Interaction of 2,4,5-trichlorophenylsulphonylmethyl thiocyanate with fungal spores

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Abstract

Interaction of a new fungicidal compound, viz. 2,4,5-trichlorophenylsulphonylmethyl thiocyanate with spores of Fusarium culmorum has been investigated.

The compound is readily taken up by spores and converted to the non-fungitoxic 2,4,5-trichlorophenylsulphinic acid, which is released into the ambient solution. Uptake of the thiocyanate can be markedly reduced by pretreatment of the spores with a thiol reagent like iodoacetic acid and slightly enhanced by pretreatment with a thiol, like dithiodiglycol. Moreover, the thiocyanate reacts with thiols in vitro by forming the same sulphinic acid. Hence, it is concluded that the title compound is able to react with fungal cell thiols.

However, addition of a thiol reagent does not affect the fungitoxicity of the thiocyanate. The absence of synergism suggests that the fungitoxicity of the thiocyanate is based at least partly on reaction with fungal cell thiols.

The thiocyanate investigated resembles the fungicide captan with respect to both uptake pattern and failure to give synergism with a thiol reagent. These observations suggest that the fungicidal compounds have a similar mode of action, as far as reaction with fungal cell thiols is concerned.

Introduction

Most present-day fungicides, acting as protectants, are able to inhibit germination of fungal spores. The selective toxicity of the fungicides, not harmful to the host, may be based on physical factors such as the permeability of the spore envelope. For this reason information about the interaction of fungicidal compounds with spores, as measured by several authors, may be fruitful in studies of fungicidal action.

Most fungicides, having a low toxicity to the spores, on a spore weight basis (Miller et al., 1953) act as non-specific agents on reactive groups like sulphydryl, amino or hydroxyl groups. Organic thiocyanates have been found to react with sulphhydryl groups and the reaction was put forward as a possible mechanism of action (Zsolnai, 1962; Croshaw et al., 1966).

Recently a new group of fungicidal thiocyanates was synthesized by Dolman et al. (1969) in the laboratories of Philips-Duphar. The present study was undertaken to investigate the interaction of the title compound with fungal cells with particular reference to the interaction with cell thiols. Use is made of the procedure of Richmond and Somers (1966), who investigated the interaction of N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (captan) with thiols in conidia of Neurospora crassa by pretreatment of the spores with thiol reagents or thiols.
Materials and methods

Chemicals. 2,4,5-trichlorophenylsulphonylmethyl thiocyanate, 2,4,5-trichlorophenylsulphinic acid and N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (captan) were synthesized and purified in the laboratories of Philips-Duphar. Iodoacetic acid was obtained from Schuchardt (München, W. Germany), dithiodiglycol from Koch and Light (Colnbrook, England) and N-ethylmaleimide from Fluka (Switzerland).

Buffer solutions. Media were buffered with mixtures of citric acid and disodium hydrogen phosphate. Concentrations in reaction media were 0.020/0.010 (pH 3.0), 0.016/0.017 (pH 4.0) and 0.010/0.029 (pH 5.6) M citric acid/M phosphate.

Conidia. Conidia of *Fusarium culmorum* were washed from 10-day-old malt agar cultures, cultivated at 23 °C in the dark. Conidia of *Neurospora crassa* were harvested from cultures which were incubated for 5 days at 22 °C in the light. Spores were washed five times with distilled water by centrifugation.

Uptake experiments. Spores (3.4 × 10⁶/ml) were gently shaken in a closed vessel at 20 °C with 25 ml of an aqueous solution containing buffer (pH 5.6), 1% ethanol (v/v) and a non-toxic concentration of the fungicidal compound (0.013 mM). After appropriate time intervals the loss of compound from the supernatant was determined and the uptake is expressed as a percentage of the initial concentration removed from the external solution. After uptake experiments, spores were washed and tested on viability in a slide germination test.

Pretreatment of the spores. The same spore concentration and incubator shaking conditions were used for the reagent pretreatment of the spores. Spores were pretreated for 30 min, except with dithiodiglycol, which was incubated with spores for 3 h. Pretreatment with iodoacetic acid was performed at pH 3.0 to facilitate uptake of the acid, using a citrate phosphate buffer. Spores were washed twice with distilled water before uptake experiments.

Analysis of compounds in the supernatant. After uptake by spores the thiocyanate was extracted from the supernatant with ether. Thiocyanate concentrations were determined in ether at 240 με (ε = 11400), using a recording UV spectrophotometer Optica Milano CF4R. The sulphinic acid formed was identified in the water layer. Captan was determined in the supernatant by the method of Burchfield and Schechtman (1958).

Slide germination test. Conidia were diluted with suspensions of the compound to be tested or with water in case of a viability test and a constant amount of cherry juice to a density of 10,000 spores/ml. Drops of the suspensions were put onto glass slides. Each slide was placed in a separate closed tube and incubated at 23 °C. After 24 h the minimum concentration at which germination was inhibited completely (MIC) was determined.

Assessment of mycelial growth. The compounds were finely divided and suspended in

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