Fungicidal Activity of Mercury Compounds Co-existing With Protein in Rice Plant

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イネ体内でたんぱく質と共存する水銀化合物の抗菌性

達　山　和　紀

Introduction

A number of experiments have been attempted to make clear the effects of mercury compounds on the control of rice blast disease from chemotherapeutic view point (16). Mercury compounds were absorbed and translocated easily by rice plant and proved to be quite effective as the control agents of rice blast (8, 9, 10, 14, 17). Considering the results of these observations, the chemotherapeutic action of mercury compounds seems to be direct to the causal fungus in rice plant. On the other hand, the fungicidal activity of the compounds is inactivated by mixing with the macerated juice of rice tissues (1, 7, 15, 18, 19). And it has been clearly shown by some investigators that inactivation was due to the formation of the mercaptide of mercury group with thiol-group of protein (18, 10). This fact seems to be contradictory to the prominent chemotherapeutic effects of the mercury compounds in the plant.

One of the well-known hypotheses on the chemotherapeutic action of mercury compounds is as follows (6) : When the causal fungus invades the plant tissue and make contact with mercury which have been absorbed and have combined with the plant components probably with the rice protein, the mercury getting free from the rice protein combine with fungal components. After the mycelial growth of the fungus is prevented, disease development is checked. This is an interesting idea, but in order to prove the hypothesis, fungicidal action of mercury co-existing with protein should be examined elaborately. The present study was designed to secure information in relation to this problem.

I) Antifungal activity of protein solution which was dialyzed against mercury chloride solution

Mercury compounds were supplied to protein solutions through cellulose membrane in the present investigations, for, the chemicals which have been applied to the plant in field, are probably diffused successively from the outside of the treated plant to the inner tissues. Cellulose tube with 2 ml crude protein solution from macerated juice of rice seedlings was suspended in a beaker of mercury chloride solution, and the outer liquid was exchanged in every 24 hours. After this procedure, the solution was dialyzed again for 24 hours against running water to remove the mercury un-combined with protein. Antifungal activity of the dialyzed solution was assayed with slide-germination method, and conidial germination and growth of germ-tube of *Cochliobolus miyabeanus* were recorded.

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Antifungal activities of the dialyzed crude protein solutions were shown in Table 1. The protein, mixed with the mercury chloride solutions, did not show antifungal activity at any concentration of mercury chloride, but got an antifungal activity when the liquid was dialyzed more than 24 hours against mercury chloride solution. Then, pure albumin from bovine serum was used in place of the crude protein. Table 2 and 3 show the experimental results carried out on the antifungal activity of the protein solutions dialyzed under various combinations and concentrations of protein and mercury chloride. The antifungal activity of dialyzed solution was under the control of total quantity of mercury chloride which had been made contact with protein. When sufficient time for the dialysis is allowed, protein solution gets an antifungal activity by the dialyses against mercury chloride solution even at low concentration of mercury chloride.

Table 1. Conidial germination of *Cochliobolus miyabeanus* in crude protein solutions from rice seedlinge dialyzed against mercury chloride solutions

Conc. of mercury chloride	Dialyzed time (hours)				
	0※	24	48	72	120
10^{-4} M	###※※	−	−	−	−
¼ × 〃	###	+	±	−	−
$¼^2$ × 〃	###	###	###	###	##
$¼^3$ × 〃	###	###	###	###	###
0	###				

※ Means the protein solutions mixed with concentrated mercury chloride solution to give the final concentrations just before the dialyses against running water.

※※ Length of germ tube　###……above 200 μ
##……100 μ to 200 μ　+……20 μ to 100 μ
±……20 μ and less　−……non-germination

Table 2. Conidial germination of *C. miyabeanus* in albumin solutions dialyzed against ¼ × 10^{-4} M mercury chloride solutions

Conc. of albumin	Dialyzed time (hours)				
	0	24	48	72	120
16 mg/ml	###※	##	+	±	±
4 〃	###	±	±	−	−
1 〃	###	−	−	−	−
¼ 〃	+	−	−	−	−

※ Length of germ tube : see Table 1

Table 3. Conidial germination of *C. miyabeanus* in 4 mg/ml albumin solutions dialyzed against mercury chloride solutions at different concentrations

