. Pflanzenphysiol.* (in press).

† Abbreviations: MVA, mevalonate; IPP, isopentenyl pyrophosphate; DMAPP, 3,3-dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; NPP, neryl pyrophosphate; FPP, 2-*trans*,6-*trans*-farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate.

RESULTS AND DISCUSSION

Cell-free extracts

In our initial experiments the extraction and incubation media developed for obtaining geranylsynthetase from *Tanacetum vulgare* [19] were used. A crude homogenate from pea seeds that had been germinated for 3 days was fractionated into $S_{0.8}$, S_{104} (S_n = supernatant after centrifugation at $n \times 10^3$ g), microsomal, mitochondrial and chloroplast fractions. Practically all (>90%) FPP-synthetase activity (IPP + GPP → FPP; taken as indicative of prenyltransferase) resided in the S_{104} fraction. Under standardized conditions (see Experimental), *ca* 0.46% incorporation of tracer from [4-^{14}C]-IPP occurred and this was high for such a crude cell-free extract from a higher plant (cf. [19]). Combinations of the subcellular fractions did not increase this level of activity: this finding differed from that reported for similar systems from 10-day-old pea seedlings [17, 20].

Extensive screening to improve the media [21] led to the development of a cell-free extract (see Experimental) which gave a S_{104} fraction that incorporated up to 10% (in certain experiments, but generally incorporated *ca* 8%) of tracer from IPP into FPP. In particular, the extraction medium was not improved by addition of PVP which indicates that phenolics were not appreciably present at this early stage of development, and the incubation medium did not require Mn^{2+}, although addition of Mg^{2+} was necessary for maximum activity. By use of these media the following observations were made: (a) Maximum FPP-synthetase activity was extracted from seedlings 3 days after germination (see Fig. 1). Germination and subsequent development in darkness gave the same pattern but with lower activities. Such germination conditions had been claimed to increase the formation of

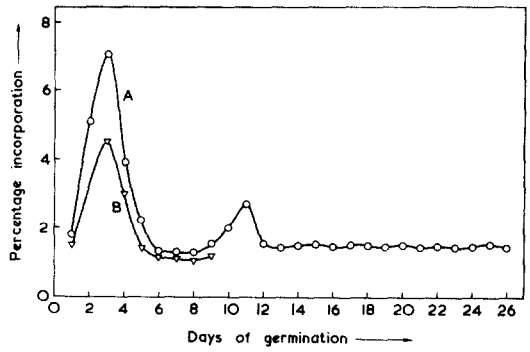

Fig. 1. FPP-synthetase activity during development. Enzyme activity measured as % incorporation of [^{14}C]-IPP under standard conditions for cell-free system (see Experimental). Curves A and B represent profiles for seed germinated and developed in natural light and darkness, respectively.

triterpenoids in peas [20]. (b) Other pea varieties (e.g. cvs Kelvedon Wonder and Gradus) gave the same pattern and percentage incorporations (± 5%). (c) Ungerminated ripe pea seed contained extractable FPP-synthetase at a level *ca* 0.5% that in 3-day-old seedlings. (d) Growth regulators, such as kinetin, GA_3, 2-CEPA, β-ionone and DPA, injected into seed 1 day after germination (at concentrations typically used for studies of growth regulations, cf. [22]) had marginal (± 10%) effects on the level of FPP-synthetase from either 3- or 5-day-old seedlings. In contrast, 2,4-D caused cessation of growth and the activity extracted from 3-day-old seedlings fell to *ca* 10% that of controls. (e) Intermediates of terpene biosynthesis injected into seed as in (d) generally reduced the extractable enzyme levels, viz.: control 100; NPP 82; IPP 14; GPP 35; GPP + IPP 121; FPP 11. The last is noteworthy as a possible example of feedback control.

