FINAL REPORT

PRAWN ALLERGEN IDENTIFICATION AND PURIFICATION

Ron Marschke

Queensland Government
Department of Primary Industries

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

SEAFOOD SERVICES AUSTRALIA

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FELIX

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NON-TECHNICAL SUMMARY

Crustaceans such as prawn, crab and lobster are recognised as a common cause of allergic reactions to seafood. Overseas studies have shown that the major allergen in prawn is the heat-stable muscle protein tropomysin. A similar protein has been found in other crustaceans.

In the current investigation, a survey was carried out to determine the incidence of tropomyosin in Australian crustaceans and one imported prawn species. Electrophoretic analysis of boiled extracts of ten prawn, six crab and three lobster samples showed that a protein with molecular weight of 37.9- 40.6 kDa was present in all crustaceans except Mantis shrimp (*Squilla mantis)*, while low levels of the protein were found in sand crab (*Ovalipes australiensis*). The protein, presumed to be tropomyosin by its molecular weight, was one of several major proteins occurring in boiled crustacean extracts.

The commonly-farmed prawn species, *Penaeus monodon,* was selected for the isolation of tropomyosin. The protein was purified from a boiled homogenate of *P. monodon* using a combination of anion-exchange chromatography, gel filtration and preparative denaturing electrophoresis. Purity of the tropomyosin was confirmed by polyacrylamide gel electrophoresis. Tropomyosin exhibited two forms by electrophoresis with molecular weights of 38.0 and 39.0 kDa.

An amount of 29 mg of tropomyosin was obtained from the purification. This material will be used as an antigen in the production of antiserum for the development of an enzyme-linked immunosorbent assay (ELISA). The techniques developed in this study could be applied to future production of tropomyosin from any species of crustacean.

BACKGROUND

Ingestion of prawn is a common cause of allergic reaction in hypersensitive individuals. Symptoms can range from mild oral reactions to fatal anaphylactic shock. The prevalence of seafood allergies is difficult to estimate but is generally considered to be around 0.07% of the population. In Australia this means that around 14 000 people may be affected by allergies to seafood.

At least 13 different allergens have been identified in raw prawns (Morgan *et al.* 1990) Although many of these allergens are destroyed by heat during cooking, a heat-stable muscle protein, tropomyosin, has been identified by overseas researchers as the major prawn allergen (Shanti *et al.* 1993; Daul *et al.* 1994). This protein, which has a reported molecular weight of 34 to 39 kDa, has been isolated from several prawn species (Nagpal *et al.* (1989); Lin *et al.* 1993; Daul *et al.* 1994;) and other crustaceans (Leung *et al.* 1998a; Leung *et al.*1998b). Based on amino acid composition and partial sequence information, it appears that the protein is similar between prawn species. In addition, serum which is reactive to prawn tropomyosin has been shown to react with a similar molecular weight protein from other crustaceans, demonstrating the presence of shared allergenic determinants (Lin *et al.* 1993; Lopata and Potter, 2000).

The purification of tropomyosin from prawn, squid and oyster has been reported (Nagpal *et al.* 1989; Miyazawa *et al.* 1996; Ishikawa *et al.* 1997). Although methods vary, purifications usually involve a combination of anion exchange chromatography, gel filtration and HPLC. Jeoung *et al.* (1997) used preparative electrophoresis for the purification of tropomyosin for the production of monclonal antibodies. Most of the purification and allergenicity studies on prawn have been conducted in the United States and India on the species *Penaeus aztecus* and *P. indicus*.

The present study examines the incidence and levels of tropomyosin in a range of Australian prawn, crab and lobster species, and describes the purification of tropomyosin from *P. monodon*.

NEED

A sensitive method for the detection of heat-stable prawn allergen in food is not available in Australia. While immunological test kits may be available overseas, they are not based on antigens to prawn or crustacean species consumed in this country. A commercially available test would enable Australian food manufacturers, food service operators and analytical laboratories to detect and quantify crustacean allergen transfer during cooking processes, and to analyse food which may be contaminated with such allergens.

The need for the current research project is to confirm the presence of the allergen tropomyosin in a range of Australian crustaceans and to isolate tropomyosin from a selected prawn species to commence development of a test kit for prawn allergen.

OBJECTIVES

To survey local and imported prawn species and other crustaceans for the presence and levels of tropomyosin.

To purify tropomyosin from one prawn species for use in developing an ELISA test for prawn allergen.

METHODS

Protein survey

Samples

Samples of uncooked crustaceans including eight prawns, six crab and three lobster species were obtained from various sources in Australia (Table 1). Paradise prawn (*P. stylirorostus)* was imported from New Caledonia. One sample of Greasyback prawn (*Metapenaeus bennettae*) was cooked prior to collection. Samples were stored at –20°C before extraction.

Table 1. Crustacean species for tropomyosin survey.

Crustacean	Common name	Species name	Source
Prawn	Brown Tiger	*Penaeus esculentus*	Clarence R. Co-op NSW
	Endeavour	*Metapenaeus endeavouri*	Clarence R. Co-op NSW
	Grooved Tiger	*Penaeus semisulcatus*	Clarence R. Co-op NSW
	Western King	*Penaeus latisulcatus*	Kailis WA
	Black Tiger	*Penaeus monodon*	Gold Coast marine hatchery QLD
	Southern Velvet	*Metapenaeopisis palmensis*	Kailis WA
	Mantis shrimp	*Squilla mantis*	Morgans Seafood QLD
	Eastern King	*Penaeus plebejus*	Sam's Seafood QLD
	Paradise	*Penaeus stylirorostus*	Sontari Foods, NSW (imported ex New Caledonia)
Crab	Redclaw	*Cherax quandricarinatus*	Aquaculture Calliope QLD
	Mud crab	*Scylla serrata*	Morgans Seafood QLD
	Sand crab	*Ovalipes australiensis*	Sams Seafood QLD
	Rock crab	*Charybdis natator*	Sams Seafood QLD
	Coral crab	*Charybdis feriata*	Sams Seafood QLD
	Three Spot crab	*Portunus sanguinolentus*	Sams Seafood QLD
Lobster	Yabbie	*Cherax destructor*	Aquaculture, Redlands QLD
	Moreton Bay bug	*Thenus orientalis*	Sams Seafood QLD
	Western Rock	*Panulirus cygnus*	Kailis WA
Prawn (cooked)	Greasyback	*Metapenaeus bennettae*	Sontari Foods, NSW

Extraction

Uncooked samples were thawed, shelled and cut into small pieces which were thoroughly mixed. For prawn extracts, at least six meats were used for each species, but only one meat of each species was used for crab and lobster. Samples (10 g) were homogenised in 100 ml of phosphate buffered saline (PBS) pH 7.4 for 1 min at high speed in a Waring blender. The homogenate was transferred to a 250 ml Schott bottle and held at 95-100°C for 10 min in a boiling water bath. Heat treatment was not applied to the cooked sample of Greasyback prawn. After cooling on ice the homogenates were centrifuged at 15000 rpm for 15 min at 5°C and filtered through Whatman No 1 paper. The clarified extracts were stored at -20°C before analysis. The extraction method ensured all soluble, heat-stable proteins were retained in the extract.

