COMPARTMENTATION OF ISOPENTENYL PYROPHOSPHATE ISOMERASE AND PRENYL TRANSFERASE IN DEVELOPING CASTOR BEAN ENDOSPERM[1]

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Summary: Isopentenyl pyrophosphate isomerase and prenyl transferase are present in the proplastid and mitochondrial fractions of developing castor bean endosperm. Three forms of prenyl transferase have also been separated in extracts of germinating seeds. One of these enzymes, farnesyl transferase, is present in the proplastid. The precise subcellular locations of the other two, geranyl transferases I and II, have not yet been determined. These results are consistent with the proposal that enzyme segregation plays an important role in governing the flow of carbon in isoprenoid pathways.

Goodwin and Mercer proposed that enzyme segregation between the chloroplast and cytosol is a factor in regulation of isoprenoid metabolism in higher plants (1). Experiments on whole tissue (2-4) and on crude chloroplast fractions (5-9) support this hypothesis. In yeast, Momose and Rudney demonstrated the presence of IPP[3] isomerase and prenyl transferase in mitochondria (10). MVA kinase is present in chloroplasts (5) but little evidence has been presented for other enzymes of isoprenoid biosynthesis in specific subcellular organelles. In this study the presence of multiple forms of prenyl transferase has been demonstrated in germinating castor bean (Ricinus communis L.) seedlings, and the localization of IPP isomerase and prenyl transferase activities in subcellular organelle fractions derived from developing castor bean endosperm is examined.

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[3] Abbreviations: MVA, mevalonic acid; IPP, isopentenyl pyrophosphate; DMAPP, dimethyallyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; SDH, succinic dehydrogenase; TPI, triose phosphate isomerase.

Materials and Methods:

[^{14}C]IPP was prepared and isolated from yeast autolysates using (R,S)-[2-^{14}C]MVA and ATP as described by Green and Baisted (11). GPP was obtained from Dr. George Popjak, Department of Biological Chemistry, UCLA, and geranylgeraniol was a gift of Dr. Walter Adolf, Department of Chemistry, UCLA. Other biochemicals and enzymes were purchased from the Sigma Chemical Company. Pure trans, trans-farnesol was isolated from commercial farnesol by spinning band vacuum distillation under 4 mm Hg at 190° and FPP was prepared and isolated from this as described by Popjak (12).

Whole tissue homogenates used in the isolation of prenyl transferase enzyme activity were prepared as described by Green and West (13), except the homogenizing buffer was 50 mM potassium phosphate in 10 mM potassium pyrophosphate, pH 6.3. The homogenate was centrifuged for 10 min at 27,000 x g and the supernatant fractionated by ammonium sulfate precipitation. Prenyl transferase activity precipated between 40 and 60% saturated ammonium sulfate. The pellet was resuspended in 10 mM Tris-maleate, 10 mM potassium pyrophosphate, 100 mM KCl, 25% glycerol, 0.02% NaN_3 buffer, pH 6.3, and then passed through a 5 x 100 cm G-100 Sephadex column equilibrated in the same buffer. Prenyl transferase was further purified by sievorptive chromatography (14) on a 5 x 50 cm DEAE A-25 Sephadex column equilibrated with 10 mM piperazine-HCl, 25% glycerol, 5 mM 2-mercaptoethanol, pH 6.0. An intrinsic gradient was generated with 0.5 M $(NH_4)_2SO_4$.

Developing castor bean seeds were harvested from plants growing in the Botanical Garden at UCLA. Endosperm tissue (8 g) was homogenized in 12 ml of extraction medium as previously described (15). The 5,000 g pellet was taken up in 2 ml of extraction medium, centrifuged at 500 x g for 2 min, layered on a discontinuous sucrose gradient and centrifuged as described by Reid et al. (16). The gradients were collected dropwise from the bottom in approximately 1.2 ml fractions.