Cone. of mercury chloride	Dialyzed time (hours)				
	0	24	48	72	120
10^{-3} M	±※	−	−	−	−
10^{-4} M	+	−	−	−	−
¼ × 〃	###	±	−	−	−
$¼^2$ × 〃	###	##	+	±	−
$¼^3$ × 〃	###	###	###	##	+

※ Length of germ tube : see Table 1

II) Antifungal activity of protein solution which was dialyzed against solution of metallic compound

Solutions of several metallic compounds were used as outer liquids in place of mercury compound solution. Albumin solutions were dialyzed for 24 hours against silver nitrate or some metallic compound solutions at the concentration of 10^{-4}M, and antifungal activity of the dialyzed solutions was examined.

There was close correlation between poisoning owing to the metallic compounds and antifungal activity of the dialyzed solution (Table 4), but, silver, copper or chromiun, as well as mercury, lose their antifungal activity forming the mercaptide of metallic compounds with thiol-group of the protein easily, besides, metallic compounds uncombined with protein do not exist in the dialyzed solutions after the dialyses against running water. Therefore, the antifungal activity of the protein solution was probably due to the action of metallic compounds adsorbed to the protein, but not due to the

action of metallic compouds combined with thiol-group of the protein.

Ⅲ) Antifungal activity of precipitate contained in the dialyzed solution

When metallic compound was supplied to protein solution more than enough to combine with the thiol-group of the protein, the metallic compound will be adsorbed to the protein and become insoluble to water. The fungicidal action of the dialyzed solution may be related to the antifungal activity of the precipitate. The following experiment was carried out to make clear this point.

Table 4. Conidial germination of *C. miyabeanus* in 4 mg/ml albumin solutions dialyzed for 24 hours against several metallic compounds solutions at a concentration of 10^{-4} M

Metallic compounds	Serial dilutions of the dialyzed solutions				
	1	1/4	$1/4^2$	$1/4^3$	$1/4^4$
$HgCl_2$	−※	−	−	±	+
$AgNO_3$	−	−	−	±	±
$CuSO_4$	−	−	±	++	+++
CrO_3	+	++	++	+++	+++
$CdCl_2.2\frac{1}{2}H_2O$	++	+++	+++	+++	+++
$Pb(NO_3)_2$	+++	+++	+++	+++	+++
$Ni(NO_3)_2.6H_2O$	+++	+++	+++	+++	+++
$FeCl_3$	+++	+++	+++	+++	+++
$MnCl_2$	+++	+++	+++	+++	+++

※ Length of germ thbe : see Table 1

Solution of 4 mg/ml albumin was dialyzed against $1/4 \times 10^{-4}$ M solution of mercury chloride. After the dialysis against running water, the solution was centrifuged at 12,000 rpm for 30 minutes to separate the precipitate from the solution. The supernatant was poured off, and the precipitate was washed thoroughly and resuspended in distilled water, and antifungal activity of the suspension was examined. Conidia of *C. miyabeanus* germinated normaly in the supernatant, but did not germinate in the suspension of the precipitate. Judging from the experimental results, the antifungal activity of the dialyzed solutions was surely due to the antifungal action of the precipitate.

Ⅳ) Behavior of the mercury adsorbed to protein

The following experiment was carried out to make clear the mechanism of antifungal action of the precipitate.

If the precipitate was made contact with thiol-rich compounds, the adsorbed mercury, probably, getting free from the protein, combine with the thiol-group of the chemicals. After albumin powder was added to the antifungal suspension and mixed well, the antifungal activity was examined after 30 minutes of mixing.

Mixing with albumin powder, the suspension became transparent, and their fungicidal activities dropped down (Table 5). As the mercury which had been adsorbed to the

Table 5. Conidial germination of *C. miyabeanus* in the antifungal suspensions mixed with albumin powder

Final albumin conc. in the mixture	Antifungal suspensions			
	Made from 4 mg/ml albumin		Made from 1 mg/ml albumin	
	Before addition	After addition	Before addition	After addition
40 mg/ml	−※	++	−	+++
20 〃	−	+	−	++
10 〃	−	+	−	+
5 〃	−	−	−	±

※ Length of germ tube : see Table 1

Table 6. Conidial germination of *C. miyabeanus* in the antifungal suspensions boiled for 5 minutes

Water	Antifungal suspensions			
	Made from 4 mg/ml albumin		Made from 1 mg/ml albumin	
	Before boiling	After boiling	Before boiling	After boiling
＋＋※	−	＋	−	＋＋

※ Length of germ tube : see Table 1

precipitate, probably combined with thiol-group of the added albumin, the suspension sbecame transparent and lost their antifungal activities at the same time.

