Prenyltransferase compartmentation in cells of *Vitis vinifera* cultivated in vitro

G. Feron, M. Clastre and C. Ambid

Ecole Nationale Supérieure Agronomique, 145 avenue de Muret, F-31076 Toulouse cédex, France

Received 19 June 1990; revised version received 10 August 1990

Two prenyltransferases were located in cell cultures of *Vitis vinifera*. A geranyl pyrophosphate synthase (EC 2.5.1.1) was associated with plastid-like membranes whereas a farnesyl pyrophosphate synthase (EC 2.5.1.10) was found to be soluble.

Prenyltransferase; Geranyl pyrophosphate synthase; Compartmentation; Monoterpene synthesis; Cell culture

1. INTRODUCTION

Prenyltransferases (dimethylallyl*trans*transferases) are known to catalyse the reaction of isopentenyl pyrophosphate (IPP) with an allylic substrate. They have received some attention as they function at the branch points in the isoprenoid biosynthetic pathway and may thus have a regulatory function [1]. In recent years, studies have focused on their purification and characterization. Interesting observations are now emerging concerning the assignment of distinct biosynthetic sequences to individual subcellular compartments. Present knowledge indicates that plastids, in plants, are the site of geranyl geranyl pyrophosphate synthase [2,3] and apparently of a geranyl pyrophosphate (GPP) synthase [4,5]. At present, no evidence is available to suggest the existence in these organelles of two individual transferases for the C10 and C20 moieties, respectively. Their existence would be important for the functioning and regulation of the monoterpene pathway. In an attempt to investigate several aspects of the biosynthesis of terpenes by *Vitis vinifera* cv Muscat de Frontignan cell suspensions, we now report the partial purification of farnesyl pyrophosphate (FPP) and GPP synthases in this material and their localization within the cells.

2. EXPERIMENTAL

2.1. *Preparation of organelles*

Previously established [6] *Vitis vinifera* cv Muscat de Frontignan cell suspensions were used. Cell culture was initiated by inoculating 60 ml (packed cell volume 40%) of 14-day-old suspension into 200 ml of fresh medium as previously described [6].

Protoplasts, isolated according to the procedure of Bouzayen et al. [7], were resuspended in the extract medium (5×10^6 cells/ml) containing 50 mM tricine-NaOH buffer (pH 7.8), 0.5% (w/v) polyvinylpyrrolidone (PVP), 0.1% (w/v) bovine serum albumin and 350 mM sucrose, and broken through a 10 μm nylon cloth. The resulting homogenate was centrifuged at 400 × *g*, for 5 min to remove nuclei, cell debris and intact protoplasts. Centrifugation of the supernatant at 4000 × *g* for 10 min pelleted most of the plastids. This pellet was carefully resuspended and layered at the top of a sucrose gradient (0.75, 0.9, 1.1, 1.3, 1.5 M in 50 mM tricine-NaOH buffer, pH 7.2) and centrifuged at 100 000 × *g* for 60 min. Each interface was assayed for enzyme activity. The 4000 × *g* supernatant was also centrifuged for 60 min at 100 000 × *g* and the resulting pellet and supernatant were tested for prenyltransferase activity.

2.2. *Enzyme preparation*

Prenyltransferase purification was performed according to the procedure of Dogbo and Camara [3] with the following modifications. Lyophilized cells were frozen with liquid N_2 and ground to a fine powder. The material (5 g) was stirred for 30 min at 4°C in 110 ml of extract medium containing 50 mM tricine-NaOH buffer (pH 7.6), 0.5% (w/v) PVP and 10 mM 2-mercaptoethanol. The mixture was centrifuged for 30 min at 100 000 × *g*. The supernatant was subjected to PEG 6000 precipitation. The pellet of the 20–30% fraction was dissolved in 50 mM Tris-HCl buffer pH 7.6 containing 20% (v/v) glycerol and chromatographed through a 1.6 × 17 cm DEAE-Sephacel column (continuous gradient 0.05–0.18 M NaCl in the same buffer). Fractions (3 ml) were collected and tested for enzyme activities.

