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# A hemolysin from the mushroom *Pleurotus eryngii*

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**Abstract** A monomeric 17-kDa hemolysin designated as eryngeolysin was isolated from fresh fruiting bodies of the mushroom *Pleurotus eryngii*, using a protocol that involved gel filtration on Superdex 75, ion exchange chromatography on Mono Q and gel filtration on Superdex 75. Its N-terminal sequence demonstrated striking homology to that of its counterparts ostreolysin from the oyster mushroom *Pleurotus ostreatus* and aegerolysin from the mushroom *Agrocybe cylindracea*. Its hemolytic activity was unaffected over the pH range 4.0–12.0, but no activity was observed at pH 13 and at and below pH 2. The hemolysin was stable between 0 and 30 °C. At 40 °C, only residual activity was detectable. At and above 50 °C, activity was indiscernible. Eryngeolysin exhibited cytotoxicity toward leukemia (L1210) cells but not toward fungi. The hemolysin was inactivated by treatment with trypsin. It exhibited antibacterial activity against *Bacillus* sp. but not against other species. It inhibited basal as well as ConA-stimulated mitogenic response of murine splenocytes. *N*-Glycolyneuraminic acid was the only sugar capable of inhibiting the hemolytic activity. Eryngeolysin-induced hemolysis was osmotically protected by polyethylene glycol (PEG) 10000 with a mean hydrated diameter dose to 9.3 nm. However, no protection was offered by PEG 10000 to the anti-mitogenic and antiproliferative activities of eryngeolysin. The susceptibility of erythrocytes from different classes of vertebrates to eryngeolysin was mammalian > avian > reptilian > piscine.

## Introduction

Mushrooms have captured the attention of a large number of investigators because of their economic value. They have a high protein content and a variety of proteins have to date been isolated from mushrooms. Lectins (Wang et al. 1996, laccases (Giardina et al. 1999), antifungal proteins (Lam and Ng 2001), and hemolysins (Berne et al. 2002) are examples of biologically active mushroom proteins. Some of these mushroom proteins such as lectins have antitumor and antiproliferative activities (Wang et al. 1996) while antifungal proteins and laccases have potential applications and biotechnological importance, respectively.

Hemolysins have been isolated from very few mushroom species including *Pleurotus ostreatus* and *Agrocybe cylindracea* (Berne et al. 2002). Thus, it would be a worthwhile undertaking to isolate a hemolysin from another species, the eryngii mushroom *Pleurotus eryngii*. Very few bioactive proteins have been purified hitherto from the eryngii mushroom. The present investigation would add biochemical data to this edible mushroom with increasing economic importance. The biological activities of previously isolated mushroom hemolysins have not been extensively characterized. The present study was undertaken with a view to examine the biological activities of *P. eryngii* hemolysin in detail.

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## Materials and methods

### Isolation of eryngeolysin

Fresh fruiting bodies of *P. eryngii* (1.5 g) were extracted. The crude extract was subjected to gel filtration in 20 mM $NH_4HCO_3$ buffer (pH 9.4) by fast protein liquid chromatography (FPLC) on a Superdex 75 HR 10/30 column (Amersham Biosciences). The first peak eluted was further purified by ion exchange chromatography on a 1-ml Mono Q column (Amersham Biosciences) in 20 mM $NH_4HCO_3$ buffer (pH 9.4) by FPLC. After elution of unadsorbed proteins, adsorbed proteins were desorbed using two successive linear NaCl concentration gradients (0–0.3 M followed by 0.3–1 M). The second adsorbed peak was chromatographed on a Superdex 75 column in 20 mM $NH_4HCO_3$ buffer (pH 9.4).

### Molecular mass determination and sequence analysis

The purified hemolysin was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli and Favre 1973) and gel filtration on a calibrated FPLC-Superdex 75 HR 10/30 column to determine its molecular mass. Its N-terminal sequence was determined by Edman degradation.

