新規殺菌剤ピリベンカルブの作用機構と選択性

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Original Article

Mechanism of action and selectivity of a novel fungicide, pyribencarb

Satoshi KATAOKA, Makiichi TAKAGAKI, Koichiro KAKU and Tsutomu SHIMIZU*

Life Science Research Institute, Kumiai Chemical Industry Co., Ltd., Kikugawa-shi, Shizuoka 439–0031, Japan

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Pyribencarb is a novel benzylcarbamate-type fungicide, which is active against a wide range of plant pathogenic fungi. In this paper, the inhibitory effects of this fungicide on the electron transport system of fungi, plants, rat and carp were examined to elucidate its mode of action and selectivity. Pyribencarb potently inhibited succinate-cytochrome c reductase (SCR) activities of *Botrytis cinerea* (cucumber gray mold), *Corynespora cassiicola* (leaf spot) and decylubiquinol-cytochrome c reductase (UCR) activity of *B. cinerea*. Pyribencarb inhibited the UCR of *B. cinerea* in an uncompetitive manner with respect to decylubiquinol, which was the same as strobilurin fungicides, and the substrate-dependent inhibition constant was found from calculation to be 13 nM. These results suggested that the target site of pyribencarb is cytochrome *b* of complex III in the electron transport system of the respiratory chain. On the other hand, the inhibitory potency of pyribencarb on SCR activities of plants, rats and carp was relatively weak compared with that of strobilurin fungicides, indicating that pyribencarb is a Qo inhibitor of cytochrome *b*, whose properties are superior to well-known Qo inhibitor fungicides in terms of target. The binding site of pyribencarb on cytochrome *b* was assumed to be a little different from that of strobilurin fungicides, because pyribencarb inhibited SCRs of strobilurin fungicide-resistant strains of *B. cinerea* and *C. cassiicola* with relatively low concentrations. The binding site was also discussed through comparison of amino acid sequences of plants, rats, carp, yeast and fungi, including *B. cinerea*, which was elucidated in this paper.

Keywords: cytochrome *b*, complex III, *Botrytis cinerea*, pyribencarb, Qo inhibitor, QoI

Introduction

Pyribencarb, methyl {2-chloro-5-[(1*E*)-1-(6-methyl-2-pyridylmethoxyimino)ethyl]benzyl}carbamate (experimental code number, KUF-1204 or KIF-7767), which has been developed by Kumiai Chemical Industry Co., Ltd. and Ihara Chemical Industry Co., Ltd., is a novel benzylcarbamate-type fungicide active against a wide range of plant pathogenic fungi, especially gray mold cased by *Botrytis cinerea* and stem rot caused by *Sclerotinia sclerotirum*.[1,2] Pyribencarb expresses preventive and curative controls for the fungi, while having no phytotoxicity to a variety of crops.[2–5]

This chemical compound has a similar chemical structure to strobilurin fungicides, such as kresoxim-methyl and azoxystrobin,[6,7] but a different chemical structure regarding the substitution of the carbonyl moiety on the benzene ring (Fig. 1). In the present paper, the inhibitory effects of pyribencarb on the electron transport system of fungi, plants, rat and carp were examined to elucidate its mode of action and selectivity. The binding site on the target was also discussed through comparison of amino acid sequences of the target protein.

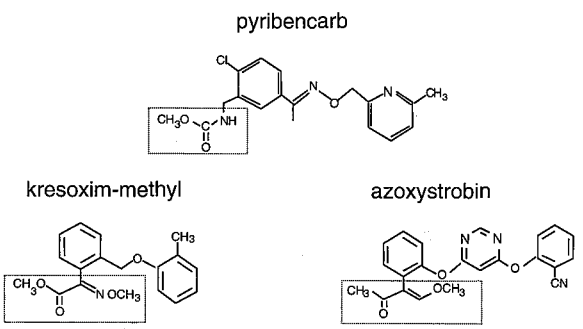

Fig. 1. Chemical structures of pyribencarb- and strobilurin-type fungicides.

* To whom correspondence should be addressed.
E-mail: t-shimizu@kumiai-chem.co.jp
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Materials and Methods

1. Materials

Wild-type strains of *B. cinerea* and *Corynespora cassiicola* were maintained on a potato-dextrose (PD) agar slant in our laboratory. Resistant strains of *B. cinerea* and *C. cassiicola* to strobilurin fungicides were obtained from Mr. Masanori Kansako of Wakayama Research Center of Agriculture, Forestry and Fisheries, Fruit Tree Experimental Station and Dr. Hideo Ishii of National Institute for Agro-Environmental Science, respectively. These strains have been elucidated to have a substitution of glycine for alanine at position 143 (G143A mutation) in cytochrome *b*.[5,8] Fischer rats F344/DuCrlCrlj (*Rattus norvegicus*) and carp (*Cyprinus carpio*) were obtained from Charles River Laboratories Japan Inc. (Kanagawa, Japan) and Kato Koi Farm Company (Gifu, Japan), respectively. Etiolated seedlings of crops [cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* Mill.), kidney bean (*Phaseolus vulgaris* L.), and soybean (*Glycine max* Merr.)] were grown in the dark for 5 to 7 days at 27°C.