2.3. *Enzymic assay*

The reaction mixture contained 100 mM Tris-HCl buffer pH 7.2, [1-^{14}C]IPP (35 μM; 18.5 kBq), dimethylallyl pyrophosphate (DMAPP) (200 μM), mercaptoethanol (10 mM), $MnCl_2$ (2 mM), $MgCl_2$ (15 mM) in a final volume of 610 μl. The reaction was started by the addition of enzyme and, after 1 h of incubation, was stopped by shaking the mixture with 2 × 1.5 ml of diethyl ether. After extraction of oxygenated compounds, 500 μl of alkaline phosphatase solution (2 U) in 100 mM glycine-NaOH buffer (pH 10), were added to the incubation medium to hydrolyse diphosphate compounds. After 4 h of incubation at 36°C, the terpenes released were extracted as described above.

Correspondence address: C. Ambid, Ecole Nationale Supérieure Agronomique, 145 avenue de Muret, F-31076 Toulouse cédex, France

Abbreviations: GPP, geranyl pyrophosphate; PVP, polyvinylpyrrolidone; FPP, farnesyl pyrophosphate; IPP, isopentenyl pyrophosphate

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

Table I

Distribution of specific activities and purification factor of prenyl transferase, UDP galactosyl transferase, SDH and catalase in the 4000 × g pellet and fractions collected at the interfaces of a discontinuous sucrose gradient

Gradient interface		Prenyl transferase		UDPGal. transferase		SDH		Catalase	
Sucrose mol.	prot. (mg)	spec. act.	purif. factor	spec. act.	purif. factor	spec. act.	purif. factor	spec. act.	purif. factor
Pellet 4000 × g without gradient	5.2	0.42	1	0.014	1	683	1	3833	1
0.35/1.1	2.3	-	-	-	-	-	-	-	-
1.1/1.3	0.5	0.83	2	0.023	1.6	-	-	5330	1.4
1.3/1.5	0.4	1.6	3.8	0.050	3.5	500	0.7	3666	1
Gradient pellet	1.85	0.63	1.5	0.022	1.6	1750	2.3	8243	2.1

Enzyme units refer to pkat·mg^{-1} protein.

2.4. *Analysis of compounds*

Extracts were analysed with a gas chromatograph (HP 5700A) equipped with a flame ionization detector and coupled with a proportional counter (Packard model 894). The samples were run through an inox column (2.5 × 0.63 cm) packed with 10% FFAP on chromosorb WHP 80–100 mesh; temperature programme 90–240°C (8°C/mn). Alcohols (isopentenol, dimethylallyl alcohol, geraniol and farnesol) were added as carriers to the different samples. Terpene and radioactive peaks were obtained simultaneously on a two-channel recorder and the radioactivity of each compound was calculated from the corresponding peak area.

2.5. *Marker enzymes*

Succinate dehydrogenase (SDH) (EC 1.3.99.1) and catalase (EC 1.11.1.6) were assayed as described by Singer et al. [8] and Lück [9], respectively. The method described by Douce [10] was used for UDP galactosyl transferase (EC 2.4.1.22). Protein was determined by the BioRad protein reagent.

3. RESULTS

After differential centrifugation, the maximum rate of incorporation of IPP into isoprenoid compounds was obtained with the soluble fraction (100 000 × g supernatant). Part of the enzyme activity was also located in the 4000 × g pellet. After purification of this pellet in the discontinuous sucrose gradient, the highest specific prenyltransferase activity was recovered in the 1.1–1.3 M and 1.3–1.5 M interfaces (Table I). A more accurate examination of these membranes with enzyme markers of mitochondria (succinate dehydrogenase), peroxisomes and glyoxisomes (catalase) and plastids (UDP galactosyl transferase) indicated that they had a plastid origin. Effectively, in spite of contamination by other organelles, the prenyltransferase activity was associated, in the 1.3–1.5 M interface, with membranes which only exhibited an increase in UDP galactosyl transferase activity. Moreover the product of the prenyl reaction in plastids was mainly identified as GPP. In contrast, the soluble fraction showed an incorporation of [1-^{14}C]IPP into farnesol and FPP (Table II). As

Table II

Nature of compounds elaborated by 100 000 × g supernatant and plastid fraction during incubation with [1-^{14}C] IPP (35 μM) and unlabelled DMAPP (200 μM)

	Supernatant	Plastid fraction interface 1.3-1.5 M
	nmole IPP incorporated·h^{-1}·mg^{-1} protein	
Geraniol	-	-
Farnesol	7	-
Geranyl-PP	-	6
Farnesyl-PP	26	0.1

