THE ISOLATION AND CHARACTERIZATION OF THREE LACTOSE BINDING PROTEINS IN THE HAEMOLYMPH OF THE PROTOCHORDATE *Botrylloides leachii*

Samuel F. Schluter, B.Sc. (Hons.) (Adelaide)
Department of Microbiology and Immunology
The University of Adelaide

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ABSTRACT

This thesis is concerned with the isolation and characterization of three lactose-binding proteins from haemolymph of the protochordate *Botrylloides leachii*. Initially, the haemagglutinating activity of the haemolymph for various erythrocytes was extensivley investigated and it was concluded from the results of cross-adsorption and sugar inhibition experiments together with gel filtration data that two haemagglutinins were present. Although both molecules were inhibited by lactose, their binding specificities were shown to be different both by their affinity for different types of erythrocytes and their susceptibility to inhibition by different sugars. The haemagglutinins were purified by a combination of affinity chromatography using an adsorbent consisting of lactose-substituted acid-treated Sepharose, and gel chromatography. During the purification a third protein was specifically eluted from the affinity column. This molecule was shown to bind specifically to guinea pig erythrocytes without causing haemagglutination and could be inhibited by lactose. The three proteins were designated Haemagglutinin One (HA-1), Haemagglutinin Two (HA-2) and Lactose Binding Protein Three (LBP-3). The purity of each preparation was confirmed by SDS-PAGE and immunochemical analyses.

The major findings concerning the nature of these proteins were as follows:

1) HA-2 molecules, which agglutinated every type of erythrocyte tested, have only one type of binding site and this recognises lactose (i.e., D-Gal-β1→4-D-Glu) moieties. The agglutinin is a globular (f/f_o = 1.34) protein and occurs as a non-covalently linked dimer (mol. wt. = 65,000) composed of identical subunits (apparent mol. wt. = 33,400).

2) HA-1 molecules (mol. wt. = 152,000) are non-globular (f/f_o = 1.8), covalently linked polymers which appear to be composed of two types of sub-units, HA-1/1a (apparent mol. wt. = 29,600) and HA-1/1b (apparent mol. wt. = 28,200). This molecule agglutinated only guinea pig erythrocytes and its activity required Ca^{++} ions. It appeared to have only a single type of binding site which bound lactose, melibiose, D-galactose and D-fucose with similar affinity.

3) LBP-3 molecules (mol. wt. = 140,000 - 160,000) exhibited a binding specificity similar to that of HA-1 molecules and their activity also required Ca^{++} ions. They are covalently linked polymers composed of three types of subunits, LBP-3/1a (apparent mol. wt. = 29,600, LBP-3/1b (apparent mol. wt. = 28,200) and LBP-3/2 (apparent mol. wt. = 23,600). It is proposed that the LBP-3/1a and 1b subunits are identical to the HA-1/1a and 1b subunits and that these subunits probably contain the carbohydrate binding site.

4) The HA-1 molecules appear to exist as pentamers

comprising one or the other of the HA-1/1a and 1b subunits. LBP-3 molecules appear to be hexamers comprising no more than two LBP-3/2 subunits and approximately four of either the LBP-3/1a or 1b subunits.

Preliminary studies of the haemagglutinin in *B. schlosseri* haemolymph indicated the presence of a haemagglutinin that was very similar to *B. leachii* HA-1.