2. Chemical compounds

Pyribencarb was provided as a reagent-grade product by KI Chemical Research Institute Co. Ltd., (Shizuoka, Japan). Strobilurin fungicides: kresoxim-methyl and azoxystrobin, antimycin A, decylubiquinone and other chemicals were purchased commercially.

3. Preparation of submitochondrion fraction

Submitochondrion fractions of *B. cinerea* and *C. cassiicola* were prepared as follows. *B. cinerea* and *C. cassiicola* were grown to the stationary phase (96 hr) in 150 ml PD media at 23°C. Hyphae were then collected by centrifugation at 8,000×*g* for 15 min. After filtering mashed hyphae with a nylon gauze, 10 g hyphae were suspended in 30 ml of 20 mM 3-(N-morpholino)-2-hydroxy-propanesulfonic acid (MOPS)-KOH buffer (pH 7.1) containing 0.3 M mannitol, 0.1% (w/v) bovine serum albumin (BSA), and 1 mM EDTA, and homogenized with 20 g sea sand in a mortar at 4°C. The homogenate was centrifuged at 2,000×*g* for 10 min. The supernatant was centrifuged at 15,000×*g* for 20 min and the membrane fraction was re-suspended in 30 ml of 10 mM MOPS-KOH buffer (pH 7.1) containing 0.25 M sucrose and 1 mM EDTA. The suspension was centrifuged again at 15,000×*g* for 20 min and the resultant precipitate was re-suspended in 2 ml of 10 mM MOPS-KOH buffer (pH 7.1) containing 0.25 M sucrose, 30% (v/v) glycerol and 1 mM EDTA, and stored at −80°C.

Submitochondrion fractions of etiolated crop seedlings were prepared as follows, basically in accordance with the method of Nawa and Asahi.[9] Fifty grams of chopped etiolated seedlings were suspended in 100–150 ml of 50 mM Tris-HCl buffer (pH 7.3) containing 0.3 M sucrose, 0.1% (w/v) BSA, 1% (w/v) sodium isoascorbate and 1 mM EDTA, and homogenized with 25 g sea sand in a mortar at 4°C. The homogenate was centrifuged at 700×*g* for 10 min. The supernatant was centrifuged at 15,000×*g* for 20 min and the membrane fraction was re-suspended in 30 ml of 50 mM Tris-HCl buffer (pH 7.3) containing 0.5 M sucrose and 0.1% (w/v) BSA. The suspension was centrifuged again at 15,000×*g* for 20 min and the resultant precipitate was re-suspended in 5 ml of 50 mM Tris-HCl buffer (pH 7.3) containing 0.5 M sucrose and 0.1% (w/v) BSA, and stored at −80°C.

Submitochondrion fractions of rat liver and carp hepatopancreas were prepared as follows, basically in accordance with the method of Johnson and Lardy.[10] Five grams of chopped liver and hepatopancreas were suspended in 30 ml of 20 mM MOPS-KOH buffer (pH 7.1) containing 0.3 M mannitol, 0.1% (w/v) BSA and 1 mM EDTA, and homogenized with Teflon homogenizer at 4°C. The homogenate was centrifuged at 700×*g* for 10 min. The supernatant was centrifuged at 15,000×*g* for 20 min and the membrane fraction was re-suspended in 30 ml of 10 mM MOPS-KOH buffer (pH 7.1) containing 0.25 M sucrose and 1mM EDTA. The suspension was centrifuged again at 15,000×*g* for 20 min and the resultant precipitate was re-suspended in 3 ml of 10 mM Tris-HCl buffer (pH 7.1) containing 0.25 M sucrose, 30% (v/v) glycerol and 1 mM EDTA, and stored at −80°C.

Protein concentrations of submitochondrion suspensions were determined by the Bradford method in accordance with the manufacturer's protocol (Bio-Rad, CA, USA).

4. Enzyme assays for the electron transport activity of the respiratory chain

Succcinate-cytochrome *c* (SCR) activity reflects electron transfer from succinate to complex III *via* complex II (complex II and III activities), while decylubiquinol-cytochrome *c* (UCR) activity reflects electron transfer confined to complex III (complex III activity only). Both activities were assayed by measuring the increase of the absorbance at 550 nm resulting from the reduction of cytochrome *c*.[11] SCR reaction mixtures contained submitochondrion suspension (50–100 μg protein), 60 mM potassium phosphate (pH 7.5), 2 mM sodium azide, 50 μM cytochrome c and 20 mM sodium succinate in a final volume of 3 ml. UCR reaction mixtures contained submitochondrion suspension (50–100 μg protein), 60 mM potassium phosphate (pH 7.5), 2 mM sodium azide, 50 μM cytochrome c and 10 μM decylubiquinol in a final volume of 3 ml. Decylubiquinol was prepared from decylubiquinone in accordance with the method of Fisher *et al.*[12] Rection mixtures without submitochondrion suspension were pre-incubated for 5 min at 30°C. The reaction was started by addition of submitochondrion suspension and monitored at 30°C.

