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Purification of α-Sarcin and an Antifungal Protein from Mold (*Aspergillus giganteus*) by Chitin Affinity Chromatography

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A simple method for preparation of ***α***-sarcin and an antifungal protein (AFP) from mold (*Aspergillus giganteus* MDH 18894) has been developed. ***α***-Sarcin and AFP were purified simultaneously by chitin affinity column chromatography and gel filtration. By this method, 4.5 mg of pure ***α***-sarcin and 6.9 mg of pure AFP were obtained from 2 liters of culture medium. Compared with other purification methods such as ion-exchange column chromatography, this procedure was very simple and specific. The purified ***α***-sarcin and AFP were homogeneous as characterized by SDS–polyacrylamide gel electrophoresis. Both ***α***-sarcin and AFP exhibited the binding activity to generated chitin. Soluble glycochitin decreased the intensity of fluorescence of ***α***-sarcin and made the λ_{em}m shift from 340 to 347 nm. Titration of ***α***-sarcin with *N*-bromosuccinimide under native conditions revealed that two tryptophans (Trps) were all located in the core part of ***α***-sarcin molecule. This indicated that Trps were not involved in the binding of ***α***-sarcin to chitin. Glycochitin in the culture medium increased the expression of ***α***-sarcin, while it had no effect on the expression of AFP. Unlike other ligands such as Cibacron blue for the affinity purification of ***α***-sarcin and AFP, glycochitin increased the nuclease activity of ***α***-sarcin.

Key Words: antifungal protein (AFP); *Aspergillus giganteus* MDH 18894; *N*-bromosuccinimide; chitin affinity column chromatography; glycochitin; ***α***-sarcin.

The mold *Aspergillus giganteus* MDH 18894 can produce two extracellular proteins (1, 2). One of them, α-sarcin, is a specific ribonuclease that inhibits protein synthesis by inactivating ribosomes (3, 4) and thus named as ribosome-inactivating protein (RIP).[2] The other one, an antifungal protein (AFP), is a highly basic and small protein (51 amino acids), which can inhibit the growth of many filamentous fungi (5, 6).

The molecular mechanism of action of α-sarcin is to cleave a phosphodiester bond between G-4325 and A-4326 of 28S RNA in rat ribosomes, producing an RNA fragment (called α/R-fragment) from the 3′end of the 28S ribosomal RNA (7). Another large group of RIPs from higher plants such as ricin and cinnamomin are RNA *N*-glycosidase that inhibit protein synthesis by removing an adenine base at the position 4324 of 28S RNA in rat ribosome (8, 9). The phosphodiester bond cleaved by α-sarcin is adjacent to the adenine base removed by ricin and this structure is thus named as "sarcin/ricin" domain. This domain is crucial to the structure and function of the ribosome since it is the site where the eukaryotic elongation factors bind. For this reason, α-sarcin is used as a powerful molecular probe to detect the structure and function of ribosome (10). In addition, α-sarcin shows a selective and high toxicity against cells with altered or damaged cell membranes, particularly sarcoma and carcinoma cell. It is a promising candidate for the treatment of cancer and viral infections such as AIDS (11, 12). AFP is one of the most potent fungistats and shows activity against many

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[2] Abbreviations used: RIP, ribosome-inactivating protein; AFP, antifungal protein; AIDS, acquired immunodeficiency syndrome; Trp, tryptophan; NBS, *N*-bromosuccinimide; BSA, bovine serum albumin.

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pathogenic fungi (1, 5). Although the mechanism of action of AFP is still unknown, its strong antifungal activity and stability suggest several technological uses of this protein such as food preservation, development of antifungal drugs, or the design of plants with resistance against phytopathogenic fungi (13).

Due to the above-mentioned applications of α-sarcin and AFP, it is necessary to develop an effective purification procedure by which large amounts of these proteins can be easily obtained. Previous procedures for this purpose usually adopted the ion-exchange, gel filtration, and blue affinity column chromatography (1, 5, 14). In this paper, chitin affinity column chromatography for the purification of α-sarcin and AFP was developed. Moreover, the chitin binding ability of α-sarcin and AFP revealed by this method might shed light on the unknown function of α-sarcin in *A. giganteus* cells and the unclear antifungal mechanism of AFP.

MATERIALS AND METHODS

Materials

Chitosan from crab shell was obtained from Sigma. Gel filtration column (P10 column) was the product of Bio-Gel. Cinnamomin was purified by the method as described earlier in our laboratory (15). L-[^{14}C]leucine was purchased from Amersham. The plant pathogen, *Fusarium oxysporum* was generously provided by Professor Z. Gong. All other chemicals were of analytical purity.

