RESULTS

Part I Partial Purification of Lectin from Hemolymph of *Penaeus merguensis*

1. Hemagglutinin Activity in *Penaeus merguiensis* Hemolymph

Among the erythrocytes tested, the serum of *P. merguiensis* agglutinated human A, B, O, AB and pig and chicken erythrocytes. It did not react with horse erythrocytes. The trypsinisation of human A, B, O, AB erythrocytes enhanced their susceptibility for agglutination by the serum (Table 4).

Table 4 Hemagglutinating activity (HA) of serum from *Penaeus merguiensis* against different types of mammalian and animal erythrocytes.

Erythrocytes	HA (titer)	Erythrocytes	HA (titer)
Human A	64	Horse	0
Human A Trypsin treated	256	Sheep	128
Human B	64	Pig	4
Human B Trypsin treated	256	Rabbit	128
Human O	64	Guinea pig	64
Human O Trypsin treated	256	Rat	64
Human AB	64	Mouse	256
Human AB Trypsin treated	128	Goose	256
		Chicken	16

2. Factors Affecting Hemagglutinating Activity

The hemagglutinating activity of the serum showed resistance in some degrees to heating. The initial activity of 64 titer was lowered to half by heating at 60°C for 30 min. While the activity of 2 titer was still observed after heating at 70°C. Heating at 80°C was required to completely destroyed the activity (Table 5). The

hemagglu- tinating activity was maintained at pH ranging from 7.5-10 (Table 6). The activity was less than a quarter of the original activity at pH below 6. The activity of *P. merguiensis* serum was increased by the addition 128 times of 10 mM $CaCl_2$ (Table 7). $MgCl_2$ and $MnCl_2$ at 10 mM had no effect to the hemagglutinating activity.

Table 5 Effects of temperature on hemagglutination

Temperature (°C)	HA(titer)
4	64
25	64
35	64
60	8
70	2
80	0
100	0

Table 6 Effects of pH on hemagglutination

pH	HA(titer)
4	4
5	8
6	8
7.5	64
8	64
9	64
10	64

Table 7 Effects of divalent cation on hemagglutination

Condition	HA(titer)
Before dialysis	128
After dialysed against TBS	128
After dialysed against EDTA (control)	2
10 mM $CaCl_2$	256
10 mM $MgCl_2$	4
10 mM $MnCl_2$	2

3. Hemagglutination Inhibition assays

Various carbohydrates were tested for the ability to inhibit the hemagglutination of human A erythrocytes. A lectin from the serum and affinity purified lectin of *P. merguiensis* was most effectively inhibited by sialo-glycoprotein, mucin at 0.005 mg/ml. By contrast amino derivatives carbohydrates, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine inhibited only the lectin from serum activity at 75 and 50 mM respectively, while the simple sugars were not inhibited an agglutination (Table 8).

Table 8 The sugar specificity of lectin from serum and affinity purified lectin of *Penaeus merguiensis*

Sugars and glycoproteins	Minimum inhibitory concentration	
	Serum[a]	Affinity purified lectin[a]
***Sugars* (mM)**		
D-fructose	N	N
D-Ribose	N	N
D-Xylose	N	N
L-Arabinose	N	N
D-Galactose	N	N
D-Glucose	N	N
D-Mannose	N	N
D-Rhamnose	N	N
L-Fucose	N	N
D-Sorbitol	N	N
D-Galactosamine	N	N
D-Glucosamine	N	N
N-Acetyl-D-galactosamine	75 (16.59 mg/ml)	N
N-Acetyl-D-glucosamine	50 (11.06 mg/ml)	N
α-Methyl-D-mannopyranoside	N	N
Cellobiose	N	N
Lactose	N	N
Maltose	N	N
Melibiose	N	N
Sucrose	N	N
Raffinose	N	N
***Glycoproteins* (mg/ml)**		
PSM (Mucin type II from porcine stomach)	0.0065	0.0130
Fetuin (from fetal calf serum)	N	N

[a] Inhibitory activity was estimated with the crude extract (serum) and affinity purified lectin diluted to titer of 16 against human A erythrocytes.

N, no inhibitory activity at 100 mM (sugars) or 0.125% glycoproteins)

4. Purification of lectin from *Penaeus merguiensis*

Based on the hemagglutinating inhibition results demonstrated an apparent specificity of serum agglutinin of *P. merguiensis* for mucin (PSM), therefore the single-step purification was attempted by affinity chromatography using mucin-CNBr activated Sepharose 4B. A typical column profile depicting the purification of *P. merguiensis* agglutinin on PSM-CNBr activated Sepharose 4B is shown in Figure 8. The serum (20 ml; HA titer = 32 (human A)) passed through the affinity matrix and the effluent was collected during subsequent washing of this matrix with 50 mM Tris-HCl buffer saline/ 0.01M $CaCl_2$ (TBS/Ca-2). Affinity chromatography on PSM Sepharose 4B gave two lectin peaks. The major lectin peak was eluted from the column with 50 mM Tris-HCl buffer saline/ 25 mM EDTA (TBS /EDTA), and tested for hemagglutinin with human A as shown in Figure 8. Active fractions were combined and concentrated by lyophylization.