Protein content

The protein content of the boiled crustacean extracts was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 15% acrylamide mini-slab gels (BioRad). The gels were stained in 0.25% Coomassie Blue and documented on a desktop scanner (Scan Jet 3C, Hewlett Packard). Molecular weights of the tropomyosin band were estimated on 12% acrylamide analytical gels using standards supplied by BioRad.

Purification of tropomyosin

A flow chart of the purification steps used in the isolation of tropomyosin is shown in Appendix 1.

Extraction

Uncooked Black Tiger prawns (*P. monodon*) were obtained from a marine hatchery on the Gold Coast, Queensland. The prawns were peeled and washed in PBS pH 7.4 and cut into small pieces. An amount of 300 g of prawn flesh was homogenised for 2 min in a Waring blender in a total of 1.5 L of cold PBS (3 x100 g of prawn). The homogenate was transferred to 500-ml Schott bottles (250 ml per bottle) and heated at 95-100°C for 10 min in a boiling water bath. After cooling on ice, the homogenate was centrifuged at 5000 rpm for 30 min at 5°C and the supernatant collected as the crude extract. A 30-60% ammonium sulphate fraction was obtained and the precipitate resuspended in 50 mM Tris HCl pH 8.0 and dialysed against the same buffer.

Anion exchange chromatography

The dialysed extract was applied to a column of DEAE Sepharose Fast Flow (Amersham Pharmacia) with bed dimensions 90 mm x 50 mm diameter and equilibrated in 50 mM Tris HCl pH 8.0 at a flow rate of 5 ml/min. The bound material was washed with 50 mM Tris HCl pH 8.0 and eluted initially with a linear gradient of 0-0.1 M NaCl in Tris buffer (150 ml) followed by isocratic elution at 0.1 M NaCl (150 ml). Tropomyosin was eluted at approximately 0.2 M using a linear gradient of 0.1-0.3 M NaCl (700 ml). Fractions (10 ml)

containing tropomyosin, as determined by the presence of a 38-kDa band on SDS-PAGE, were pooled and concentrated using a stirred cell (Amicon) fitted with a PM10 membrane.

Gel filtration

Gel filtration was performed initially on a column of Sephacryl S-200 HR (column bed 890 mm x 26 mm) equilibrated in 50 mM Tris HCl pH 8.0 containing 0.2 M NaCl at a flow rate of 2 ml/min. A concentrated sample from anion exchange (12 ml) was applied and 4.5-ml fractions were collected. In a subsequent experiment, gel filtration was carried out on a column of Sephadex G75 Superfine of similar dimensions, and using the same Tris buffer. A slower flow rate of 0.25 ml/min was used due to higher back pressure. Fraction size was 2 ml.

Gel filtration was also performed on a column of Sephacryl S-200 HR (column bed 880 mm x 16 mm) equilibrated with 50 mM Tris HCl pH 8.0 containing 4 M urea and operated at a flow rate of 0.5 ml/min. The sample was dialysed in the same buffer and concentrated on a PM10 membrane prior to loading. Fraction size was 1.7 ml.

Lectin affinity chromatography

Affinity chromatography was done on Concanavilin A Sepharose using a 10 mm x 100 mm column packed to a bed height of 60 mm and equilibrated at 0.5 ml/min with 20 mM Tris HCl at pH 7.4. A linear gradient of 0-0.5 M α-D-methylglucoside was used to elute bound protein which was collected in 1 ml fractions.

Preparative SDS-PAGE

Purification of tropomyosin by electroelution was performed on a Prep Cell 491 system (BioRad) using denaturing SDS-PAGE. Proteins were separated on a 11% acrylamide separating gel that was cast in a 37 mm diameter cell (gel length 12 cm, monomer volume 100 ml). A 2-cm length stacking gel was used. Concentrated sample (1.5 ml in 20 mM Tris HCl pH 7.4) was digested with 4.5 ml of loading buffer, and electrophoresed for 16 h at 12 W constant power. Buffer systems were as described in the BioRad instruction manual. After 13 h, fractions (3.0 ml) were collected from the elution stream which was set at 0.75 ml/min. Migration of the front of the tropomyosin band through the separating gel was at an average rate of 0.9 cm/h. Fractions containing pure tropomyosin were identified on SDS-PAGE mini-gels.

Five preparative electrophoresis runs were carried out and, in each case, fractions were pooled and dialysed against demineralised water. A total of 630 ml of dialysed tropomysin solution was concentrated to 50 ml using a PM10 membrane. This solution was analysed for protein content and further dialysed against microfiltered water. The protein was subsequently lyophilised and stored desiccated at –20°C.

RESULTS AND DISCUSSION

Protein survey

The protein levels in water soluble extracts of boiled crustaceans are shown in Table 2. Excluding the cooked Greasyback prawn, soluble protein levels ranged from 1.2 to 4.7 mg/ml with Black Tiger prawn giving the highest protein level. Since the extracts are 10% (w/v) these data also approximate the percentage of soluble, heat stable proteins in crustacean flesh. In the case of prawn, the total protein content is around 20% (published data), so it would appear that 10-24% of the total protein is water soluble after boiling.

Table 2. Protein analyses of crustacean extracts.

