Reversal of Self-Inhibition by Peroxidase and Hydrogen Peroxide in Wheat Stem Rust Uredospores: Mode of Action

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ABSTRACT

Peroxidase and hydrogen peroxide stimulate the germination of uredospores of *Puccinia graminis* var. *tritici* by conversion of the self-inhibitor to an inactive compound, apparently a dimer of methyl ferulate.

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Uredospores of the wheat stem rust fungus fail to germinate if floated in dense populations on water because of the presence of methyl *cis*-ferulate, the endogenous self-inhibitor (1, 5, 6). In contrast, a number of low-molecular-weight compounds such as *n*-nonanol stimulate germination of self-inhibited wheat stem rust uredospores (3), and it has been reported that the self-inhibition is also reversed by floating uredospores on peroxidase-hydrogen peroxide ($PO\text{-}H_2O_2$) solutions (7). In either case, nothing is known about the mode of stimulation of germination. Our purpose here was to determine how $PO\text{-}H_2O_2$ stimulates the spores to germinate in the presence of self-inhibitor. Such information is needed to understand the mechanism of this stimulation, and also for studies on host resistance, since it has been reported that the activity of peroxidase increases in resistant wheat infected by the wheat stem rust fungus (2, 7).

Uredospores of the wheat stem rust fungus (*Puccinia graminis* var. *tritici* Eriks. & E. Henn.) race 56 were increased on wheat (*Triticum aestivum* 'Little Club') in the greenhouse. The germination assay, preparation of synthetic self-inhibitor (methyl *cis*-ferulate) and analytical techniques have been published (6).

Germination of uredospores was completely inhibited on water solutions of methyl *cis*-ferulate at concns above 10 ng/ml (Fig. 1-A). Peroxidase (Type I, Sigma Chemical Co., 10 μg/ml) and 5 μM hydrogen peroxide (Mallinckrodt) added separately to the same concns of the self-inhibitor did not stimulate germination (Fig. 1-A). On the other hand, if both peroxidase and hydrogen peroxide were in solution with the inhibitor, spores were stimulated to germinate (Fig. 1-B). The addition of $PO\text{-}H_2O_2$ restored germination of the spores to the level of the control in the presence of up to 1,000 ng/ml of the inhibitor. This suggested that the inhibitor could be converted by the peroxidase system to a compound(s) which is not active as a germination inhibitor. Such a product could be a dimer of methyl ferulate, since there are numerous reports in the literature that phenylpropanoid compounds are readily dehydrogenated by $PO\text{-}H_2O_2$ to their dimers (4, 8). To test this possibility, methyl *cis*-ferulate (1,000 ng/ml) was stirred with peroxidase (10 μg/ml) and hydrogen peroxide (5 μM) in water for 30 min at 20 C. The reaction mixture was then extracted with ether and chromatographed along with guide spots of untreated methyl *cis*-ferulate on preparative (0.5 mm)

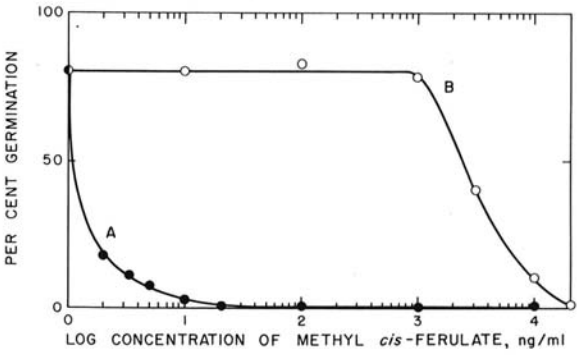

Fig. 1. Germination of wheat stem rust (*Puccinia graminis* var. *tritici*) uredospores on water solutions of the synthetic self-inhibitor (methyl *cis*-ferulate), peroxidase and hydrogen peroxide. **A)** Germination curve in the presence of the inhibitor alone and with either peroxidase (10 μg/ml) or 5 μM hydrogen peroxide. **B)** Germination curve in the presence of the inhibitor with both peroxidase (10 μg/ml) and hydrogen peroxide (5 μM).

silica gel thin-layer plates in a mixture of benzene and diethyl ether (80:20, v/v).

One major band (R_f 0.5) and traces of two bands (R_f 0.3 and 0.2) were visible under UV light. Neither the *cis*- nor *trans*-isomer of methyl ferulate was found at the corresponding R_f of 0.6 as detected by UV light, by a biotest of the extract from a 1-cm zone at this R_f, and by gas chromatography. Germination tests of extracts from 1-cm bands scraped from the rest of the plate also showed no inhibitory activity. Deletion of either peroxidase or hydrogen peroxide from the reaction mixture, resulted in full recovery of the methyl *cis*-ferulate using the same procedure.

The mass spectrum of the material extracted from the major band after TLC of the complete reaction mixture indicated a molecular weight of 414. This is compatible with the suggestion that the principal product of reaction of methyl *cis*-ferulate with PO-H_2O_2 is a dimer of the inhibitor. This compound has no apparent inhibitory activity. Thus, the present study, together with that reported previously (7), leads to the conclusion that the mode of action of PO-H_2O_2 in stimulating wheat stem rust uredospore germination is the conversion of the self-inhibitor to an inactive compound, probably a dimer of methyl ferulate. Whether such a system is operative in vivo, particularly in resistant wheat infected with wheat stem rust fungus, is not known.

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