Isolation and Partial Characterisation of Lectin from Turbo sp.

R Anantha Rajan, S Suganthi, A Sabitha, G Prakash Vincent

Citation

R Anantha Rajan, S Suganthi, A Sabitha, G Prakash Vincent. *Isolation and Partial Characterisation of Lectin from Turbo sp.*. The Internet Journal of Genomics and Proteomics. 2006 Volume 3 Number 1.

Abstract

A calcium dependent lectin was purified from Turbo sp. using DEAE column chromatography. Minimal Ca^{2+} concentration required for the haemagglutination activity was 0.25M. Muscle extract of Turbo sp. showed maximum heamagglutination activity with chicken erythrocytes with titre value of 24. It showed heamagglutination activity with titre value of 3 for A and B and 6 for O human erythrocytes. The haemagglutination activity for the DEAE column purified lectin was observed between pH of 6 and 9 and not at lower pH and was high between 30°C and 40°C. The molecular weight of Turbo sp. lectin was about 46kDa.

INTRODUCTION

The recognition of carbohydrate moieties in macromolecules by lectins has important applications in cell to cell interactions, signal transduction, cell growth and differentiation (Lis, & Sharon, 1991). Mollusc has variates of lectin with different biotechnological applications. Fenelson et al. (1987) studied the formalin fixed, paraffin embedded tissue from 100 consecutive cases of breast carcinoma binding with Helix pomatia and Ulex europeus lectin. Aplysia gonad lectin isolated from the mollusc Aplysia depilans was successfully conjugated to colloidal gold and used for ultrastructural detection of galacturonic acids in some pathogenic fungi (Benhamou et al., 1989). Helix pomatia lectin binding is a useful prognostic indicator in colorectal carcinoma (Schumacher et al., 1994). Considering the easy availability of Turbo sp. along the Kanyakumari (8.08° N 77.57° E) coast lectin was extracted, purified and partially characterised.

MATERIALS & METHODS

Turbo sp. collected off Arokyapuram, Kanyakumari was brought alive to the laboratory and immediately preserved at -20°C. Extract was prepared by grinding 1g muscle sample in 1ml Tris base saline (TBS) (50mM Tris-Hcl, pH 7.5, 150mM NaCl, 5mM CaCl2) and centrifuging at 6000 rpm for 15 minutes at 4°C. Haemagglutination assay was carried out as described by Ravindranath et al., (1985). A sample of 25µl was diluted up to 1:32 by two fold dilution method with TBS. To each well, 25µl of 1.5% erythrocyte was added, mixed and kept at 30.0 ° C for one hour. After incubation period the wells were examined microscopically. For the heamagglutination inhibition, lactose, galactose, glucose, mannitol, sucrose, fructose, maltose, mannose, melibiose and N-acetyl neuraminic acid were diluted in sample up to 1: 32 dilution. After 1h, 25µl of erythrocytes was added and incubated for 1h at 30.0 ° C.

500µl of the Turbo sp. extract was applied to the DEAE cellulose column. Protein was eluted by stepwise gradient buffer elution using different concentration from 0.1M to 1M. The flow rate was set at 250µl per minute and was collected in eppendorff tubes. The eluted fractions were estimated for protein by UV spectrometer at 280nm. Agglutination activity was carried out for each column fraction. Apparent molecular weights and purification of protein subunits were determined by using SDS/PAGE following the methods of Laemmli, (1970).

In order to find out the optimum temperature for maximum activity and temperature tolerance the samples were incubated at different temperatures from 20°C to 90°C using water bath for 20 minutes and haemagglutination activity was determined. To find out the optimum pH, the extracts was dialyzed at pH 3 to 9 for 24 h and then in TBS for 24 h at pH 7.6. The samples were dialysed against TBS without calcium, with Ca^{2+} and Mg and after 24 h haemagglutination assay was carried out. The sample was dialysed the TBS with 0.25M EDTA and haemagglutination activity was carried out and the same sample was dialysed with TBS after which the haemagglutination activity was estimated.

