

FUNGAL CHOLINE BIOSYNTHESIS – A TARGET FOR CONTROLLING RICE BLAST

Yasuhiko Uesugi, formerly of the National Institute of Agro-Environmental Sciences Japan, discusses the mode of action and biological activity of phosphorothiolate and related fungicides

Introduction

The phosphorothiolate fungicides, including iprobenfos and edifenphos, were introduced in Japan as rice blast fungicides in 1963 and are still being used as one of the major groups of rice blast fungicides; a related dithiolane fungicide, isoprothiolane, has been used against blast since 1975 (Figure 1). Iprobenfos and isoprothiolane have systemic action and are used mainly as granules for application on the surface of paddy water (soil application). On the other hand, edifenphos seems to be less soluble in water and less stable in plants, and hence is most effective by foliar application.

This short article outlines the development of our knowledge of the mode of fungicidal action of phosphorothiolates and isoprothiolane as choline biosynthesis inhibitors (CBIs), and relates this to their influence on mammals and insects.

Mode of action of phosphorothiolate and related fungicides

Initially the mode of action of phosphorothiolate fungicides was proposed to be interference with biosynthesis of chitin, a component of the fungal cell wall, but this was later rejected as a secondary effect of the fungicides. During these studies, disorganization of the fungal cell membrane, with leakage of cytoplasmic substances from the fungal cells, was often observed, leading to the conclusion that the mode of action is inhibition of the biosynthesis of phosphatidylcholine, an important component of the fungal cell membrane (Kodama *et al.*, 1979, 1980).

Two major pathways have been known for phosphatidylcholine biosynthesis (Figure 2). In Greenberg's pathway, the transmethylation occurs at the final step, while in Kennedy's pathway, it occurs at the preliminary step yielding choline, a precursor to be further incorporated into phosphatidylcholine. Using ¹³C labeling studies, Yoshida showed that Kennedy's pathway is the principal route of phosphatidylcholine biosynthesis in the rice blast fungus (Yoshida, 1984), and that the transmethylation yielding choline is inhibited by phosphorothiolates and isoprothiolane (Figure 2). Phosphorothiolates and isoprothiolane were thus classified as choline biosynthesis inhibitors (CBIs).

Interesting relationships have been observed between phosphorothiolate and other rice blast fungicides (Uesugi and Takenaka, 1993). Laboratory fungal mutants resistant to phosphorothiolates have proved to be specifically sensitive to a group of experimental fungicides having phosphoramidate structure. The phosphoramidates exhibited, however, little activity against normal wild-type strains of the fungus. This negative cross-resistance has been studied in relation to

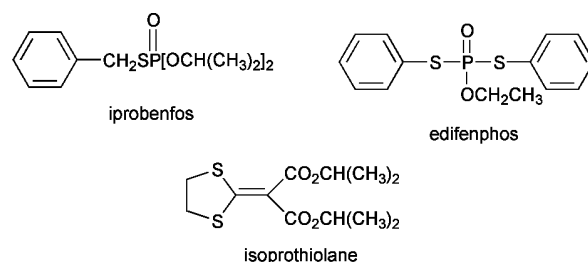


Figure 1. Chemical structures of CBIs and related fungicides

fungal metabolism of both groups of fungicides. The wild-type strains detoxified phosphoramidates by *N*-demethylation and hydroxylation of the higher alkyl group at the (ω -1) position and seemed to activate phosphorothiolates by a metabolism accompanied by cleavage of the P-S bond. These detoxifying and activating metabolic reactions were mediated by mixed function oxygenases. In the laboratory mutants these reactions were almost lost, and this loss of the metabolism may explain the negative cross-resistance between phosphorothiolates and phosphoramidates. With this type of fungal mutant, cross-resistance between isoprothiolane and phosphorothiolates and negative cross-resistance between isoprothiolane and phosphoramidates were also observed, so that similar activation in the fungicidal action of isoprothiolane has been suggested, though the details of the metabolism have not been studied.