### Assay of hemolytic activity (Berne et al. 2002)

Rabbit erythrocytes were washed with 10 mM Tris-buffered saline (TBS, pH 7.5) three to four times and adjusted to a final concentration of 4% (*v*/*v*) in TBS. A sample solution (0.2 ml) was mixed with rabbit erythrocytes (0.2 ml) and incubated at room temperature for 1 h before centrifugation at 1,000×*g* for 5 min. The amount of hemoglobin released from disrupted erythrocytes was determined spectrophotometrically. One hundred percent hemolysis was defined as $OD_{540}$ of hemoglobin released from erythrocytes treated with 0.1% Triton X-100. One hemolysin unit (HU) was defined as the amount of hemolysin eliciting 50% hemoglobin release.

### Effect of different sugars on hemolytic activity

The hemolysis inhibition tests to investigate inhibition of hemolysin-induced hemolysis by various carbohydrates were performed in a manner analogous to the hemolysis test erythrocyte suspension. Sugar in the final reaction mixture which completely inhibited 16 HU of the hemolysin preparation was calculated (Berne et al. 2002).

### Effects of temperature and pH on hemolytic activity

Hemolytic activity of eryngeolysin (8 HU) was routinely assayed at 37 °C. To determine the effect of temperature on hemolytic activity, a suspension of the washed 4% rabbit erythrocytes (0.2 ml) was incubated with eryngeolysin (8 HU in 0.2 ml of TBS) for 5–60 min at different temperatures, and then $OD_{540}$ of the supernatant was measured (Berne et al. 2002).

### Osmotic protection experiments

For these experiments, 0.2 ml of a 4% rabbit erythrocyte suspension containing an osmotic protectant was mixed with 0.2 ml of eryngeolysin solution (8 HU). polyethylene glycol (PEG) 400, PEG 1500, and PEG 4000 were used as the osmotic protectants at a final concentration of 30 mM. PEG 6000, PEG 10000, and PEG 20000 were used at a final concentration of 15 mM. Protection from hemolysis was calculated as follows: protection (%)=(1−hemolysis rate in the presence of osmotic protectant/hemolytic rate without osmotic protectant)×100 (Berne et al. 2002).

### Assay of eryngeolysin for antiproliferative activity toward leukemia cells (Wang et al. 1996)

The results would reveal whether eryngeolysin produces an adverse effect on cells other than erythrocytes.

### Assay of eryngeolysin for anti-mitogenic activity (Wang et al. 1996)

The assay of anti-mitogenic activity was performed using splenocytes isolated from BALB/c mice.

### Assay of eryngeolysin for antibacterial activity

A liquid growth inhibition assay was performed. Bacteria grown in Luria–Bertani (LB) medium was collected in the exponential phase of growth and resuspended with phosphate-buffered saline, pH 6.0, at a density of $1\times10^8$ cells/ml. Samples were suspended in 200 μl of 0.2% (*w*/*v*) bovine serum albumin and then incubated in 190 μl of LB medium with 10 μl of bacterial suspension and shaking for 3 h at 37 °C. $OD_{600}$ was measured.

### Assay of eryngeolysin for antifungal activity

The results would reveal whether eryngeolysin produces an adverse effect on cells other than erythrocytes. The assay for antifungal activity, a potentially exploitable effect, was carried out as described by Lam and Ng (2001).

## Results

### Purification and sequence determination of eryngeolysin

The fruiting body extract was fractionated on a Superdex 75 column into several small peaks and a large, sharp peak

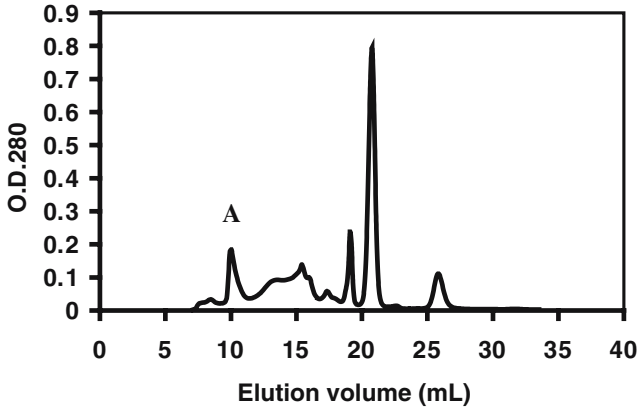


**Fig. 1** Gel filtration of crude extract of *P.eryngii* fruiting bodies on a Superdex 75 HR 10/30 column in 20 mM $NH_4HCO_3$ buffer (pH 9.4) at a flow rate of 0.4 ml/min. Only the peak labeled *A* exhibited hemolytic activity