Molecular mass of the purified lectin was determined by gel filtration. The 0.5 ml of purified and concentrated lectin was applied on a Sephacryl S-200 column (1.5 x 100 cm). The elution profile by the analytical gel column showed one lectin peak (PML) as shown in Figure 9. The molecular mass of the lectin (PML) was estimated to be approximately 112 kDa in gel filtration and consisted of 30.09 (PML1) and 28.01 kDa (PML2) subunits by reducing SDS-PAGE (Figure 10)

Lectin from hemolymph was purified by affinity chromatography on PSM conjugated CNBr-activated Sepharose 4B and gel filtration on Sephacryl S-200. The lectin purified from hemolymph had the purity folds of 4,000 and 50,000 respectively (Table 9).

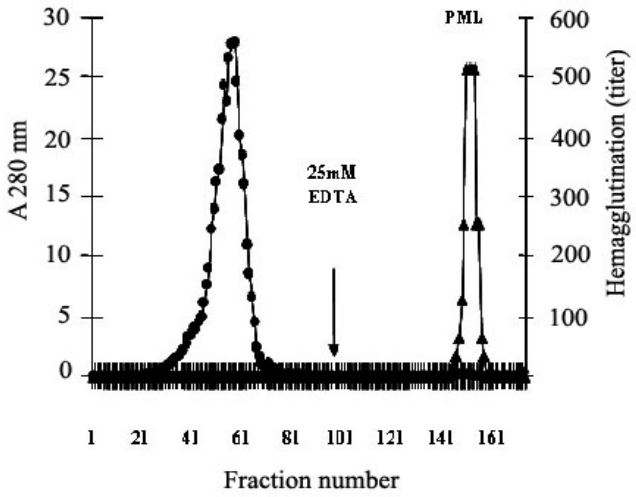

Figure 8 Affinity chromatography of the *P. merguiensis* lectin on mucin CNBr-activated Sepharose 4-B. ●-● , absorbance at 280 nm, ▲-▲, hemagglutinin titer against trypsin treated humanA erythrocytes

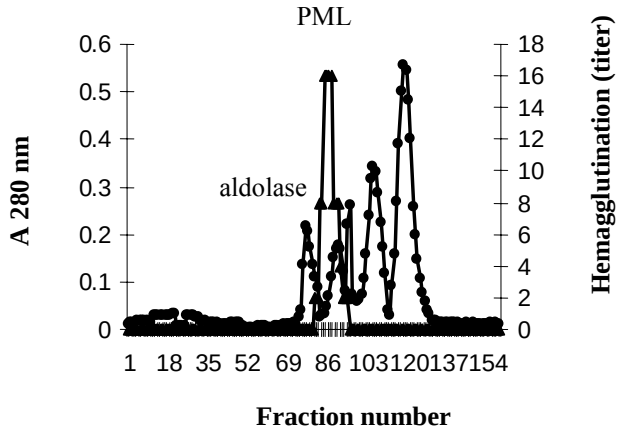

Figure 9 Gel filtration of the *P. merguiensis* lectin on Sephacryl S-200, ●-● absorbance at 280 nm, ▲-▲ hemagglutinin (titer) against trypsin treated human A erythrocytes. The standard proteins used were aldolase (158 kDa), albumin (67 kDa), ovalbumin (43 kDa) and chymotrypsinogen (25 kDa)

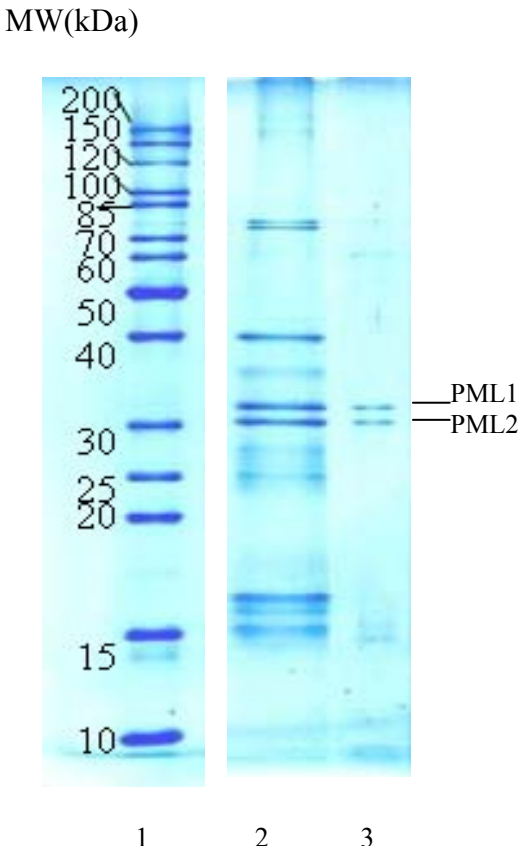

Figure 10 SDS-PAGE (12.5%) of the *P. merguiensis* lectin eluted from PSM-Sepharose 4B column and Sephacryl S-200 column. Molecular weight marker (lane1), affinity purified lectin (lane 2) and gel filtration purified PML (lane3).

Table 9 Purification of *Penaeus merguiensis* lectin

Fraction	Total Volume (ml)	Total Protein (mg)	Total HA Activity (U)	Specific Activity (U/mg)	Purification fold
Serum	20	1,344	204,800	152.38	1
Mucin-Sepharose 4B	18	0.2896	184,320	636,464	4,177
Sephacryl S-200	15	0.0088	72,000	8,181,818	53,694