Production and Analysis of Proteins

As described by Olson and Goerner (1), *A. giganteus* MDH 18894 was cultured at 30°C in 1-liter flasks each containing 250 ml of culture medium composed of 1.5% beef extract, 2.0% peptone, 2.0% corn starch, and 0.5% NaCl. All were made by mass/volume. The aeration and agitation for this fermentation were provided by a rotary shaker. After 70–80 h, the culture medium was collected for subsequent purification of α-sarcin and AFP. To test the effect of chitin on the expression of α-sarcin and AFP, 0.2% glycochitin was included in the culture medium and the other conditions were kept unchanged. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE, 12%) was carried out according to the method of Laemmli (16). SDS–PAGE (15%) was performed by the method of Schagger and Von Jagow (17) in the Tris–tricine system that allowed a good separation of proteins with molecular mass <10 kDa. The protein bands on the gel were stained by silver to examine the purity of proteins (18). Protein concentration was determined according to the method of Bradford (19).

Preparation of Chitin Affinity Column

Regenerated chitin was freshly prepared according to the method of Molano *et al.* (20). The amounts of reagents were given per gram of chitosan used. The chitosan was grounded in a mortar with 20 ml of 10% acetic acid and kept overnight. Then 90 ml of methanol was added slowly with stirring and the solution was filtered through glass wool on a Buchner funnel. To the filtrate 1.5 ml of acetic anhydride was added with stirring. After 1 min, the gel was allowed to stand for about 30 min and was cut into small pieces. The liquid that oozed out was removed and the gel fragments were transferred to a homogenizer. After covering with methanol, the suspension was homogenized for 1 min at maximum speed. The finely dispersed chitin was filtered with a medium porosity sintered glass funnel and washed with water to neutrality. The regenerated chitin was autoclaved and filled in a column (4 × 10 cm).

Purification of α-Sarcin and AFP

α-Sarcin and AFP have been purified from the culture medium of *A. giganteus* MDH 18894 by the following procedure. The culture medium was filtered through eight layers of cheesecloth and the filtrate was centrifuged (12,000*g*, 15 min, 4°C) to remove mycelia debris and spores. The supernatant was adjusted to 20 mM $NaHCO_3$, pH 8.4, and then the solution was loaded on a regenerated chitin column (4 × 10 cm) at the flow rate of 0.5 ml/min. The first washing was performed with buffer A (20 mM $NaHCO_3$, pH 8.4) until the eluate was free of proteins as detected by the absorbance at 280 nm. The second washing was done with buffer B (20 mM acetate buffer, pH 5.6) for 90 min. Finally, the proteins were released from the chitin matrix with 10 mM acetic acid (pH 3.3) and immediately dialyzed against water. After dialysis, the protein solution was dried in a vacuum concentrator. Then the dried protein was dissolved in buffer C (50 mM Tris/HCl buffer, pH 7.0, 0.15 M NaCl) and subjected to a Bio-Gel P10 column (3 × 100 cm) preequilibrated with buffer C. Two peaks were eluted from the column by buffer C; the first one contained α-sarcin and the second one was AFP.

Assay for Ribonuclease Activity

Ribosomes were isolated from rat liver as described by Spedding (21). A total of 1.5 A_{260} units of ribosomes (27 pmol) were incubated with α-sarcin in 100 μl of buffer D (40 mM malonate buffer, pH 3.0–5.0, 40 mM KCl, 5 mM EDTA) at 37°C for 15 min. The reaction was stopped immediately by adding 10 μl of 10% SDS solution and then kept in an ice bath. After phenol–chloroform extraction and ethanol precipitation, ribosomal RNAs were separated by 8 M urea-denatured

polyacrylamide gel (3.5%) electrophoresis. The α/R-fragments were quantitated by scanning the bands stained by methylene blue in an UVP GDS-8000 complete documentation analysis system. In the assay for effect of chitin on the ribonuclease activity, reaction buffer E (40 mM Tris/HCl, pH 7.6, 40 mM KCl, 5 mM EDTA) contained soluble glycochitin with the concentration from 0.05 to 0.20%.