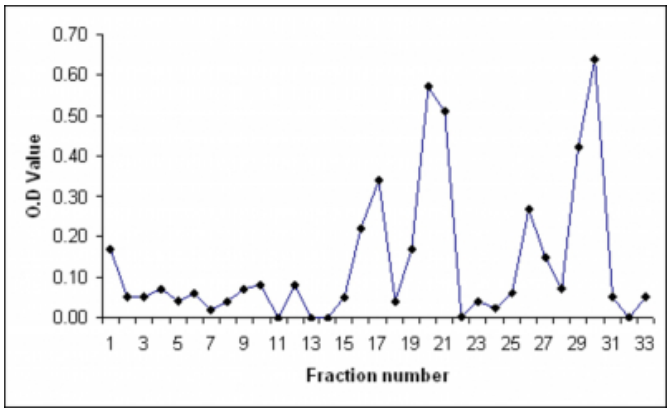
RESULTS AND DISCUSSION

Muscle extract of Turbo sp. showed maximum titre value of 24 for chicken erythrocyte and 1.5, 6 and 6 for sheep, cow and rabbit erythrocytes respectively. The titre value was 3 for A and B and 6 for O human erythrocytes. The above results co-related the haemolymph of the snail, Bulinus nastus (Harris et al., 1993), hard clam, Mercenaria mercenaria (Tripp, 1992) and Pacific oyster, Crassostrea gigas (Olafsen et al., 1992) that showed specific agglutination to some of the red blood cells. Garber et al. (1985) purified the gonad lectin from Aplysia depilans. This lectin strongly reacted with rabbit and human erythrocytes.

In the haemagglutination inhibition assay, the inhibitors Lactose, Glucose, Galactose, Fructose, Maltose, Mannitol, Mannose, N-acetyl neuraminic acid were used. None of them inhibited the agglutination. Muscle extract purified by

Figure 1

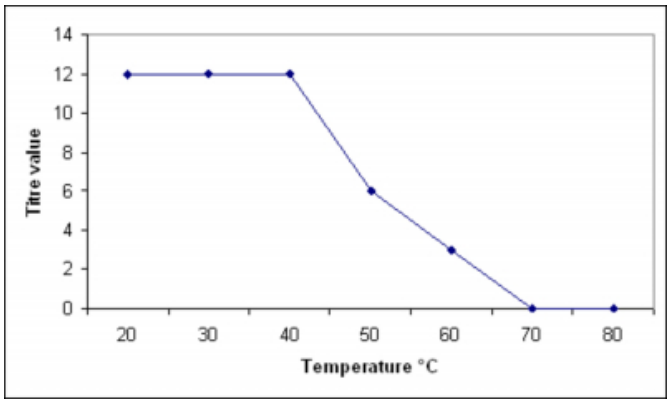
Figure 1: Purification of muscle proteins using DEAE ion exchange column chromatography.



DEAE cellulose chromatography showed 4 peaks in 17, 20, 26 and 30 fractions as given in Fig. 1. Among these, the 7 th fraction showed titre value of 12 for rabbit erythrocytes. The extract of Turbo sp. did not show haemagglutination in the absence of Ca^{2+} and the minimal Ca^{2+} concentration for the haemagglutination activity was 0.25M. The haemagglutination activity of the column fractions of Turbo sp. was sensitive to temperature and pH. The maximum agglutination was observed at 30°C and 40°C as in fig.2.

Figure 2

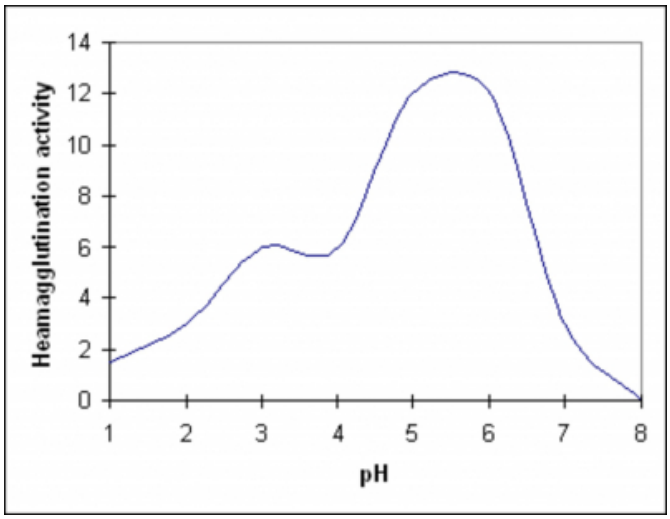
Figure 2: Effect of temperature on column purified lectin of



