Communication to the Editor

EBELACTONES INHIBIT CUTINASES PRODUCED BY FUNGAL PLANT PATHOGENS

Sir:

The plant cuticle is a continuous layer of lipid material covering the outer walls of epidermal cells. It consists of a soluble mixture of waxes embedded in a polymer named cutin. The structure of this polymer is best described as a polyester of ω-hydroxyfatty acids[1]. Most plant pathogenic fungi invade their hosts by breaching the intact plant surface, and the cuticle including the polymeric cutin comprises the first barrier to be penetrated. For several host-pathogen interactions it has been demonstrated that cutin-hydrolyzing enzymes excreted by the invading pathogen are a crucial requirement for the penetration of plant cuticles[2,3]. Consequently, cutinase inhibitors were shown to block this initial step of plant infection and, thus, protect plants from disease[2,3].

All fungal cutinases have been identified as serine hydrolases[2~5], although enzymatic dissimilarities exist. For example, the optimal pH for cutin hydrolysis can be either slightly acidic or strongly alkaline[3,5]. Recent evidence indicates a potential relationship between this enzymatic parameter and the tissue specificity of the respective pathogen[6]. Ebelactones A and B, both members of the mycolic acid β-lactones produced by actinomycetes, have been shown to act as potent inhibitors of serine hydrolases such as liver esterase or pancreas lipase[7,8]. In determining the inhibitory activity of the ebelactones against fungal cutinases, two representative enzymes purified from *Venturia inaequalis* (pH-optimum 6.5)[5] and *Rhizoctonia solani* (pH-optimum 9.5)[6] were employed. The purification of cutinase from *R. solani* was accomplished similar to the procedures established for other cutinases (unpublished results). Ebelactones A and B (purity >99%) was obtained from Calbiochem (San Diego, California, U.S.A.). Stock solutions (1 mg/ml) were prepared in ethanol. For assays of cutinase activity with *p*-nitrophenyl butyrate (PNB) as a model substrate, the method used previously[5] was modified as follows: 2.8 ml of 0.2 M phosphate buffer containing 0.2% Triton X-100 (pH 8.0), 0.03 ml of ethanol with or without inhibitor and 0.03 ml of enzyme preparation was incubated for 5 minutes at 20°C. Substrate (0.1 ml of 10 mM PNB in water containing 0.2% Triton X-100) was added, and the initial velocity was determined spectrophotometrically at 400 nm. The amount of cutinase was adjusted to absorbance changes of 0.2 to 0.3 per minute in nontreated samples.

Both cutinases were inhibited by ebelactones A and B (Fig. 1). However, differences between the two enzymes were apparent. Ebelactone B was more active than ebelactone A by a factor of over 300 when tested with cutinase prepared from *R. solani* (Table 1). This greatly elevated inhibitory potency of ebelactone B has been reported before for mammalian esterase and lipase (Table 1). In contrast, cutinase derived from *V. inaequalis* was

Fig. 1. Inhibition of cutinases from *Rhizoctonia solani* and *Venturia inaequalis* by ebelactones.
(A) *R. solani*, (B) *V. inaequalis*. ● ebelactone A, ○ ebelactone B.

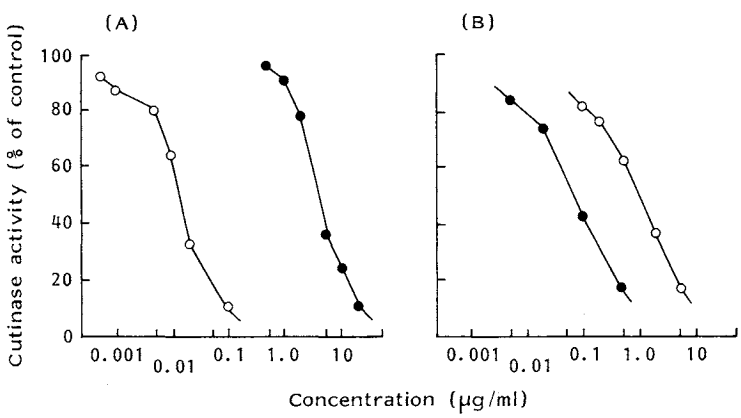

Table 1. Hydrolase-inhibitory activity of ebelactones.

Inhibitor	IC_{50} (μg/ml)[a]			
	Cutinase		Esterase[b] hog liver	Lipase[b] hog pancreas
	V.i.	*R.s.*		
Ebelactone A	0.08	4.0	0.056	0.003
Ebelactone B	0.67	0.013	0.00035	0.0008

[a] IC_{50} is concentration for 50% inhibition.
[b] Data from UMEZAWA *et al.*[7].
Abbreviations: *V.i.*, *Venturia inaequalis*; *R.s.*, *Rhizoctonia solani*.

almost equally inhibited by both compounds, with ebelactone A slightly more active (Table 1). These distinct patterns of cutinase inhibition substantiate the enzymatic diversity of cutinases produced by fungal plant pathogens.

Organo phosphate esters, like ebelactone inhibitors of serine esterases, were shown to inhibit fungal cutinases and, thus, to protect plants from disease by acting as antipenetrants[9,10]. The results presented in this report represent the first example of cutinase inhibitors produced by microorganisms. In the past, mycolic acid β-lactones have been investigated with regard to their effects on the immune response[7,8,11]. Their potency as inhibitors of fungal cutinases might open an novel field of application as plant protectants in agriculture.

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