5. Inhibition studies of pyribencarb on electron transport activity of the respiratory chain

Pyribencarb and strobilurin fungicides were added to the reaction mixtures of SCR and UCR as acetone solutions. The final

concentration of acetone was under 1%. Inhibitory activity by each fungicide was represented as the fungicide concentration required for 50% inhibition (I_{50}) and the inhibition constants (Ki and Ki′). I_{50} values were determined by calculating the inhibition data by Probit analysis. Resistance/susceptible (RS) ratios for SCR inhibition were obtained from calculation using I_{50} values of the wild-type strain and the resistant strains to strobilurin fungicides. The ratios of I_{50} values for SCR inhibition of plants to that of *B. cinerea* and the ratios of I_{50} values for SCR inhibition of rat and carp to that of *B. cinerea*, were also determined by the following equation.

$$\text{Ratio} = (I_{50} \text{ values of plant, rat or carp}) / (I_{50} \text{ value of } B.\ cinerea)$$

6. Determination of nucleotide sequence of cytochrome b of B. cinerea

Genomic DNA of *B. cinerea* was isolated from hyphae using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Part of the DNA sequence of the cytochrome b gene of *B. cinerea* was amplified by PCR using the known primers (RSCBF1 and RSCBR2)[13] and sequenced. Upstream and downstream sequences were determined by genome walking using the Universal GenomeWalker Kit (BD Biosciences, San Diego, CA, USA) and DNA Walking SpeedUp Kit (Seegene, Seoul, South Korea).

Total RNA of *B. cinerea* was isolated from hyphae using the RNeasy Plant Mini Kit (Qiagen). cDNA was prepared by SuperScript II Reverse Transcriptase with an adapter primer (5′-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTT-TT-3′) (Invitrogen, Carlsbad, CA, USA).

PCR was performed in 50 μl with 5 units of Expand Hifi polymerase (Roche Diagnostics, Mannheim, Germany), 1×Expand Hifi PCR Buffer (containing $MgCl_2$), 0.2 mM dNTPs and 0.02 mM each of upstream primer BOTO-CYTB-33 (5′-GACTTTCACGCTTGCACAAG-3′, annealed on 5′-UTR) and downstream primer BOTO-CYTB-28 (5′-AATCC-GAGATACCAGTAGCG-3′, annealed on 3′-UTR), by which the whole cytochrome b sequence was covered. The PCR regime was 95°C for 30 s, 55°C for 1 min and 72°C for 2 min for 40 cycles, followed by a final extension step at 72°C for 7 min. DNA was sequenced on an ABI DNA sequencer (Model 301) by cycle sequencing using the BigDye Terminator Cycle Sequencing FS Ready Kit ver.1 (Applied Biosystems, Foster City, USA).

Results

1. Effects of pyribencarb on SCR and UCR activities of B. cinerea *and on SCR activity of* C. cassiicola

Pyribencarb inhibited SCR activity of *B. cinerea* at low concentrations, as did strobilurin fungicides (Table 1, Fig. 2). The potency of pyribencarb for the inhibition of UCR activity of *B. cinerea* was approximately 3-fold stronger than that for SCR activity (Table 1). Pyribencarb also inhibited SCR of *C. cassiicola* at low concentrations, but its potency was approximately one-fourth of that against *B. cinerea* (Table 1). SCRs

Table 1. Concentration required for 50% inhibition of SCR and UCR activities.

Mitochondrion Source	I_{50} for SCR (μM)[a]			
	Pyribencarb	Kresoxime-methyl	Azoxystrobin	Antimycin A
B. cinerea (wild type)	0.079	0.057	0.210	0.003
B. cinerea (resistant)[b]	5.100	40.000	62.000	0.002
C. cassiicola (wild type)	0.340	0.033	0.031	—
C. cassiicola (resistant)[c]	5.600	19.000	36.000	—
Cucumber	1.700	0.040	0.210	—
Tomato	5.600	0.140	1.300	—
Kidney bean	30.000	0.420	1.300	—
Soybean	5.500	0.200	1.200	—
Rat liver	1.400	0.450	0.620	—
Carp hepatopancreas	5.100	0.220	0.770	—
Mitochondrion Source	I_{50} for UCR (μM)			
	Pyribencarb	Kresoxime-methyl	Azoxystrobin	Antimycin A
B. cinerea (wild type)	0.0230	0.0042	0.0530	0.0015

[a]: I_{50} values were obtained from probit analysis of inhibition data with 5 concentrations of each chemical compound. [b],[c]: strobilurin-resistant strains with G143A mutation in cytochrome *b*.

