Inhibition of Microbial Cell Wall Synthesis by Lipopeptin A

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Lipopeptin A is a selective inhibitor of *in vitro* peptidoglycan synthesis of *E. coli* Y-10. In the study here it inhibited the formation of lipid intermediates from UDP-[U-¹⁴C]GlcNAc and UDP-MurNAc-L-Ala-D-Glu-*meso*-DAP-D-Ala-D-Ala, but did not inhibit the formation of MurNAc-pentapeptide-*p-p*-lipid from UDP-MurNAc-L-Ala-D-Glu-[³H]*meso*-DAP-D-Ala-D-Ala. Lipopeptin A also did not have a significant effect on polymerase reaction. Therefore, the inhibition of the formation of GlcNAc-MurNAc-pentapeptide-*p-p*-lipid from MurNAc-pentapeptide-*p-p*-lipid and UDP-GlcNAc is concluded to be the site of action.

Lipopeptin A inhibits fungal growth, causing swelling in mycelia. It did not significantly inhibit the incorporations of ¹⁴C-labeled glucosamine, thymidine, uridine, phenylalanine, and sodium acetate into TCA insoluble fraction of mycelial suspension of *Piricularia oryzae*. In *in vitro* test, however, it inhibited the transfer of mannose from GDP-[U-¹⁴C]mannose (ID₅₀ = 250 μg/ml) and GlcNAc from UDP-[U-¹⁴C]GlcNAc (ID₅₀ = 100 μg/ml) into proteoheteroglycan with a particulate enzyme of *Piricularia oryzae*. It also slightly inhibited chitin synthesis (ID₅₀ = 750 μg/ml) in the same enzyme system, but did not inhibit β-1,3-glucan synthesis.

In the screening for inhibitors of bacterial peptidoglycan synthesis, lipopeptin A was isolated from the culture filtrates and mycelia of *Streptomyces* sp. No. AC-69, which resembles *Streptomyces violaceochromogenes*.¹⁾

Lipopeptin A is a member of peptolide antibiotics having a fatty acid side chain. The structure is shown in Fig. 1. The determination of the structure is reported in a separate paper.²⁾ Amphomycin³⁾ and enduracidin^{4~6)} belonging to this group of antibiotics inhibit peptidoglycan synthesis and the site of action was reported to be the inhibition of the

formation of lipid intermediates.^{7,8)}

Lipopeptin A inhibits *in vitro* peptidoglycan synthesis of *Escherichia coli* Y-10 but shows only weak inhibitory activity against bacterial cells. On the other hand, it shows inhibitory activity against some species of phytopathogenic fungi inducing characteristic swelling of mycelia.¹⁾

This paper describes the site of action of lipopeptin A in peptidoglycan synthesis of *E. coli*. *In vitro* studies on cell wall glycan synthesis of *Piricularia oryzae* is also described.

FIG. 1. Structure of Lipopeptin A.

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MATERIALS AND METHODS

Radiochemicals. UDP-[U- ^{14}C]GlcNAc, UDP-[U- ^{14}C]glucose, GDP-[U- ^{14}C]mannose, [U- ^{14}C]uridine, [2- ^{14}C]thymidine, D-[1- ^{14}C]glucosamine, and L-[U- ^{14}C]phenylalanine were purchased from Radiochemical Centre, Amersham/Searle. [2- ^{14}C]Sodium acetate was purchased from New England Nuclear Corporation. UDP-MurNAc-([^3H]DAP)-pentapeptide was prepared as previously described.⁹⁾

Organisms and growth conditions. *Bacillus cereus* T and *Escherichia coli* Y-10 were grown in bouillon medium (peptone 1%, meat extract 0.3%, NaCl 0.5%, Eiken Chemical Co.) at 37°C on a rotary shaker. *Piricularia oryzae* was grown in Y.G. liquid medium (yeast extract 0.4%, glucose 2%) at 27°C on a rotary shaker.

In order to obtain UDP-MurNAc-pentapeptide, *B. cereus* T was grown in a 30 liter jar fermentor containing 15 liters of bouillon medium.

Preparation of particulate enzyme of *E. coli* Y-10. Particulate enzyme was prepared by grinding cells of *E. coli* Y-10 with sea sand (200~400 mesh) according to the procedure previously reported.⁹⁾

Preparation of UDP-MurNAc-L-Ala-D-Glu-meso-DAP-D-Ala-D-Ala. UDP-MurNAc-pentapeptide was obtained after inducing its accumulation in cells of *B. cereus* T by treatment with 12.5 µg of vancomycin per ml according to the procedure previously reported.⁹⁾ From 15 liters of the culture, approximately 500 O.D. unit (A_{260}) of UDP-MurNAc-pentapeptide was isolated.

