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Mechanism of action of *Pseudomonas syringae* phytotoxin, syringomycin. Interaction with the plasma membrane of wild-type and respiratory-deficient strains of *Saccharomyces cerevisiae*

Lei Zhang [a] and Jon Y. Takemoto [b]

Departments of [a] Chemistry and Biochemistry and [b] Biology, Utah State University, Logan, UT 84322-0300 (U.S.A.)

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The effects of the phytotoxin, syringomycin, produced by *Pseudomonas syringae* pv. *syringae*, were examined on cells of a wild-type and a respiratory-deficient (*rho*^{0}) mutant of *Saccharomyces cerevisiae*. The growth of both strains in liquid culture was inhibited by 0.5 μg syringomycin per ml and higher. Uptake rates of tetraphenylphosphonium and dimethyloxazolidine ions in cell suspensions of both strains increased when 1.5 μg per ml syringomycin was added. These responses were kinetically and quantitatively similar in the two strains and indicated increases in electrical potential (cell interior negative) and pH differences (cell interior alkaline) across the plasma membrane. Glucose (0.1 M) enhanced the effect on the electrical potential, was required for the pH changes, and increased the cellular ATP levels. These results show that the effects of syringomycin are energy-dependent and are due to alterations of plasma membrane and not to mitochondrial function.

The phytotoxin syringomycin is a small peptide-containing molecule produced by the phytopathogen, *Pseudomonas syringae* pv. *syringae* [1–3]. Syringomycin is a virulence factor in several plant diseases and affects fungi as well as plants [4–7]. Previous observations suggested that the plasma membrane is a cellular target site of syringomycin [8,9]. Another investigation on mitochondria isolated from etiolated maize shoots [10] showed that syringomycin works as an uncoupler of oxidative phosphorylation. These studies show that syringomycin affects membranes. However, it is not clear which membrane is primarily affected and what is the mechanism of membrane dysfunction.

In a recent report [11], we described several observations which suggest that in the fungus *Rhodotorula pilimanae*, the plasma membrane is a primary site of syringomycin action. In this organism, the toxin alters the electrical potential and the pH difference across the membrane and stimulates the proton pump ATPase. However, *R. pilimanae* is a strict aerobe and a possible role for mitochondria in the responses to syringomycin cannot be determined from whole cell studies. In this report, we examine the effects of syringomycin on cells of a wild-type and a respiratory-deficient mutant of *Saccharomyces cerevisiae*, a facultative aerobe. The electrical potential and pH gradient changes were measured using the permeable molecules, tetraphenylphosphonium (TPP) and dimethyloxazolidine (DMO), respectively [12]. The results show that syringomycin affects the plasma membrane electrical potential and pH gradient in both the wild-type and mutant strains of *S. cere-*

Abbreviations: TPP, tetraphenylphosphonium; DNO, dimethyloxazolidine.

Correspondence address: Dr. J.Y. Takemoto, Department of Biology, Utah State University, Logan, UT 84322-0300, U.S.A.

visiae as with *R. pilimanae*, and that the effects are related to the cellular ATP levels. We conclude that mitochondria are not involved in these responses to syringomycin.

S. cerevisiae D273-10B/Al (rho^+) and a respiratory-deficient petite mutant derivative (rho^0) lacking mitochondrial DNA [13] were provided by Dr. A. Tzagaloff (Columbia University). They were grown in PDB medium (24 g Difco potato dextrose in 1 liter) at 30°C with rotary shaking (100 rpm) in a New Brunswick G76 shaker. When an absorbance of 3–4 measured at 600 nm (Bausch and Lomb Spectronic 20 photometer) was attained, the cells were harvested by centrifugation at 3000 *g* for 10 min, washed twice with distilled water, and then used immediately for TPP and DMO uptake and cellular ATP measurements. Oxygen uptake rates in the rho^0 mutant were 70-fold lower than in the rho^+ strain. *R. pilimanae* was grown and harvested as previously described [11].

