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Short Communication

Perspective of Sialic Acid Lectin from Freshwater Crab *Paratelphusa Jacquemontii* in Innate Immunity

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Introduction

Sialic acids are diverse forms of 9-carbon carboxylated sugars positioned on the terminal end of glyconjugates on the cell surface of deuterostomes and in developmental larval stages of protostomes [1], fungi [2] and some microbes [3]. The fresh water crab *Paratelphusa jacquemontii* a protostome that lack the biosynthetic pathway of sialic acid possess lectin specific to *O*-acetyl neuraminicacid with potential application as biomarker, and therapeutic research. The isolation and biological function of the lectin are described.

Isolation of lectin by affinity chromatography

The freshwater crab *P. jacquemontii* used for experimental purpose were of either sex, uninjured, in intermoult stage and 30-55g in weight. Hemolymph was collected from the incised dactylus allowed to bleed and serum prepared by centrifuged (10,000 rpm,30min, 4°C) to obtain clarified serum by sedimentation of hemocyanin (15X10^4 Xg for 2 h at 4°C).

Clarified serum applied to BSM-activated sepharosein an Biorad column previously equilibrated with TBS at 4°C and successively washed with high salt buffer (HSB: 50 mM Tris, 1 M NaCl, 10 mM $CaCl_2$, pH 7.5) and low salt buffer (LSB: 50 mM Tris, 300 M NaCl, 10 mM $CaCl_2$, *p*H 7.5) until the A_{280} of the effluent was <0.002. The lectin was eluted with buffer containing 10 mM EDTA, dialysed and stored [4].

Structure of the lectin

The isolated lectin was analyzed in discontinuous polyacrylamide gel electrophoresis (PAGE-7%) under non-denaturing conditions following Maurer (1971), and appeared as a single band at molecular weight of 320kDa. In discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions following Laemmli (1970), performed using a 4% stacking gel (*p*H 6.8), and a 10% separating gel (*p*H 8.8) in tris-glycine buffer (*p*H 8.3) showed a single band at 75kDa. The molecule of lectin was constituted of a single protein of homogenous subunits.

Hemagglutination ability

The hemagglutination activity of the agglutinin in the serum and the isolated lectin was tested with different mammalian erythrocytes and the procedure followed was as recommended for crab humoral lectin by Ravindranath and Paulson [5]. The HA profile of the agglutinin or lectin showed specific binding affinity to erythrocyte with sialylated cell surface, mice, horse, buffalo and rabbit erythrocyte. The sialidase treated erythrocyte failed to agglutinate whereas with trypsin treatment the hemagglutination activity remained unchanged.

Sugar specificity

The sugar specificity of the humoral agglutinin and isolated lectin was determined by hemagglutination inhibition assay using 100mM concentration each of monosaccharides, disaccharides, N-acetylglucosamine, N-acetylgalactosamine, N-acetylmannosamine and N-acetylneuraminic acid and N-glycolylneuraminic acid 5mg/ml of glycoproteins such as Bovine Submaxillary Mucin (BSM), transferrin, porcine and bovine thyroglobulin, fetuin and porcine stomach mucin as described by Ravindranath et al., [6]. The lectin showed avid binding to BSM with inhibitory potency of 0.931 x 10-6mg.ml-1and on neuraminidase treatment or de-*O*-acetylation failed to inhibit.

Induction of lectin activity

Challenging the crabs with erythrocyte possessing sislylated cell surface enhanced hemagglutination activity within 2h upto 8h. Whereas the neuraminidase treated erythrocyte failed to respond. This implied that the lectin in the serum of crab possessed the ability to recognize the sialic acid on pathogen cell surface. The elicitation of lectin activity in response to sialic acid however lacking the biosynthetic pathway for it arguably explains the recognition mechanism in innate immunity of the prostomial crab.