On analysis of the products produced after incubation of the S_{104} fraction with [^{14}C]-IPP + GPP, *ca* 44% of the radioactivity was recovered in 'lipid' and 'pyrophosphate' fractions. The residue contained water-soluble components that could not be easily extracted: presumably they were the products of epoxidation and hydration processes that have been characterized in other higher plants [23]. The bulk of extracted tracer was unreacted substrate but the following incorporations (%) into products were observed: FPP 8; GPP 1.2; GGPP 0.4; phytyl pyrophosphate 0.3; squalene 10.6. The fraction is thus a useful source of squalene synthetase. No isomerization of FPP to its *cis* isomer was observed; no higher terpenoids except the three listed were found (cf. [18, 19]); and no nerolidol or its pyrophosphate (the allylic isomer of FPP) was detected. The presence of the last compound in cell-free extracts [9] may be caused by non-enzymic isomerization of FPP catalysed by Mn^{2+} added to the medium [24]: this ion was not added to our incubation systems.

The leaves and flowers from some 20 plant species, many of which accumulate oils containing sesquiterpenes, were assayed for FPP-synthetase using the S_{104} fraction of the crude homogenate. Activity levels were uniformly < 1% those in pea except for petals of *Rosa dilecta* cv Lady Seton 82 (pea 100), *R. dilecta* leaves 17 and *Mentha pulegium* leaves 20. Young and mature (6–12 weeks old) plants were screened and these low levels, compared with pea seedlings, may reflect developmental differences or the presence of phenolics that denature enzymes during the process of extraction. We hope to repeat this work with 1- to 28-day-old seedlings of the *Rosa* and other species.

Metabolic pools

Incubation of the S_{104} fraction with [^{14}C]-IPP as the sole substrate resulted in FPP being formed in yields *ca* 12% those obtained from (IPP + GPP) under the same conditions. This could be the result of the presence of IPP-

Table 1. Labelling patterns in FPP

Precursor*	Degradation† products	Distribution of tracer (%)‡ Uniform	C-5 pool	C-10 pool	Observed§
[1-^{14}C]-IPP	Me_2CO	0	0	0	2
	Levulinic acid	67	50	0	46
	$(COOH)_2$	33	50	100	47
[4-^{14}C]-IPP	Me_2CO	33	0	0	2
	Levulinic acid	66	100	100	95
	$(COOH)_2$	0	0	0	0

* Precursor incubated with S_{104} fraction under conditions specified in Experimental.

† FPP was converted into farnesal and this was ozonized to yield Me_2CO, levulinic acid and $(COOH)_2$ (see Fig. 2).

‡ Percentage of tracer in degradation products. 'Uniform' are the calculated proportions if all three C-5 units are derived from the labelled precursor; 'C-5 pool' are those if two IPP units are incorporated, and 'C-10 pool' refers to incorporation of one IPP unit.

§ Observed values are ± 2%. All values are independent determinations. Specific radioactivities in degradation products were 2×10^3–3×10^4 dpm/mmol.

isomerase (EC 5.3.3.2) which provides the 'starter' DMAPP unit and/or the existence in the extract of a soluble metabolic pool of the biogenetic equivalent of DMAPP. Such a pool, probably protein-bonded, has been inferred to be present in cell-free extracts from *T. vulgare* and other plant species [25]. Addition to the incubations of iodoacetic acid, iodoacetamide, or the drugs SKF-525A and SKF-3301A (0.1–5 mM), all potent inhibitors of the isomerase from liver [26], inhibited *ca* 60% of the incorporations of IPP. The consequent inference that a metabolic pool of DMAPP or its biogenetic equivalent was present, and also the interesting additional possibility that a similar pool for GPP existed, was investigated using both [1-^{14}C]- and [4-^{14}C]-IPP. FPP, biosynthesized from these substrates under conditions where the isomerase was inhibited, was cleaved to farnesol, and this after dilution with carrier was oxidized to farnesal, and then ozonized and worked up under oxidizing conditions to yield acetone, levulinic acid (2-oxo-pentanoic acid) and oxalic acid. The distribution of tracer between these cleavage products (for which a good isotope balance was achieved) could be compared with that expected if DMAPP or GPP pools (or their biogenetic equivalents) were present (Table 1). The conclusion is clear: the former, but not the latter, pool occurs and the pool size can be calculated to be *ca* 10 nmol/mg protein in the extract.

