ISOLATION AND PURIFICATION OF LECTIN TARGETING TUMOR ASSOCIATED SIALIC ACID FROM A SLUG

FINAL REPORT OF THE MAJOR RESEARCH PROJECT

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SUMMARY OF THE FINDINGS

Invertebrates lack an adaptive immune system, and rely exclusively on their innate immune system to defend themselves against a variety of invading pathogens. Agglutinins or lectins, the multivalent sugar binding proteins have been suggested to participate in innate immunity of these invertebrates by inducing agglutination of the invading foreign materials.

A perusal of literature on the availability of lectins/agglutinins revealed the presence of sialic acid specific lectins among gastropodan mollusks. So preliminary studies were made to screen the naturally occurring agglutinins in the various tissues of three different gastropodan species *Pila globosa*, *Lymnaea stagnalis and Laevicaulis alte* by hemagglutination (HA) assay using mammalian erythrocytes of diverse origin.

Natural agglutinins capable of agglutinating diverse species of mammalian erythrocytes were recognized in the hemolymph, mucus and various tissues of the molluscan representatives, the freshwater apple snail *Pila globosa*, the great pond snail *Lymnaea stagnalis* and the tropical land slug *Laevicaulis alte* by hemagglutination assay.

Agglutinin with specific affinity for the glycocalyx of rabbit erythrocytes is identified in the hepatopancreas of the freshwater apple snail *Pila globosa*, whole body extract and excreta of the great pond snail *Lymnaea stagnalis* and the mucus of the tropical land slug *Laevicaulis alte*. Since the HA titer was observed to be high in the mucus of the tropical land slug *Laevicaulis alte*, it was selected for further analysis and physico-chemical characterization.

Cross adsorption of the mucus of the tropical land slug *Laevicaulis alte* with rabbit, sheep, buffalo, Pig and dog erythrocytes abolished the ability of the agglutinin to agglutinate any of the tested erythrocytes revealing the presence of a single agglutinin.

Although pH did not have a significant influence on HA, maximum HA titer was observed at pH 7.5 and hence pH 7.5 was considered as the optimum pH for the mucus agglutinin of the tropical land slug *Laevicaulis alte*.

The agglutinability of the mucus agglutinin of the tropical land slug *Laevicaulis alte* showed a gradual increase along with every 10^{o}C rise in temperature till 55^{o}C, beyond which it got reduced and became negligible at 70^{o}C.

Increase in HA titer following addition of Calcium reveals that the mucus agglutinin of the tropical land slug *Laevicaulis alte* depends on Calcium for its activity. Calcium dependency is further confirmed following reduction in HA titer of the agglutinin with rabbit erythrocytes with the inclusion of EDTA.

Sialoglycoproteins Fetuin and α acid glycoprotein were identified as the potent inhibitors of the mucus agglutinin of the tropical land slug *Laevicaulis alte*. However other sialoglycoproteins Bovine submaxillary mucin, Procine thyroglobulin, Lactoferrin and Apotransferrin also inhibited agglutination of rabbit erythrocytes with great potency.

Reduction in HA titer in HA assay carried out using desialylated rabbit erythrocytes and decrease in HAI titer in HAI assays carried out using asialoglycoproteins affirm the sialic acid specificity of the mucus agglutinin of the tropical land slug *Laevicaulis alte*.

The sialic acid specific lectin purified by affinity column chromatography on fetuin sepharose 4 B from the mucus of the tropical land slug *Laevicaulis alte* showed a molecular mass 40 kDa. The lectin could be used as a valuable tool in glycoconjugate research, identification of pathogenic bacteria and neoplastically altered cells.