Methods for determining TPP and DMO uptake and cellular ATP levels were described in a previous paper (11). Cells were suspended in 2 mM Tris-Mes buffer (pH 6.5) to an absorbance (600 nm) of 1.0 (about 0.7 mg cell dry wt. per ml). When appropriate, glucose was added to a final concentration of 0.1 M at the beginning of the measurements. Syringomycin was added from a stock solution (20 mg per ml H_2O) to a final concentration of 1.5 μg per ml. The reactions were started with the addition of cells.

The effects of syringomycin on growth of *S. cerevisiae* cells were measured using 25 ml cell suspensions in PDB medium and in 250 ml Erlenmeyer flasks. The flasks were incubated at room temperature on a rotary shaker (70 cycles per min). Growth was monitored spectrophotometrically at 600 nm.

Syringomycin purified from *P. syringae* pv. *syringae* strain B301D was provided by R.C. Bachmann of this laboratory.

All experiments were repeated at least three times with similar results. Representative results from single experiments are presented.

Syringomycin, added at cell inoculation, inhibited the growth of the wild-type (rho^+) and respiratory mutant (rho^0) strains to the same extent. At 0.5 μg per ml, both began to show decreases in growth rates after 5 h following syringomycin addition. At 2 μg per ml growth was completely inhibited. These levels were similar to those that inhibited growth of *R. pilimanae* [11].

The membrane-permeable cation, TPP, was used to monitor the electrical potential change across the plasma membrane. Syringomycin (1.5 μg per ml) stimulated the cellular uptake of [^{3}H]TPP by both strains of *S. cerevisiae* (Fig. 1A and B). The kinetics of stimulation were the same for the two strains, and by 50 min after exposure to the toxin, the amount of TPP accumulated was 4- to 9-fold higher with syringomycin than without it. With *R. pilimanae*, maximum uptake was attained in 20 min which was 10 to 15-fold higher than without syringomycin (Fig. 1C) [11].

Addition of 0.1 M glucose to the incubation medium stimulated syringomycin's effect on TPP accumulation in both strains of *S. cerevisiae* (Fig. 1A and B) . The TPP uptake increased 10- to 20-fold in both strains (by 50 min) with syringomycin and glucose. Without syringomycin, glucose increased the TPP uptake rate in both strains only 20 to 40%. In contrast, glucose decreased syringomycin's stimulatory effect on TPP uptake in *R. pilimanae* (Fig. 1C).

To assess changes in pH across the plasma membrane, the rates of [^{14}C]DMO uptake were measured. Without glucose, syringomycin had little effect on DMO uptake in either strain of *S. cerevisiae* (Fig. 2A and B). However, with glucose, syringomycin stimulated rates of DMO uptake in both cases 7- to 9-fold, reaching a maximum in about 40 min. This indicated that the cytoplasm became more alkaline with syringomycin addition. Without syringomycin, a slightly lower rate of DMO uptake was observed with the addition of glucose. With *R. pilimanae*, syringomycin markedly increased the rate of DMO accumulation in the absence of glucose (Fig. 2C) (11), and adding glucose did not enhance the syringomycin-stimulated uptake of DMO. A significant efflux occurred approx. 30 min after syringomycin exposure.

The influence of glucose on the ATP levels of these cells were determined (Table I). Adding glucose to *R. pilimanae* did not significantly change the cellular ATP level within 20 min, but the ATP levels later decreased. In contrast, adding