Purification of FPP-synthetase

The enzyme was prepared from a batch (3 kg) of 3-day-old seedlings by the procedure outlined in Table 2. The purification was limited by the necessity of maintaining pH in the range 5.0–8.5: outside this, the enzyme activity was rapidly lost (*ca* 60% in 1 hr). The final product (106-fold purification from S_{104} fraction; 0.29% yield) was best stored freeze-dried at −20°: under these conditions *ca* 70% activity was retained after 14 days. Storage in the presence of glycerol or pyrophosphate ion (cf. [3]) had no effect on the stability. The purified product exhibited a specific activity of 150 nkat/mg for IPP with GPP as co-substrate at the pH optimum and under the conditions for incubation described in the Experimental. This compares very favourably with the activities recorded for highly-purified prenyltransferase from other sources: thus, the enzyme from yeast has an activity of *ca* 80 nkat/mg at its optimum [4]. These previous studies have yielded purification factors of 300- to 600-fold, i.e. values 3- to 6-fold greater than achieved in the present work. However, it is worth noting that our starting material was chosen to contain maximum enzymic activity (cf. Fig. 1): if 4- to 6-day-old, or older, seedlings had been selected, a considerably greater purification factor would have been probably achieved, for the amount of total protein per seedling (w/w) did not vary greatly over the period 3–6 days after germination.

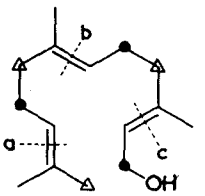

Fig. 2. Labelling and degradation of farnesol. ●, represents label from incorporation of [1-^{14}C]-IPP; △, represents label from incorporation of [4-^{14}C]-IPP. Cleavage at (a), (b), and (c) yields Me_2CO, levulinic acid and the latter together with hydroxyacetic acid, respectively.

The enzymically-active band from the final (DEAE-52) column of the purification procedure had a constant (± 10%) specific activity (units/mg) across the band. The protein in the band was eluted from a calibrated Sephadex G-200 column essentially as a single species (>95% of total) with MW 96000 ± 4000. This compares with values of 60000–86000 for the analogous enzymes from liver, yeast, and bean [2–5]. SDS-polyacrylamide gel electrophoresis gave a MW of 45000 ± 3000, and no other protein was unambiguously detectable by the staining method used (i.e. >93% was protein of this MW). However, starch-gel electrophoresis at pH 6.9, 7.5 and 8.5 revealed two main components (comprising >95% of the protein) and disc electrophoresis on polyacrylamide at pH 7.5 indicated four bands in the same total percentage. Attempts to correlate enzyme activity with the bands from electrophoresis failed because only *ca* 30% of the applied activity could be recovered from the gels and that recovered was not localized in the bands, but was smeared. These findings suggest that extensive denaturation occurred: indeed this has been reported for prenyltransferase from castor bean under similar conditions [5]. The homogeneity of our preparation is thus uncertain. We suggest that the levels of activity (see before), the results from the DEAE-52 and Sephadex G-200 columns, and from SDS-gel electrophoresis suggest that it is at least 90% pure (probably better) and that the electrophoretic data imply that complexes occur between different subunits that have similar molecular weights. Evidence has been previously presented to suggest that liver prenyltransferase exists in two similar but non-identical subunits that could not be separated by SDS-electrophoresis [3].

