

## REGULATION OF TERPENOID BIOSYNTHESIS IN TAPPED LATEX

George J. Piazza, Edward J. Saggese, and Marvin P. Thompson

Agricultural Research Service  
U. S. Department of Agriculture  
Philadelphia, PA 19118

## INTRODUCTION

The tapped latex of Euphorbia lathyris can convert acetate to several structurally different triterpenols (TOH) and their fatty acid esters (TE) (1). Simple low speed centrifugation of the latex affords a pellet that utilizes mevalonic acid as a triterpenoid precursor (2). Biosynthetic activity in the latex pellet is absolutely dependent upon the presence of an osmoticum (0.4 M sorbitol), indicating that biosynthesis occurs in an osmotically sensitive organelle. Calcium constitutes about 7.5% of dry latex weight, and the addition of  $\text{Ca}^{2+}$  to an EGTA-treated latex pellet stimulates triterpene biosynthesis. This study was undertaken to investigate whether the calcium binding protein, calmodulin, mediates  $\text{Ca}^{2+}$  effects upon triterpenoid biosynthesis.

## METHODS

Triterpene biosynthesis. Freshly tapped latex (100  $\mu\text{l}$  per assay tube) was diluted three-fold with cold buffer containing 10 mM Na-phthalate (pH 5.5), 10 mM  $\text{MgCl}_2$ , 10 mM KCl, 30 mM  $\text{CaCl}_2$  and 0.4 M sorbitol, and centrifuged for 5 min at 8,800 g. The pellet was resuspended in 220  $\mu\text{l}$  buffer containing 1.0 nmol (0.01 mCi) R-(5- $^3\text{H}$ )-mevalonic acid, 0.66  $\mu\text{mol}$  dithiothreitol, and 22 nmol S-adenosylmethionine. Radiolabel in triterpenes was determined as described (1).

Phosphodiesterase Activity. Assays were performed at room temperature as described (3) except that  $\text{CaCl}_2$  (18  $\mu\text{M}$ ) and calmodulin (0.1  $\mu\text{g}$  or as indicated) were present.

Isolation of Calmodulin. Upper stems and leaves of E. lathyris were homogenized in the buffer (pH 6.5) described by Schreiber et. al. (4), also containing 20 mM mercaptoethanol. After centrifugation, the supernatant was heated to 85°C for 5 min, rapidly cooled, and recentrifuged. The supernatant was slurried with DE-52 cellulose, and the suspension was poured into a glass column. Elution of protein was achieved with a salt gradient of 0.1-0.5 M NaCl. The calmodulin fraction was purified to homogeneity by chromatography on Sephadex G-100 at pH 7.5 with a buffer containing EGTA.

The addition of calmodulin antagonists (compounds I, II, III) to the latex pellet at a concentration of 200  $\mu$ M severely inhibits TOH and TE biosynthesis.  $I_{50}$  values for compounds II and III are 150 and 55  $\mu$ M, respectively, consistent with an action upon calmodulin, rather than a nonspecific mode of inhibition. Inhibition of biosynthesis by phenoxyalkylamines (compounds IV, V, VI) increases with increasing chlorine substitution. Assays using calmodulin-stimulated phosphodiesterase show that antagonism toward calmodulin increases in the same order.

A direct test of calmodulin involvement by an observed stimulation of biosynthetic activity by exogenously added calmodulin is not feasible due to the presence of a limiting membrane around the site of biosynthesis, and to date we have not observed biosynthesis in broken organelles.

Our goal was to demonstrate the presence of calmodulin in tapped latex. To obtain an appropriate standard, calmodulin was isolated and purified to homogeneity from *E. lathyris*. The isolated calmodulin is very acidic and has a molecular weight of about 17 KDa, similar to calmodulins isolated from other plants (5). The amino acid analysis of *E. lathyris* calmodulin is shown following with the values in parentheses being those of spinach calmodulin: Asp 27(24), Thr 7(9), Ser 7(4), Glu 30(27), Pro 4(2), Gly 11(10), Ala 10 (11), Cys 1(1), Val 7(8), Met 5(8), Ile 6(7), Leu 9(11), Tyr 1-2(1), Phe 7(9), His 1(1), TML 1(1), Lys 9(9), Trp 0(0), Arg 4(5).

Fig. 1 shows that *E. lathyris* calmodulin is a good stimulator of bovine heart phosphodiesterase. The data were best fitted with two distinct dissociation constants, indicating an interaction by two different modes. In contrast, bovine brain calmodulin interacts with phosphodiesterase in a way that does not reveal any heterogeneity in binding sites.

When whole tapped latex is subjected to polyacrylamide gel electrophoresis in the presence of urea, a protein band corresponding to authentic calmodulin is observed (arrow, Fig. 2). Calmodulin is also detected when the latex is treated with EGTA. Calmodulin is not visible in the electrophoresis of untreated latex, indicating that it remains bound to its target protein(s).