Protein Synthesis in the Cell-Free System

Rabbit reticulocyte lysate was prepared as described by Sambrook *et al.* (22). One-hundred microliters of the reaction mixture, containing 10 mM Tris/HCl (pH 7.4), 100 mM ammonium acetate, 2 mM magnesium acetate, 1 mM ATP, 0.2 mM GTP, 15 mM phosphocreatine, 4 μg of creatine phosphokinase, 50 μM 19 amino acids (minus leucine), 10 μl of lysate, 0.125 μCi of L-[^{14}C]leucine (303 mCi/mmol), and various amount of α-sarcin, was incubated at 30°C for 15 min, and then a 20-μl aliquot was withdrawn and absorbed onto Whatman 3MM filter paper which was put in a centrifuge tube and washed with 1.0 ml of hot 10% trichloroacetic acid at 90°C and 1.0 ml of cold 10% trichloroacetic acid at room temperature respectively for 10 min. Then the filter paper was dehydrated with 1.0 ml of 95% ethanol and dried at room temperature. The radioactivity of the acid-insoluble substances on the filter paper was measured by a liquid scintillation counter. One unit of activity of α-sarcin was the amount of protein causing 50% inhibition of protein synthesis in 100 μl of rabbit-reticulocyte lysate system.

Assay for Antifungal Activity

Antifungal activity was assayed under sterile conditions using a hyphal extension–inhibition method as described by Roberts and Selitrennikoff (23). Fungal mycelia were harvested from actively growing fungal plates and placed in the center of petri dish containing PDA medium (20% potato, 2% dextrose, and 1.5% agar, all made by mass/volume) that was used for maintaining the growth of fungi under test. After incubating these dishes at 30°C for 48 h to allow for the mycelial growth, sterile filter paper disks were placed on the agar surface in front of the advancing fungal mycelium. Then 20 μl of protein solution containing 0.5, 1.0, or 1.5 μg of AFP in buffer F (20 mM malonate buffer, pH 2.0) was applied to each disk. The plates were incubated at 30°C for an additional 24 h and then photographed.

For a quantitative assay, fungal spores were grown on PDA plates at 24°C for 1–3 weeks. Spores were washed off plates with sterile water and collected by centrifugation (5000*g*, 10 min, 4°C). In a microplate well, 20 μl of sterile protein solution was added to 100 μl of spore suspension (2×10^4 cells/ml) in PD medium (20% potato and 2% dextrose, all made by mass/volume). The control well contained 20 μl of sterile water and 100 μl of the fungal spore suspension. The $A_{495\ nm}$ of the culture medium was measured after 30 min and 48 h. Percentage of growth inhibition was calculated according to Xu and Reddy (24). One unit of activity of AFP was the amount of protein inducing 50% inhibition of *F. oxysporum*.

Measurement of Fluorescence and Titration of Trp with NBS

Fluorescence measurements were performed on a Hitachi 4010 fluorescence spectrophotometer at 25°C with a circulating water bath. The emission and excitation slits were all set at 5 nm. Fluorescence emission spectra of the native α-sarcin and α-sarcin bound to glycochitin were taken over 310–400 nm with excitation at 295 nm to minimize tyrosine emission. All the solution had been filtered to minimize the possible disturbance from the pollution of dust particles. Titration of tryptophan (Trp) with *N*-bromosuccinimide (NBS) was carried out at room temperature according to the method of Spande *et al.* (25). A solution of α-sarcin with the concentration of 0.33 mg/ml in buffer G (0.05 M acetate buffer, pH 5.0) was pipetted into a quartz cell, and then the cell was placed in a spectrophotometer. Ten aliquots of NBS (1.78×10^{-3} M) in buffer G were added to the protein solution with rapid mixing and the changes of A_{282} were measured. Oxidized Trp residues were calculated from the equation of Spande *et al.* (25).

Chitin Binding Assay

Chitin binding assay was performed as described by Bormann *et al.* (26). The reaction mixture for binding assay containing 4 mg of regenerated chitin and various amounts of proteins in 0.5 ml buffer H (0.1 M Tris/HCl, pH 7.4, 150 mM NaCl) were incubated in an ice bath for 1 h with stirring every 15 min. The mixture that contained unabsorbed protein and regenerated chitin was centrifuged (12,000*g*, 10 min, 4°C) and the protein content in the supernatant was measured by the Bradford method (19).

RESULTS AND DISCUSSION

Purification and Characterization of α-Sarcin and AFP

As shown in Fig. 1, chitin affinity column could efficiently separate the α-sarcin and AFP from other proteins. The first elution with buffer B was necessary to assure the purity of α-sarcin and AFP, since it could desorb the proteins that bound unspecifically to the chitin affinity column. In this way, the chitin affinity column could efficiently increase the purity of α-sarcin

