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PURIFICATION AND SOME PHYSICO-CHEMICAL PROPERTIES OF A LECTIN FROM THE SEEDS OF *TRICHOSANTHES ANGUINA*

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Lectins constitute a class of proteins and glycoproteins, of non- immune origin, possessing a specific binding affinity to carbohydrates, and hence, can interact with polysaccharides and glycoconjugates (1). Lectins occur in a wide variety of plants, micro-organisms, invertebrates and vertebrates. During our studies on the seeds of local plants for their lectins, we detected lectin activity in the extract of the seeds of snake gourd, *Trichosanthes anguina*.

In haemagglutination assay (2) the phosphate buffered saline (PBS, pH 7.4) extract of snake gourd seeds agglutinated the erythrocytes of human A,B and O groups as well as goat, rabbit, rat and mouse erythrocytes. Sugar inhibition assay (3) revealed lactose to be the best inhibitor of haemagglutination by the lectin. When the seed extract was subjected to ammonium sulphate salt fractionation (3) the lectin activity was found mainly in the precipitate obtained at 60% ammonium sulphate saturation. The precipitate was dissolved in PBS and dialysed extensively against PBS. The lectin was isolated in pure form by affinity chromatography (3) on a column of immobilised lactose ('Selectin 2', Pierce Chemical Company, USA). The bound fraction from the column was eluted with PBS containing 50 mM lactose. The eluted protein was dialysed extensively against PBS to remove lactose and then concentrated. The material obtained from the affinity column retained about 80% of the haemagglutination activity observed in the seed extract. The lectin was found to be electrophoreticaly pure, migrated as a single sharp protein band when subjected to polyacrylamide gel electrophoresis under non reducing conditions. Gel filtration of the lectin on a column of sephadex G-200 yielded a single protein peak having a molecular mass of about 49 KDa. On sodium dodecyl sulphate-polyacrylamide gel electrophoresis the lectin yielded a single protein band of 48 KDa in the absence of beta mercaptoethanol, and two bands of 30, 200 Da and 17, 800 Da in presence of beta-mercaptoethanol. These results suggested that the lectin molecule contains two polypeptide chains linked by disulphide bonds. Periodic acid- Schiff's staining of SDS-PAGE gel and also the Phenol-H_2SO_4 reaction, intended for detecting any carbohydrate moeity, yielded negative results revealing the absence of covalently linked sugars in the

lectin molecule. The snake gourd lectin was found to be thermally stable with the optimum haemagglutinating activity between 25^0C and 45^0C. Beyond 45^0C haemagglutinating activity decreased gradually but some activity was retained even after incubation for 30 minutes at 95^0C. The optimum pH range of the lectin is between 4.5 and 7.5. We have not come across any report on the isolation of a lectin from the seeds of *Trichosanthes anguina*. A few lectins have been reported from the phloem exudates of certain plants belonging to Cucurbitaceae family. Comparison of the properties of these lectins with the snake gourd lectin revealed that the present lectin is different from those reported so far from the members of Cucurbitaceae.

References

1. Goldstein I J, Hughes R C, Monsigny M, Osawa T and Sharon N (1980). Nature (London) 285:66.
2. Beena C, Shanavas K R and Vasudevan D M (1993). Isolation and some properties of a lectin from the seeds of *Artocarpus hirsuta*. Pro. Vth Kerala Science Congress. P. 374-376.

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