Crustacean	Protein (mg/ml)	Molecular weight of tropomyosin (kDa)	Report reference
Brown Tiger	2.9	38.0	Fig. 1
Endeavour	3.5	-	Fig. 1
Grooved Tiger	1.9	38.4	Fig. 1
Western King	3.9	-	Fig. 2
Black Tiger	4.7	38.8, 37.9	Fig. 2
Southern Velvet	3.5	37.9	Fig. 2
Mantis shrimp	3.6	-	Fig. 3
Eastern King	2.9	38.0	Fig. 3
Paradise	3.3	-	Fig. 3
Redclaw	2.1	39.5	Fig. 4
Mud crab	2.8	40.6	Fig. 4
Sand crab	3.2	-	Fig. 4
Rock crab	2.0	39.6	Fig. 5
Coral crab	3.0	40.1, 38.7	Fig. 5
Three Spot crab	2.3	-	Fig. 5
Yabbie	3.0	-	Fig. 6
Moreton Bay bug	2.8	38.8	Fig. 6
Western Rock	1.2	38.1	Fig.6
Greasyback	0.7	38.3	Fig. 7

The protein fingerprints of water soluble proteins in boiled crustaceans are shown in Figures 1-7. The major band in the 38 to 40 kDa region was presumed to be tropomyosin. This protein band occurred in all species except Mantis shrimp. Although present in sand crab, the level of tropomyosin in this species was relatively low. In several cases, the tropomyosin band appeared as a doublet (Figures 2, 5, and 7) which suggests that the protein occurs in isomeric forms. Another major protein band is seen at 22-24 kDa in most species, but the identity of this protein is unknown.

The molecular weights of tropomyosin were estimated for several species using 12% analytical gels. In different species of prawn the tropomyosin molecular weights were similar (Table 2; Figures 1-3) and ranged from 37.9 to 38.8 kDa. The molecular weights of tropomyosin in crabs, notably mud crab, were slightly higher (38.7 to 40.6 kDa) than in prawns (Figures 4 and 5). In these gels the Black Tiger prawn is shown as the control

sample in Lane 2. For lobsters, the molecular weights of tropomyosin were similar to prawn (Figure 6.). A comparison of tropomyosin across different species is shown in Figure 7 where mud crab (Lane 6) has the highest molecular weight.

The molecular weights of prawn tropomyosin reported in overseas studies are in the range of 34 to 39 kDa (Lin *et al.* 1993; Shanti *et al.* 1993). Our results lie at the upper end of this range, while some data for crab are slightly higher.

The present survey of soluble heat-stable proteins in crustaceans has confirmed the presence of a tropomyosin-like protein in all species except for Mantis shrimp. However this study does not prove unequivocally that this protein is tropomyosin and does not give any indication of its allergenicity in the respective species.

Purification of tropomyosin

The prawn species *P. monodon,* was selected for the purification of tropomyosin. This species is commonly farmed in Australia and south-east Asian countries. Tropomyosin was purified to homogeneity from a boiled homogenate of *P. monodon.* The yield from 300 g prawn flesh was 0.01% with 29 mg of lyophilised tropomyosin produced. Based on a total protein of 10.5 g in the boiled crude extract, the tropomyosin protein yield was 0.3%. Since tropomyosin constitutes around 20% of soluble protein in boiled crude extract (O' Neil and Lehrer, 2000) the recovery of tropomyosin in the purification was 1.4%. The low product yield was attributed to losses during multiple chromatography steps in which attempts to separate tropomyosin from other proteins were unsuccessful. Effective purification of tropomyosin was achieved by preparative electrophoresis under denaturing conditions.

Anion exchange chromatography

Suitable conditions were developed for the elution of a tropomyosin enriched fraction from DEAE Sepharose (Figure 8). The procedure was useful in removing proteins with molecular weights higher than tropomyosin, as well as some low molecular weight proteins. However, many proteins co-eluted with tropomyosin and resolution could not be achieved by changing the slope of the salt gradient. Figure 8 also shows a large peak which eluted above 250 mM NaCl. This material did not contain protein and was identified as DNA from a wavelength scan.

Gel filtration

Three gel filtration procedures were investigated in an attempt to separate low molecular weight proteins from tropomyosin. Elution profiles of two methods using Sephacryl S-200 and Sephadex G75 Superfine are shown in Figures 9 and 10. Both chromatography gels failed to resolve tropomyosin from low molecular weight proteins. Sephadex G75 Superfine, with a narrow fractionation range of 3-70 kDa, showed no improvement over the broader range Sephacryl S-200, even though adequate resolution was achieved on Sephadex G75 for a mixture of low molecular weight protein standards. Since other workers have reported success with gel filtration (Nagpal *et al.* 1989; Ishikawa *et al.* 1997), we concluded that the proteins in the prawn extract had self-associated and could not be resolved by simple size exclusion. A subsequent trial using 4 M urea to dissociate the proteins, followed by gel filtration on Sephacryl S-200, was also unsuccessful. Higher levels of urea were not investigated.

Lectin affinity chromatography

As tropomyosin is a glycoprotein containing about 4% carbohydrate (Hoffman *et al.* 1981), its purification was examined on the affinity resin Concanavalin A Sepharose. All proteins including tropomyosin failed to bind to the resin under the conditions described by the manufacturer (Amersham Pharmacia). This provided further evidence of self-association of proteins in the load sample, which may have prevented interaction of glycosylated sites of tropomyosin with the affinity resin.

Preparative SDS-PAGE

Under the denaturing conditions of SDS-PAGE, tropomyosin was separated completely from other prawn proteins. This procedure involved pretreatment of the sample to dissociate proteins by heating at 95°C for 5 min in the presence of SDS detergent and reducing agent β-mercaptoethanol. As a result of this treatment it is not known if the purified tropomyosin has retained its native structure which is predominantly α-helical. Unfolding of the protein may affect its properties as an antigen.

Preparative electrophoresis using the Prep Cell 491 produced pure tropomysosin but the method required long run times (15 h) and produced a low amount of protein (5-6 mg) per run at maximum capacity. A total of five runs were carried out to produce the final output of 29 mg of tropomyosin.

The protein fingerprints of the prawn extract at various stages of the purification of tropomyosin are presented in Figure 11. These results demonstrate the effectiveness of each of the purification steps. Initial fractionation with ammonium sulphate at 30-60% had little effect on the overall protein profile of the crude prawn extract except for reducing the level of a 23 kDa protein (Figure 11, Lane 3). Hence this step could be replaced with a single 60% ammonium sulphate precipitation to concentrate the extract. Anion-exchange chromatography removed high molecular weight proteins above 40 kDa (Lane 4) but subsequent gel filtration steps made no contribution to the purification (Lanes 5 and 6). The most effective step in the purification was preparative SDS-PAGE which resulted in pure tropomyosin (Lane 8).