**Table 1** Purification of hemolysin from *P. eryngii* fruiting bodies

| | Purification step | Protein yield (mg/1.5 g mushroom) | Total hemolytic activity (HU) | Specific hemolytic activity (HU/mg) | Yield of hemolytic activity (%) |
|---|---|---|---|---|---|
| I | Crude extract | 28 | 31,444 | 1,123 | 100 |
| II | Superdex 75 | 7.6 | 27,078 | 3,563 | 86.1 |
| III | Mono Q | 1.3 | 19,067 | 14,667 | 60.6 |
| IV | Superdex 75 | 0.45 | 7,327 | 16,283 | 23.3 |

(Fig. 1). Hemolytic activity was located in the first small peak A (Table 1). Peak A was resolved on Mono Q into two unadsorbed peaks devoid of hemolytic activity and three adsorbed peaks eluted in the first NaCl concentration gradient (Fig. 2). Hemolytic activity was detected only in the second adsorbed peak B (Table 1). B was already highly purified as judged by gel filtration on Superdex 75 which revealed essentially a single peak (data not shown). This peak exhibited a molecular mass of 17 kDa in SDS-PAGE (Fig. 3). There was an approximately 15-fold increase in specific hemolytic activity of the hemolytic principle as a result of this purification procedure. The yield of the hemolysin designated as eryngeolysin was 300 μg/g fruiting bodies (Table 1). The results of N-terminal sequence of eryngeolysin revealed a single peak in each cycle indicating homogeneity of the preparation. Eryngeolysin exhibited 80% N-terminal sequence identity to ostreolysin and aegerolysin when the first 40 amino acid residues were compared. Sequence identity to hemolysins from *Aspergillus fumigatus* and *Clostridium bifermentans* was less extensive (Table 2).

## Effect of sugars, trypsin digestion, pH, and temperature on hemolytic activity of eryngeolysin

Among the various salts examined, only $Na_2CO_3$, $Na_3PO_4$, $FeCl_2$, and $CuCl_2$ inhibited the hemolytic activity of eryngeolysin. The $ID_{50}$ causing 50% inhibition was, respectively, 8, 16, 1.2, and 2.1 mM. No inhibitory effect was detected when the following compounds were tested at 50 mM. NaCl, $NaHCO_3$, $Na_2S_2O_5$, $Na_4S_2O_7$, $NaH_2PO_4$, KBr, KI, $KH_2PO_4$, $AgNO_3$, $Ag_2SO_4$, $ZnSO_4{\cdot}7H_2O$, $MgSO_4$, $MgCl_2{\cdot}4H_2O$, $CuSO_4Pb(CH_3COO)_2{\cdot}3H_2O$, $NiCl_2$, EDTA, SDS, sodium tartrate, L-ascorbic acid, urea, and succinic acid. *N*-Glycolylneuraminic acid at 7 mM concentration inhibited the hemolytic activity of

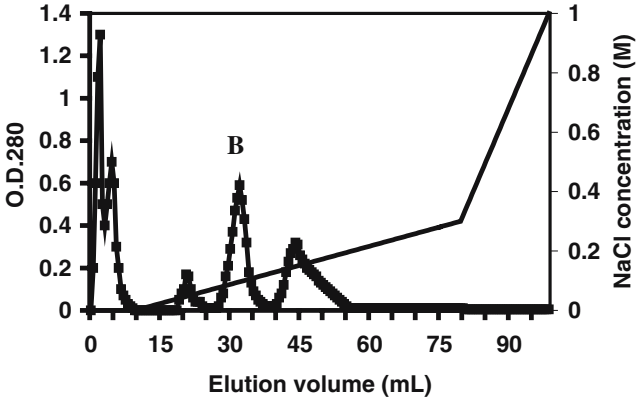


**Fig. 2** Fast protein liquid chromatography of peak A on a 1-ml Mono Q column in 20 mM $NH_4HCO_3$ buffer (pH 9.4) at a flow rate of 1 ml/min. The two *slanting lines* across the chromatogram indicate the two linear NaCl gradients used to elute adsorbed proteins. The peak labeled *B* was the only peak showing hemolytic activity