Succinic dehydrogenase was measured from the reduction of 2,6-dichlorophenol-indophenol (DCIP) as described by Reid et al. (16) except 0.3 mM DCIP was used. The assay for triose phosphate isomerase contained 0.23 mM R,S-glyceraldehyde-3-phosphate, 0.1 mM NADH, 7 units of α-glycerophosphate dehydrogenase, and 50 mM triethanolamine buffer, pH 7.9 in a total volume of 1.0 ml. Enzyme activity was assayed by measuring the decrease in absorbance at 340 nM.

IPP isomerase was measured from the conversion of [^{14}C]IPP into acid-labile extractable [^{14}C]products. A typical assay was carried out in a final volume of 0.6 ml in 30 mM potassium phosphate, 25% glycerol, 3 mM $MgCl_2$, 0.1% Triton X-100, and 2.9 μM [4-^{14}C]IPP (7.15 μCi/ M). The reaction was terminated by the addition of 2-3 ml benzene followed by rapid mixing and removal of the benzene layer. Residual [^{14}C]acid-labile products in the reaction mixtures, and geranyl and farnesyl transferase activities, were all assayed by previously described methods (13); routine assays of farnesyl transferase employed FPP in place of GPP, 25% glycerol was included in the assay and the buffer was adjusted to pH 6.3.

[^{14}C]Farnesol and [^{14}C]geranylgeraniol were identified by co-chromatography with authentic samples of farnesol and geranylgeraniol by reverse phase chromatography (17).

Results and Discussion:

Two forms of prenyl transferase, geranyl transferases I and II, which catalyze the elongation of DMAPP to FPP by two steps of addition of IPP, are present in germinating castor bean seed (13). A farnesyl transferase activity

which catalyzes the reaction of FPP and IPP to form GGPP has also been identified from this source. The latter activity also catalyzes the elongation of DMAPP and GPP to GGPP. It can be separated from geranyl transferases I and II by sievorptive chromatography (Fig. 1). The purification and characterization of farnesyl transferase will be presented elsewhere (18).

The presence of IPP isomerase in the proplastid and mitochondrial fractions of castor bean endosperm is shown in Fig. 2. Triose phosphate isomerase (TPI) (16) and succinic dehydrogenase (SDH) were used as marker enzymes for proplastids and mitochondria, respectively. The absence of SDH activity in tubes 7 and 8 indicates that the main proplastid fraction is free of mitochondria. IPP isomerase is therefore a component of the proplastid. Tubes 19 and 20, which correspond to the mitochondrial fraction, have TPI activity and are therefore contaminated by proplastids. However, the ratio of IPP isomerase to TPI

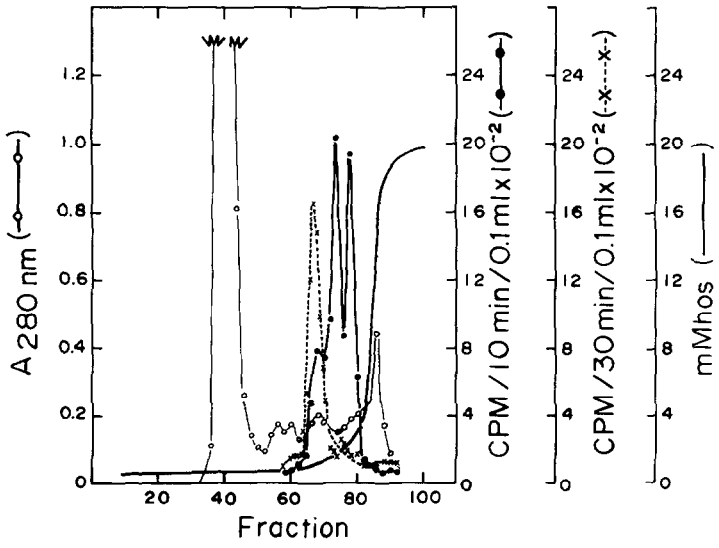

Figure 1. Sievorptive chromatography of G-100 Sephadex purified prenyl transferases on DEAE A-25 Sephadex in 10 mM piperazine-HCl, 25% glycerol, 5 mM 2-mercaptoethanol, pH 6.0. Farnesyl (-x---x-) and geranyl (-●-●-) transferase enzyme activities were assayed as described in the text. The intrinsic gradient (—) was generated with 0.5 M $(NH_4)_2SO_4$.