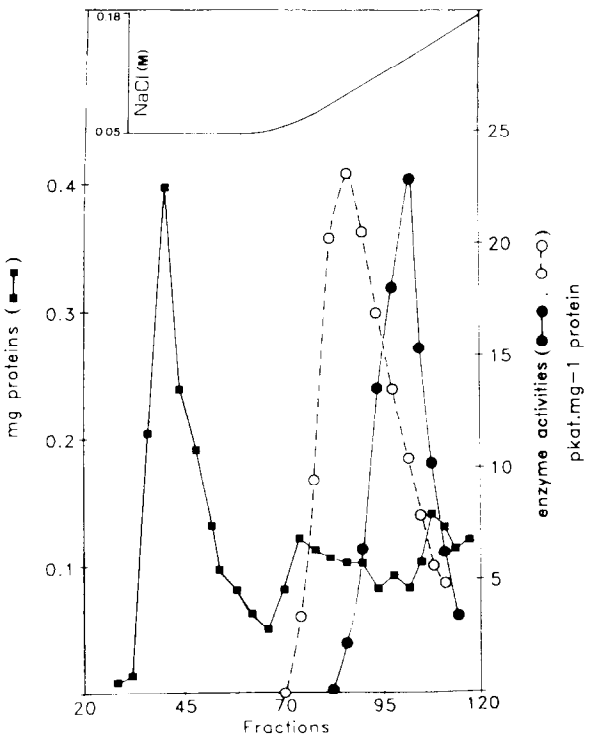

Fig. 1. Elution of FPP synthase (○- - -○) and GPP synthase (●——●) activities from a DEAE-Sephacel column with a linear NaCl gradient.

these data suggested the presence of two distinct prenyltransferases, a purification procedure was initiated. The results (Fig. 1) showed that elution with a 0.05–0.18 M NaCl gradient on a DEAE-Sephacel column separated a FPP synthase and a GPP synthase.

4. DISCUSSION

The results presented in this paper constitute the first report of the subcellular location of a GPP synthase. In cell culture of Muscat grapes., GPP synthase was associated with plastid-like membranes. This finding is of interest for the concept of monoterpene biosynthesis since the presence, in plastids, of a specific prenyltransferase involved in synthesis of GPP, had been in doubt. Moreover, in spite of the presence of GPP synthase in our system, the specific compartmentation of this enzyme could be an explanation for the lack of production of monoterpenes often observed in plant cell tissue cultures [11,12].

Further work is in progress to study the location of this enzyme within plastids in order to understand the relationship between GPP synthase segregation and the specific membrane permeability for the enzyme substrates (IPP and/or DMAPP).

Acknowledgements: We are grateful to Professor M. Gleizes and Dr. G. Pauly (Université Bordeaux I) for helpful suggestions and assistance in the chemical synthesis of IPP and DMAPP. This work was supported by the Ministère de l'Education Nationale, de la Jeunesse et des Sports (Grant 88-1994).

REFERENCES

[1] West, C.D., Dudley, M.W. and Dueber, M.T. (1978) in: Recent Advances in Phytochemistry, vol. 13 (Swain, T. and Waller, G.R., eds) pp. 163–198, Plenum Press, New York.
[2] Spurgeon, S.L., Sathyamoorthy, N. and Porter, J.W. (1984) Arch. Biochem. Biophys. 230, 446–454.
[3] Dogbo, O. and Camara, B. (1987) Biochim. Biophys. Acta 920, 140–149.
[4] Gleizes, M., Pauly, G., Carde, J.P., Marpeau, A. and Bernard-Dagan, C. (1983) Planta 159, 373–381.
[5] Kleinig, H. (1989) Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 39–59.
[6] Ambid, C., Moisseeff, M. and Fallot, J. (1983) Physiol. Veg. 21, 87–92.
[7] Bouzayen, M., Latche, A. and Pech, J.C. (1990) Planta 180, 175–180.
[8] Singer, T.P., Oestreicher, G., Hogue, P., Contreiras, J. and Brandao, I. (1973) Plant Physiol. 52, 616–621.
[9] Lück, H. (1965) in: Methods of Enzyme Analysis (Bergmeyer, H.U., ed.) pp. 895–897, Academic Press, New York.
[10] Douce, R. (1974) Science 183, 852–853.
[11] Banthorpe, D.V., Branch, S.A., Njar, V.C.O., Osborne, M.G. and Watson, D.G. (1986) Phytochemistry 25, 629–636.
[12] Cormier, F. and Ambid, C. (1987) Plant Cell Rep. 6, 427–430.