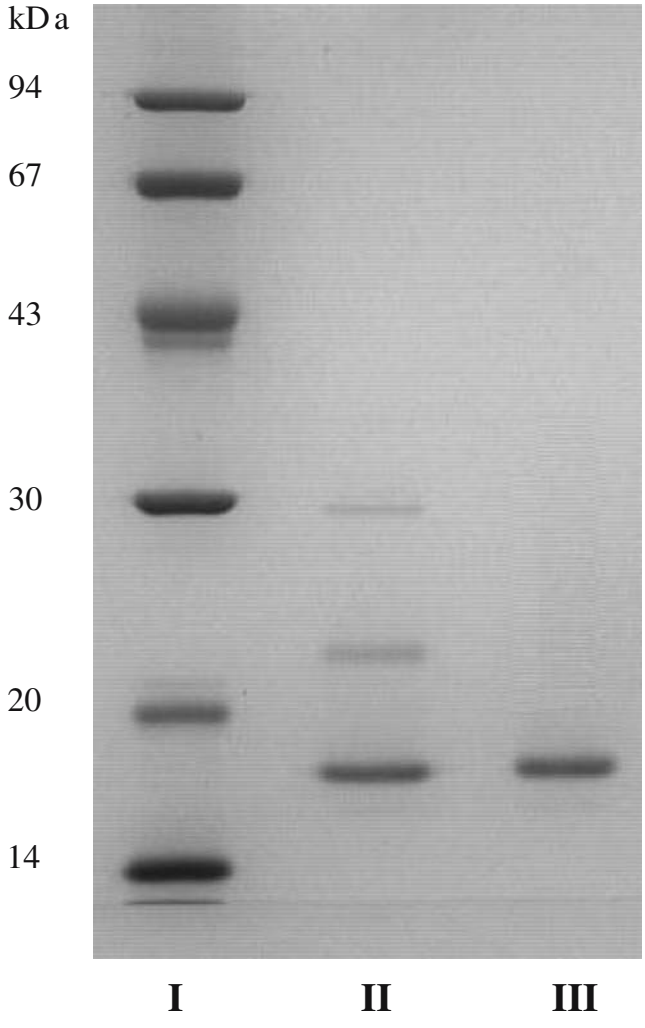


**Fig. 3** SDS-polyacrylamide gel electrophoresis followed by staining with Coomassie blue. *Lane I*, molecular mass marker proteins; *lane II*, 10 μg protein from peak A obtained in first gel filtration step; *lane III*, 10 μg purified hemolysin from single peak obtained in second gel filtration step

**Table 2** N-terminal sequence comparison of *P. eryngii* hemolysin (eryngeolysin) with other hemolysins

| Origin of hemolysin | Sequence | Total no. of amino acids |
|---|---|---|
| *Pleurotus eryngi* (eryngeolysin) | (1) AYAQWV I I I I HNVGSKDVKIVNLKPSWGKLSAAGDLQTEV(40) | |
| *Pleurotus ostreatus* (ostreolysin) | (1) AYAQWVIIIIHNVGSQDVKIKNLKASWGKLHADGDKDAEV (40) | 138 |
| *Agrocybe aegerita* (aegerolysin) | (9) AYAQWVIIIILHNVGSSPFKI ANLGLSWGKLYADGNKDKEV (48) | 145 |
| *Aspergillus fumigatus* | (6) AYAQWVTVHLI NSMSSETLSIKNASL-SWGK (35) | 131 |
| *Clostridium bifermentans* | (20) AYRQWVKFHIEAVHSNG–LKIRNASL TWGKFHDPYNKDIPI (58) | 152 |
| *Vibrio fluvialis* | (1) LKLNSYAATTNALCSLSASGNVFADIHDPVCAAVSILSQVSD (42) | 740 |