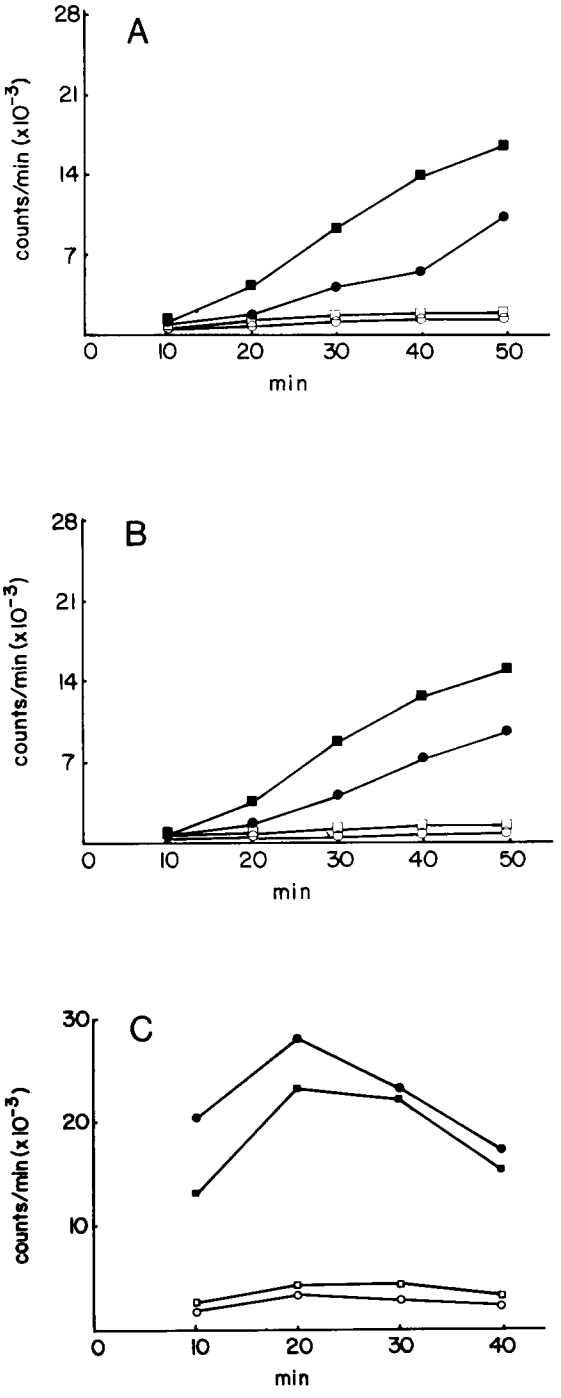

Fig. 1. The effects of glucose and syringomycin on TPP uptake by cell suspensions. Syringomycin was added at concentration of 1.5 μg/ml (●; ■) in the presence (■; □) and in the absence (●; ○) of 0.1 M glucose. (A) *S. cerevisiae* (*rho*$^+$); (B) *S. cerevisiae* (*rho*^{0}); (C) *R. pilimanae*.