Characterisation of tropomyosin

The molecular weight of the purified tropomyosin was determined on SDS-PAGE. Using a lower (5-fold) protein load than in Figure 11, two forms of the protein were apparent with molecular weights of 38.0 and 39.0 kDa (Figure 12). It is possible that these are isomeric forms of tropomyosin having slight differences in amino acid residues or the level of glycosylation. The molecular weights are comparable to those reported elsewhere for prawn tropomyosin (Hoffman *et al.* 1981; Nagpal *et al.* 1989; Lin *et al.* 1993). However, the presence of two forms of tropomyosin has not been shown in other studies.

Low levels of aromatic amino acids in tropomyosin, particularly tryptophan (Leung *et al.* 1994), resulted in low absorbance of the pure protein at 280 nm and low apparent protein content using the Lowry protein assay. For example a 1 mg/ml solution of pure tropomyosin gave an A_{280} of 0.35 and a Lowry protein of 0.38 mg/ml. Using an average molecular weight of 38.5 kDa for tropomyosin, the extinction coefficient for tropomyosin at 280 nm was calculated as 13 480/M/cm.

BENEFITS

The present research has provided useful information on the incidence of tropomyosin in Australian crustaceans, and has produced antigen material for the development of a test for the tropomyosin allergen. A sensitive test procedure to detect this allergen in food would benefit a number of potential end users. These would include food processors, food safety professionals, food service operators and analysts in pathology laboratories. Some applications for a tropomyosin test kit would be for the detection and quantification of heat-stable allergen in crustacean products, and for monitoring the degree of transfer of allergenic material to non-crustacean food during cooking. This would enable food manufacturers to modify cooking processes to avoid contamination of non-crustacean food or to appropriately label potentially allergenic products. In addition a test kit would be a useful tool for pathology laboratories to determine if a suspect food has been contaminated with crustacean material, thereby facilitating the diagnosis of an affected patient.

FURTHER DEVELOPMENT

Following the successful purification of tropomyosin in the present work, the next step would be the production of a polyclonal antiserum in rabbits or sheep. The antiserum would be screened for its reactivity with the pool of frozen extracts obtained in the protein survey of the current study. If screening results show adequate cross-reactivities with other prawn and crustacean species, an ELISA will be developed using the antiserum.

Since completing the work for this stage of the project, we have investigated the use of reverse phase HPLC (RP-HPLC) as a non-denaturing procedure to purify tropomyosin. Initial studies using mobile phases 10 mM phosphate buffer pH 6.0 and acetonitrile showed that tropomyosin could be isolated by preparative RP-HPLC, but further optimisation of gradient conditions would be necessary. Advantages of an RP-HPLC method would be the production of undenatured tropomyosin with higher throughput per run than preparative SDS-PAGE.

CONCLUSIONS

- A protein, presumed to be tropomyosin, is present at significant levels in most crustacean species found in Australia.
- Preparative electrophoresis is a suitable method for obtaining pure tropomyosin from prawn extract. Disadvantages of the method are its low throughput per run and its possible denaturing effects on the purified protein.
- Sufficient tropomyosin (29 mg) has been produced to enable the next stage of ELISA development to proceed.
- The objectives of the project have been met.

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APPENDIX 1

Flow chart of purification of tropomyosin

Raw prawn
(300g)
|
Boiled homogenate
(10 min)
|
Crude extract
|
$(NH_4)_2SO_4$ precipitation
(30-60%)
|
Anion exchange chromatography
(DEAE Sepharose Fast Flow)
|
Gel filtration
(Sephacryl S-200 & Sephadex G75)
|
Affinity chromatography
(Concanavalin A)
|
Preparative electrophoresis
(SDS-PAGE)
|
Pure tropomyosin
(29 mg)

APPENDIX 2

Intellectual property

Information generated in the project will be exclusively for the use of Elisa Systems Pty Ltd for a period of eighteen months following completion of the project, subject to ongoing development of the ELISA kit test.

APPENDIX 3

Staff

Ron Marschke, Senior Scientist, Principal Investigator
Jacquie Edwards, Seafood Technologist
Christine Gore, Laboratory Technician
Paul Exley, Laboratory Technician
Jeff Herse, Laboratory Technician
Suzette, Pyke, Laboratory Technician
Sandra Jarrett, Laboratory Technician
Darren Leighton, Technical Assistant

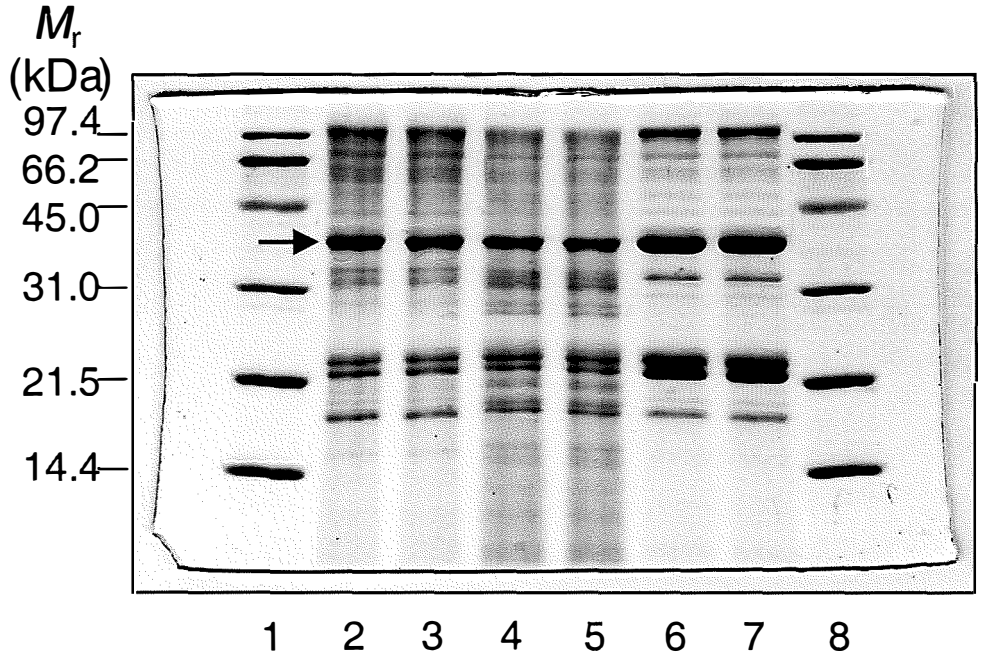

Figure 1. SDS-PAGE of boiled prawn extracts. Lanes: (1) molecular weight marker; (2) and (3) Brown Tiger; (4) and (5) Endeavour; (6) and (7) Grooved Tiger; (8) molecular weight marker. Arrow indicates tropomyosin.