Aegereolysin (9) refers to A being the ninth residue in the hemolysin. Identical corresponding amino acid residues are underlined. The sequence of *P. eryngii* hemolysin is from this study, those of *P. ostreatus* and *Agrocybe aegerita* hemolysins are from Berne et al. (2002), and that of *V. fluvialis* hemolysin is from Han et al. (2002)

hemolysin by 50%. Other sugars tested at 50 mM had no effect. They included α-D(+)-melibiose, polygalacturanose, α-L(−)fructose, L(+)-arabinose, α-lactose, D-galactose, 3′-*N*-acetylneuraminic lactose, *N*-acetyl-D-galactosamine, L(−)-fucose, D(+)galactosamine, D-mannosamine, L(−)-mannose, α-methyl-β-D-glucoside, and rhamnose (data not shown). The hemolytic activity of eryngeolysin after incubation with an equal weight of trypsin at 37 °C for 0, 5, 10, 15, and 20 min was, respectively, 15,823±158, 9,950±50, 3,108±28, 160±4, and 6 HU/mg. Hemolysis induced by eryngeolysin was osmotically protected by a mean hydrated diameter close to 9.3 nm (PEG 10000), but not by PEG 400, PEG 1500, PEG 4000, PEG 6000, or PEG 20000 (Fig. 4). Eryngeolysin was stable over the pH range 4.0–12.0. There was a precipitous decrement in activity when the pH was raised to 13 or lowered to 3. About 20% activity remained at pH 3 while there was no activity at pH 2 and 3 (data not shown). The protein was stable between 0 and 30 °C, but its activity underwent a precipitous decline when the temperature was raised to 40 °C. Only about 5% activity remained at 40 °C. At and beyond 50 °C, no activity was detectable (data not shown). All of the aforementioned thermal treatments lasted for 30 min.

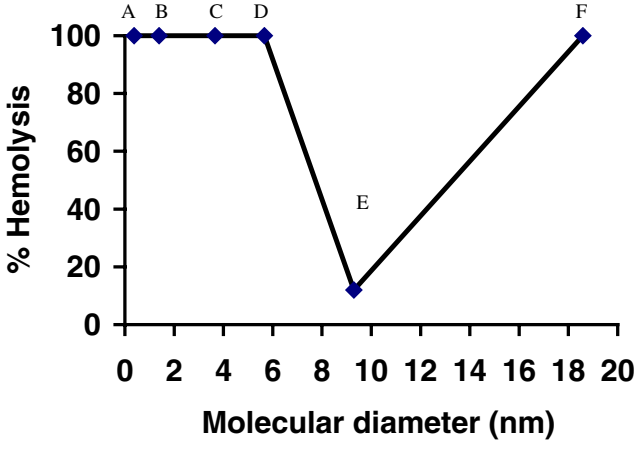


**Fig. 4** Inhibitory effect of osmotic protectants on hemolysis induced by eryngeolysin. The protectants used were **a** PEG 400 molecular diameter (0.37 nm), **b** PEG 1500(1.39 nm), **c** PEG 4000 (3.66 nm), **d** PEG 6000 (5.66 nm), **e**: PEG 10000 (9.29 nm), and **f** PEG 20000 (18.59 nm)

## Effect of eryngeolysin on erythrocytes of different origins, tumor cells, and spleen cells

Erythrocytes from different animals had different susceptibilities to eryngeolysin (Table 3). Eryngeolysin reduced the viability of leukemia L1210 cells (Fig. 5). Its anti-mitogenic action on mouse splenocytes (Fig. 6) could not be offset by PEG 1000. The ConA-stimulated mitogenic response in mouse splenocytes was attenuated by eryngeolysin (Fig. 6). *N*-Glycolylneuraminic acid did not abolish the anti-mitogenic effect of eryngeolysin on splenocytes and its inhibitory effect on leukemia cells (Figs. 5 and 6). Eryngeolysin did not manifest any antifungal activity toward *B. cinerea*, *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Physalospora piricola*, and *Rhizoctonia solani* (data not shown). However, eryngeolysin exerted antibacterial activity toward *Bacillus megatarium* and *Bacillus subtilis* with $IC_{50}$ values of 110±10, 175±15, and 25±5 μM (mean±SD, $n$=3), respectively. No effect was noted toward other bacterial species including *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Mycobacterium phlei*, and *Proteus vulgaris*. PEG 10000 was able to protect the bacteria from the antibacterial action of eryngeolysin. No antibacterial effect was observed in the presence of PEG 10000 alone or eryngeolysin plus PEG 10000.