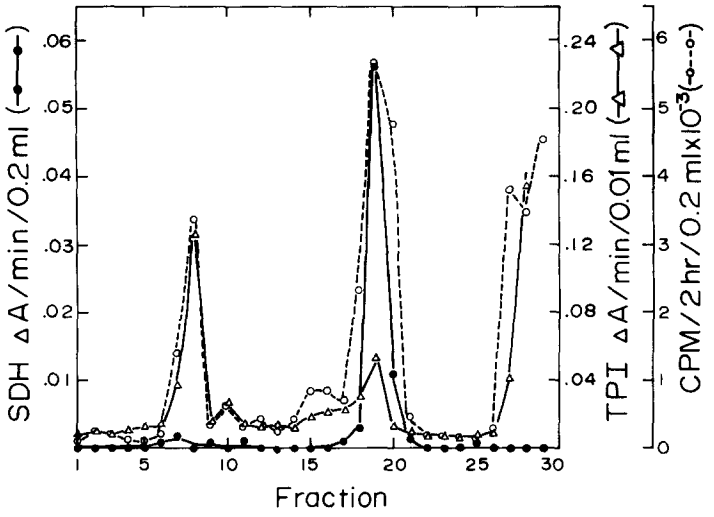

Figure 2. Distribution of SDH (-●-●-), TPI (-Δ-Δ-) and IPP isomerase (-o--o-) on a discontinuous sucrose gradient. Fraction 1 constitutes the bottom and fraction 30 the top regions of the gradient.

activity is four-fold greater in tubes 19 and 20 than tubes 7 and 8 suggesting that the mitochondria themselves contain IPP isomerase. IPP isomerase in the soluble fraction (tubes 27-30) is a mixture of enzymes from broken organelles and cytosolic enzyme carried over from the unwashed pellet.

The presence of prenyl transferase activity within the proplastid and mitochondrial fractions was determined by showing that [^{14}C]farnesol and [^{14}C]-geranylgeraniol were formed from [^{14}C]IPP (Table I). Since [^{14}C]geranylgeraniol is produced in the proplastid, farnesyl transferase must be present in this organelle. The farnesyl transferase activity in the mitochondrial fraction may result from proplastid contamination. Since this enzyme also exhibits geranyl transferase activity, it is difficult to determine if geranyl transferases I and II are in these organelles.

The possibility of IPP crossing the proplastid membrane was determined by incubations with intact and broken proplastids. The same activity of IPP isomerase was found in the proplastid fraction whether it was assayed directly

or after prior lysis by sonication or the addition of 0.1% Triton X-100. IPP isomerase activity measured directly in the mitochondrial fraction was less (ranging from 30 to 100%) than that in the presence of Triton X-100. The mitochondrial membrane is, therefore, probably less permeable to IPP than the proplastid membrane. The degree of permeability to IPP of the mitochondria and proplastids may be another parameter in the regulation of the isoprenoid pathway. The importance of this observation cannot be evaluated until pure fractions of mitochondria and proplastids have been isolated and the intactness of these organelles determined.