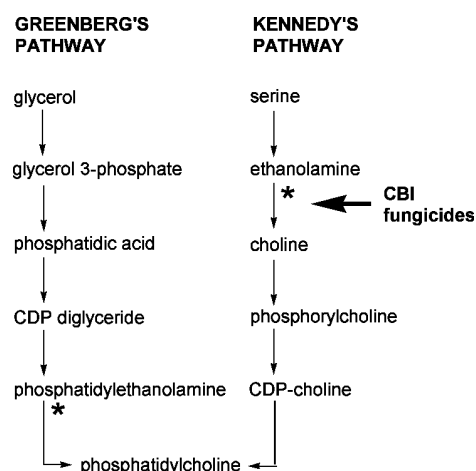


Figure 2. Two major pathways of phosphatidylcholine biosynthesis, showing the site of action of CBI fungicides (* = transmethylation steps)

The fungal activation of phosphorothiolates was further ascertained by antagonistic action on wild-type fungal strains with another group of fungicides – the demethylation inhibitor (DMI) type of sterol biosynthesis inhibiting (SBI) fungicides which are thought to be inhibitors of mixed function oxygenases. Possibly DMIs antagonize the fungicidal activity of phosphorothiolates by interfering with enzymatic activation. Activation of phosphorothiolates was thus suggested, but the activated form of the fungicides seemed to be an unstable intermediate or a by-product produced during the course of cleavage of the P–S bond.

Fungicidal spectrum of CBIs

CBIs act against several diseases of rice plants, but their effectiveness against diseases other than rice blast is not reliable enough to enable them to be used as major protectants. The rice blast fungus seems to have peculiar physiological characteristics yielding sensitivity to CBIs. Possible hypotheses are that the fungicides are specific inhibitors of choline biosynthesis, and phosphatidylcholine biosynthesis proceeds exclusively through the Kennedy's pathway in this fungus, but not in most other fungi, and/or that only this fungus is equipped with an enzymatic system to activate phosphorothiolates and isoprothiolane; these hypothesis have not been examined.

Resistance to CBI fungicides among various strains of the rice blast fungus has been an important problem in practice and also an interesting physiological phenomenon. As stated before, laboratory resistance to CBIs seemingly caused by decreased activation has been observed, and this resistance was accompanied by sensitivity to phosphoramidates by decreased detoxification. Resistance to CBIs was later observed in the field, but the field resistance differed from the laboratory resistance in that negative cross resistance to phosphoramidates was not observed in most resistant field isolates. In CBI-resistant field isolates, activation of phosphorothiolates accompanied by P–S cleavage decreased, but another mode of metabolism, S–C cleavage, increased. Detoxification of phosphoramidates still remained in the resistant field isolates though the detoxifying metabolism was slightly modified, decreasing *N*-demethylation and increasing hydroxylation. In other words, the field resistance seemed to be caused by a change in the mode of fungicide metabolism by mixed function oxidases, not by total loss of it.

Field resistance to CBI fungicides in the rice blast fungus has been observed when this group of fungicides were used successively and exclusively, but it is not a problem when fungicides with different modes of action are used in turn.

Low mammalian toxicity of CBIs

Choline is an important component for animals, including mammals and insects, in transmission of stimuli within their nervous systems. It also plays other roles, such as prevention of dysfunction diseases like fatty liver. Biosynthesis of choline in mammals, however, seems poor, so that choline has been classed as a nutrient or vitamin for mammals; this explains why CBIs display very low mammalian toxicity. This situation is comparable to that of sulfonylurea and imidazolinone herbicides which inhibit biosynthesis of branched chain amino acids such as valine, leucine and

isoleucine and to that of glyphosate, which interferes with biosynthesis of aromatic amino acids such as phenylalanine, tyrosine and tryptophan. Inhibitors of the biosynthesis of mammalian vitamins or nutrients, like choline and the above amino acids, are useful candidate pesticides with low mammalian toxicity.

Influence of CBIs on insects

CBI fungicides sometimes control leafhoppers and planthoppers on rice plants; e.g. isoprothiolane was registered as an insecticide to control brown planthoppers on rice plants in Japan. It is not a quick-acting insecticide, but reduces insect populations. Could this be related to the inhibitory action on choline biosynthesis? Choline is known to accelerate growth of insects such as rice stem borer in laboratory studies, but its necessity as an insect nutrient has not been demonstrated. Moreover, symbiotic microbes, which are common in insects, may synthesize choline and supply it to host insects. If choline biosynthesis is vital to insects and/or their symbiotic systems, its inhibition may be lethal to them, though this has not been established as the mode of action of CBI fungicides on insects.

Future outlook

CBI fungicides, such as phosphorothiolates and isoprothiolane, were developed following the discovery that sensitivity to chemical inhibition of fungal choline biosynthesis is a specific characteristic of the rice blast fungus; there is a possibility of development of new fungicide molecules with a similar action. Although existing CBI fungicides are already old, this group of fungicides have been, and will continue to be, necessary as one of the major fungicide classes used against the disease, and required to give the diversity of action needed in the fight against resistance development. The development of new CBIs is also encouraged by their low toxicity to mammals and their secondary insecticidal action, reducing the population of insects such as plant hoppers and leaf hoppers.

References

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