**Table 3** Susceptibility of erythrocytes of various species to eryngeolysin

| Animal species | Hemolytic activity (HU/mg) | Sensitivity factor |
|---|---|---|
| Pig | 18,207±226 | 1.15 |
| Rabbit | 15,823±158 | 1 |
| Human | 14,937±132 | 0.94 |
| Mouse | 11,029±80 | 0.70 |
| Chicken | 7,505±107 | 0.47 |
| Soft-shelled turtle | 2,863±59 | 0.18 |
| Fish (white pomfret) | 2,257±24 | 0.13 |

Data represent means±SD ($n$=3)

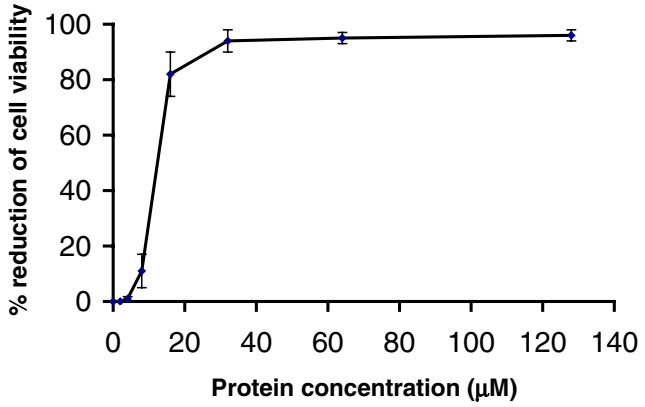


**Fig. 5** Cytotoxicity of eryngeolysin on leukemia (L1210) cells (data represent means±SD, *n*=3). *N*-Glycolylneuraminic acid (50 mM) did not have any effect on the cytotoxicity of eryngeolysin

## Discussion

A simple procedure is described herein for the isolation of a hemolysin from fruiting bodies of the mushroom *P. eryngii*. The procedure involves gel filtration on Superdex 75, ion exchange chromatography on Mono Q, and gel filtration again on Superdex 75. A procedure that comprised $(NH_4)_2SO_4$ precipitation, gel filtration on Sephadex G50, and ion exchange chromatography on High Q and Resource Q has previously been used for isolating other mushroom hemolysins (Berne et al. 2002). The $(NH_4)_2SO_4$ precipitation step was omitted from the present purification scheme to make it simpler. Eryngeolysin can be obtained at a high yield (300 mg/kg) from the fruiting bodies of the eryngii mushroom, similar to the yields of ostreolysin and aegerolysin (Berne et al. 2002). The specific hemolytic activity of purified eryngeolysin is similar to that of *V. fluvialis* hemolysin (Han et al. 2002). The purification of both hemolysins was monitored with the same hemolysis assay. A nearly 50-fold purification was achieved in case of *V. fluvialis* hemolysin (Han et al. 2002) whereas an approximately 15-fold, 22-fold, and 30-fold purification was obtained for eryngeolysin, aegerolysin (Berne et al. 2002), and ostreolysin (Berne et al. 2002), respectively.