Table 2. Purification of prenyltransferase

Fraction	Protein (mg)	Total Act. (10^4 × units*)	Spec. Act. (Units/mg)	Purif. (*n*-fold)	Yield (%)
S_{104}	2344	27.3	117	1	100
$(NH_4)_2SO_4$ fraction	1905	11.5	606	5	81
ex CM-52	27.0	15.3	5680	48	1
ex Sephadex G-200	8.20	10.0	12200	104	0.3
ex DEAE-52	7.01	8.69	12400	106	0.29

* The practical unit of activity was defined as the amount of enzyme catalysing the incorporation of 100 dmp of [^{14}C]-IPP into FPP per min under the conditions described in the Experimental.

The purified protein catalysed (under optimum conditions) the reactions of IPP with GPP, DMAPP and NPP in the ratio 100:88:0.8. No activities of GGPP-synthetase, phytyl pyrophosphate-synthetase, squalene synthetase, farnesol- or farnesyl-pyrophosphate isomerase (2-*trans*-FPP → 2-*cis*-FPP) or of MVA-kinase were detected, but IPP-isomerase was present at *ca* 3% level of FPP-synthetase. Pyrophosphatase activity (1.5% of maximum FPP-synthetase) was detected: such activity has been reported to be an integral property of highly-purified prenyltransferase [27]. However, in our experiments pyrophosphate ion had no activation effect and NaF (1–30 nM; a known inhibitor of phosphatase) reduced the phosphatase level by 60%: these findings differed from those previously found [27].

Our preparation had a sharp pH optimum at 7.6; half-maximum at 6.3 and 8.2. K_m(IPP) was 14 μM (measured when GPP was in large excess). Values of from 0.5 to 50 μM have been recorded for prenyltransferases from other sources, and that from bean was claimed to vary 10-fold over a 4-fold increase in substrate concentration—allegedly owing to reversible interactions between two forms of the enzyme [5]. Such variations were not observed in our experiments. Addition of reduced glutathione or cysteine hydrochloride (0.1–20 nM) had no effect on the activity of our enzyme. Iodoacetic acid (1 nM) and β-hydroxymercuribenzoate (1 mM) caused 5 and 11% inhibition, respectively. ATP (3 mM) and Mg^{2+} (6 mM) were found (by screening over the range 1–100 mM) to be required for optimal activity.

In summary, prenyltransferase from pea differs considerably in its properties from the enzyme isolated from bean and from non-plant sources; at present we are carrying out studies on the enzyme from various species of bean.

EXPERIMENTAL

Materials. All products had satisfactory IR, MS ^{1}H NMR and elemental analyses (cf. [21]). [4-^{14}C]-IPP (17 mCi/mmol) was obtained (60% from *R*-MVA) from *RS*-[2-^{14}C]-MVA using an enzyme fraction from latex of *Hevea brasiliensis* [28]. [1-^{14}C]-IPP (20%; 12 mCi/mmol) was prepared by carbonation with $^{14}CO_2$ of the Grignard reagent prepared from 1-bromo-2-methylprop-2-ene followed by reduction ($LiAlH_4$) and pyrophosphorylation [29]. [^{14}C]-FPP (50%; 20 mCi/mmol) was prepared from [2-^{14}C] by means of enzymes from liver [30]. The purified compounds (>98% by TLC on several systems) were stored on Whatman No. 7 paper at −20° under desiccation.

IPP, DMAPP, GPP, NPP and FPP were obtained by standard procedures from the corresponding alcohols [31–33]. The crude products were separated by PC on Whatman 3MM with elution by downward-displacement with *iso*-PrOH–NH_3(0.88)–H_2O (8:1:1) for 72 hr and were detected by an acid molybdate spray; R_f (approx.); monophosphates 0.8, pyrophosphates 0.35, triphosphates 0. The pyrophosphates were cut out and eluted by downward displacement with aq. NH_4OH (1%). The eluate (*ca* 130 ml) was concd (1 ml) and successively subjected to PC on Whatman No. 1 with (a) *iso*PrOH–NH_3(0.88)–H_2O (6:3:1) and (b) *iso*-PrOH–*s*-BuOH–NH_3(0.88)–H_2O (40:20:1:39) to give products (5.20% overall yield) that were >98% pure (TLC on various systems: cleavage to alcohols and GLC; assay for PPi). Organic pyrophosphates were determined spectrophotometrically [34].