Antifungal activity of the suspension dropped down also by boiling for 5 minutes (Table 6).

V) **Action of the adsorbed mercury when the mercury has made contact with fungal body**

If the adsorbed morcury, getting free from the precipitate, combine easily with thiol-group of fungal components in the living plant, the mereury will play an important role on the disease control effects. Action of the adsorbed mercury, which had been made contact with fungal body, was examined in the following experiment.

Dialyzed albumin solution was centrifuged at 2,500 rpm for 5 minutes, and the supernatant was used as antifungal solution, because, in the following procedure, the solution will be centrifuged under the same condition before the starting of the antifungal test. The supernatant have kept the antifungal activity, for, some amount of antifungal substances, which had not been precipitated, remained in the supernatant. 2 ml of the supernatant were poured into test tube, in which *C. miyabeanus* had been cultured for two weeks on potato sucrose agar. After 30 minutes the supernatants were centrifuged at 2,500 rpm for 5 minutes to remove the suspended fungal bodies.

Antifungal activity of the supernatant dropped down in contacting with the fungal bodies as shown in Table 7. These experimental results suggest that, the adsorbed mercury, getting free from the rice protein, combine easily with fungal components, when the causal fungus invades the plant tissues.

Table 7. Conidial germination of *C. miyabeanus* in the antifungal supernatants made contact with fungal body

Water	Antifungal supernatants			
	Made from 4 mg/ml albumin		Made from 1 mg/ml albumin	
	Before contact	After contact	Before contact	After contact
＋＋※	−	±	±～−	＋

※ Length of germ tube : see Table 1

Discussion

Substantial understanding of the fungicidal mechanism is possible only when one can give an insight to the mutual relations among the fungicides, suscept cells, and fungus concerned (16). As fungicidal activity of mercury compounds is closely related to protein in the rice

plant (1, 15, 18, 19), fungicidal activity of mercury co-existing with protein was examined in the present investigation.

Protein solutions get antifungal activity by dialyzing against mercury chloride solution. The dialyzed solutions against silver nitrate, copper sulfate or chromium trioxide solution also get the antifungal activity. The antifungal activity was probably due to the mercury adsorbed to protein. Further experiments should be carried out on the mechanism of adsorptions, however, the mercury may have been adsorbed to protein electrostatically. When thiol-rich substance made contact with the adsorbed mercury, the mercury getting free from the protein combine with the thiol-group of the substance. Dropping of the antifungal activity of the precipitate shown in Table 6 also was due to combining of the mercury with the exposed thiol radicals of the protein by boiling. When protein is boiled or treated with some chemicals, intramolecular masked radicals are exposed as the results of unfolding or unaggregating of protein molecules (4, 5, 11).

