BIOLOGIA PLANTARUM 47 (3): 477-479, 2003/4

BRIEF COMMUNICATION

Activities of soluble and microsomal farnesyl diphosphatases in *Datura stramonium*

S.B. HA, D.E. LEE, H.J. LEE, S.J. SONG and K. BACK*

Department of Biotechnology, Agricultural Plant Stress Research Center, Chonnam National University, Gwangju, 500-757, South Korea

Abstract

Farnesyl diphosphatase (FDPase; EC 3.1.7.1) produces farnesol from farnesyl diphosphate (FDP) in a reaction that does not require Mg^{2+}. This report shows that FDPase is constitutively expressed at a high level in the soluble and the microsomal fractions of *Datura stramonium*. Soluble and microsomal FDPase have a similar pH optimum (5.0 - 6.0) and a similar substrate specificity. Geranyl diphosphate (GDP) and geranylgeranyl diphosphate (GGDP) compete for FDP, but isopentenyl diphosphate (IDP), ATP, and para-nitrophenyl phosphate do not. Soluble FDPase activity was highest in fruit and flower followed by root, and leaf.

Additional key words: ATP, farnesol, farnesyl diphosphate, geranyl diphosphate, isopentenyl diphosphate, thorn apple.

Farnesol (C15 sesquiterpene allylic alcohol) is formed by hydrolysis of farnesyl diphosphate (FDP). Recent studies provide some insight into the physiological and biochemical role of farnesol in mammals and plants. For example, application of farnesol to tobacco cells inhibited growth, increased a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity and resulted in cell death (Hemmerlin and Bach 2000). The precise role played by farnesol in regulating HMG-CoA reductase is not yet clear (Keller *et al.* 1996); however, the reversible conversion of FDP into farnesol may play an important role in regulating isoprenoid metabolism (Thai *et al.* 1999, Nah *et al.* 2001). FDP is converted into farnesol by farnesyl diphosphatase (FDPase) which is localized in the microsomal fraction in rice cells (Nah *et al.* 2001). FDPase activity is relatively low in the microsomal fraction, which makes FDPase's purification difficult and has hampered its molecular and genetic characterization. However, our recent experiments show that soluble and microsomal FDPase activity can be detected in cellular extracts of *Datura stramonium*.

FDPase enzyme assay was carried out as described previously (Nah *et al.* 2001). Tissues from *Datura stramonium* L. (thorn apple) were homogenized in a mortar and pestle with 1 cm^3 homogenization buffer: 80 mM Tris/HCl (pH 7.0), 20 % (m/v) glycerol, 10 mM sodium metabissulfate, 10 mM sodium ascorbate, 1 % (m/v) polyvinylpyrrolidone, 5 mM β-mercapthoethanol, and 2 mM EDTA. The homogenate was filtered through two layers of Miracloth (*CalBiochem*, La Jolla, USA), centrifuged for 10 min at 3 000 *g*, and the supernatant centrifuged for 60 min at 100 000 *g* to obtain a crude microsomal pellet. The pellets were dissolved in 0.1 cm^3 homogenization buffer and used for microsomal fractions. The 100 000 *g* supernatant extracts were employed as soluble proteins. Soluble and membrane bound fractions (0.01 cm^3) were assayed in a total of 0.05 cm^3 containing 100 mM Tris/HCl (pH 7.0), 5 mM EDTA, and 36 μM [1-^{3}H]FDP or [1-^{3}H]GGDP (108 Bq mol^{-1}) (*Amersham Biosciences*, Piscataway, USA). After 30 min incubation at 35 °C, the reaction was stopped with 0.1 cm^3 95 % ethanol : 40 % KOH (1:1). *Dowex* anionic

Received 11 November 2002, *accepted* 22 April 2003.

Abbreviations: FDPase - farnesyl diphosphatase; FDP - farnesyl diphosphate; GDP - geranyl diphosphate; GGDP - geranylgeranyl diphosphate; IDP - isopentenyl diphosphate; HMG-CoA - 3-hydroxy-3-methylglutaryl-coenzyme A.

Acknowledgements: This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the Agricultural Plant Stress Research Center (R11-2001-09203001-0).

*Author for correspondence; fax: (+82) 62 5302169, e-mail: kback@chonnam.ac.kr

exchange resin 1×2-200 (*Sigma*, St. Louis, USA) was added to absorb unused substrate. The mixture was vortexed and allyl alcohols were extracted in 0.2 cm^3 petroleum ether. The organic layer of 0.1 cm^3 was removed and radioactivity measured by scintillation counting. FDPase activity was expressed as radioactive allyl alcohol product g^{-1}(protein) s^{-1}.

The dependence of enzyme activity on the pH was measured between pH 3.0 and 8.5. Buffers used are 0.1 M citric acid-sodium citrate (pH 3.0 - 5.0), 0.1 M MES (pH 5.5 - 6.5) and 0.1 M Tris-HCl (pH 7.0 - 8.5). As for effects of various substrates on FDPase activity, assays were performed as described previously (Nah *et al.* 2001). In the presence of nonspecific substrates, FDPase activities were assayed and expressed as a percentage relative to the activity measured in the absence of nonspecific substrates.