Geraniol was purified from commercially available (ex BBA Ltd., London) material via its complex with $CaCl_2$ [35]. Commercial farnesol (BDH Ltd., London) was fractionated into the 2-*trans*,6-*trans*; 2-*cis*,6-*trans* + 2-*trans*,6-*cis*; and 2-*cis*,6-*cis* isomers (95:3:2) by GLC on FFAP (10% on Cellosorb W; 2.1 m × 0.5 cm; 220°, N_2 3.6 l./hr) or TLC on Si gel F_{254} with C_6H_6–EtOAc (85:15); R_f 0.35, 0.50, 0.65; or Si gel H $AgNO_3$ (5:1) with EtOAc; R_f 0.47, 0.58, 0.60; (cf. [36]). Both TLC separations were at 2° and the separated isomers were identified by IR and ^{1}H NMR [37]. The predominant (and biosynthetically significant) 2-*trans*,6-*trans* isomer formed a diphenylurethane, mp 63° (ex EtOH). *cis*-Nerolidol was obtained by fractionation of Cabreuva oil (ex BBA Ltd. London) using a Büchi spinning-band column (1 m): 66%, 167.5–167.9°/20 mm Hg (>99.9% via GLC on FFAP or Carbowax 20M).

Pea seeds were purchased from local nurseries, and were cultivated on water-saturated vermiculite in a greenhouse at 25° under natural illumination (period: March–September).

Cell-free systems and assays. The preparation of the cell-free extracts was performed at 4°, and the appropriate boiled enzyme controls were included in all the assays. Seed coats were removed from the plants (10 g) which were homogenized (20 sec; full speed) in a Waring–Bendix blender in Pi buffer [150 ml, pH 7.5, 0.1 M; containing 2-mercaptoethanol (1 mM)]. The homogenate was centrifuged (800 g; 0.5 hr) and the $S_{0\text{-}8}$ fraction (S_n = supernatant from centrifugation at $10^3 \times n$ g) was further centrifuged to give S_8, S_{10}, S_{45} and S_{105} fractions. The corresponding ppts were subjected to centrifugation in a discontinuous sucrose gradient [38] to obtain subcellular fractions which were characterized as rich in microsomes, mitochondria, and chloroplasts by assay for the marker enzymes catalase, succinic dehydrogenase and triosephosphate isomerase, respectively [39–41]. Protein was estimated by a modified Lowry method [42]: typically, biosynthetically active fractions contained 5–8 mg/ml. Fractions (1 ml) were incubated with IPP (86 mM; 10^5 dpm) and GPP (200 mM) in Pi buffer (1 ml; pH 7.5; 0.1 M) containing ATP (2 mM), $MgCl_2$ (5 mM) and nicotinamide (20 mM) at 30°. The plateau for % incorporation into products was achieved within 2.5 hr. At the completion of the incubation, EtOH (3 ml) was added and the soln was boiled (5 min), the precipitated protein removed and washed with aq. EtOH (20:80), and the washings combined with the supernatant. $CHCl_3$–MeOH (1:1) was also an effective quenching agent. The pH of the soln was then adjusted to >10 (aq. NH_4OH) and the EtOH removed at 40° under reduced pressure.