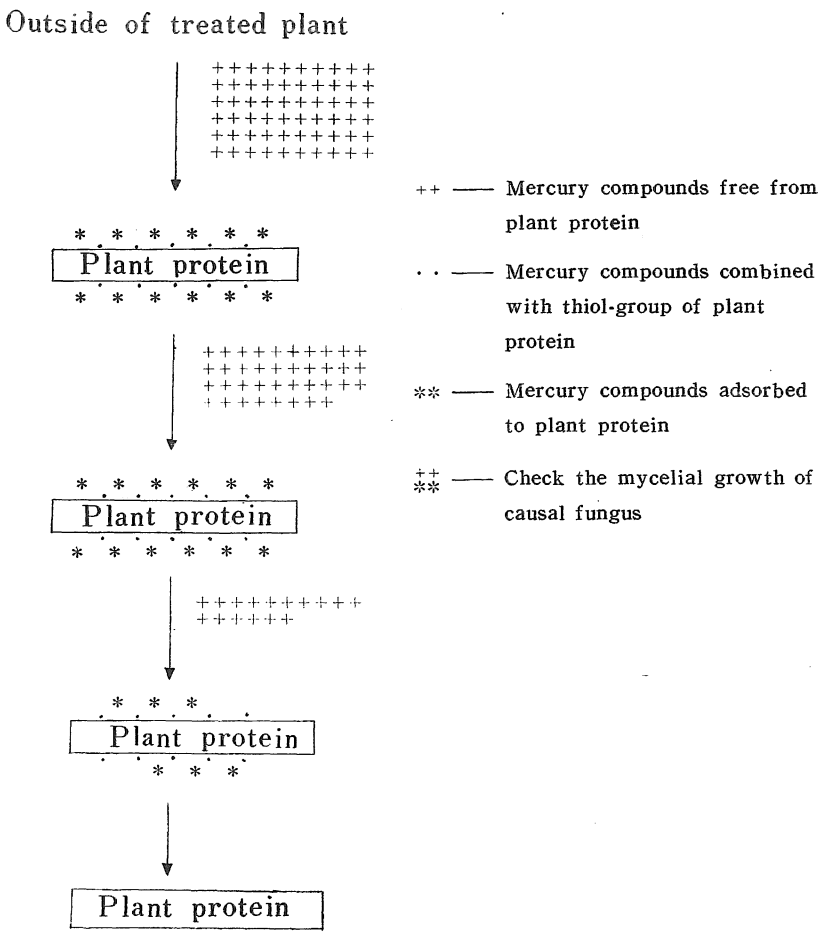

Fig. 1 Diffusion of mercury compounds in living plant

Table 2 and 3 suggest that if mercury compouds are diffused successively from the outside of the plant to the inner cells or tissues, the antifungal precipitate will be formed gradually in the plant. Mercury compounds are observed as particles scattered in plant tissues when the compounds were supplied to plant (9, 20). The writer can not tell whether the particles observed in the plant tissues are the same substance with the antifungal precipitate or not.

Mercury poisoning of cells is due to inactivation of enzyme system through reaction between mercury and thiol-group which form biologically inactive complex (2, 3, 12, 13). If mercury combine mainly with thiol-group of protein concerned in the constructions of plant cells or tissues without remarkable injury to the plant before making contact with enzyme system of plant cells, besides, in those places, the mercury are adsorbed to the protein, the adsorbed mercury will play an important role on the disease control effect as suggested in Table 7. Diffusion of mercury comnounds in treated plant is illustrated schematically in Fig. 1.

Summary

Since fungicidal activity of mercury compounds applied to the plant against pathogen is closely related to the protein in the plant, the fungicidal activity of mercury chloride co-existing with protein has been examined to make clear the disease control action of the compounds.

Mercury compounds were supplied to protein solutions through cellulose membrane in the present investigations, for, the chemicals which have been applied to the plant in field are probably diffused successively from the outside of the treated plant to the inner tissues. Protein solutions got an antifungal activity when mercury compouds were supplied to the protein solutions more than enough to combine with the thiol-group of the protein. The antifungal activity was probably due to the action of mercury compounds adsorbed to the protein, but not due to the action of mercury compounds combined with the thiol-group of protein.

Mercury compounds adsorbed to plant protein may play an important role on the disease control effect, for, the adsorbed mercury combine with thiol-group of the protein of causal fungus easily.

摘　　要

植物疾病防除のために植物に施用される水銀剤の抗菌力は，植物体内のたんぱく成分と密接な関係があるので，水銀剤の疾病防除作用を明らかにするために，たんぱく質と共存している状態での水銀剤の抗菌性について実験を行なった．

植物体に施用された水銀剤は，その植物の外表から内部組織へむかって徐々に侵透するものと思われるので，本実験においては透析膜を用いて水銀を継続的にたんぱく質と接触させた．すなわち，たんぱく液を塩化第二水銀溶液中で透析したが，たんぱく質がそのチオール基を満たしてなお過剰の水銀との接触が行なわれた場合，たんぱく液は抗菌性を得た．この抗菌性はチオール基と結合している水銀によるものではなく，たんぱく質に吸着されている水銀によるものと思われた．この水銀は病原菌菌体中のたんぱく質と容易に結合するので，植物体内での疾病防除効果に重要な役割を持つものと考えられる．

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