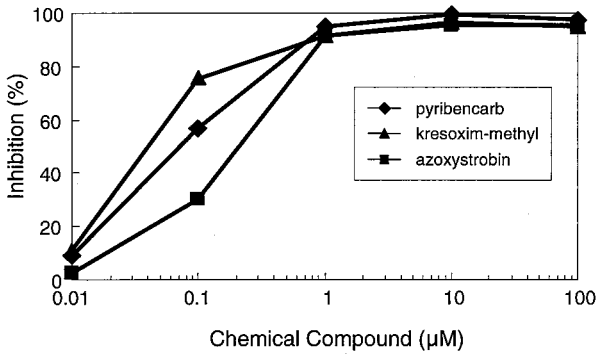

Fig. 2. Inhibitions of SCR activity of *B. cinerea* by pyribencarb- and strobilurin-type fungicides.

Table 2. RS ratios found from calculation of data shown in Table 1

Chemical Compound	RS ratio	
	B. cinerae	*C. cassiicola*
Pyribencarb	65.00	16.00
Kresoxime-methyl	700.00	580.00
Azoxystrobin	300.00	1200.00
Antimycin A	0.67	—

of strobilurin fungicide-resistant strains of *B. cinerea* and *C. cassiicola* were highly resistant to kresoxim-methyl and azoxystrobin, but less resistant to pyribencarb, with the result that the RS ratios of pyribencarb for the inhibition of SCR activities of *B. cinerea and C. cassiicola* were smaller than those of kresoxim-methyl and azoxystrobin (Table 2).

2. Kinetics for the inhibition of UCR activity of B. cinerea *by pyribencarb*

Pyribencarb inhibited the UCR activity of *B. cinerea* in an uncompetitive manner, and the substrate-dependent inhibition constant (Ki′) was found from calculation to be 13 nM (Fig. 3A). Kresoxim-methyl and azoxystrobin also inhibited it in the uncompetitive manner (Fig. 3B, 3C). The Ki′ of kresoxim-methyl and azoxystrobin was found from calculation to be 2.3 nM and 30 nM, respectively. On the other hand, the Qi inhibitor, antimycin A, inhibited it in a mixed manner, which included non-competitive and competitive components (Fig. 3D), and the substrate-independent inhibition constant (Ki) and Ki′ values were found from calculation to be 1.0 nM and 3.6 nM, respectively.

3. Effects of pyribencarb on SCR activities of crops, rats and carp

Inhibition of SCR activities of crops by pyribencarb was much weaker than by kresoxim-methyl and azoxystrobin (Table 1). The I_{50} value of pyribencarb for the inhibition of SCR activity of each crop was compared with that of *B.*

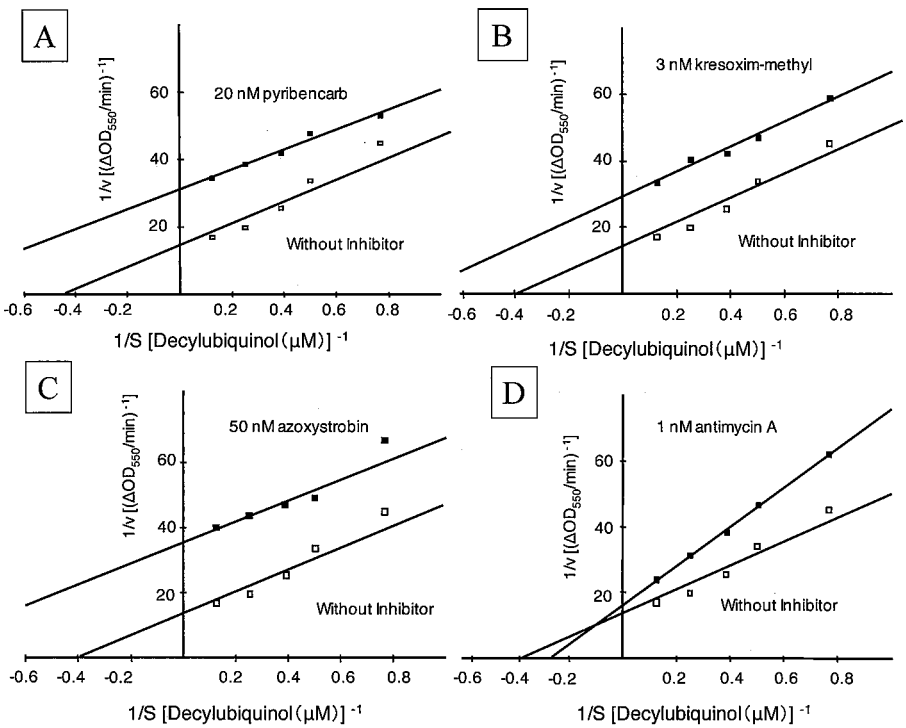

Fig. 3. Lineweaver-Burk plots for the inhibition of UCR activity of *B. cinerea* by pyribencarb and kresoxim-methyl, azoxystrobin and antimycin A with respective to decylubiquinol. A, pyribencarb; B, kresoxim-methyl; C, azoxystrobin; D, antimycin A.