Lectin as an opsonin in phagocytosis

The rabbit erythrocyte was taken as the target cell for the study of in vitro phagocytosis in hemocytes of the crab *P. jacquemontii*. The monolayer of hemocytes prepared on a glass slide (23°C) was overlaid with rabbit erythrocyte and observed under phase optics at 40 x magnifications. The target cell appeared to be internalized by ten minutes. The hemocytic phagocytosis rate of 16.48 ± 2.31% increased to 17.54 ± 1.5% with serum coated erythrocyte, 19.50 ± 2.43% with clarified serum coated erythrocyte and to 35 ± 3.25% with Pjlec lectin coated erythrocyte. The trypsin treated erythrocyte remained the same as untreated erythrocyte (control) whereas the glycosidase and neuraminidase treated erythrocyte failed to display phagocytosis.

The microscopic observations were supported by flow cytometry evaluation displaying rapid engulfment of lectin coated erythrocyte. The phagocytic activity of hemocytes against untreated rabbit erythrocyte was 33.74% and the trypsin treated erythrocyte 68.29% and with lectin coated rabbit erythrocyte 97% [7].

Potential of sialic acid binding lectin

The sialic acid binding lectins are molecules of recognition in innate immunity process. Altered sialylations on cell surface glycoproteins and glycolipids have been implicated in tumorogenesis

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and metastasis. In pathogenesis also the sialic acid antigenecity are crucial in therapeutic treatment. The sialic acid specific lectin from the crab *P. jacquemontii* with enhanced polysaccharide antigenic response and opsonic function augments for research to advance its use as a tool for diagnosis and therapy. To identify and mark the alteration in *O*-acetylation of sialic acid in human melanoma, the lectin from the marine crab *Cancer antennarius* has been applied [8,9]. Altered sialylation of oligosaccharides of glycoproteins and glycolipids in cell surface have been implicated in tumor progression and metastases [10]. In the cervical cancer correlated with poor prognosis and lymph node metastasis in cervical cancer [11].

References

1. Saito M, Kitamura H, Sugiyama K. Occurrence of gangliosides in the common squid and pacific octopus among protostomia. Biochim Biophys Acta. 2001; 1511: 271-280.
2. Alviano CS, Travassos LR, Schauer R. Sialic acids in fungi: a mini review. Glycoconj J. 1991; 16: 545-554.
3. Vimr ER, Kalivoda KA, Deszo E, Steenbergen SM. Diversity of microbial sialic acid metabolism. Microbiol Mol Biol Rev. 2004; 68: 132–153.
4. Denis M, Mercy PD, Bai RN, Suriya SJ. Purification and characterization of a sialic acid specific lectin from the hemolymph of the freshwater crab Paratelphusa jaquemontii. Eur J Biochem. 2003; 270: 4348-4355.
5. Ravindranath MH, Paulson JC. O-acetyl sialic acid specific lectin from the crab Cancer antennarius Methods. Enzymol. 1987; 138: 520-527.
6. Ravindranath MH, Higa HH, Cooper EL, Paulson JC. Purification and characterization of an O-acetyl sialic acid specific lectin from a marine crab Cancer antennarius. J Biol Chem. 1985; 260: 8850-8856.
7. Denis M, karthigayani T, Ramasamy SM, Munusamy A. Opsonic function of sialic acid specific lectin in freshwater crab Paratelphusa jacquemontii. 2015; 4: 601.
8. Cheresh DH, Reisfeld R A, Varki AP. O- acetylation of isialoganglioside by human melanoma cells creates a unique antigenic determinant. Science. 1984; 225: 844-846.
9. Ravindranath MH, Paulson JC, Irie R F. Human melanoma antigen O-acetylated ganglioside GD3 is recognized by Cancer antennarius. J Biol. Chem. 1988; 263: 2079-2086.
10. López-Morales D, Reyes-Leyva J, Santos-López G, Zenteno E, Vallejo-Ruiz V. Diagnostic Pathology. 2010; 5: 74.
11. Wang PH, Li YF, Juang CM, Lee YR, Chao HT, Tsai YC, et al. Altered mRNA expression of sialyltransferase in squamous cell carcinomas of thecervix. GynecolOncol. 2001; 83: 121-127.

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