The demonstration in proplastids of IPP isomerase and farnesyl transferase indicates that the isoprenoid pathway is present in this organelle. MVA kinase is present in a related organelle, the chloroplast (5). Acetyl-CoA for iso-

TABLE I

Reverse Phase TLC Analysis of Isoprenoid Products Derived from [4-^{14}C]IPP*

	Exp. 1	Exp. 2	Exp. 3
Activity	Proplastid Fraction	Proplastid Fraction	Mitochondrion Fraction
SDH (Δ O.D./min/200 µl)	.006	0	.085
TPI (Δ O.D./min/10 µl)	.037	.128	.046
farnesol synthesis	183 CPM	115 CPM	169 CPM
geranylgeraniol synthesis	522 CPM	584 CPM	688 CPM

* 0.2 ml enzyme aliquots of each fraction were mixed with [^{14}C]IPP (21,000 CPM) and phosphate buffer, pH 6.3, made up in 0.1% Triton X-100 in a total volume of 0.6 ml and allowed to stand overnight at room temperature. [^{14}C]Farnesol and [^{14}C]geranylgeraniol were identified in acid hydrolysates of each sample by reverse phase TLC as described in Methods.

prenoid biosynthesis in proplastids may be supplied by pyruvate dehydrogenase (16) and there is also evidence for the glycolytic pathway (19).

Since a mitochondrial fraction free of other organelles was not obtained it is not possible to unambiuously determine if this organelle has the capacity for isoprenoid biosynthesis. The permeability studies with IPP and the specific activity results would indicate that the mitochondrion contains IPP isomerase and possibly at least one of the prenyl transferases (Table I). It would be logical to propose that the mitochondrion has one geranyl transferase, and the other geranyl transferase is in the cytosol, although no evidence to support this hypothesis is yet available.

The separation of isoprenoid biosynthesis in three cell compartments would allow independent regulation. The flux through these pathways may be different and the pool sizes may also vary. Control of permeability of intermediates through the organelle membranes may also be a factor in regulation. The results presented in this report support the concept of Goodwin and Mercer (1) that enzyme segregation and metabolic channelling of carbon is an important regulatory feature of isoprenoid metabolism.

References:

1. Goodwin, T.W., and Mercer, E.I. (1963) Biochem. Soc. Symp., No. 24, 37-41.
2. Threlfall, D.R., and Goodwin, T.W. (1967) Biochem. J. 103, 573-588.
3. Threlfall, D.R., and Griffiths, W.T. (1967) Biochemistry of Chloroplasts, T. W. Goodwin, ed., Vol. 2, 254.
4. Griffiths, W.T., Threlfall, D.R., and Goodwin, T.W. (1968) Europ. J. Biochem. 5, 124-132.
5. Rogers, L.H., Shaw, S.P.J., and Goodwin, T.W. (1966) Biochem. J. 99, 381-388.
6. Charlton, J.M., Treharne, K.J., and Goodwin, T.W. (1967) Biochem. J. 105, 205-212.
7. Shah, D.V., Feldbruegge, D.H., Houser, A.R., and Porter, J.W. (1968) Arch. Biochem. Biophys. 127, 124-131.
8. Stoddart, J.L. (1969) Phytochem. 8, 831-837.
9. Murphy, G.J.P., and Briggs, D.E. (1974) Phytochem. 14, 429-434.
10. Momose, K., and Rudney, H. (1972) J. Biol. Chem. 247, 3930-3940.
11. Green, T.R., and Baisted, D.J. (1972) Biochem. J. 130, 983-995.
12. Popjak, G. (1969) Methods in Enzymology 15, 385-390.

13. Green, T.R., and West, C.A. (1974) Biochem. 13, 4720-4729.
14. Kirkegaard, L.H. (1974) Biochem. 12, 3627-3632.
15. Drennan, C.H., and Canvin, D.T. (1969) Biochem. Biophys. Acta 187, 193-200.
16. Reid, E.E., Lyttle, C.R., Canvin, D.T., and Dennis, D.T. (1975) Biochem. Biophys. Res. Comm. 62, 42-47.
17. Oster, M.O., and West, C.A. (1968) Arch. Biochem. Biophys. 127, 112-123.
18. Green, T.R., and West, C.A., In Preparation.
19. Dennis, D.T., and Green, T.R. (1975) Biochem. Biophys. Res. Comm., See preceding paper.