The lectin from Achatina fulica was found to be temperature sensitive and it agglutinated rabbit and human umbilical cord erythrocytes only at low temperature (Sarkar et al., 1984). The agglutination activity of lectin from the haemolymph of cephalopod Octopus vulgaris was completely destroyed at 90 °C.

Figure 3

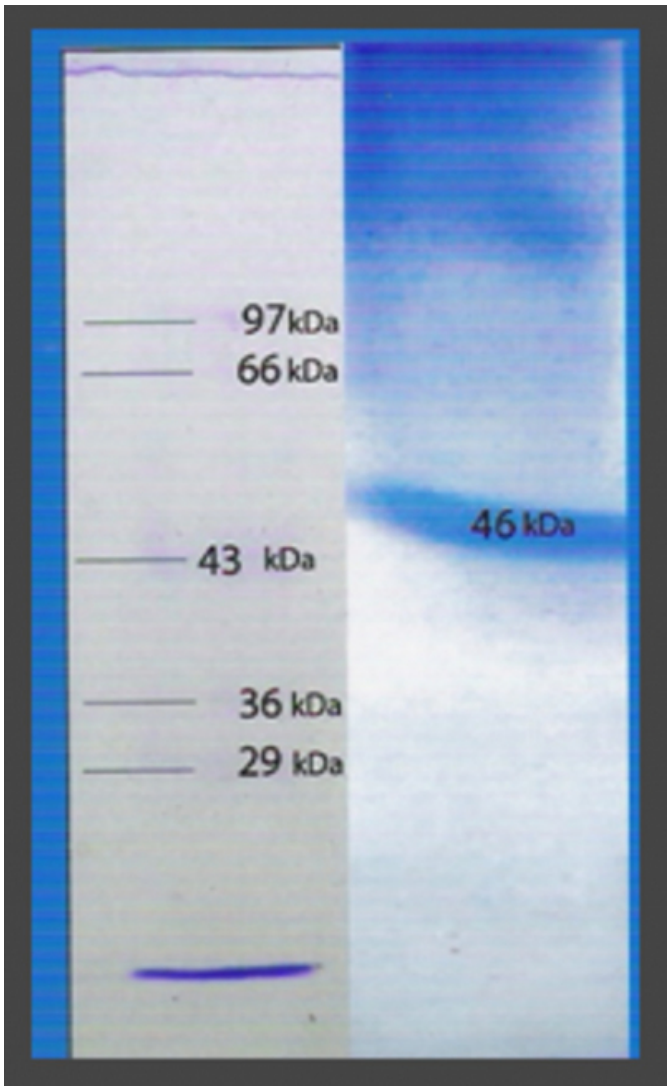
Figure 3: Haemagglutination activity of column purified lectin of in different pH.



Haemagglutination activity was inactivated in lower pH observed in higher pH of 7 and 8 as in fig 3. The haemagglutinating activity of extracts of mollusk, Pomacea flagellata was stable between pH 4.0 and pH 10.0. Espinosa et al (1997) reported that the haemagglutination activity of the protein from Aplysia californica was unstable below pH 5.

Figure 4

Figure 4: Protein profile of column purified . lectin.