Table 1. Effect of Calmodulin Antagonists on Triterpene Biosynthesis

COMPOUND	TRITERPENE BIOSYNTHESIS (control = 100%)	
		%
I Chlorpromazine	TOH	9 $\pm$ 2
	TE	7 $\pm$ 1
II Fluphenazine	TOH	31 $\pm$ 3
	TE	26 $\pm$ 8
III Trifluoperazine	TOH	3 $\pm$ 1
	TE	1 $\pm$ 1
IV 2-(2,6-dichlorophenoxy) ethyl N,N-diethylamine	TOH	87 $\pm$ 6
	TE	72 $\pm$ 8
V 2-(2,4,5-trichlorophenoxy) ethyl N,N-diethylamine	TOH	17 $\pm$ 6
	TE	20 $\pm$ 4
VI 2-(pentachlorophenoxy) ethyl N,N-diethylamine	TOH	1 $\pm$ 1
	TE	2 $\pm$ 1

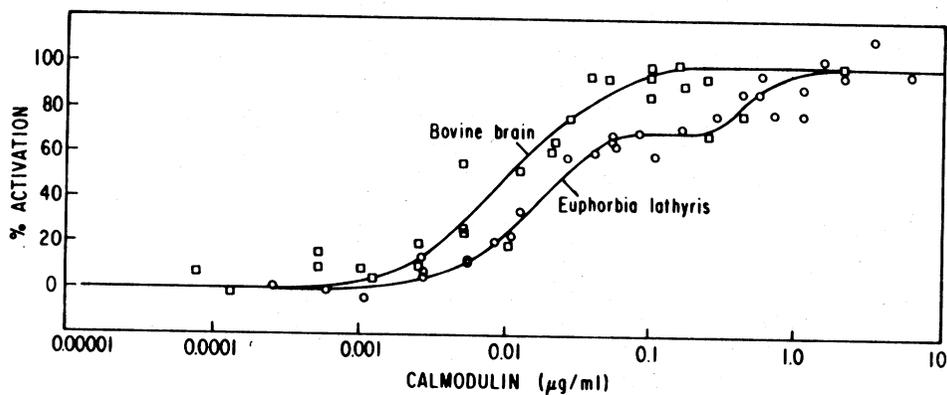


Figure 1. Activation of bovine heart phosphodiesterase-catalyzed hydrolysis of cAMP.

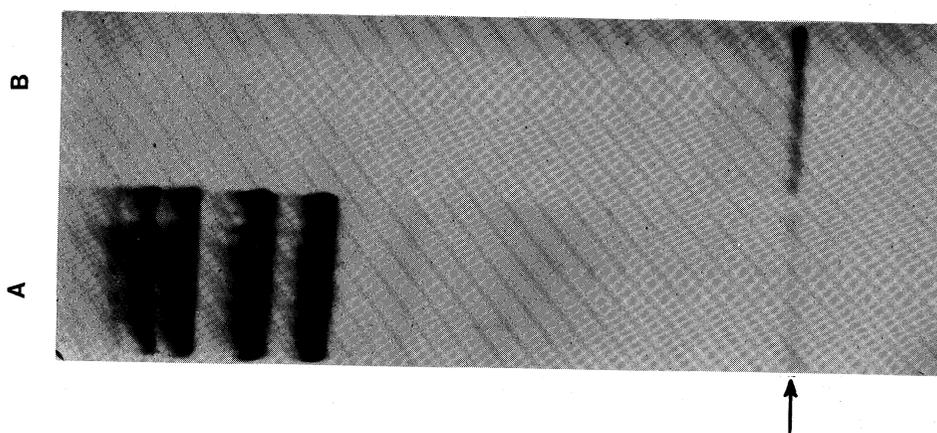


Figure 2. Polyacrylamide gel electrophoresis of whole latex (lane A) and purified *E. lathyris* calmodulin (lane B). Gel conditions: 4.5 M urea, 12% acrylamide. Running buffer: Tris-glycine, pH 8.3. Samples were dissolved in running buffer containing tracking dye, glycerol, 4.5 M urea, and 1.2 mM mercaptoethanol.

#### REFERENCES

1. E.K. Nemethy, C. Skrukrud, G.J. Piazza, and M. Calvin, Terpenoid biosynthesis in *Euphorbia latex*, *Biochim. Biophys. Acta* 760:343 (1983).
2. G. Ponsinet and G. Ourisson, Aspects particuliers de la biosynthese des triterpenes dans le latex d'*Euphorbia*, *Phytochemistry* 7:757 (1968).
3. R.W. Butcher, Cyclic 3',5'-nucleotide phosphodiesterase from bovine heart, *Methods in Enzymology* 38:218 (1974).
4. W.E. Schreiber, T. Sasagawa, K. Titani, R.D. Wade, D. Malencik, and E.H. Fisher, *Biochemistry* 20:5239 (1981).
5. G.J. Piazza, Calmodulin in plants, in: "Calcium Binding Proteins," M.P. Thompson, ed., CRC Press, Boca Raton, FL., in press.