Peptidoglycan synthesis and lipid intermediate formation from UDP-[U- ^{14}C]GlcNAc and UDP-MurNAc-pentapeptide. A typical incubation mixture contains 5 µmol of Tris-HCl buffer (pH 7.5), 1 µmol of MgCl_2 , 0.033 µmol of UDP-[U- ^{14}C]GlcNAc (Sp.Act. 300 µCi/µmol, 13000 cpm), 10 µmol of UDP-MurNAc-pentapeptide, and 5 µl of the particulate enzyme in a final volume of 50 µl was incubated at 37°C for 10~120 min. After incubation, the reaction mixture was heated in a steam bath for 1 min and then spotted on Whatman 3 MM paper. After descending paper chromatography with isobutyric acid-1 N NH_4OH (5:3) for 16 hr, spots on the paper corresponding to peptidoglycan (R_f 0) and lipid intermediate (R_f 0.9) were cut out and radioactivity was measured using toluene scintillation cocktail by a Packard Tricarb model 3330 liquid scintillation counter.

Formation of MurNAc-pentapeptide-p-p-lipid. The lipid intermediate was prepared by the method described by Tanaka *et al.*⁷⁾ A reaction mixture (50 µl) containing 2×10^{-4} M UDP-MurNAc-L-Ala-D-Glu-[^3H]-meso-DAP-D-Ala-D-Ala (Sp. Act. 17.6 µCi/µmol, 12000 cpm), 0.1 M Tris-HCl buffer (pH 7.5), 20 mM MgCl_2 and 5 µl of the

particulate enzyme was incubated for 10~30 min. After incubation, the lipid intermediate was extracted with 150 µl of *n*-butanol-6 M pyridinium acetate, pH 4.2 (2:1). The extracts were transferred into a scintillation vial and evaporated to dryness. The radioactivity was measured using dioxane scintillation cocktail.

Isolation of GlcNAc-MurNAc-pentapeptide-p-p-lipid and its utilization for polymerase reaction. The lipid intermediate was isolated by the method described by Anderson *et al.*¹⁰⁾

For polymerase reaction, 0.1 ml of the extract of the lipid intermediate was dried under nitrogen stream and other components of the reaction mixture; 0.1 M Tris-HCl buffer (pH 7.5), 200 µM MgCl_2 , and 10 µl of the particulate enzyme were added to it to give the final volume of 100 µl. After incubation for 2 hr at 37°C, the reaction mixture was heated for 1 min and spotted on Whatman 3 MM paper. After descending paper chromatography with isobutyric acid-1 N NH_4OH (5:3) for 16 hr, radioactivity at the origin was measured.

Chitin synthesis. A reaction mixture contained 16.7 mM Tris-maleate-NaOH buffer pH 7.2, 0.6 mM UDP-[U- ^{14}C]GlcNAc (Sp. Act. 300 µCi/µmol, 13000 cpm), 2.7 mM MgCl_2 , 0.6 mM β -mercaptoethanol, 0.06 mM EDTA, 17 mM GlcNAc, and 10 µl of the particulate enzyme of *P. oryzae*, prepared according to the procedure of Hori *et al.*¹¹⁾ in a total volume of 60 µl. Incubation was carried out for 2 hr at 25°C and the reaction was terminated by heating in a boiling water bath for 1 min. The reaction mixture was spotted on Whatman 3 MM paper and paper chromatography was performed overnight in isobutyric acid-1 N NH_4OH (5:3). Radioactivity at the origin was cut out and measured using toluene scintillator.

β -1,3-Glucan synthesis. β -1,3-Glucan synthesis was measured according to the procedure developed recently in our laboratory.¹²⁾

A reaction mixture containing 3.41×10^{-3} M UDP-[U- ^{14}C]glucose (Sp. Act. 239 µCi/µmol, 15000 cpm), 20 mM sodium cacodylate-HCl buffer pH 6.2, 0.6 mM cellobiose, 2.4 mM MgCl_2 , and 10 µl of *P. oryzae* particulate enzyme in a total volume of 50 µl was incubated at 25°C for 1 hr. After incubation, the reaction mixture was boiled for 2 min and then spotted on Whatman 3 MM paper. After descending paper chromatography by ethanol-1 N acetic acid (7:3) overnight, radioactivity at the origin was measured.

