

# Lectin agglutination



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Experiment 6 : Lectin Agglutination Aim: To study the agglutination pattern of different strains of *Pseudomonas aeruginosa* to different sialic acid-binding lectins. Abstract: The cell membrane of two strains of *Pseudomonas aeruginosa*, P22 and P201 were treated the sialic acid lectins SNA I, WGA, MAL and HAA to confirm the presence of sialic acid carbohydrates in the cell surface membranes of these strains. This was done by using two sets of samples-one treated with trypsin and the other without.

The sample treated with trypsin showed agglutination for all lectins while the untreated sample showed little to no agglutination. A control was also used with the cells being incubated with buffer instead of lectins. The lectin pattern of agglutination varied based on the specific sialic acid available on each cell surface membrane of the two strains investigated. Introduction: Lectins are carbohydrate proteins or glycoproteins of non-immune origin which are often specific in the agglutination or binding to cell membrane glycoproteins similar in structure to the lectin.

Lectins can be derived from the extracts of plants, animal viruses and microorganisms. They form precipitates with glycoconjugates and are useful for identifying or separating oligosaccharides with identical sugar compositions such as galactose, mannose or glucose. It can also be used in blood typing when they agglutinate or bind to glycoproteins on red blood cells. These agglutinins can select types according to blood group activities utilizing sugar binding mechanisms. The real function of lectins in plants is still unclear but is believed to be the binding of glycoproteins on the surface of cells.

Lectins containing sialic acid are also known to bind conjugate glycoproteins containing sialic acid. Sialic acid is a generic term for the N- or O-substituted derivatives of neuraminic acid, a nine-carbon monosaccharide. It is also the name for the most common member of this group, N-acetylneuraminic acid (Neu5Ac or NANA). Sialic acids are found widely distributed in animal tissues and in bacteria, especially in glycoproteins. Sialic acid rich glycoproteins on cell membranes help to keep the water on the surface of cells. This aids in cellular fluid uptake and maintains the negative charge on the cell membrane.

Sialic acid is also used to evade phagocytosis in as sialic acid binding lectins can hide mannose antigens on the surfaces of host cells or bacteria from mannose binding lectins. The lectins used in this experiment are mostly derived from plants. SNA I is derived from the stem bark, leaves, flowers, fruits and root extracts from the deciduous shrub *Sambucus nigra*. The WGA lectin is derived from the wheat germ of *Triticum vulgare*. The other lectins MAL and HAA are derived from plants and all contain sialic acid and have specificity to bind with a certain carbohydrate on the cell membrane.

In this experiment these lectins are used to observe the agglutination pattern of the lectins to the lectins contained on the cell surface membrane on two strains of *P. aeruginosa*. Lectins are very volatile and must be stored at low temperatures in phosphate buffered saline (PBS) before use. Two sets of cells are used, labelled treated and untreated. The treated cells were incubated in trypsin to partially hydrolyse the proteoglycans which will make more lectins available for lectin agglutination. The cell suspensions are then

heated to degrade surface structures and to dissolve more glycoconjugates from the cell wall.

A negative control is also included with buffer instead of lectin. The agglutination can identify the presence of sialic acid on the membranes (which is linked to virulence factors). The effectiveness of treating cells with trypsin to expose glycoproteins can also be assessed. This test may also be used to differentiate between the two strains of *P. aeruginosa*. Method: As outlined on pg. 21-22 of the BC34M Laboratory manual Results: Table 1: The agglutination patterns of sialic acid lectins to sialic acid carbohydrates for strain of *P. aeruginosa*. *P. aeruginosa* Control SNA WGA