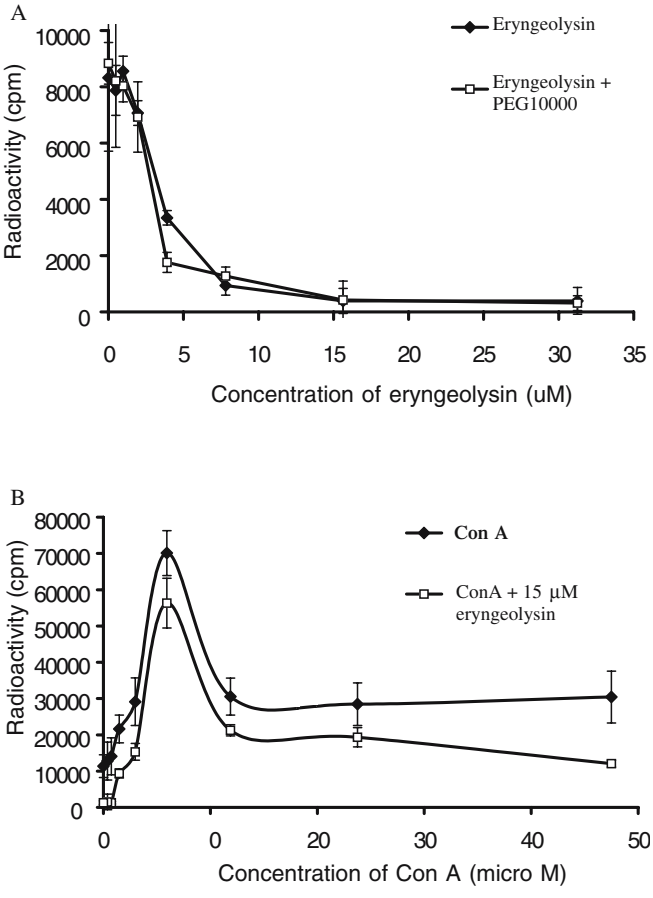


**Fig. 6** Anti-mitogenic activity of eryngeolysin on splenocytes as reflected in reduction in uptake of [methyl-$^3$H] thymidine. *N*-Glycolylneuraminic acid (50 mM) did not have any effect on the anti-mitogenic activity of eryngeolysin

The hemolysin from *P. eryngii*, designated eryngeolysin, manifests impressive sequence homology to osteolysin, its counterpart from a closely related mushroom, the oyster mushroom *Pleurotus ostreatus* (Berne et al. 2002). The molecular masses of eryngeolysin and ostreolysin are similar. However, lectin (hemagglutinin) is absent from *P. eryngii* whereas both lectin and hemolysin can be isolated from *P. ostreatus* (Berne et al. 2002) as well as from the mushroom *A.cylindracea* (Wang et al. 1996, Berne et al. 2002). Eryngeolysin also closely resembles aegerolysin from *A. cylindracea* in N-terminal sequence as well as in molecular mass. N-terminal sequence similarity to hemolysins from the fungus *A. fumigatus* and the bacterium *C. bifermentans* is less extensive despite similarity in molecular mass. However, marked differences in amino acid sequence and molecular mass from hemolysins of the bacteria *Vibrio fluvialis*, *Vibrio cholerae*, *Vibrio mimicus*, and *Vibrio anguillarum* (Han et al. 2002) are observed.

Eryngeolysin is not stable at temperatures above 40 °C, unlike the thermostable hemolysin from *Vibrio parahemolytics* (Raimondi et al. 2000). It is also susceptible to inactivation by trypsin. These observations suggest that hemolysin in the eryngii mushroom would be inactive after cooking for human consumption and passage through the gastrointestinal tract. Ostreolysin and aegerolysin are also heat labile (Berne et al. 2002).

The influence of cations on the activity of mushroom hemolysins has not been studied. Hemolysis induced by ostreolysin is not enhanced by $Zn^{2+}$ (Berne et al. 2002), unlike *A.fumigatus* hemolysin (Sakaguchi et al. 1975). Ostreolysin is inhibited by $HgCl_2$ (Berne et al. 2002). *V. fluvialis* hemolysin is inhibited by the chlorides of the divalent $Zn^{2+}$, $Ni^{2+}$, $Cd^{2+}$, and $Cu^{2+}$ cations but not by the chlorides of monovalent cations such as $Li^+$ and $Cs^+$ (Han et al. 2002). The hemolytic activity of eryngeolysin is similarly not enhanced by $ZnSO_4$ and not inhibited by the salts of a variety of monovalent cations except $Na_3PO_4$, $Na_2CO_3$, and $Na_2S_2O_3$. It is instead inhibited by $CuCl_2$ and $FeCl_2$. The ability of $Na_2CO_3$ but not $NaHCO_3$ to inhibit the hemolytic activity may be due to an effect of pH. Overall speaking, eryngeolysin is only inhibited by certain

chemicals out of the long list tested. The effect of sugars on the hemolytic activity of hemolysins has not been extensively studied previously. The present investigation discloses that *N*-glycolylneuraminic acid inhibits the hemolytic activity of eryngeolysin, implying that the interaction of eryngeolysin with *N*-glycolylneuraminic acid present on the erythrocyte membrane may play a role in preventing its hemolytic action. The inability of *N*-glycolylneuraminic acid to inhibit the anti-mitogenic activity of eryngeolysin toward mouse splenocytes and antiproliferative activity toward leukemia cells suggests that the two types of activities of eryngeolysin do not depend on its interaction with *N*-glycolylneuraminic acid on the plasma membrane of spleen cells and leukemia cells. It is worth mentioning that the mitogenic activity but not the antiproliferative activity of glucose/mannose knife bean lectin, whereas both activities of ConA are inhibited by glucose.