Routine product analyses were made for FPP. Little farnesol (<10% FPP) was formed: presumably very little endogenous phosphatase was present. All analyses were carried out in triplicate (s.e. ±3%). The pyrophosphates in the incubation medium were extracted with *n*-BuOH (2 × 1 ml) after EDTA (1 M; 50 μl) had been added [43], and the conc extract was separated on three systems in succession: (a) TLC on Si gel H with *iso*-PrOH–NH_3(0.88)–H_2O (6:3:1), (b) PC on Whatman No. 1 with the same solvent; and (c) PC on Whatman No. 1 with *iso*-PrOH–*s*-BuOH–NH_3(0.88)–H_2O (40:20:1:39). The papers were washed before use according to standard recipes [44]. FPP had R_f 0.30, 0.48 and 0.86 in these systems and the spots were located by radiochromatogram-scanning or by a molybdate spray [45]. These procedures effected a complete separation of FPP (2-*trans*,6-*trans* isomer) from the other FPP isomers and from nerolidol: recoveries of 95% of FPP were achieved at each step. This routine method of assay was checked in several cases by addition of carrier farnesol to the incubation medium after FPP had been hydrolysed, and isolation of the alcohol as its dephenylurethane, mp 63°, and recrystallization of this to constant specific radioactivity. Values obtained by the routine analysis were *ca* 95% of those obtained by the more rigorous method. In a few examples a more complete product analysis was

carried out; the aq. residue from the extraction with *n*-BuOH was extracted with petrol (bp 40–60°) and this extract was subject to TLC as described for the previous assay, followed by GLC on FFAP. Prior to these separations, isopentenol, 3,3-dimethylallylalcohol, geraniol, nerol, farnesol, phytol and squalene were added (50 mg) as carrier. Finally, the GLC cuts were separated by TLC on Si gel H with (a) petrol (bp 40–60°)–liq. paraffin (19:1) and (b) MeOH–H_2O (3:2) saturated with liq. paraffin. The residue from the extraction with petrol was incubated with apyrase (EC 3.6.1.5) and alkali phosphatase (phosphomonoesterase ex *E. coli*; EC 3.1.3.1) in $KHCO_3$ buffer (0.1 M; pH 10.4) with $MgCl_2$ (10 mM) for 3 hr at 37°. After denaturation and removal of the protein, the resulting alcohols were assayed by TLC and GLC as described above. Geranylgeraniol was not obtainable as a standard, and its presence was inferred from published R_f values [17, 20].

Studies with additives. Growth inhibitors, potential activators of enzyme levels, etc. were injected (Hamilton syringe; 10 μl Pi buffer pH 7.5 containing 10 μg of additive) into 1-day-old seedlings. After a further 48 hr, the seedlings were assayed for FPP-synthetase. C-5 or C-10 terpenoids, when used as additives, were removed by passage through a column of Sephadex G-25 before preparation of the cell-free extracts.

Degradation of farnesol. Farnesol (500 mg) was added as carrier to a large scale (100 ml) cell-free extract that had been incubated under the usual conditions. After hydrolysis of the biosynthetically formed pyrophosphate, and recovery of the alcohol, this was oxidized to the aldehyde by MnO_2, and the latter cleaved to (Me_2CO, levulinic acid, and $(COOH)_2$ by ozonolysis and these products were purified by the methods used for analogous degradations of geraniol [46]. Yields (%) were: levulinic acid 55; Me_2CO 80; $(COOH)_2$ 10.

Radiochemical methods. Generally, butyl-PBD (0.8%) in toluene was used as scintillant. This had a counting efficiency of *ca* 90% for ^{14}C (as calibrated by an external standard using the channels-ratio method) with a background of 40–70 dpm, and a shelf-life of 4 months, if stored in the dark. Aq. solutions were assayed using butyl-PBD (0.8%) in toluene–MeOH (1:1) (efficiency *ca* 85%) or Bray's solution [47] (efficiency, 90%). Typically, aliquots containing 1×10^3–5×10^3 dpm were assayed, and 40000 disintegrations were accumulated to ensure that 2σ was ±1%. Radiochromatograph scanning was made with a Tracerlab 525B scanner (2π for TLC; 4π for PC).