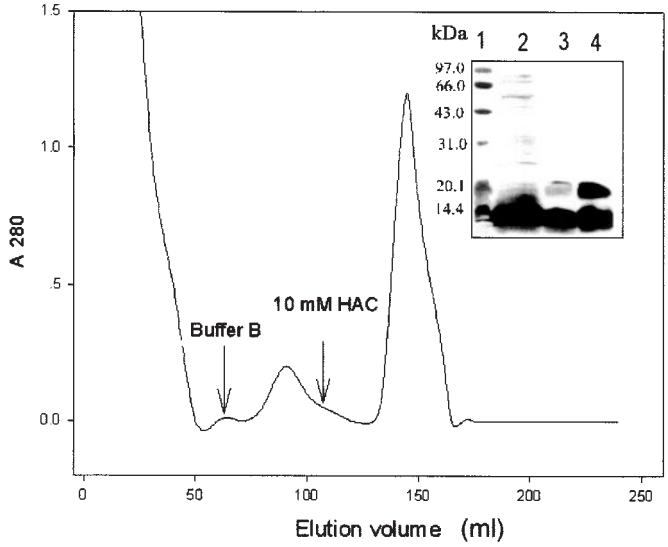

FIG. 1. Purification of α-sarcin and AFP by chitin affinity column chromatography. Proteins bound to the chitin affinity column (4 × 10 cm) were eluted with buffer B and 10 mM acetic acid (pH 3.3) sequentially. (Inset) Characterization of the purity of α-sarcin and AFP by SDS–PAGE (12%) and silver staining. Lane 1, protein markers (the molecular masses are indicated in the left margin); lane 2, culture medium (40 μl); lane 3, α-sarcin and AFP eluted by 10 mM acetic acid (5 μg); lane 4, α-sarcin and AFP eluted by 10 mM acetic acid (10 μg).

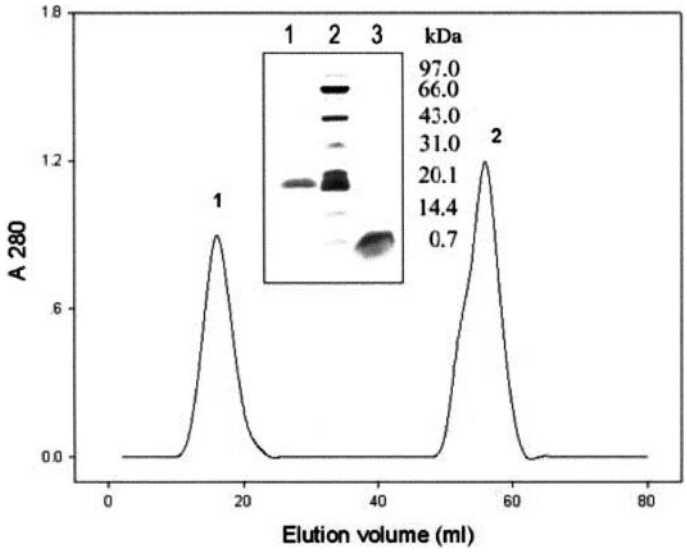

FIG. 2. Separation of α-sarcin and AFP by gel filtration. α-Sarcin and AFP eluted from chitin affinity column (3 × 100 cm) were further resolved by Bio-Gel P10 column chromatography. (Inset) Characterization of the purity of α-sarcin and AFP by SDS–PAGE (15%) and silver staining. Lane 1, purified α-sarcin (peak 1, 4 μg); lane 2, protein markers (the molecular masses are indicated in the right margin); lane 3, purified AFP (peak 2, 4 μg).

and AFP up to 434 and 464-fold, respectively (Table 1). α-Sarcin and AFP obtained from this step could be separated well by the gel filtration chromatography on Bio-Gel P10 column. The purity of α-sarcin and AFP was very high as characterized by SDS–PAGE and silver staining (Fig. 2). From 2 liters of culture medium, about 4.5 mg of pure α-sarcin and 6.9 mg of homogeneous AFP were obtained by this procedure.

Previous procedures for purifying α-sarcin and AFP usually adopted the ion-exchange column chromatography as the first step (1, 5, 14). The ion-exchange column chromatography such as CM-cellulose 52 had low specificity and reproducibility. Here, a novel procedure for the simultaneous purification of α-sarcin and AFP through chitin affinity column chromatography was developed for the first time. Compared with other approaches, this method is simple, rapid, and highly specific. Moreover, it is easy to obtain chitin from various sources, and the chitin affinity column can be prepared conveniently in the laboratory. This affinity column can be regenerated by simple elution with 8 M urea and used repeatedly for many times without loss of specificity. With these virtues, this procedure can be adopted to purify simultaneously α-sarcin and AFP on a large scale. This economical purification procedure is especially meaningful to AFP, which has promising practical application in many fields.