Incorporation of ^{14}C -mannose and ^{14}C -GlcNAc into proteoheteroglycan. A reaction mixture containing 1.4×10^{-3} M GDP-[U- ^{14}C]mannose (Sp.Act. 146 µCi/µmol) or 6.7×10^{-4} M UDP-[U- ^{14}C]GlcNAc (Sp.Act. 300 µCi/µmol), 20 mM sodium cacodylate-HCl buffer pH 6.5, 10 mM MnCl_2 , 1 mM β -mercaptoethanol, and 10 µl of *P. oryzae* particulate enzyme in a total volume

of 50 μ l was incubated at 25°C for 30 min. After incubation, reaction was terminated by adding 100 μ l of chloroform-methanol (2:1) and spotted on Whatman 3 MM paper. After descending paper chromatography with isobutyric acid-1 N NH_4OH (5:3) overnight, radioactivity at the origin was measured.

RESULTS

Effect of Lipopeptin A on peptidoglycan synthesis

By a particulate enzyme system of *E. coli* Y-10, peptidoglycan is synthesized from UDP-MurNAc-pentapeptide and UDP-GlcNAc via two lipid intermediates, e.g., MurNAc-pentapeptide-*p-p*-lipid and GlcNAc-MurNAc-pentapeptide-*p-p*-lipid. Effect of lipopeptin A on lipid intermediates and peptidoglycan syntheses was examined with this system.

The time courses of syntheses of lipid intermediate and peptidoglycan from UDP-[U- ^{14}C]GlcNAc and UDP-MurNAc-pentapeptide are shown in Fig. 2. Lipopeptin A inhibited both syntheses completely at the concentration of 500 $\mu\text{g}/\text{ml}$. At 150 $\mu\text{g}/\text{ml}$ of lipopeptin A, both syntheses were inhibited by 50%, as calculated from the initial reaction velocity (Fig. 3). This finding suggests that the inhibition of peptidoglycan synthesis is caused by the inhibition of lipid intermediate formation. Two steps are known in the formation of lipid intermediates. The first is the formation of MurNAc-pentapeptide-*p-p*-lipid and the second is the formation of GlcNAc-MurNAc-pentapeptide-*p-p*-lipid from the former and UDP-GlcNAc. If UDP-GlcNAc is omitted from the reaction mixture, only the first step should proceed without the second step. As clearly shown in Fig. 4, lipopeptin A did not inhibit the formation of the first lipid intermediate, MurNAc-pentapeptide-*p-p*-lipid from UDP-MurNAc-([^3H]DAP)-pentapeptide. We also tested the effect of lipopeptin A on polymerase reaction using crude GlcNAc-MurNAc-pentapeptide-*p-p*-lipid obtained according to the procedure of Anderson *et al.*⁽¹⁰⁾

Inhibition of peptidoglycan synthesis by

FIG. 2. Effect of Lipopeptin A on Lipid Intermediate and Peptidoglycan Syntheses.

A reaction mixture containing 0.33 μM of UDP-[U- ^{14}C]GlcNAc (Sp. Act. 300 $\mu\text{Ci}/\mu\text{mol}$), 50 μM of Tris-HCl buffer pH 7.5, 10 μM of MgCl_2 , 100 μM of UDP-MurNAc-pentapeptide, and 50 μl of *E. coli* Y-10 particulate enzyme in a total volume of 500 μl was incubated at 37°C. Periodically, 50 μl aliquots were taken up, inactivated, and subjected to paper chromatography using isobutyric acid-1 N NH_4OH (5:3), which separated peptidoglycan (origin) and lipid intermediate (*Rf* 0.9). Radioactivity was measured in a liquid scintillation counter.

○, lipid intermediate; —, no antibiotic; ●, peptidoglycan; ---, lipopeptin A, 500 $\mu\text{g}/\text{ml}$.

lipopeptin A from this lipid intermediate was found to be very weak, if there was any at all. In the same conditions, vancomycin, a selective inhibitor of this polymerase reaction, showed almost complete inhibition at the concentration of 125 $\mu\text{g}/\text{ml}$ (Table I).

These results indicate that lipopeptin A inhibits selectively *N*-acetylglucosamine transferase, which catalyzes the formation of GlcNAc-MurNAc-pentapeptide-*p-p*-lipid from MurNAc-pentapeptide-*p-p*-lipid and UDP-GlcNAc.