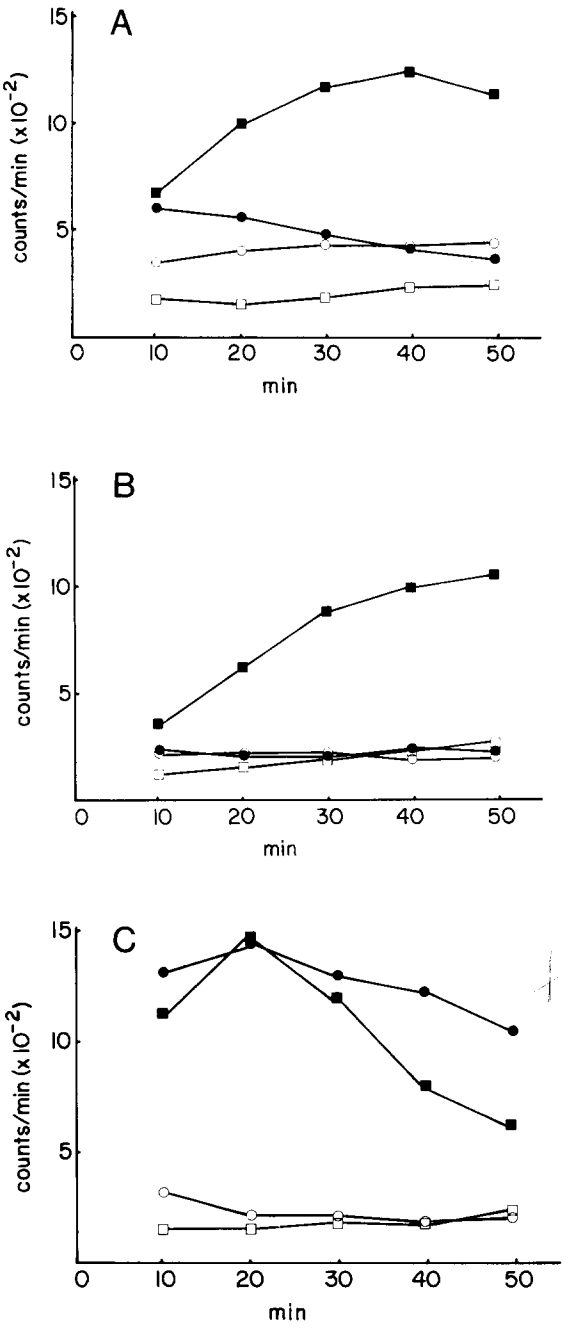

Fig. 2. The effects of glucose and syringomycin on DMO uptake by cell suspensions. The symbols and lines are the same as those used in Fig. 1.

glucose increased the cellular ATP levels of both *S. cerevisiae* strains. The absolute values of cellular ATP concentration varied between experi-

TABLE I

THE EFFECT OF GLUCOSE ON CELLULAR ATP LEVELS IN *R. PILIMANAE* AND *S. CEREVISIAE*

Cells were incubated with or without 0.1 M glucose for 50 min. The values are expressed in nmol per mg cell dry weight. n.d., not determined.

Expt.	ATP level					
	R. pilimanae		*S. cerevisiae* (*rho*$^+$)		*S. cerevisiae* (*rho*^{0})	
	−glucose	+glucose	−glucose	+glucose	−glucose	+glucose
1	10.38	3.92	6.22	8.92	5.8	10.16
2	7.59	5.74	4.85	8.37	2.43	4.11
3	9.53	8.05	5.92	9.10	0.00	5.99
4	n.d.	n.d.	n.d.	n.d.	2.35	3.72

ments, but glucose consistently caused these changes (Table I). Syringomycin addition did not alter the ATP levels in either strain of *S. cerevisiae*.

The results show that the two strains respond identically to the toxin. The growth of both was inhibited by less than 2 μg per ml of syringomycin, and both showed kinetically and quantitatively similar increases in TPP and DMO uptake with the same amount of syringomycin. Thus, the effects of syringomycin on cellular membrane potential and proton efflux were not due to disturbances of mitochondrial function. We conclude that syringomycin has a primary effect on the plasma membrane of *S. cerevisiae*, as well as *R. pilimanae*, which results in an increased membrane potential (cytoplasm negative) and pH difference (cytoplasm alkaline) across this membrane. It is very likely that these effects are coupled to the stimulation of the plasma membrane ATPase proton pump [11].

The effects of glucose further support our idea [11] that the syringomycin effects are energy-dependent and are related to cellular ATP levels. The addition of glucose to *S. cerevisiae* cells elevated ATP levels. It appears that the increased cellular concentration of ATP increased the capacity to pump protons via the ATPase when syringomycin was added. This response is consistent with the observations on *R. pilimanae* ATP levels. ATP levels decreased after the addition of glucose, and the stimulation of TPP and DMO uptake by syringomycin was concomitantly decreased.

Our work raises the question of whether the mitochondria are primary sites of syringomycin action in plants infected with *P. syringae* [10]. In addition, it should be determined whether plant cells and fungi exhibit similar primary responses to this toxin.

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