TABLE 1

Purification of α-Sarcin and AFP from *A. giganteus* MDH 18894

Procedure	Protein (mg)	Activity[a] (units)		Sp act (units/mg)		Yield (%)		Purification (fold)	
		α-Sarcin	AFP	α-Sarcin	AFP	α-Sarcin	AFP	α-Sarcin	AFP
Culture medium[b]	6078	106,900	1021	18	0.17	100	100	1	1
Chitin column	11.8	92,200	927	7,814	79	86.2	90.8	434	464
P10 column									
Peak 1	4.5	81,900		18,200		76.6		1,011	
Peak 2	6.9		821		119		80.4		700

[a] One unit of α-sarcin activity was the amount of protein causing 50% inhibition of protein synthesis in 100 μl of rabbit-reticulocyte lysate system. One unit of AFP activity was the amount of protein inducing 50% inhibition of *F. oxysporum* in the quantitative assay as described under Materials and Methods.

[b] Results refer to 2 liters of culture medium.

Activity of α-Sarcin

To evaluate the enzymatic activity of the α-sarcin purified by this new method, both the inhibitory activity to *in vitro* protein synthesis and the cleaving activity to 28S RNA of rat ribosomes were assayed. The inhibitory activity of the purified α-sarcin to the protein synthesis in the rabbit lysate system is shown in the Fig. 3A. In 100 μl of reaction mixture, 30 ng of the purified α-sarcin could display inhibitory activity to protein synthesis. When 120 ng of α-sarcin was added, the protein synthesis was almost entirely inhibited. The IC_{50} of α-sarcin purified by this method was 32 nM, which was close to that of α-sarcin purified by another method (14).

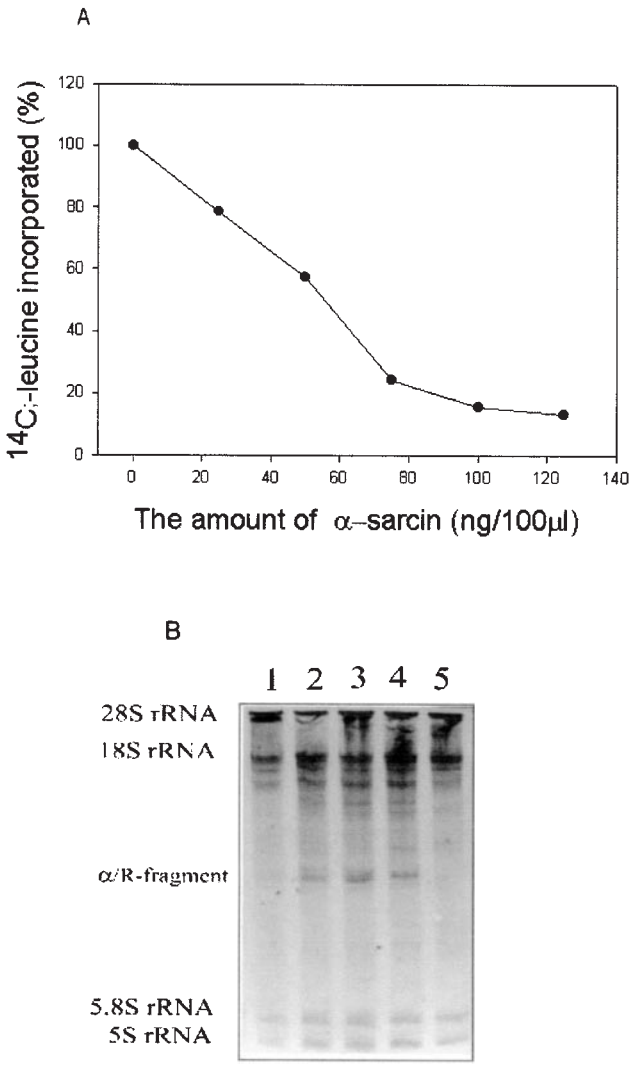

FIG. 3. Activity of purified α-sarcin. (A) Effect of α-sarcin on the protein synthesis in the cell-free system of rabbit reticulocyte. The protein synthesis system contained the indicated amount of α-sarcin in a final volume of 100 μl of reaction mixture as described under Materials and Methods. The control value of [^{14}C]leucine incorporated is 18,760 cpm. (B) Cleaving activity of α-sarcin to the 28S RNA in rat ribosome. Lane 1, control; lane 2, ribosomes treated with cinnamomin (5×10^{-9} M); lanes 3–5, ribosomes treated with α-sarcin (5×10^{-8} M), respectively, at pH 5.0, pH 4.0, and pH 3.0.