The pH dependence of the hemolytic activity of ostreolysin, aegerolysin and *V. fluvialis* hemolysin has not been examined, and the temperature dependence of the activity has not been investigated over the entire temperature range of 0 °–100 °C. This study reveals the stability of eryngeolysin all the way from pH 4 to 12. Eryngeolysin is stable only between 0 and 30 °C. Only residual activity is observed at 40 °C. *V. fluvialis* hemolysin contrariwise demonstrates optimal activity at 37 °C and its activity is indiscernible at 0 °C (Han et al. 2002). Boiling ostreolysin for 5 min totally inactivates the hemolysin (Berne et al. 2002).

Eryngeolysin-induced hemolysis is osmotically protected by a mean hydrated diameter in the vicinity of 9.3 nm as revealed by the effects of osmotic protectants on hemolysis. Hemolysis induced by *V. fluvialis* hemolysis is osmotically protected by a mean hydrated diameter of 2.8 to 3.7 nm (Han et al. 2002).

Ostreolysin is devoid of insecticidal activity (Berne et al. 2002). Eryngeolysin lacks antifungal activity toward a variety of fungal species including *Botrytis cinerea*, *F. oxysporum*, *M. arachidicola*, *P. piricola*, and *Rhizoctoria solani*, indicating that it does not have a lytic action on fungal cells. It is noteworthy that eryngeolysin exhibits a suppressive action on leukemia cells.

Eryngeolysin forms pores in erythrocyte membrane and the diameter of the pores is estimated by using osmotic protectants to be about 9.3 nm, larger than that formed by *V. fluvialis* hemolysin (Han et al. 2002).

Eryngeolysin-induced inhibition of growth in *Bacillus* species is reversed by treatment with PEG 10000 but not inhibition of leukemia cell proliferation and mitogenic response of splenocytes in response to eryngeolysin, indicating that eryngeolysin action on bacteria, but not its action on leukemia cells and splenocytes, involves pore formation. It is known that hemolysins display a variety of mechanisms of action in different cell types in addition to pore formation, e.g., increasing production of cyclic adenosine monophosphate (Fuji et al. 2003), binding to low-density lipoprotein (Kudo et al. 2001).

Hemolysin production is associated with virulence for many clinical microorganisms such as bacteria, producing septicemia and diarrhea (Raimondi et al. 2000). Hemolysin causes lysis of erythrocytes, mast cells, neutrophils, and polymorphonuclear cells and increases virulence by producing tissue damage or by dissolving materials that would prevent pathogen spreading throughout the tissue (Raimondi et al. 2000). By contrast, the expression of ostreolysin is undetectable during mycelial growth, occurs during formation of primordia and fruiting bodies but declines during maturation (Kudo et al. 2001). It is likely that eryngeolysin is responsible for fruiting initiation in the eryngii mushroom. *Aspergillus* hemolysin (Sakaguchi et al. 1975) is expressed during sporulation. Whether hemolysin plays a common role in bacteria, ascomycete fungi like *Aspergillus* species, and basidiomycete fungi including mushrooms remains to be elucidated.

In summary, the isolation of a hemolysin, with N-terminal sequence similarity to previously reported mushroom hemolysins, using a simplified protocol is reported herein from the eryngii mushroom. It bears little or no N-terminal sequence resemblance to bacterial hemolysins. The present study on eryngeolysin furnishes the following additional information about hemolysins. *N*-Glycolylneuraminic acid inhibits the hemolytic action of eryngeolysin, and eryngeolysin exhibits an antiproliferative action against leukemia cells and anti-mitogenic activity toward mouse splenocytes. Eryngeolysin is stable in the range of pH 4–12. Although eryngeolysin demonstrates a specific antibacterial action against *Bacillus* species, it is devoid of antifungal action.

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