Effect on fungal cell wall synthesis

Lipopeptin A inhibits growth of *P. oryzae* at the concentration of 150 $\mu\text{g}/\text{ml}$ ⁽¹⁾ and the

FIG. 3. Inhibition of Lipid Intermediate and Peptidoglycan Syntheses by Lipopeptin A.

The reaction mixture is the same as that shown in Fig. 2. ○, lipid intermediate (37°C, 10 min); ●, peptidoglycan (37°C, 30 min).

Initial reaction velocity was measured from the radioactivities of peptidoglycan or lipid intermediate formed in 30 min and 10 min respectively, in the absence or presence of various concentrations of lipopeptin A. Percent inhibition = (radioactivity incorporated in the absence of lipopeptin A - radioactivity incorporated in the presence of lipopeptin A / radioactivity incorporated in the absence of lipopeptin A) × 100.

FIG. 4. Effect of Lipopeptin A on the Formation of MurNAc-pentapeptide-*p-p*-lipid.

A reaction mixture contained 2×10^{-4} M UDP-MurNAc-L-Ala-D-Glu-[^3H]-*meso*-DAP-D-Ala-D-Ala (Sp.Act. 176 $\mu\text{Ci}/\mu\text{mol}$, 12000 dpm), 0.1 M Tris-HCl buffer pH 7.5, 0.02 M MgCl_2 , and 5 μl of *E. coli* Y-10 particulate enzyme in a total volume of 50 μl . After incubation at 37°C for the time indicated, lipid intermediate was extracted with 150 μl of *n*-butanol-6 M pyridinium acetate pH 4.2 (2:1) and transferred in a scintillation vial, evaporated and radioactivity was measured.

○, no antibiotic; ●, lipopeptin A 500 $\mu\text{g}/\text{ml}$; ◐, lipopeptin A 125 $\mu\text{g}/\text{ml}$.

TABLE I. THE EFFECT OF LIPOPEPTIN A AND VANCOMYCIN ON POLYMERASE REACTION

Each reaction mixture contained crude [^{14}C]GlcNAc-MurNAc-pentapeptide-*p-p*-lipid (18000 cpm), 0.1 M Tris-HCl buffer pH 7.5, 200 μM MgCl_2 , antibiotic as indicated, and 10 μl of *E. coli* Y-10 particulate enzyme in a total volume of 100 μl . Incubation was conducted for 2 hr at 37°C

Antibiotic added	Radioactivity at the origin (cpm)	% Inhibition
None	99	
Lipopeptin A 500 $\mu\text{g}/\text{ml}$	46	53
Lipopeptin A 125 $\mu\text{g}/\text{ml}$	116	0
Vancomycin 125 $\mu\text{g}/\text{ml}$	18	82

swelling of mycelia was observed at little below this concentration.

This indicates that the mechanism of action of this antibiotic is related to the inhibition of cell wall biosynthesis. For example, selective inhibitors of chitin synthetase, polyoxins caused swelling of mycelia and spores of phytopathogenic fungi.¹³⁾ Therefore, we tested the effect of lipopeptin A on incorporation of glucosamine, a component of fungal cell wall, into TCA insoluble fraction of mycelial suspension of *Piricularia oryzae*. As shown in Fig. 5, lipopeptin A inhibited the incorporation of D-[1- ^{14}C]glucosamine only slightly at the concentration of 1 mg/ml. Incorporation of other radiochemicals, e.g., [^{14}C]uridine, [^{14}C]thymidine, L-[^{14}C]phenylalanine, and [^{14}C]sodium acetate were also not significantly inhibited (data not shown).

Next, we tested the effect of lipopeptin A on *in vitro* synthesis of cell wall components of *P. oryzae* using a particulate enzyme system. Nakajima *et al.*¹³⁻¹⁵⁾ reported that the cell wall of *P. oryzae* constitutes of at least three different polysaccharides; proteo-heteroglycan containing D-mannose, D-galactose, β -1,3-glucan, and chitin. Of the cell wall components, β -1,3-glucan occupies more than 60%, while the contents of heteroglycan and chitin are 10% and 13% respectively.

FIG. 5. Effect of Lipopeptin A on the Incorporation of Glucosamine into TCA Insoluble Fraction of *Piricularia oryzae*.

At 30 min incubation after the addition of lipopeptin A into the mycelial suspension, D-[1-¹⁴C]glucosamine 0.1 µCi (5.88 µCi/µmol) was added and the incubation was started.