Assays for microsomal and soluble squalene synthetase and sesquiterpene cyclase were performed as previously reported (Vogeli *et al.* 1988). The squalene synthetase activity was measured by counting the radiolabeled squalene product on a TLC which was separated from radiolabeled FDP in the assay reaction. The sesquiterpene cyclase activity was determined by measuring the hexane-extractable hydrocarbon products from radiolabeled FDP substrate as described previously (Back and Chappell 1995). The 100 000 *g* supernatants were used as soluble extracts and their corresponding pellets were employed as microsomal pellets for these assay. Soluble protein levels were determined by the Bradford method using the *Bio-Rad* protein assay dye (Hercules, CA, USA). Microsomal protein concentration was determined by the *Bio-Rad DC* protein assay by using bovine serum albumin as a standard. Both enzymatic activities were represented by reaction product(s) g^{-1}(protein) s^{-1}.

The soluble and microsomal FDPase activities from *D. stramonium* leaves have a similar specific activity of approximately 2055 - 2083 and both activities are Mg^{2+}-independent (Table 1). The soluble FDPase activity has not been previously found in mammals or plants. In attempt to address that the existence of soluble FDPase was not ascribed to the contamination of microsomal fraction, we had measured the squalene synthetase activity, a representative membrane bound enzyme of isoprenoid biosynthetic pathway. The squalene synthetase activity was detected only in the microsomal fraction not in the soluble fraction (Table 1). The squalene synthetase specific activity (54) measured in the soluble fraction represented negligible activity. By contrast, a sesquiterpene cyclase which catalyzes the FDP conversion into bicyclic terpenes is a well known soluble enzyme. The sesquiterpene cyclase activity showed the peak in the soluble fraction, but no activity was measured in the microsomal fraction (Table 1). These data clearly suggested that the Mg^{2+}-independent FDPase was present in the soluble fraction and in the microsomal as well.

Table 1. Specific activity [pmol g^{-1}(protein) s^{-1}] of squalene synthetase, sesquiterpene cyclase, and FDPase. Means of three replicates ± SD.

Enzymes	Microsomal fraction	Soluble fraction
Squalene synthetase	1805 ± 138	54 ± 27
Sesquiterpene cyclase	83 ± 55	1055 ± 83
FDPase	2083 ± 184	2055 ± 83

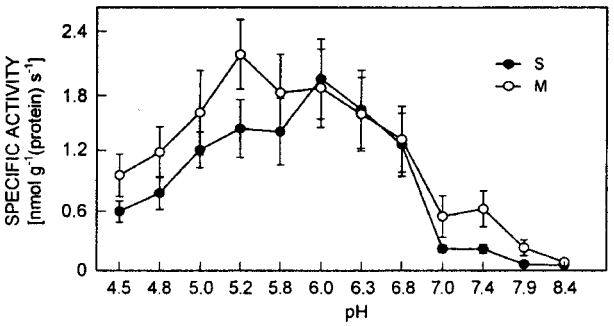

Fig. 1. pH profile of *D. stramonium* soluble (S) and microsomal (M) FDPase. Means of three replicates ± SD.

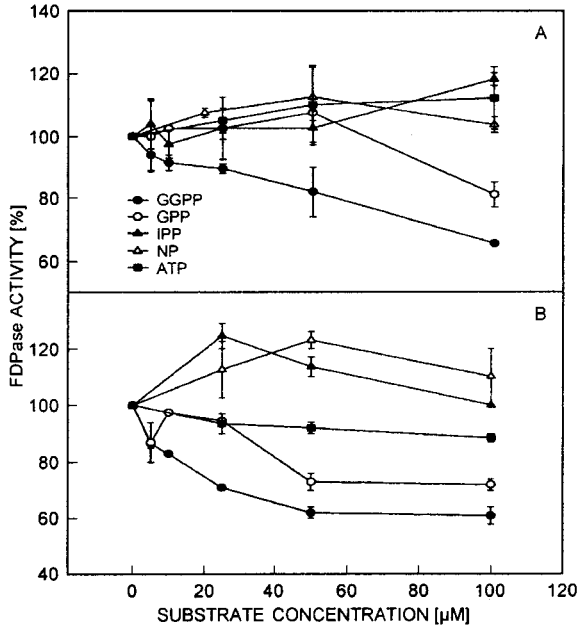

Fig. 2. Effects of various unlabeled competitor substrates on FDPase activities (*A* - soluble FDPase, *B* - microsomal FDPase). The rate at which [1-^{3}H]FDP was converted to [1-^{3}H]farnesol was measured in the presence of unlabeled competitor substrates. FDPase activity is expressed as a percentage relative to the activity in the absence of competitor. Means of three replicates ± SD.

The pH optima of these enzymes was analyzed from pH 3.0 to 8.5. The relative specific activity of microsomal and soluble FDPase was similar through the entire pH range (Fig. 1). Both enzymes prefer an acidic pH (5.2 - 6.8). The pH optimum of soluble FDPase is approxi-

mately 6.0, while for the microsomal FDPase pH 5.2. These pH optima differ slightly from rice FDPase (Nah *et al.* 2001) which has pH optimum of 6.8.