Table 3. Ratios of selectivity found from calculation of data shown in Table 1

Chemical Compound	Ratio of selectivity					
	Cucumber	Tomato	Kidney bean	Soybean	Rat liver	Carp hepatopancreas
Pyribencarb	22.00	71.00	380.00	70.00	18.00	65.00
Kresoxime-methyl	0.70	2.50	7.40	3.50	7.90	3.90
Azoxystrobin	1.00	6.20	6.20	5.70	3.00	3.70

cinerea. When similar analysis was performed for kresoxim-methyl and azoxystrobin, it was found that the selectivity ratios of pyribencarb were over 10-fold greater than those of kresoxim-methyl and azoxystrobin (Table 3).

The I_{50} values of pyribencarb for the inhibition of SCR activities of rats and carp were also compared with that of *B. cinerea*. When similar analysis was performed for kresoxim-methyl and azoxystrobin, it was found that the selectivity ratios of pyribencarb were greater than those of kresoxim-methyl and azoxystrobin (Table 3).

4. Nucleotide sequence of the cytochrome b gene of B. cinerea*, and comparison of amino acid sequences of cytochrome b among fungi, crops, rat and carp*

The nucleotide sequence of the cytochrome *b* gene of *B. cinerea* was determined by genome walking of mitochondrial DNA around the region coding cytochrome b, followed by the amplification of cytochrome *b* cDNA by PCR utilizing specific primers, each annealing on 5′-UTR and 3′-UTR. The cytochrome *b* gene of *B. cinerea* consisted of four exons divided by three introns on mitochondrial DNA (Fig. 4). Each exon was 201 bp, 194 bp, 95 bp and 683 bp. The nucleotide sequences of cytochrome b of *B. cinerea* are now available from DDBJ, EMBL and GenBank databases with accession numbers of AB262969 and AB262970 for genomic DNA and cDNA, respectively. The deduced amino acid sequence of cytochrome b of *B. cinerea* consisted of 391 amino acid.

Homologies of the amino acid sequence of *B. cinerea* cytochrome *b* with other fungi, such as scab (*Venturia inaequalis*) and powdery mildew (*Blumeria graminis*, partial sequence) were 87% and 80%, respectively. On the other hand, when compared with cytochrome b of crops, such as cucumber (*Cucumis sativus*), rapeseed (*Brassica napus*), tobacco (*Nicotiana tabacum*), rice (*Oryza sativa*) and corn (*Zea mays*), homologies of the amino acid sequence of *B. cinerea* cytochrome b were 53%, 55%, 54%, 53% and 53%, respectively. Also, when compared with cytochrome b of rat (*Rattus norvegicus*) and carp (*Cyprinus carpio*), homologies were 50% and 50%, respectively (Fig. 5).

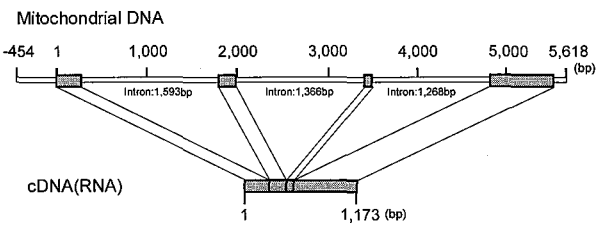

Fig. 4. Comparison of the gene structures of cytochrome b of *B. cinerea* on mitochondrial DNA and cDNA. Exons of cytochrome *b* of *B. cinerea* are shown in a gray box.

Discussion

From inhibition studies on SCR and UCR activities of *B. cinerea* and *C. cassiicola*, the target site of pyribencarb existed in complex III of the respiratory electron transport system of fungi. Three different kinds of inhibitors are known to inhibit quinone mediated electron flow in complex III. Two are inhibitors of the o-center in the quinone cycle (Qo inhibitors), and the other is an inhibitor of i-center (Qi inhibitors).[6] Representative of Qo inhibitors are kresoxim-methyl, azoxystrobin, metominostrobin and undecylhydroxy-dioxobenzothiazol. The former three fungicides are categorized as strobilurin fungicides and inhibit the oxidation of ubiquinol to ubiquinone in the o-center in the quinone cycle, which is the same as natural compounds, strobilurin A and myxothiazole A,[7,14,15] and the latter inhibits electron flow from the Rieske iron-sulfur protein to cytochrome c_1.[16] An example Qi inhibitor is cyazofamid, which inhibits the reduction of ubiquinone to ubiquinol in the i-center of the quinone cycle, like a natural compound, antimycin A.[17] Kinetic studies on the inhibition of UCR activity of *B. cinerea* by pyribencarb, kresoxim-methyl, azoxystrobin and antimycin A, suggested that pyribencarb did not bind to the same site as antimycin A, but bound in the neighborhood of kresoxim-methyl and azoxystrobin. These results indicated that the binding site of pyribencarb existed on the o-center of the quinone cycle in cytochrome b in complex III.