○, no antibiotic; ●, lipopeptin A 1 mg/ml; ◐, lipopeptin A 100 µg/ml.

Lipopeptin A inhibited β -1,3-glucan synthesis only by 17% at the concentration of 1 mg/ml. This result indicates that the primary site of action of lipopeptin A is not the inhibition of synthesis of β -1,3-glucan, major component of cell wall.

Lipopeptin A inhibited chitin synthesis at high concentration (Fig. 6). Concentration required for 50% inhibition was estimated to be 750 µg/ml. This is a much higher value compared to the minimal inhibitory concentration (150 µg/ml) in *in vivo* testing.

Although details of fungal proteo-heteroglycan synthesis is not known, we studied the incorporation of sugars from GDP-mannose and UDP-GlcNAc into proteo-heteroglycan by a particulate enzyme from *P. oryzae*. The enzyme was found to catalyze the incorporation of mannose from GDP-[U-¹⁴C]mannose and GlcNAc from UDP-[U-¹⁴C]GlcNAc into proteo-heteroglycan. This reaction is dependent on

FIG. 6. Effect of Lipopeptin A on Chitin Synthesis.

Each reaction mixture contained 0.6 mM UDP-[U-¹⁴C]GlcNAc (Sp. Act. 300 µCi/µmol), 16.7 mM Tris-maleate-NaOH buffer pH 7.2, 2.7 mM MgCl₂, 0.6 mM β -mercaptoethanol, 0.06 mM EDTA, 17 mM GlcNAc, antibiotic as indicated, and 10 µl of *P. oryzae* particulate enzyme in a total volume of 60 µl. Incubation was conducted at 25°C for 2 hr. The arrow shows the concentration for 50% inhibition.

FIG. 7. Effect of Lipopeptin A on the Transfer of Mannose from GDP-[U-¹⁴C]Mannose into Proteo-heteroglycan.

Each reaction mixture contained 1.4×10^{-3} mM GDP-[U-¹⁴C]mannose (Sp. Act. 146 µCi/µmol), 20 mM sodium cacodylate-HCl buffer pH 6.5, 10 mM MnCl₂, 1 mM β -mercaptoethanol, and 10 µl of *P. oryzae* particulate enzyme in a total volume of 50 µl. Incubation was conducted at 25°C for 60 min.

FIG. 8. Effect of Lipopeptin A on the Transfer of GlcNAc from UDP-[U- 14 C]GlcNAc into Proteo-heteroglycan.

Each reaction mixture contained the same components as shown in Fig. 7 except 6.7×10^{-4} mM UDP-[U- 14 C]GlcNAc. Incubation was conducted at 25°C for 30 min.

concentration of Mn^{++} (data not shown). As shown in Fig. 7 and Fig. 8, lipopeptin A inhibited the transfer of mannose and GlcNAc and the ID_{50} values were found to be 250 µg/ml and 100 µg/ml, respectively.

From these results, we conclude that lipopeptin A inhibits the synthesis of proteo-heteroglycan and to lesser extent, chitin synthesis. The primary site of action of this antibiotic may be the inhibition of the transfer of GlcNAc into proteo-heteroglycan. Although the total structure of proteo-heteroglycan of *P. oryzae* is not established yet, it may be that it has diacetylchitobiose structure *N*-glycosidically linked to an asparagine residue of protein as seen in yeast mannan.¹⁷⁾ If this is the case, the inhibition of transfer of GlcNAc into this moiety by lipopeptin A would also result in the inhibition of transfer of mannose which is linked successively to the diacetylchitobiose.

DISCUSSION

Lipopeptin A has a long chain fatty acid as seen in other antibiotics which inhibit cell wall synthesis, *e.g.*, tunicamycin,¹⁸⁾ enduracidin,^{4~6)} amphomycin,³⁾ and moenomycin,¹⁹⁾

etc. Lipopeptin A resembles enduracidin in view of the lactone structure, in which the hydroxyl group of threonine participate in the lactone formation. In this paper, we showed that lipopeptin A inhibits peptidoglycan synthesis as the results of inhibition of the formation of GlcNAc-MurNAc-pentapeptide-*p-p*-lipid. Lipopeptin A does not inhibit the formation of MurNAc-pentapeptide-*p-p*-lipid. Also it does not inhibit polymerase reaction. In view of the structure-activity relationship, it is interesting that both lipopeptin A and enduracidin inhibit the same site. Enduracidin inhibits peptidoglycan synthesis and the site of action is reported to be the inhibition of the formation of GlcNAc-MurNAc-pentapeptide-*p-p*-lipid.⁷⁾

Lipopeptin A also caused swelling of mycelia of fungi, as seen in polyoxins,¹³⁾ selective inhibitors of chitin synthetase. However, lipopeptin A inhibited the incorporation of glucosamine into TCA insoluble fraction of mycelial suspension of *P. oryzae* only slightly. Incorporations of uridine, thymidine, phenylalanine, or sodium acetate *in vivo* were also not inhibited.