A substrate competition experiment was used to examine the substrate specificity of soluble and microsomal FDPase. The rate at which [1-^{3}H]FDP was converted to [1-^{3}H]farnesol was measured in the presence of unlabeled competitors (Fig. 2). Assays were carried out at the appropriate pH for each enzyme. Microsomal and soluble FDPase were inhibited by isoprenoid intermediates such as GGDP and GDP but not by nonspecific intermediates. ATP and para-nitrophenyl phosphate (NP) did not inhibit soluble or microsomal FDPase at a concentration of 100 μM. In contrast, 50 μM ATP or NP stimulated soluble FDPase 8 or 10 %, respectively (Fig. 2*A*). Similarly, microsomal FDPase was not inhibited by ATP or NP up to 100 μM while NP seemed to stimulate its activity by 20 % at 50 μM (Fig. 2*B*). The isoprenoid intermediate IDP stimulated soluble (20 % increase at 100 μM) and microsomal FDPase (20 % increase at 20 μM). In contrast, these compounds do not stimulate FDPase from mammals or rice. The most potent FDPase inhibitor was GGDP, inhibiting 16 % and 38 % of soluble and microsomal FDPase at 50 μM, respectively. GDP inhibited soluble FDPase 16 % at 100 μM and inhibited microsomal FDPase 26 % at 50 μM. GGDP did not strongly inhibit *D. stramonium* FDPase, but inhibited rice FDPase 50 % at 5 μM. The inhibitory effects of GGDP and GDP were typical of FDPase activities that have been characterized to date.

The FDPase was also quantified in extracts from different *D. stramonium* tissues (Table 2). Flower and fruit exhibit the highest soluble FDPase activity, and root and leaf have lower activities. In contrast, root has the highest level of microsomal FDPase. The tissue distribution of FDPase may reflect the rate of synthesis of farnesol in different tissues; farnesol is an acyclic terpenoid which is a major component of plant essential oil (Asres *et al.* 1998). On the basis of the substrate specificity, and tissue distribution of soluble and microsomal FDPase in *D. stramonium*, it is likely that these are two distinct enzymes.

Table 2. Tissue distribution of soluble and microsomal FDPase. Data showed the specific radioactivity [pmol g^{-1}(protein) s^{-1}] measured from various tissue extracts. Means not followed by the same letter are significantly different at $P = 0.05$ as determined by a Duncan's multiple range test. Means of three replicates ± SD.

	Soluble FDPase	Microsomal FDPase
Leaf	2194 ± 444^a	3194 ± 410^a
Flower	6916 ± 2083^b	3055 ± 1055^a
Root	5194 ± 805^c	7194 ± 944^b
Fruit	7500 ± 638^b	6083 ± 861^c

Thus far, the Mg^{2+}-independent FDPase was not purified or cloned due to possible hindrance of membrane protein. Based on our discovery of the soluble FDPase in *D. stramonium*, it may be possible to purify soluble FDPase using chromatographic techniques and a conventional FDPase assay. Successful purification of this soluble enzyme in a reasonable quantity will facilitate additional molecular and genetic characterization of FDPase.

References

Asres, K., Tei, A., Moges, G., Sporer, F., Wink, M.: Terpenoid composition of the wound-induced bark exudates of *Commiphora tenuis* from Ethiopia. - Planta med. **64**: 473-475, 1998.

Back, K., Chappell, J.: Cloning and bacterial expression of a sesquiterpene cyclase from *Hyoscyamus muticus* and its molecular comparison to related terpene cyclases. - J. biol. Chem. **270**: 7375-7381, 1995.

Hemmerlin, A., Bach, T.J.: Farnesol induced cell death and stimulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in tobacco cv. Bright Yellow-2 cells. - Plant Physiol. **123**: 1257-1268, 2000.

Keller, R.K., Zhao, Z., Chambers, C., Ness, G.C.: Farnesol is not the nonsterol regulator mediating degradation of HMG-CoA reductase in rat liver. - Arch. Biochem. Biophys. **328**: 324-330, 1996.

Nah, J., Song, S.J., Back, K.: Partial characterization of farnesyl and geranylgeranyl diphosphatases induced in rice seedlings by UV-C irradiation. - Plant Cell Physiol. **42**: 864-867, 2001.

Thai, L.T., Rush, J., Maul, J., Devarenne, T., Rodgers, D.L., Chappell, J., Waechter, C.J.: Farnesol is utilized for isoprenoid biosynthesis in plant cells via farnesyl pyrophosphate formed by successive monophosphorylation reactions. - Proc. nat. Acad. Sci. USA **96**: 13080-13085, 1999.

Vogeli, U., Chappell, J.: Induction of sesquiterpenecyclase and suppression of squalene synthetase activities in plant cell cultures treated with fungal elicitor. - Plant Physiol. **88**: 1291-1296, 1988.