Pyribencarb inhibited the SCR activity of *B. cinerea* potently, while it did not inhibit the SCR activities of crops potently. The ratios of I_{50} values of pyribencarb for the inhibition of SCR activities were compared among crops and *B. cinerea*. When similar analysis was conducted for kresoxim-methyl and azoxystrobin, it was found that the selectivity of pyriben-

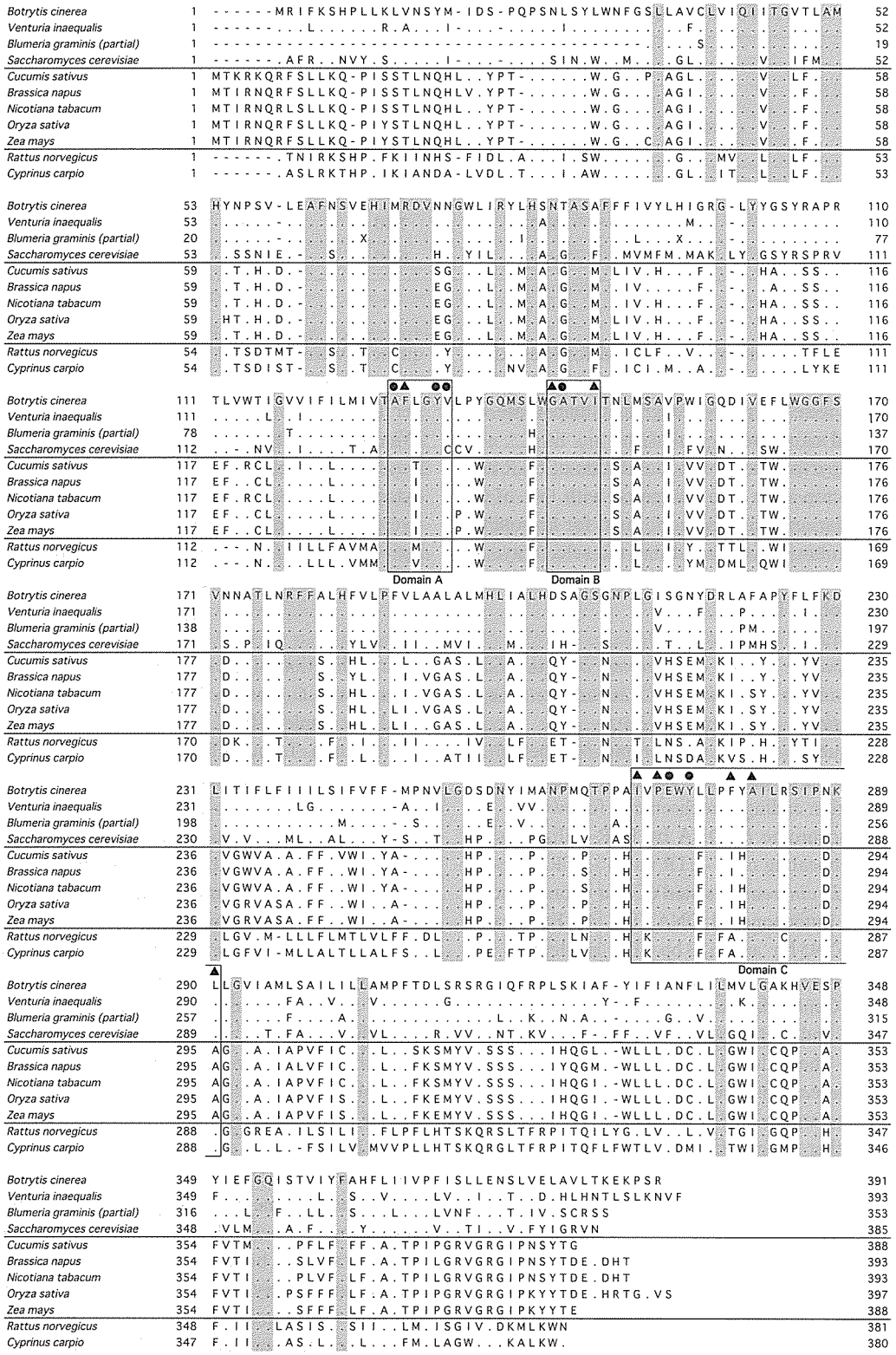

Fig. 5. Alignment of cytochrome b amino acid sequence of different fungi and plants. Amino acid sequences referred to the following DDBJ/NCBI/EMBL accession numbers: *Botrytis cinerea*, AB262969; *Venturia inaequalis*, AF047029; *Blumeria graminis* (partial sequence), AF343442; *Saccharomyces cerevisiae*, AJ011856; *Cucumis sativar*, AF288044; *Brassica napus*, AP006444; *Nicotiana tabacum*, NTU67396; *Oryza sativa*, X53710; *Zea mays*, X00789; *Rattus norvegicus*, AF295545; *Cyprinus carpio*, X61010. The same amino acids with *B. cinerea* are depicted by dots. Amino acids highlighted in gray are identical. Rsidues interacting with the toxophore of azoxystrobin are marked by black circles. Residues interacting with the carrier of azoxystrobin are marked by black triangles. Domain A, B and C are boxed.

