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A Novel Peptide with Ribonuclease and Translation-inhibitory Activities from Fruiting Bodies of the Oyster Mushroom *Pleurotus ostreatus*

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Abstract: From the fresh fruiting bodies of the oyster mushroom a peptide with a molecular weight of 9 kDa and demonstrating a novel *N*-terminal sequence GPCYLVAFYESSGRR was isolated. The isolation procedure involved ion exchange chromatography on CM-Sepharose and Mono S. The peptide was adsorbed on both types of chromatographic media. The peptide demonstrated a ribonuclease activity of 650 U/mg toward yeast transfer RNA. It inhibited cell-free translation in a rabbit reticulocyte lysate system with an IC_{50} of 15 nM. Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: mushroom; ribonuclease; translation-inhibitory; peptide

INTRODUCTION

Ribonucleases have been isolated and characterized from many different organisms [1] including a few mushroom species such as *Pleurotus ostreatus* [2], *Pleurotus tuber-regium* [3,4], *Lentinus edodes* [5,6], *Irpex lacteus* [7] and *Volvariella volvacea* [8].

The molecular weights of the various mushroom RNases differ. *P. ostreatus* RNase has a molecular weight around 11 kDa, while *P. tuber-regium* RNase and *V. volvacea* RNase are 29 kDa and 42.5 kDa in molecular weight.

A ubiquitin-like glycoprotein has been isolated from *P. ostreatus* fruiting bodies. It exhibited a molecular weight of 12.5 kDa, a rich carbohydrate content, and an *N*-terminal sequence with marked homology to ubiquitin. It demonstrated an RNase activity of 16 U/mg toward yeast tRNA and inhibited translation in a rabbit reticulocyte lysate with an IC_{50} of 160 nM [9]. In addition to this ubiquitin-like glycoprotein, a heterodimeric lectin with potent anti-hepatoma and anti-sarcoma activities has been purified [10].

The intent of the present study was to examine the fruiting bodies of *P. ostreatus*, an economically important mushroom, for other proteins and peptides. The results disclosed the presence of a novel peptide with ribonuclease and translation-inhibiting activities.

MATERIALS AND METHODS

Chromatographic Isolation

Fresh oyster mushrooms (*Pleurotus ostreatus*) were purchased from a local market. The fruiting bodies were homogenized in water. To the supernatant obtained after centrifugation NH_4OAc buffer (pH 4.6) was added until the final concentration attained 20 mM. The supernatant was then chromatographed on a column of CM-Sepharose (2.5 × 18 cm) equilibrated and eluted with the same buffer. After elution of unadsorbed material, adsorbed proteins were desorbed with a linear NaCl concentration gradient (0–0.5 M). The second adsorbed peak (CM2)

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was dialysed, lyophilized and applied on a Mono S column (1 ml) by FPLC. Unadsorbed material was eluted with 20 mM NH_4OAc buffer (pH 4.6). Adsorbed proteins were eluted with a linear gradient of 0–1 M NaCl. The small peak eluted after the main adsorbed peak was the purified *Pleurotus ostreatus* peptide.

Molecular Weight Determination by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and by FPLC-Gel Filtration

SDS-PAGE was carried out in accordance with the procedure of Laemmli and Favre [11], using a 12% resolving gel and a 5% stacking gel. At the end of electrophoresis, the gel was stained with Coomassie brilliant blue. FPLC-gel filtration was carried out using a Superdex 75 column which had been calibrated with molecular weight standards (Amersham Pharmacia Biotech).

Analysis of *N*-terminal Amino Acid Sequence

Amino acid sequence analysis was carried out using an HP Gl000A Edman degradation unit and an HP1000 HPLC system [12].

Ribonuclease Activity of *P. Ostreatus* Peptide

The activity of *P. ostreatus* peptide toward tRNA was assayed by determining the generation of acid-soluble, UV-absorbing species by the method of Mock *et al.* [13]. The RNase was incubated with 200 µg of tRNA in 150 µl 100 mM MES (pH 6.0) at 37 °C for 1 h. The reaction was terminated by introduction of 350 µl of ice-cold 3.4% perchloric acid. After leaving on ice for 15 min, the sample was centrifuged (15 000 g, 15 min) at 4 °C. The OD_{260} of the supernatant was read after appropriate dilution. One unit of enzymatic activity is defined as the amount of enzyme that brings about an increase in OD_{260} of 1/min in the acid-soluble fraction per ml of reaction mixture under the specified condition.