In *in vitro* experiment, we attempted to see the effect of lipopeptin A on chitin, β -1,3-glucan, and proteo-heteroglycan syntheses with a particulate enzyme from *P. oryzae*. β -1,3-Glucan synthesis was inhibited only by 17% at the concentration of 1 mg/ml of lipopeptin A but chitin synthesis was inhibited at the high concentration of the antibiotic (ID_{50} ; 750 µg/ml). Recently, Nakajima *et al.*¹⁶⁾ in the study of cell wall components of *P. oryzae*, clarified the partial structure of proteo-heteroglycan, which has a carbohydrate moiety composed of D-mannose, D-glucose, and D-galactose in a ratio of 6:2:1. They suggested that proteo-heteroglycan might have two kinds of carbohydrate-protein linkage, namely *N*-glycosidic and *O*-glycosidic. In yeast mannan,¹⁷⁾ a heteropolysaccharide moiety is linked *via* a diacetylchitobiose bridge by an *N*-glycosidic bond to an asparaginyl residue in the protein part and a short mannooligosaccharide is *O*-glycosidically linked to serine

and/or threonine residues in the protein. Most of mannosyl units in the mannan molecule are transferred from GDP-mannose. However, in some cases, the transfer of mannosyls to mannan does not occur directly but through lipid intermediate, dolichol phosphate mannose. In the biosynthesis of *N*-glycosidically linked mannan, *N*-acetylglucosamine is transferred from UDP-GlcNAc to dolichol phosphate to give dolichol-*p-p*-GlcNAc and dolichol-*p-p*-(GlcNAc)₂.

Biosynthesis of carbohydrate moiety of proteo-heteroglycan in *P. oryzae* is not known and existence of *N*-glycosidic and *O*-glycosidic linkages in proteo-heteroglycan is only presumptive. In the present study, we showed that lipopeptin A inhibited the transfer of mannose from GDP-mannose into proteo-heteroglycan. Lipopeptin A was also found to inhibit transfer of GlcNAc from UDP-GlcNAc. In our *in vitro* system, GlcNAc, the primer of chitin synthesis and Mg⁺⁺ ion are absent and the concentration of UDP-GlcNAc is very low compared to the *K_m* (1.43×10^{-3} M) of chitin synthase.²⁰⁾ Because chitin synthesis does not occur in this conditions, the transfer of GlcNAc from UDP-GlcNAc may reflect only proteo-heteroglycan synthesis but not chitin. This assumption was supported by the fact that ID₅₀ value for proteo-heteroglycan (100 µg/ml) of lipopeptin A was lower than that for chitin synthesis (750 µg/ml).

In conclusion, the swelling of mycelia of fungi caused by lipopeptin A may be due to the inhibition of cell wall synthesis, especially proteo-heteroglycan synthesis and the site of action is suggested to be the transfer of GlcNAc into proteo-heteroglycan.

This may explain failure of significant inhibition of glucosamine incorporation *in vivo* by lipopeptin A. If the structure of proteo-heteroglycan of *P. oryzae* has indeed diacetylchitobiose linked to an asparagine residue of protein as seen in yeast mannan, the chemical content of this *N*-acetylglucosamine should be considerably low, thus the inhibition of incorporation *in vivo* can hardly be observed.

Recently, Kang *et al.*^{21,22)} reported that

amphotycin inhibits the transfer of mannose from GDP-mannose and GlcNAc from UDP-GlcNAc to lipid linked saccharide with enzyme preparations of pig arota. Inhibition of the transfer of GlcNAc is known in tunica-mycin, which inhibits the formation of dolichol-*p-p*-GlcNAc.²³⁾ Lipopeptin A has a long chain fatty acid as seen in these two antibiotics; thus, the inhibition of proteo-heteroglycan synthesis by this antibiotic may suggest the presence of lipid intermediate in cell wall glycan synthesis in *P. oryzae*.

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