Purification of enzyme. All operations were carried out at 2°. DEAE-columns were stored with buffer containing toluene (0.03%) and Sephadex columns similarly had NaN_3 (0.02%) added to prevent growth of micro-organisms. Solns (< 200 ml) were concd by forced dialysis (rate *ca* 14 ml/hr): larger volumes were subjected to ultrafiltration in a Amicon 402 cell with a Diaflo membrane (80 ml/hr). 3-Day-old seedlings (3 kg; processed in 0.4 kg batches) were homogenized and the S_{45} supernatant (45000 g; 2.5 hr) was subjected to $(NH_4)_2SO_4$ fractionation, the pH being maintained near pH 7.5 by addition of aq. NH_4OH. The ppt. obtained between 30 and 80% saturation was collected (20000 g; 0.5 hr), dissolved in the minimum Pi buffer (pH 7.5; 10 mM) containing 2-mercaptoethanol (1 mM), dialysed against the buffer (24 hr) and concd (to 30 ml) by ultrafiltration. This concentrate was applied to a CM-cellulose CM-52 column (50 × 5 cm) that had been equilibrated with the dialysis buffer. The column was eluted with a NaCl gradient (50 mM, 0.5 l.; 150 mM, 1.5 l.; 200 mM, 2.0 l.; all in Pi buffer, pH 7.5, 50 mM) at 130 ml/hr and aliquots (10 ml) were collected. Prenyltransferase activity largely (72% of total) occurred in fractions 80–132 and these were pooled and concd (to 30 ml). This fraction was applied to a Sephadex G-200 column (40 × 2.5 cm) that had been equilibrated with, and was subsequently eluted by Pi buffer (pH 7.5, 50 mM) at 10 ml/hr. Aliquots (5 ml) were collected and the activity largely occurred in fractions 27–36. Again, these were pooled and concd, and applied to a column of DEAE-cellulose DE-52 (90 × 1.5 cm) that had been quilibrated with, and was eluted by, Pi buffer (pH 7.5, 10 mM; containing 2-mercaptoethanol (1 mM) at 40 ml/hr. Aliquots (10 ml) were collected and after an initial elution (50 ml) a NaCl gradient (1.5 M in Pi buffer) was applied. Fractions 7–42 contained the prenyltransferase activity and the traces relating quantity of protein (UV absorption) and enzymic activity were practically superimposable, i.e. the specific activity of the protein was constant ±10% across the eluted band.

Kinetic and other properties. The pH optimum, curves relating enzyme activity to $[Mg^{2+}]$, K_m, etc. were determined with IPP 860 μM; i.e. *ca* 75 K_m to ensure saturation of enzyme. Buffers used were succinate pH 4.2–6.0; MES 6.0–7.5; maleate 5.0–7.0; Pi 6.5–7.5; Tris–HCl 7.5–8.5.

Electrophoretic methods. Starch-gel electrophoresis was carried out by standard techniques [48] on protein solns concd (to 3 mg/ml) by forced dialysis. Gels were run at pH 6.9, 7.5 and 8.5 in borate buffers, cut in two, and one half stained (nigrosine, 0.5% in aq. HOAc) and the other further sectioned and assayed for enzyme. Disc-gel electrophoresis on polyacrylamide [49, 50] was carried out on protein solns (2 mg/ml in 20% aq. sucrose) at pH 7.5. The gels were cut as above and stained (naphthidine black 10B in 1% HOAc) and assayed. SDS-gel electrophoresis [51] was conventional: bromophenol blue was marker and bovine serum albumin (MW 69000), ovalbumin (41000) and myoglobin (17000) were standards. MW was determined on a calibrated Sephadex G-200 column (65 × 2.5 cm) that had been equilibrated with Pi buffer (pH 7.8, 10 mM) [52]. Protein concn was *ca* 20 mg/ml and standards were catalase (ex beef liver; MW 232000); lactic dehydrogenase (ex heart; 140000); peroxidase (ex horse radish; 43000), bovine serum albumin (69000) and alcohol dehydrogenase (ex yeast; 150000).

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