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LECTINS (ARACHIS HYPOGAEA AND VICIA FABA): PURIFICATION AND IMMUNOCHEMICAL CHARACTERIZATION

Thesis

Submitted to the Faculty of Medicine Tanta University

In partial fulfilment for the requirements of M.D. Degree in Medical Biochemistry.

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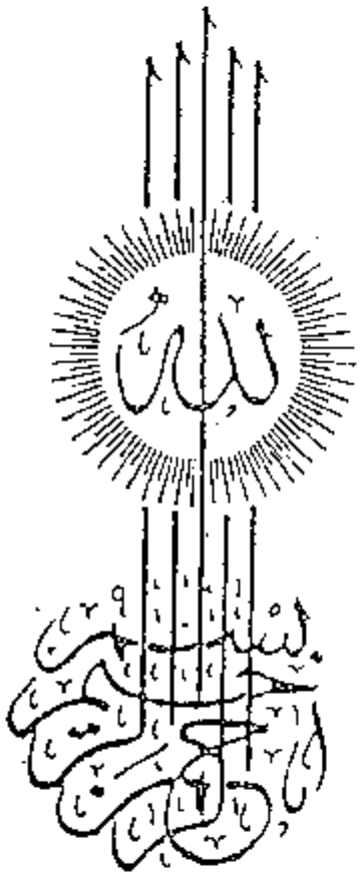

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ .

صَدَقَ اللهُ الْعَظِيمُ
البقرة - ٣٢ -

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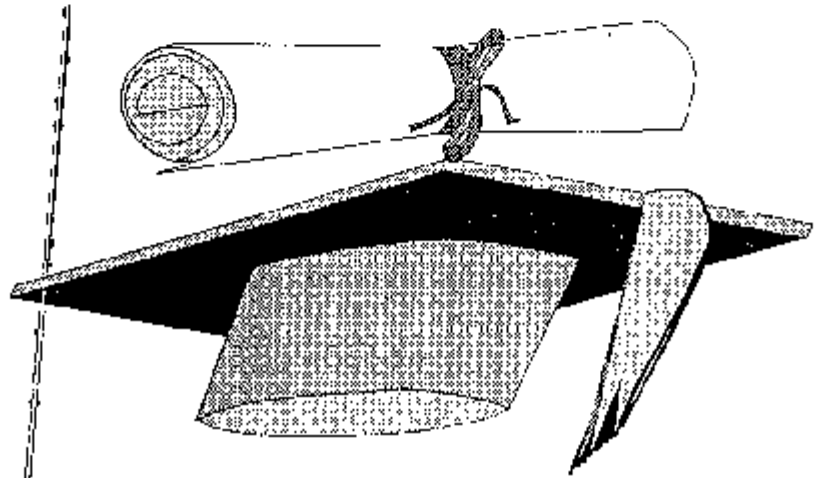

INTRODUCTION

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Lectins

History and Discovery:

It is just over a hundred years ago that the first plant lectin was described by Stillmark (1888), who obtained a preparation from castor bean (Ricinus communis) extracts which agglutinated red blood cells (1,2).

As more and more of such substances were later discovered in other plants, and as a common name for them , the term, haemagglutinins , was proposed by Elfstrand (1898) (3).

Ehrlich, who is considered to be the father of immunology, has shown that rabbits fed with small amounts of seeds containing the toxin (Ricin or Abrin) became partially immune to the toxicity, thus demonstrating that the haemagg lutinins were also antigenic (4).

Landsteiner and Raubitschek (1908) showed later that not all haemagglutinins need necessarily be as toxic as ricin or abrin, for example, the agglutinins obtained from common beans (Phaseolus vulgaris), peas (Pisum sativum), lentils (lens culinaris), etc., were relatively non-toxic , water soluble proteins.It is now known that such haemagglutinating proteins are found in all taxonomic groups of the plant kingdom and that they are not all overtly toxic (5).

The next momentous step in the history of haemagglutinins was the realization that some of the haemagglutinins agglutinated blood cells only from some groups of individuals within the ABO blood group system without affecting cells from other groups. This discovery of blood group specificity has led Boyd to coin the term, lectin, to denote this aspect of selection (in latin, legere means to select or choose) and is regarded as the starting point of modern lectinology (6).

Definition:

The first proper definition of lectins was based on the sugar specificity of the inhibition of the haemagglutination reaction. Accordingly, lectins are carbohydrate binding proteins of non-immune origin which agglutinate cells or precipitate polysaccharides or glycoconjugates (Goldstein et al., 1980) (7).

This definition was adopted by the Nomenclature Committee of the International Union of Biochemistry (Dixon, 1981) (8). The main problem with this definition is that, if it is strictly interpreted, some poorly agglutinating well –known toxins, such as ricin, abrin, etc, cannot be regarded as lectins, even though they are all known to contain lectinic subunits. Thus the first definition has since been extended to include the above toxins (Kocourek and Horejsi, 1983) (9). Moreover, as it

is now realized that some lectins contain a second type of binding site that interacts with non-carbohydrate ligands, confining the definition of lectins strictly to bivalent carbohydrate binding proteins seems to have lost its usefulness (10). The most suitable definition of lectins is that of A. pusztai who defined lectins as proteins or glycoproteins of non immunoglobulin nature capable of specific recognition of, and reversible binding to, carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognized glycosyl ligands. Thus, other sugar binding proteins, such as the various sugar – specific enzymes, hormones and transport proteins are excluded, but monovalent lectins (i.e. bacterial and plant toxins) are included (4).

Classification of lectins :

Lectins can be classified into : Plant, microbial and animal lectins(11).

I - plant lectins : -

Lectins were classified according to the inhibitory effectiveness of various mono and oligosaccharides of known composition on certain lectin reactions like haemagglutination of erythrocytes into five main groups (Goldstein and Poretz, 1986) (12) and (Damjanov, I. 1987) (13).

This is a relatively small group of lectins. Some of them are useful reagents for the detection of fucose residues in complex

hydrophobic amino acid residues in the primary polypeptide sequence(49).

Glycosylation sites:

Although most lectins are glycoproteins, there are a number of well known lectins, such as, for example, concanavalin A, lentil lectin or wheatgerm agglutinin, which contain no covalently attached carbohydrates. Rather interestingly, however, even the non-glycoprotein lectins are usually synthesized as glycosylated precursors. Thus, proconcanavalin A is an inactive glycoprotein from which the glycosidic side chain is removed during post-translational processing (52). Similarly, the non-glycoprotein wheatgerm agglutinin molecule is produced by removing a carboxyl terminal glycopeptide from the glycosylated precursor during post-translational processing (53).

All glycoprotein lectins contain a peptide sequence, asparagine – x – threonine / serine, which is characteristic for glycosylation sites. These sequences are different in the non – glycoprotein lectins. Thus, in the β-chain of Vicia faba lectin (favin) the sequence to which the glycosyl side chain is attached contains the peptide asparaginyl 169- Alanyl – threonine 171. In the non-glycoprotein, concanavalin A, this is replaced by asparaginyl-serinyl–valine. Peptide sequences, which in one glycoprotein lectin contain the glycosidic side –