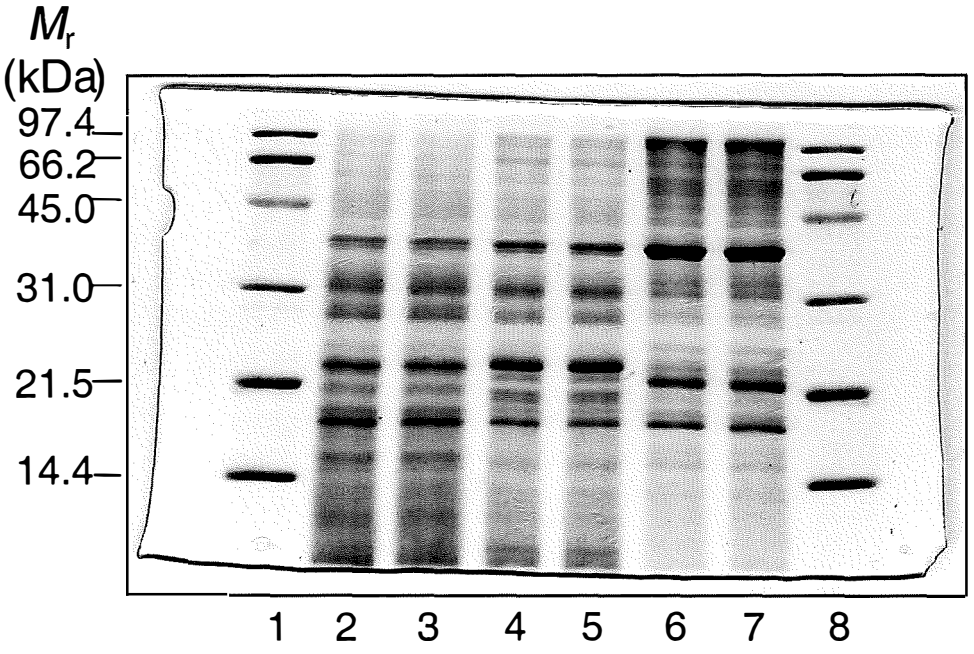

Figure 2. SDS-PAGE of boiled prawn extracts. Lanes: (1) molecular weight marker; (2) and (3) Western King; (4) and (5) Black Tiger; (6) and (7) Southern Velvet; (8) molecular weight marker.

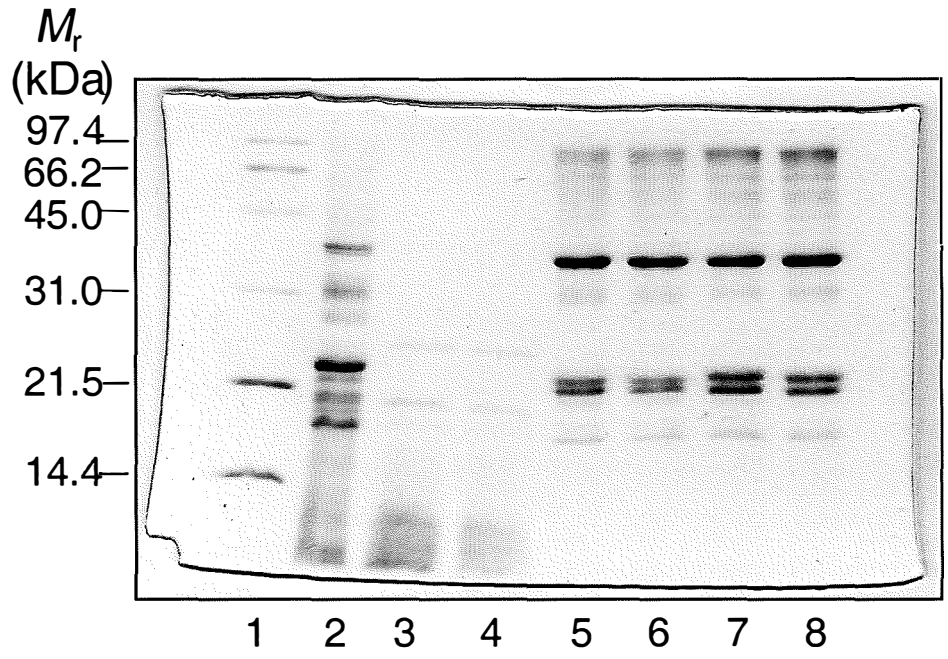

Figure 3. SDS-PAGE of boiled prawn extracts. Lanes: (1) molecular weight marker; (2) Black Tiger (3) and (4) Mantis Shrimp; (5) and (6) Eastern King; (7) and (8) Paradise.

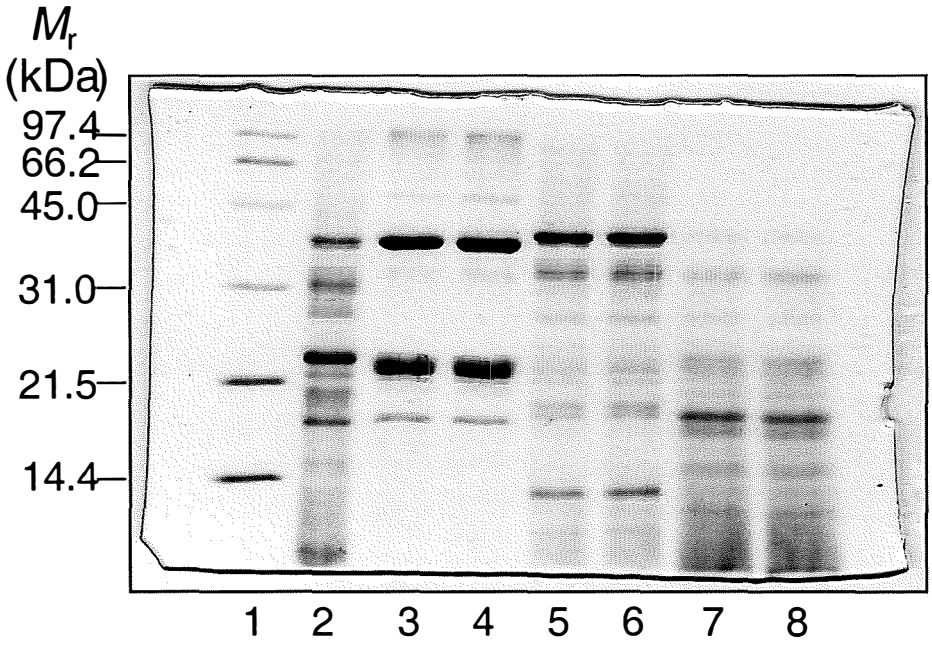

Figure 4. SDS-PAGE of boiled crab extracts. Lanes: (1) molecular weight marker; (2) Black Tiger prawn; (3) and (4) Red Claw; (5) and (6) Mud Crab; (7) and (8) Sand Crab.