Inhibitory Activity in Cell-Free Translation using a Rabbit Reticulocyte Lysate System

Rabbit reticulocyte lysate was prepared from anaemic rabbit blood. The rabbit had received phenylhydrazine treatment which made it anaemic [14]. An assay based on the rabbit reticulocyte lysate translation system [12] was used. Ten microlitres of the test sample were added to 10 µl of radioactive mixture (500 mM KCl, 5 mM $MgCl_2$ 130 mM phosphocreatine and 1 µCi L-[4,5-^{3}H] leucine) and 30 µl working rabbit reticulocyte lysate containing 0.1 µM haemin and 5 µl creatine kinase. The reaction mixture was incubated at 37 °C for 30 min, followed by addition of 330 µl 1 M NaOH and 0.2% H_2O_2. After further incubation for 10 min to allow decolorization and tRNA digestion, protein with radioactive leucine incorporated was precipitated when an equal volume of the reaction mixture was added to 40% trichloroacetic acid with 2% casein hydrolysate in a 96-well plate. The precipitate was collected on a glass fibre Whatman GF/A filter, washed and dried with absolute alcohol passing through a cell harvester attached to a vacuum pump. The filter was suspended in scintillant and counted in an LS 6500 Beckman liquid scintillation counter.

RESULTS

Ion exchange chromatography of the fruiting body extract on CM-Sepharose yielded a large unadsorbed peak. Subsequent elution with a linear NaCl concentration gradient fractionated the adsorbed proteins into three peaks of increasing sizes, CM1, CM2 and CM3 (Figure 1). Peak CM2 was separated by FPLC on Mono S into a sharp unadsorbed peak, two large adsorbed peaks and a very small adsorbed peak. The small adsorbed peak constituted purified protein designated *Pleurotus ostreatus* peptide (Figure 2).

Pleurotus ostreatus peptide demonstrated a single band with a molecular weight of 9 kDa in SDS-PAGE (Figure 3) and a single peak with the same molecular weight in FPLC-gel filtration on Superdex 75 (data not shown). Its *N*-terminal sequence is shown in Table 1. There was a certain extent of similarity with the stringent starvation protein from *E. coli* and serine protease from *Trimeresurus flavoviridis* which are, however, much larger in molecular size. Resemblance to dihydrofolate reductase from *Schizosaccharomyces pombe* and human latent transforming growth factor β-binding protein 1 precursor was detected, although again the proteins are much bigger. The yield of *Pleurotus ostreatus* peptide from 225 g fruiting bodies was 0.45 mg. It possessed an RNase activity of 651 U/mg toward yeast transfer RNA (Table 2). It caused a dose-dependent inhibition of translation in the rabbit reticulocyte lysate system. The IC_{50} was 15 nM (Table 3).