As shown in Fig. 3B, the purified α-sarcin exhibited specific ribonuclease activity. After incubation of rat 80S ribosomes with the purified α-sarcin without aniline treatment, only the specific α/R-fragment was detected by urea-denatured PAGE (3.5%). The minimal concentration of the purified α-sarcin for effectively producing the α/R-fragment was 50 nM. This showed that α-sarcin could cleave the 28S RNA in rat ribosome efficiently and specifically at the low pH value (pH 5.0 and pH 4.0). In this respect, α-sarcin was different from plant RIPs, which lost their site specificity at pH 5.0 and pH 4.0 (27, 28). When pH value of reaction buffer decreased to 3.0, α-sarcin lost the specific nuclease activity and no α/R-fragment could be detected. However, no irreversible structural changes seemed to happen in α-sarcin molecule at pH 3.0 since it exhibited the same IC_{50} value after being eluted from the chitin affinity column by 10 mM acetic acid.

Antifungal Activity of AFP

Antifungal activity of AFP was tested by inhibiting the growth of the phytopathogenic fungus *F. oxysporum*. The AFP exhibited the antifungal activity against this agronomically important pathogen with an IC_{50} of 10 μM, which was very close to that purified by other methods (5, 14). This fact demonstrated that the antifungal activity of AFP was not decreased by the elution from the chitin affinity column with 10 mM acetic acid. Direct inhibition of the growth of *F. oxysporum* by AFP is shown in Fig. 4. It was found that AFP was very stable in acidic environment and could exhibit the antifungal activity at pH 2.0 with the amount of as little as 0.5 μg. A previous report revealed that AFP was very stable under extreme conditions such as treatment with high temperatures and various proteases (13). Experimental results of this paper demonstrated that AFP was also acid stable and could exhibit the antifungal activity at the low pH value.

Chitin Binding Ability of α-Sarcin and AFP

The binding ability of α-sarcin and of AFP to chitin was examined and the results are shown in Fig. 5A. α-Sarcin and AFP exhibited high binding ability to regenerated chitin in the binding buffer. Almost all tested amount of proteins (with the concentration ranging from 5 to 160 μg/ml) could bind to the regenerated chitin. The binding ability of α-sarcin and of AFP was pH dependent, since they could be desorbed from the chitin affinity column by the low-pH solution. It should be noted that many chitin binding proteins in plants and bacteria show strong antifungal activity and the chitin binding domain was proved to be involved in the antifungal activity (24, 29). The chitin binding ability

of AFP is probably also related to its unknown antifungal mechanism.

The α-sarcin molecule has two Trps that may be involved in the chitin binding activity. To verify this speculation, steady-state intrinsic fluorescence spectra of α-sarcin were recorded with the excitation at 295 nm in the presence or absence of glycochitin. As shown in Fig. 5B, adding glycochitin to the native α-sarcin solution decreased its intrinsic fluorescence. In addition, the λ_{em}m of its intrinsic fluorescence shifted from 340 to 347 nm. This fact indicated that great changes in α-sarcin molecule were induced by glycochitin. However, Trps in the α-sarcin molecule could not be titrated by NBS under native conditions. This revealed that all these Trps were located in the core part of native α-sarcin molecule and the fluorescence changes caused by glycochitin were not the result of the direct binding of glycochitin to Trps. Structural changes perhaps happened around the Trps of the α-sarcin molecule during the binding of glycochitin to the α-sarcin. These changes might affect the fluorescence emission spectra of α-sarcin.

It was known that the binding force of proteins to carbohydrates was provided by hydrogen bond and hydrophobic interactions (30, 31). Hydrophobic interactions are due to aromatic residues (primarily Trp and Tyr) stacked along the face of pyranose rings. Concerning the binding of α-sarcin and AFP to chitin, the involvement of Trp could be excluded since the two Trps were located in the core part of the α-sarcin molecule and there was no Trp in the AFP molecule (6). Other interactions such as the hydrogen bond or hydrophobic interaction (Tyr) probably determined the binding ability of α-sarcin and AFP to chitin.