carb at the target site between *B. cinerea* and crops was over 10-fold higher than those of kresoxim-methyl and azoxystrobin. Also, the ratios of I_{50} values of pyribencarb, kresoxim-methyl and azoxystrobin for the inhibition of SCR activities were compared among rats, carp and *B. cinerea*. Consequently, it was found that pyribencarb expressed higher selectivity at the target site of rats and carp than kresoxim-methyl and azoxystrobin. It is known that kresoxim-methyl and azoxystrobin have little selectivity at the target site;[18] therefore, pyribencarb was considered to be a Qo inhibitor of cytochrome b, whose properties were superior to the well-known Qo inhibitor fungicides in terms of selectivity at the target protein.

In the present paper, we determined the nucleotide sequences of the cytochrome b gene of both the mitochondrial genome and cDNA of *B. cinerea*, and its amino acid sequence was predicted. According to a previous paper by Gisi *et al.*,[14] it is considered that Qo inhibitor fungicides interact with mainly three binding regions of yeast cytochrome b, namely domain A, B and C (Fig. 6). Among these regions, the carbonyl group of the toxophore of chemical compounds (Fig. 1) binds with a hydrogen bond to the amide group of glutamic acid at position 272 in domain C; therefore, this region plays an important role in accepting fungicides. Since substitution of glycine for alanine at position 143 (G143A) in domain B significantly lowers the affinity of strobilurin fungicides to cytochrome b,[7,8,13,14,19–23] interaction between domain B and strobilurin fungicides must be strong. On the other hand, substitution of phenylalanine for leucine at position 129 (F129L) in domain A has been shown not to significantly lower the affinity of strobilurin fungicides to cytochrome *b*.[14,21] This suggests that interaction between domain A and strobilurin fungicides might be rather weak. The result obtained here that strobilurin fungicides inhibited SCR activities of crops, rats and carp rather potently, which have different amino acid residues from those of fungi at position 130 in domain A (Fig. 6), indicated that the amino acid change at position 130 did not affect the affinity of strobilurin fungicides to domain A. This result might support a weak interaction between domain A and strobilurin fungicides; therefore, strobilurin fungicides were presumed to interact predominantly with domain B and C. On the other hand, since strobilurin fungicide-resistant strains of *B. cinerea* and *C. cassiicola*, which have G143A mutation, did not express high resistance to pyribencarb as shown above, affinity of pyribencarb to domain B might be rather low compared with strobilurin fungicides. Therefore, the different affinities to domain B between pyribencarb and strobilurin fungicides are supposed to make pyribencarb unique in terms of the efficacy for resistant fungal strains of Qo inhibitors, which have G143A mutation in domain B of cytochrome *b*.[5] Judging from the weak inhibition of pyribencarb to SCR activities of crops, rats and carp, the amino acid change at position 130 appeared to lower affinity of pyribencarb to domain A of cytochrome *b*. However, further studies will be needed to elucidate contribution of domain A for binding of pyribencarb to cytochrome b, because the amino acid (leucine) at position 130 has been shown not to interact directly with the toxophore and carrier of strobilurin fungicides (Fig. 6).[14] Crystallographic studies, as in L. Esser *et al.*,[24] provide good resolution of the contribution of domain A for the binding of pyribencarb.

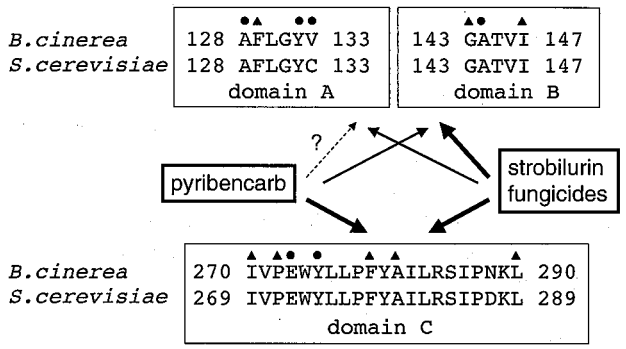

Fig. 6. Schematic view of the supposed interactions between each domain of cytochrome *b* and pyribencarb along with the strobilurin fungicide. The partial amino acid sequences of domain A, B and C of *B. cinerea* and *S. cerevisiae* are depicted. Residues interacting with the toxophore of azoxystrobin are marked by black circles. Residues interacting with the carrier of azoxystrobin are marked by black triangles. Arrows with bold, thin and dashed lines show strong, medium and uncertain interactions, respectively.