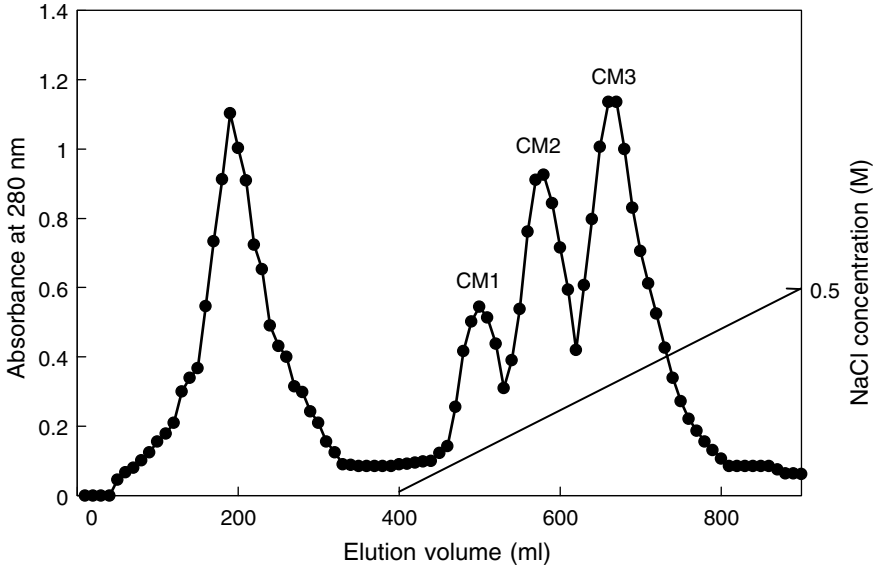

Figure 1 Ion exchange chromatography of a crude extract of *Pleurotus ostreatus* fruiting bodies on a CM-Sepharose column (2.5×18 cm). The starting buffer was 20 mM NH_4OAc buffer (pH 4.6). A linear gradient of 0–0.5 M NaCl in the starting buffer was applied. Yields: CM1, 20.4 mg; CM2, 88.8 mg; CM3, 146.9 mg.

DISCUSSION

The cell-free translation-inhibitory and RNase activities of *Pleurotus ostreatus* peptide are higher than those of PULP, the ubiquitin-like glycoprotein from the same species. PULP has a higher molecular weight (12.5 kDa) than *Pleurotus ostreatus* peptide. PULP is heavily glycosylated whereas *Pleurotus ostreatus* peptide is not. PULP and *Pleurotus ostreatus* peptide also differ markedly in *N*-terminal sequence. All evidence thereby suggest that *Pleurotus ostreatus* peptide and PULP are distinct entities.

It deserves mention that the fruiting bodies of the puffball mushroom, *Calvatia caelata*, produce a ubiquitin-like peptide (CULP) with a molecular weight of 8 kDa [15]. The *N*-terminal sequence of CULP is typical of ubiquitin-like proteins/peptides, and is dissimilar to that of *Pleurotus ostreatus* peptide. Again the translation-inhibiting and RNase activities of *Pleurotus ostreatus* peptide are more potent than those of CULP. This furnishes corroborative evidence that *Pleurotus ostreatus* peptide is not a ubiquitin-like peptide.

The novel peptide shows no sequence resemblance to *P. ostreatus* RNase [2]. There was no structural

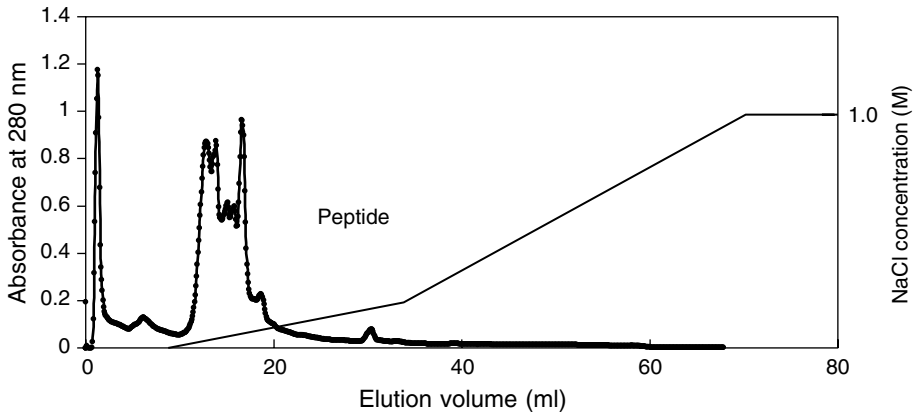

Figure 2 Ion exchange chromatography of fraction CM2 from CM-Sepharose column on a Mono S column by FPLC. The starting buffer was 20 mM NH_4OAc buffer (pH 4.6). A linear gradient of 0–1.0 M NaCl in the starting buffer was applied.

Table 1 *N*-terminal Sequence of Novel Peptide from *Pleurotus ostreatus* in Comparison with Other Proteins (Results of a BLAST Search)

	Residue No.		Length of protein
POP	1	GPCYLV•AFYESSGRR	
Stringent starvation protein B from *E. coli*	10	P•YLLRAFYE	165
POP			
Serine protease from *Trimeresurus flavoviridis*	1	GPCYLVAFYES•SGRR	260
	37	P•FLVALYDAWSGR	
Dihydrofolate reductase from *Schizosaccharomyces pombe*	37	LVSFYES•S	550
POP	1	GPCY•LVAFYESSGR	
Latent transforming growth factor beta binding protein 1 precursor (*Homo sapiens*)	351	GPCYRLV••••SSGR ••••SSGR	669

•, Space left to maximize similarity; POP, *Pleurotus ostreatus* peptide.