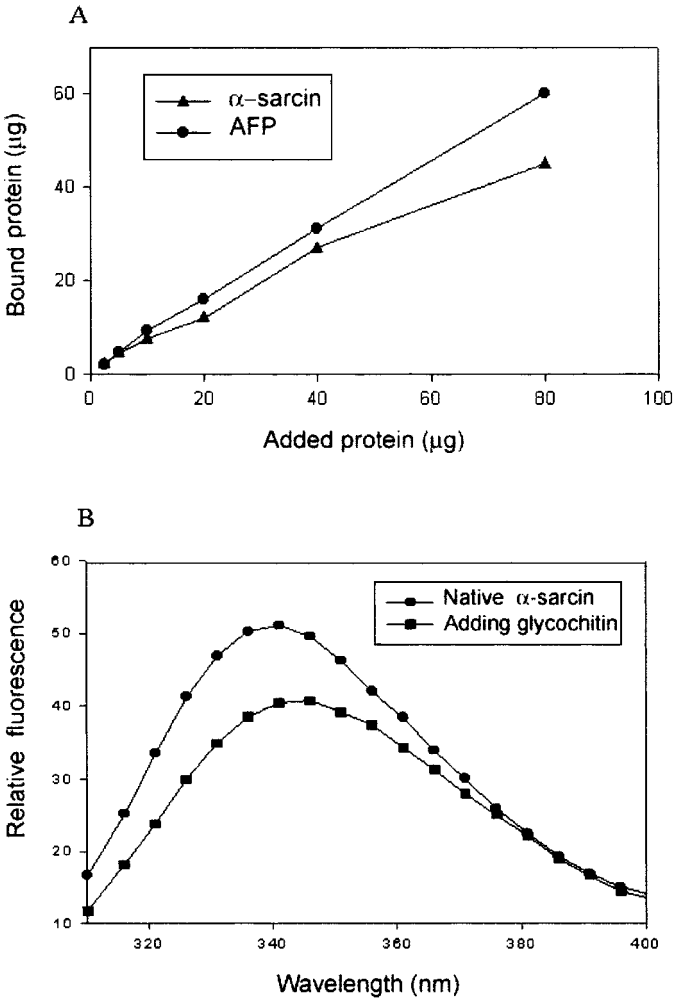

FIG. 5. Chitin binding activity of α-sarcin and AFP. (A) Various concentrations of α-sarcin and AFP were mixed with 4 mg of regenerated chitin in the binding buffer and allowed to adsorb in an ice bath for 1 h, after which the unabsorbed protein was measured by Bradford method. (B) Emission spectra of α-sarcin in the presence or absence of glycochitin. α-Sarcin (10 μM) was dissolved in binding buffer in the presence or absence of glycochitin. Both the excitation and the emission slits were set at 5 nm. Scans were performed at 25°C with excitation wavelength set at 295 nm. The emission maximums (λ_{em}m) for α-sarcin alone or with 0.20% glycochitin were 340 and 347 nm, respectively.

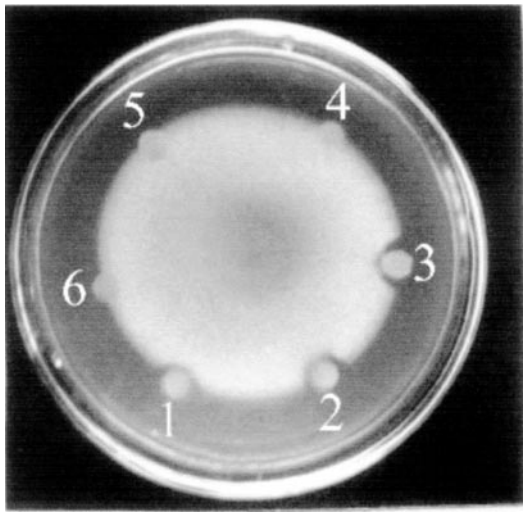

FIG. 4. Direct inhibition of fungal growth by AFP. *F. oxysporum* were cultured on potato dextrose agar plates (90 mm diameter) for 72 h. The central portion is an agar plug containing hyphae from *F. oxysporum*. Crescents surrounding disks indicate the inhibition of hyphal extension. Various amount of AFP were dissolved in 20 μl of 20 mM malonate buffer (pH 2.0). Disks 1–3, respectively, contained 0.5, 1.0, and 1.5 μg of AFP. Disks 4–6, respectively, contained 0.5, 1.0, and 1.5 μg of BSA as the control.

Effect of Glycochitin on the Expression and Activity of α-Sarcin and AFP

The effect of glycochitin on the expression of α-sarcin and AFP was studied by culturing *A. giganteus* in the presence or absence of 0.2% glycochitin. Analysis of

proteins in the culture medium by SDS–PAGE revealed that the expression of α-sarcin by *A. giganteus* cultured with the glycochitin was about 3.2-fold higher than that cultured without glycochitin (Fig. 6). SDS–PAGE could not detect the effect of glycochitin on the expression of AFP due to the disturbance of undigested peptide in the culture medium. However, the activity measurement of α-sarcin and AFP demonstrated that α-sarcin activity increased by approximately 3.2-fold while no change of AFP activity was observed after adding the glycochitin into the culture medium. The experimental results of this paper demonstrated that the glycochitin could increase expression of α-sarcin. A similar phenomenon was reported by Escott *et al.* (32) that when glycochitin was used as carbon source of *Aspergillus fumigatus* NCPF 2140, it could increase the expression of an extracellular chitinase.