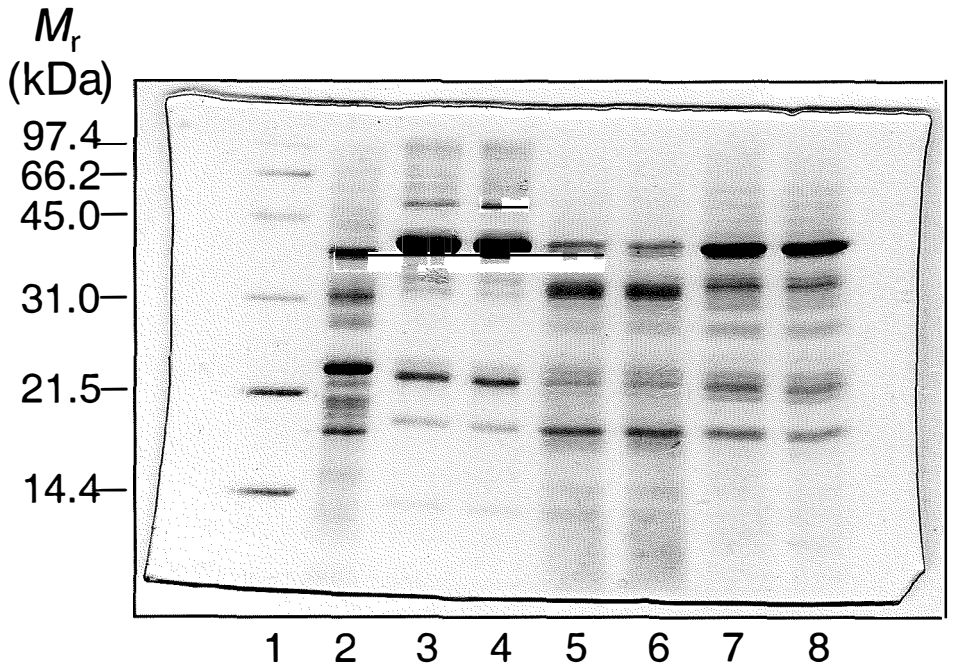

Figure 5. SDS-PAGE of boiled crab extracts. Lanes: (1) molecular weight marker; (2) Black Tiger prawn; (3) and (4) Rock crab; (5) and (6) Coral crab; (7) and (8) Three spot crab.

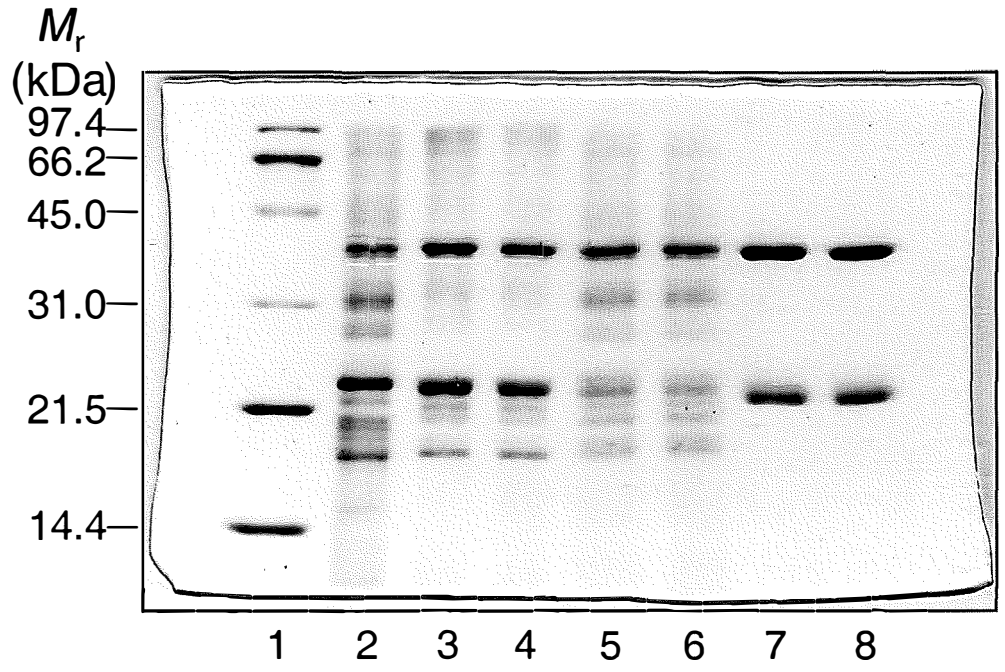

Figure 6. SDS-PAGE of boiled lobster extracts. Lanes: (1) molecular weight marker; (2) Black Tiger prawn; (3) and (4) Yabbie; (5) and (6) Moreton Bay bug; (7) and (8) Western Rock lobster.