The purified column fractions of Turbo sp. was applied on Sodium dodecyl sulphate polyacrylamide gel slab. The molecular mass of this lectin was 46 kDa. Harris et al. (1993) purified agglutinin and demonstrated the protein from haemolymph of snail, Bulinus nastus with 210 kDa by SDS poly acrylamide gel electrophoresis. Gerlach et al. (2002) purified a sialic-acid-specific lectin from the Cepea hortensis with molecular mass of about 95 kDa by gel filtration and 100 kDa by SDS electropboresis. Ozeki (1998) purified a D-galactose-binding lectin from the skin of sea hare, Aplysia Kurodai by SDS-PAGE with molecular mass of 200 kDa. Calcium dependent lectin with optimum activity between pH of 6 and 9 and 30°C and 40°C was purified. Further characterisation explores its biotechnological applications.

ACKNOWLEDGEMENT

The authors are grateful to the authorities of Manonmanium Sundaranar University, Tirunelveli, for providing required facilities.

ADDRESS FOR COMMUNICATION

Dr. R. Anantha Rajan Lecturer, Centre for Marine Science and Technology, Rajakkamangalam-629 502, Tamilnadu, India. anantharajanr@gmail.com

References

r-0. N. Benhamou, Ultrstructural study of galacturonic acid distribution in some pathogenic fungi using gold-complexed Aplysia depilans gonad lectin. Can. J. Microbiol. 35(3), 349-58 (1989)
r-1. S. Fenlon, I.O. Ellis, J. Bell, J.H. Todd, C.W. Elston, R.W. Blamey, Helix pomatia and Ulex europeus lectin binding in human breast carcinoma, J. Pathol. 152(3), 169-176 (1987)
r-2. D. Gerlach, B. Schlott, K.H. Schmidt, Cloning and expression of sialic acid binding lectin from the snail,Cepaea hortensis. FEMS Immunol. Med.Micribiol. 40(3), 215-221 (2004)
r-3. D. Gerlach, S. Wagner, B. Schlott, U. Zahringer, K.H. Schmidt, Chemical and physiochemical characterization of the sialic acid binding lectin from the snail, Cepaea hortensis, FEMS. Micribiol.Lett. 214 (1), 618 (2002)
r-4. N.G. Garber, A.J. Susswein, L. Mizrabi, D. Avichezer, Purification and charactrisation of the gonad lectin of Aplysia deplians, FEBS Lett. 181(2) 267-270 (1985)
r-5. N.G. Garber,., D.,Sudakevitz, C.Levene, L.N. Rhimi V. Yahalom, H-deficient Bombay and para Bombay red blood cells are most strongly agglutinated by the galactophillic lectins of Aplysia and Psuedomonas aeruginosa that detect I and P1 antigens. Immunohematol.,22(1), 15-22 (2006)
r-6. R.A. Harris, T.M. Preston, V.R. South, Purification of an agglutinin fro the hemolymph of the snail, Bulinus nastus and demonstrate of related proteins in other Bulinus spp. Parasitology. 106, 127- 235 (1993)
r-7. J.A. Olafsen, T.C. Fletcher, P.T. Grant, Agglutinin activity in pacific oyster (Crassostrea gigas) hemolmph following invivo Vibrio anguillarum challenge, Dev.Comp.Immunol. 16(2-3),123-138 (1992)
r-8. Y. Ozeki, Purification of a 63 KDa -D-Galactoside binding lectin from cuttle fish, Tadorodes pacificus. Biochem.Mol.Biol.Int. 41(3), 633-640 (1997)
r-9. M. Sarkar, B.K. Bachhawat, C. Mandal, A new cold agglutinin from Achatina fulica snails. Arch. Biochem.Biophys. 233(1), 286-289 (1984)
r-10. M.R. Tripp, Agglutinins in the hemolymph of the hard clam, Mercenaria mercenaria. J. Invertebr. Pathol. 59(3), 228-234 (1992)
r-11. H. Lis, N. Sharon, Lectin-carbohydrate interactions. Curr. Opin. Struct. Biol. 1, 741-749 (1991)

Author Information

R. Anantha Rajan, Ph.D.
Centre for Marine Science and Technology

S. Suganthi, M.Phil.
Centre for Marine Science and Technology

A.G. Sabitha, M.Phil
Centre for Marine Science and Technology

G. Prakash Vincent, Ph.D.
Centre for Marine Science and Technology