Table 2 Yields and Ribonuclease Activities of Various *Pleurotus ostreatus* Chromatographic Fractions toward Yeast tRNA

Chromatographic fraction	Yield from 225 g fruiting bodies (mg)	RNase activity (U/mg)
Crude extract	3096.0	48.5
CM2	88.8	237.8
Pleurotus ostreatus peptide (small adsorbed peak on Mono S)	0.45	650.8

Table 3 Inhibition of Cell-Free Translation in Rabbit Reticulocyte Lysate by *Pleurotus ostreatus* Peptide (Mean ± SEM, $n = 3$).

Concentration (μg/ml)	Inhibition (%)
0.004	5.01 ± 1.3
0.024	27.2 ± 2.6
0.12	51.2 ± 1.8
0.60	79.3 ± 0.9
3.00	94.0 ± 1.0

Note: IC_{50} = 15 nM.

similarity to RNases from *P. tuber-regium* [3,4], *L. edodes* [5], *Irpex lacteus* [7] and *V. volvacea* [8] either. The *Pleurotus ostreatus* peptide also has a smaller molecular weight than *P. ostreatus* RNase, indicating that they are separate molecules. The translation — inhibiting activity of *Pleurotus ostreatus* peptide might stem from its RNase activity. Its RNase activity is considerably lower than those of mushroom RNases. The chromatographic behaviour of the novel peptide on CM-Sepharose and Mono S is similar to that of the *P. ostreatus* ubiquitin-like glycoprotein, *P. tuber-regium* RNase and *V. volvacea* RNase in that they are all adsorbed on these or similar chromatographic media [3,8,9].

Ribosome inactivating proteins have been isolated from the mushrooms *Flammulina velutipes* [4,16], *Hypsizigus marmoreus* [17], *Lyophyllum shimeiji* [18], and *Pleurotus tuber-regium* [19]. Their molecular weights range from 14 kDa to 40 kDa. The translation-inhibitory activity of *Pleurotus ostreatus* peptide is weaker than those of the aforementioned ribosome inactivating proteins. Conversely, its RNase activity is much stronger than mushroom ribosome inactivating proteins which have little or no RNase inactivity. Its *N*-terminal sequence bears no similarity to those of the aforementional mushroom ribosome inactivating proteins. The data

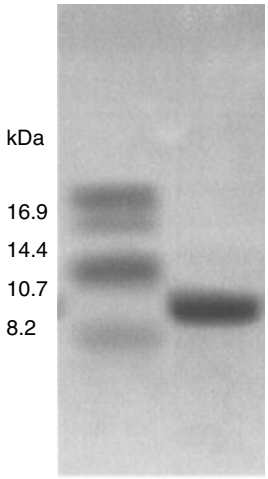

Figure 3 SDS-polyacrylamide gel electrophoresis. Left lane: molecular weight markers, from top downward: MW 16 949; MW 14 404; MW 10 700; globin, 8 159. Right lane: *Pleurotus ostreatus* peptide.

above suggest that *Pleurotus ostreatus* peptide is a novel peptide and not a ribosome inactivating protein.

The *N*-terminal sequence of *Pleurotus ostreatus* peptide manifests some homology to sequences of proteins with activities unrelated to those of the peptide. These proteins are all much larger in molecular weight than *Pleurotus ostreatus* peptide. This observation is interesting in view of the sequence dissimilarity of the peptide from ubiquitin, RNases and ribosome inactivating proteins which possess activities similar to those of *Pleurotus ostreatus* peptide.

From the fruiting bodies of *Pleurotus ostreatus*, a lectin [10], an RNase [2], a ubiquitin-like glycoprotein [9] and a metalloprotease [20] have been isolated. The present findings concerning *Pleurotus ostreatus* peptide add to the literature available on this economically important mushroom.

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