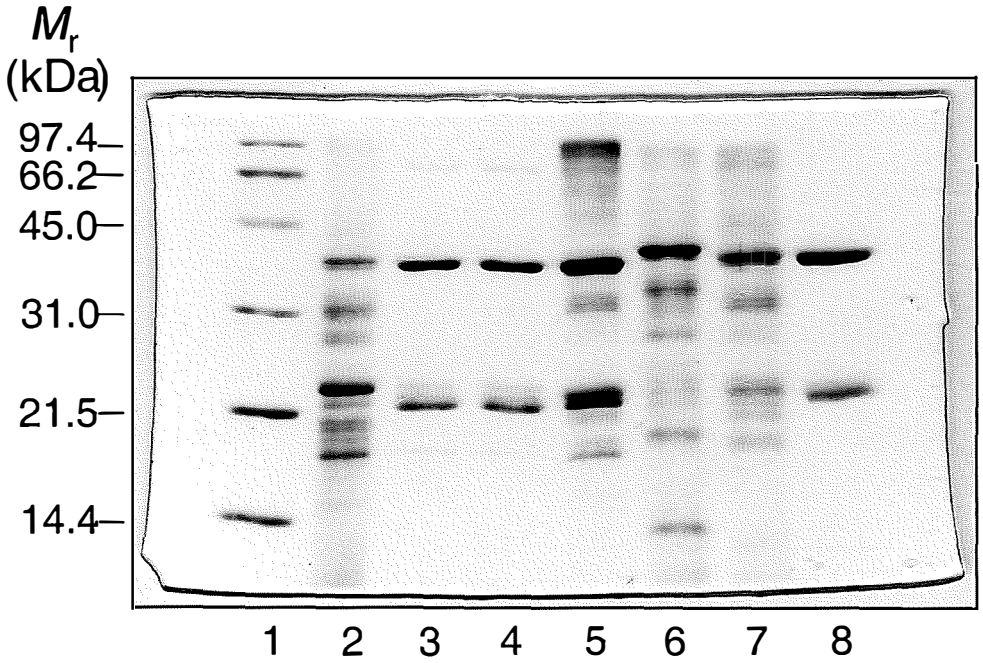

Figure 7. SDS-PAGE of boiled crustacean extracts. Lanes: (1) molecular weight marker; (2) Black Tiger prawn; (3) and (4) Greasyback prawn; (5) King prawn; (6) Mud crab; (7) Moreton Bay bug; (8) Western Rock Lobster.

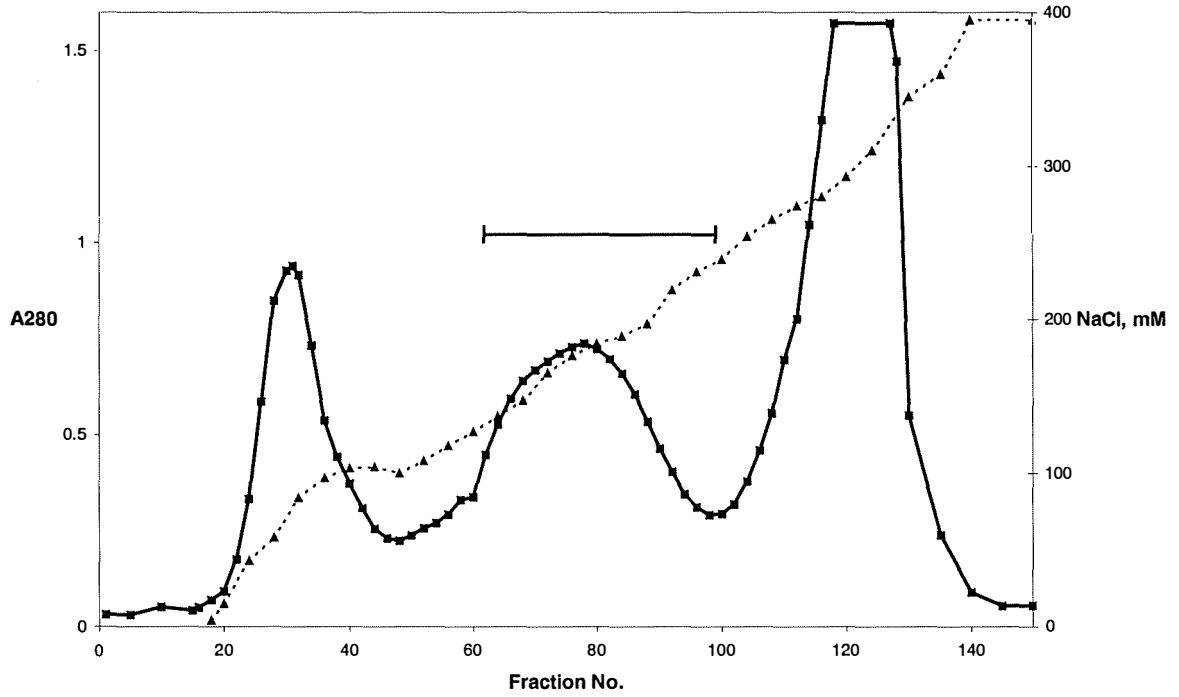

Figure 8. Anion-exchange chromatography of prawn extract on DEAE-Sepharose Fast Flow.. (■), A280; (▲), NaCl (mM). Bar represents pooled fractions.

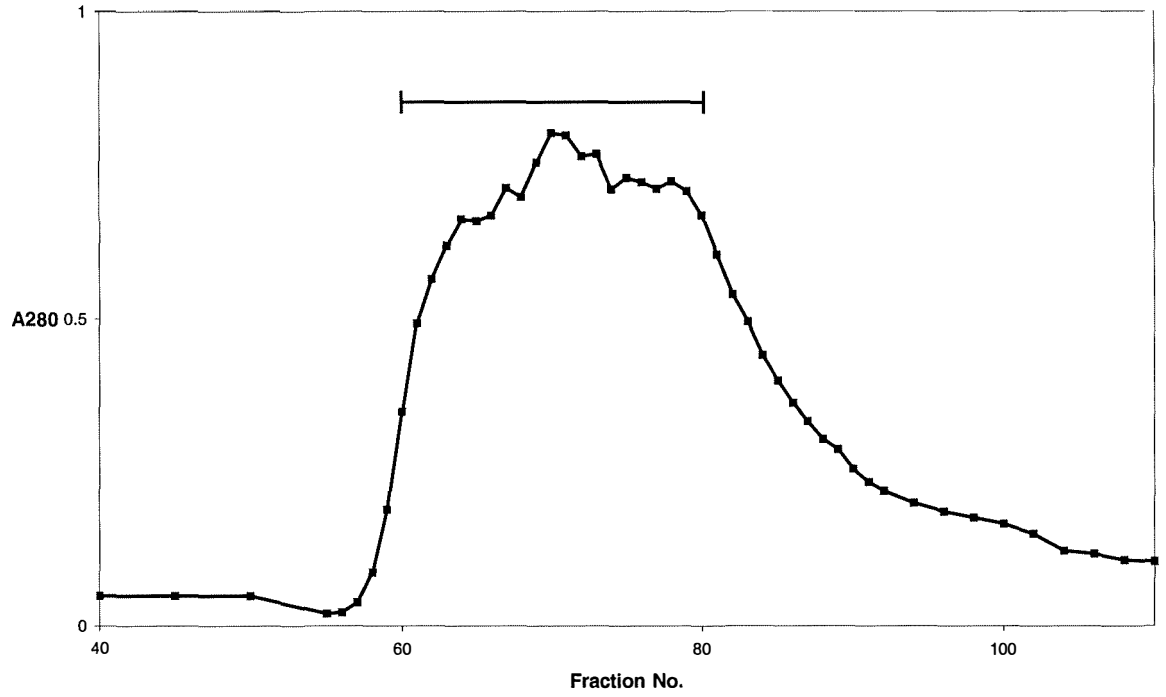

Figure 9. Sephacryl S-200 chromatography of prawn extract after anion-exchange on DEAE Sepharose.. Bar represents pooled fractions.