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英文編掲載報文・短報等の要旨

総　説

尿中代謝物の生物学的モニタリングによるピレスロイド系殺虫剤のヒト集団曝露量分析と評価について

上山　純，斎藤　勲，上島通浩

現在，ヒトにおける低用量のピレスロイド系殺虫剤曝露に関するリスク評価は，一般には食事からの残留農薬摂取量評価により行われている．ヒトを対象とした研究から得た個人曝露レベルに関する情報は少ないが，それはピレスロイド曝露の生物学的モニタリングが容易でないことに理由の一端がある．この障壁は近年，分析化学の進歩により克服されるようになった．高速液体クロマトグラフ質量分析計やガスクロマトグラフ質量分析計を用いることにより，ピレスロイド系殺虫剤の尿中代謝物の分離及び高感度の定性・定量を，今日では確実に行うことができる．本総説では，尿中ピレスロイド代謝物の生物学的モニタリングについて全体像を提示するとともに，一般生活者集団における尿中代謝物量に関する報告を整理する．そして，環境衛生学領域におけるこのモニタリング研究の将来展望について述べる．

報　文

新規殺菌剤ピリベンカルブの作用機構と選択性

片岡　智，高垣真喜一，角　康一郎，清水　力

ピリベンカルブはキュウリ灰色かび病菌呼吸鎖電子伝達系のコハク酸－チトクローム還元酵素（SCR: 複合体IIおよびIIIの活性）およびデシルユビキノール－チトクロームc還元酵素（複合体IIIの活性）を低濃度で阻害した．デシルユビキノールの濃度を変化させたときの阻害形式はアンチマイシンAとは異なりQo阻害剤のストロビルリン系殺菌剤と同様に不拮抗型であった．そしてこのときのピリベンカルブの阻害定数は13 nMであった．したがって，ピリベンカルブの作用点は複合体IIIのチトクロームb上のQoサイトであると判断された．一方，ピリベンカルブの植物，ラットならびにコイのSCR阻害は弱く，生物種間で選択性が認められた．この選択性は供試したストロビルリン系殺菌剤よりも優れたものであった．また，ピリベンカルブはストロビルリン系殺菌剤耐性となるチトクロームbの変異をもつキュウリ灰色かび病のSCRを比較的低濃度で阻害したことから，ピリベンカルブとストロビルリン系殺菌剤のチトクロームbへの結合の仕方は少し異なると考えられた．そこで，キュウリ灰色かび病菌のチトクロームb遺伝子の核酸配列を明らかにして，推定アミノ酸配列を他のカビ類，酵母，作物，ラットならびにコイの配列と比較することで，ピリベンカルブのチトクロームb上の結合部位について考察した．

数種類の幼植物に対する^{14}C標識ヘプタクロルの生物利用性

林　靖，亀代麻衣子，佐藤　清

数種類の植物に対するヘプタクロルの生物利用性を調査する目的で試験植物の根部を放射性標識ヘプタクロル含む水耕液中に72時間処理した．処理した植物の根および地上部に存在する放射性残留物の量とその特性は根部濃縮係数（RCF）および蒸散流濃縮係数（TSCF）値を算出することで調査した．RCFおよびTSCF値は，それぞれ126（トウモロコシ）から4086（レタス）および0.052（白菜）から0.494（ズッキーニ）の範囲であった．カボチャやズッキーニでは未代謝のヘプタクロルが蒸散流を経由して根部から地上部へ移行し，更なる主要な代謝は認められなかった．シス-ヘプタクロルエポシキドはトマト，キャベツ，白菜およびレタスの主要な代謝物であった．シス-ヘプタクロルエポシキドの更なる代謝は，これら植物の地上部において顕著に認められなかった．

茨城県桜川流域における水稲用農薬と主要な代謝分解物の挙動

岩船　敬，稲生圭哉，堀尾　剛，岩崎亘典，横山淳史，永井孝志

茨城県県内の水稲栽培地域を流下する桜川において，2007年および2008年の主要な11種の代謝分解物を含む39種の水稲用農薬のモニタリングを行った．河川水中での農薬の検出時期は流域内での散布時期に対応しており，一時期に広範囲で散布される除草剤では検出ピークが顕著に見られた．県内での出荷量が多く，水溶解度が高い，土壌吸着定数（Koc）が小さい，または普及率が高い除草剤の検出ピークは比較的高濃度であった．近年使用量が増加している育苗箱施用の殺菌剤と殺虫剤は移植直後に検出ピークが確認された．農薬の代謝分解物の検出は水や土壌中での親化合物の分解経路や半減期，または代謝分解物の安定性に対応しており，分解が非常に速い農薬から生成される主要な代謝分解物の中には，親化合物に比べてはるかに高い