As shown in Fig. 7, glycochitin could increase the nuclease activity of α-sarcin. In 100 μl of reaction mixture, 0.05% of glycochitin could increase the activity of α-sarcin. The nuclease activity of α-sarcin increased gradually with the increment of the concentration of glycochitin from 0.05 to 0.20%. The effect of chitin on the nuclease activity of α-sarcin was distinct from another affinity ligand, Cibacron blue, that could bind to the active site of α-sarcin and inhibit the α-sarcin activity (14). Studies through site-directed mutagenesis and nuclear magnetic resonance spectroscopy demonstrated that the fluorescence of α-sarcin was mainly contributed by the Trp of N-terminus that was linked directly to the active site (33, 34). The results of this paper demonstrated that the binding of chitin to the α-sarcin molecule induced the change in fluorescence spectrum, indicating that structural changes happened at the N-terminus of α-sarcin. These structural changes probably affected the conformation of active site, which benefited the binding of α-sarcin to the RNA substrate and thus increased the nuclease activity of α-sarcin.

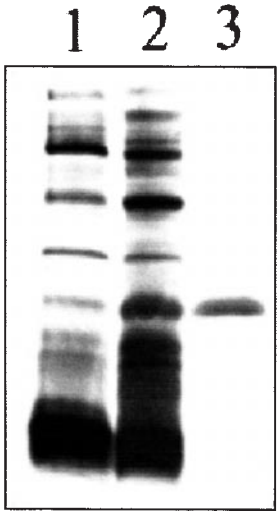

FIG. 6. Effect of chitin on the expression of α-sarcin. *A. giganteus* was cultured in the presence or absence of glycochitin and the proteins in the culture medium were separated on SDS–PAGE (12%) and visualized by silver staining. Lane 1, culture medium (40 μl) without glycochitin; lane 2, culture medium (40 μl) with 0.2% glycochitin; lane 3, the pure α-sarcin (5 μg).

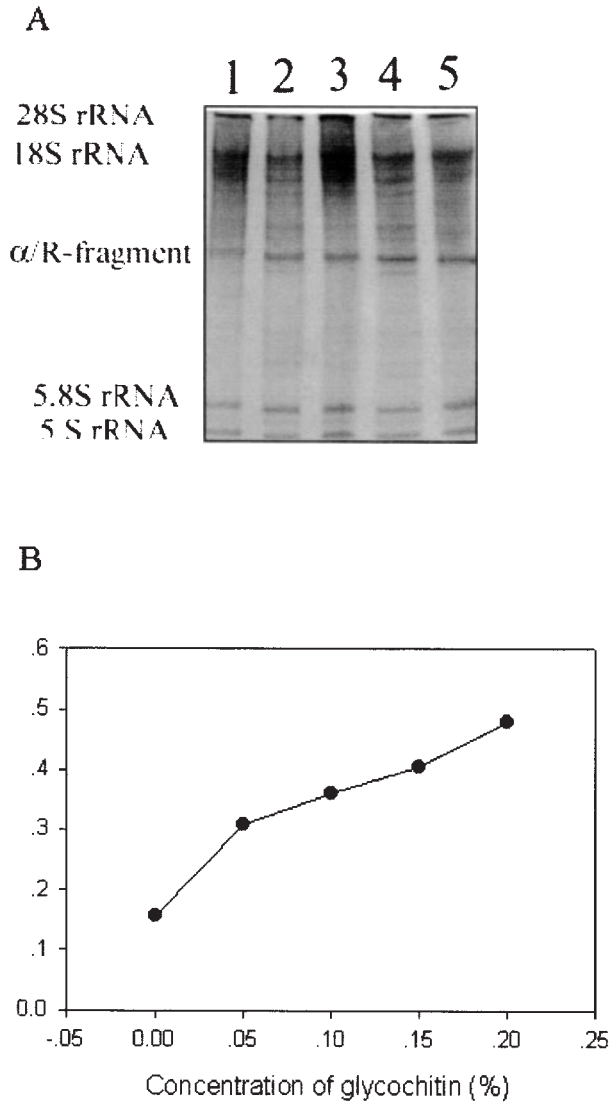

FIG. 7. Effect of glycochitin on the nuclease activity of α-sarcin. (A) 80S ribosomes (0.8 A_{260} units) were treated with α-sarcin in the presence glycochitin. The total rRNA was extracted, separated by gel electrophoresis, stained, and scanned to determined the relative amounts of α/R-fragment as described under Materials and Methods. Lane 1, control; lanes 2–5, ribosomes were treated, respectively, with α-sarcin (1×10^{-8} M) in the presence of 0.05, 0.10, 0.15, and 0.20% glycochitin. (B) The extent of the formation the α/R-fragment at each concentration of glycochitin was determined and expressed as ratio of the concentration of α/R-fragment to that of 5S rRNA.

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