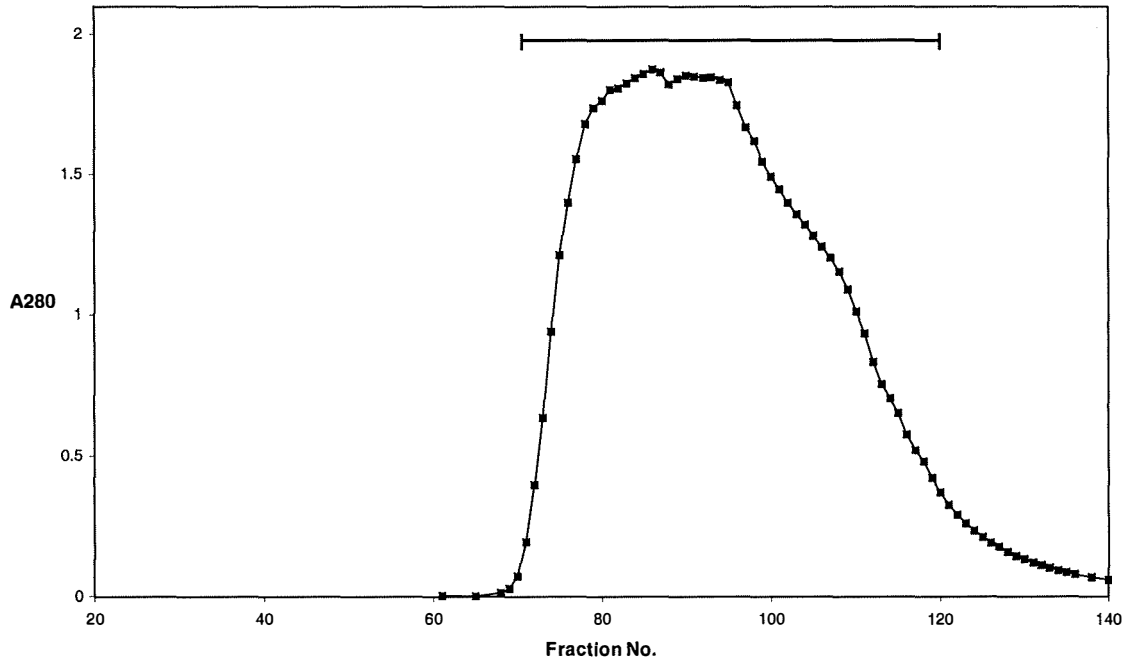

Figure 10. Sephadex G75 chromatography of prawn extract after elution from Sephacryl S200. Bar represents pooled fractions.

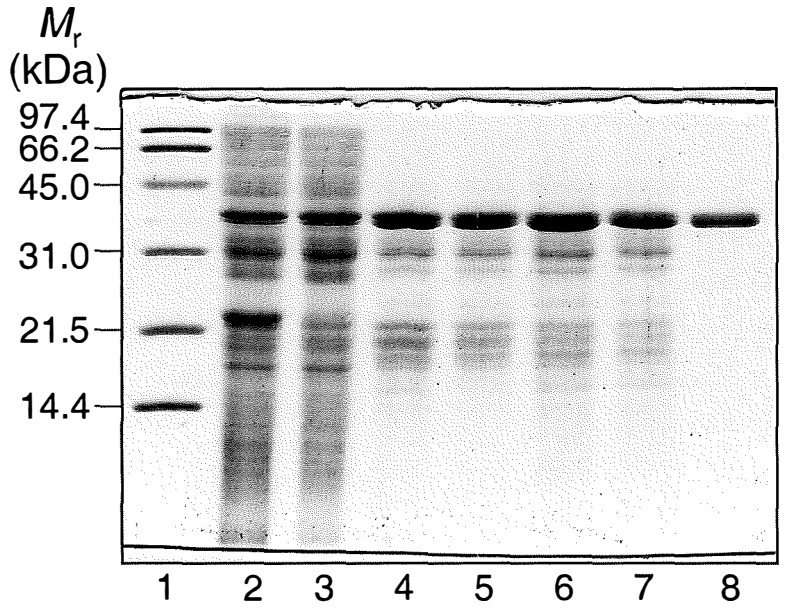

Figure 11. SDS-PAGE of the purification of tropomyosin. Lanes: (1) molecular weight marker; (2) crude prawn extract; (3) 30-60% ammonium sulphate fraction; (4) DEAE Sepharose; (5) Sephacryl S200; (6) Sephadex G75; (7) Conconavalin A; (8) pure tropomyosin after SDS-PAGE on Prep Cell 491.

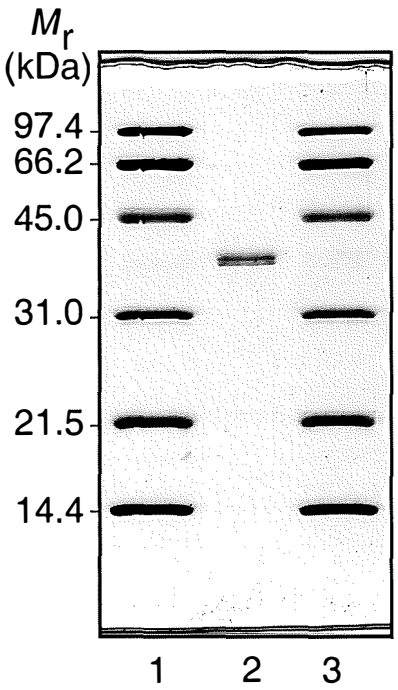

Figure 12. SDS -PAGE of pure tropomyosin isolated from *Penaeus mondon*. Lanes: (1) molecular weight marker, (2) tropomyosin, (3) molecular weight marker.