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# *Recollections*

## My Favorite Enzyme: For Love of Lectins

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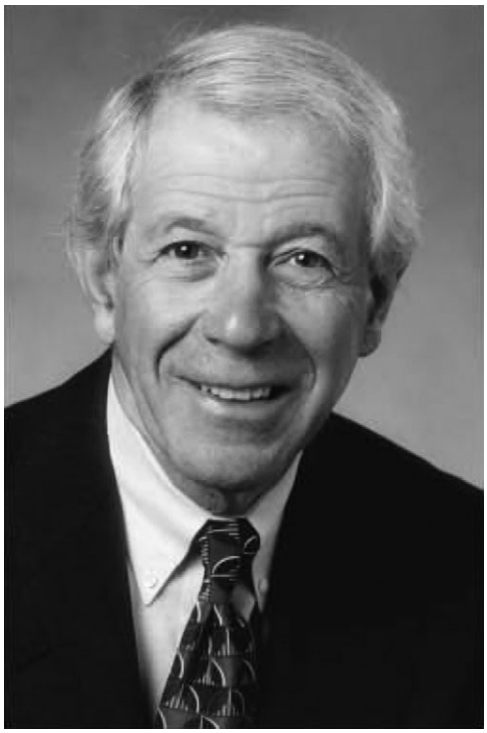

I was a student of Fred Smith at the University of Minnesota during 1952–1959. Smith was an Englishman who came to the USA to work on the Manhattan Project to build the atomic bomb. He was an outstanding carbohydrate chemist who was mentored by Sir Norman Haworth, Nobel Laureate in Chemistry for the synthesis of vitamin C (ascorbic acid). Smith was at the University of Birmingham when he received the call to come to the United States to work on "the bomb" because of his expertise in fluorine chemistry, a necessary technique for the separation of the 238 isotope of uranium as the hexafluoride.

My work in Smith's Lab involved studies on periodate oxidation products of glycosides. On receiving my PhD I stayed on as a postdoctoral fellow and instructor for two additional years. Happily for me, I was awarded a Guuggenheim Fellowship which allowed me to spend 2 years in Europe, first with Bill Whelan at the Lister Institute of Preventive Medicine in London, then with Bengt Lindberg in Stockholm. My work in both places involved the isolation, characterization, and chemical and enzymatic synthesis of oligosaccharides from natural sources. These were two wonderful years to work in and experience the scientific and cultural milieu of two different European laboratories. Bill Whelan was an enthusiastic and a helpful mentor. We both smoked at that time, and each morning "after lighting up," we discussed my research. He always provided insightful and valuable ideas and approaches to pursue.

My habit of arising early and getting to the lab in the early morning persisted while in London. At first, this did not go over well with the janitorial staff at the Lister. But after some time we became good friends and they greeted me with much enthusiasm each morning. There was another advantage from arriving early: I had the first use of equipment which was, at times, in short supply.

While at the Lister I also had the opportunity of meeting Walter Morgan and his colleague Winifred Watkins. They had been studying the chemistry of human blood group substances for many years and were in a fierce competition with Elvin Kabat, Prof. of Immunochemistry at Columbia University. They were always first to grab a copy of the latest journal to see if Kabat had scooped them. Little did I realize, at the time, that our paths would converge. They did indeed when my lab took up a study of the lima bean lectin which they had used to assay for human type A blood group substance. I had many interesting and fruitful discussions with these colleagues during the following years.

My first independent position was at the University of New York at Buffalo. To assist a colleague in the characterization of glycogen from microorganism, I decided to prepare concanavalin A from jack beans (*Canavalia ensiformis*) which was shown to precipitate glycogen. I followed the procedure of James Sumner who first prepared the protein. I decided to pass the purified protein through Sephadex, crossed-linked dextran, to assess its homogeneity. It did not emerge from the column! In an attempt to displace it from the column I suggested to a graduate student to add glucose. Pure concanavalin A was eluted!

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My graduate student and I rushed to publish this interesting result and sent it to *Biochem Biophys Res Commun*. It came back by return mail: "This represents a modest advance in an obscure area. Manuscript rejected." Subsequently it was published as a note in the *Biochem J* (*1*). Reasoning that the protein had a binding site(s) for a carbohydrate molecule, my laboratory set out on a trail of research to characterize the Con A binding site. We determined there were four carbohydrate binding sites per molecule (*2*) and that α-D-mannose (*3*) was the most avidly bound monosaccharide. Such carbohydrate-binding proteins were termed lectins by William C. Boyd, from the Latin *legere*- to pick out or to choose. We then turned our attention to find other carbohydrate binding proteins in plant material—seeds, tubers, and fungi. The study of lectins opened up many doors and avenues of research concerned with the structure, biosynthesis, and function of these fascinating proteins. The use of lectins in biochemical research also led us to many interesting applications in numerous biological fields.

It was also at this time that I received an invitation to present a seminar in the Department of Biological Chemistry at the University of Michigan as a possible candidate for the position of Associate Professor. I was flattered and excited, especially because Saul Roseman, the outstanding glycobiologist was there. I gave a seminar on my lectin research; it went over well. A week later, I was offered the position; I accepted with alacrity and have been here for the past 44 years.

Calbiochem contacted me to assist them in the preparation of Con A on a commercial level. In return for my services they sent me a quantity of *Griffonia simplicifolia* seeds obtained from Ghana. The discovery of five isolectins in these seeds was a great revelation: the isolectins were labeled $A_4$, $A_3B$, $A_2B_2$, $A_3B$, and $B_4$ to account for the specificity of the A-subunit toward *N*-acetylgalactosamine, the determinant sugar in human blood group type A substance, and the B-subunit toward galactose, of human blood group type B substance (*4*). The most valuable isolectin was $B_4$ which showed a remarkable specificity toward α-D-galactosyl end groups. That made it an extremely valuable reagent for the detection of α-D-galactosyl end groups in biological materials. We employed this $B_4$ isolectin for the assay of human type B erythrocytes (*5*) and labeled it with fluorescein, as a tool for the visualization of terminal α-D-galactosyl end groups. We discovered this carbohydrate group in Ehrlich ascites tumor cells (*6*), in cryostat sections of rat kidney cortex, in capillaries of cardiac and skeletal muscle (*7*), and in murine laminan (*8*). In its immobilized form, it proved an efficient matrix for the isolation and characterization of a series of α-D-galactosyl-containing glycoproteins from Ehrlich ascites tumor cells (*9*). The α-1,3-galactosyltransferase responsible for the syntheses of these oligosaccharide chains was purified from the Ehrlich cells (*10*). The fluorescein isothiocyanate (FITC)-labeled $B_4$ isolectin was also shown to label porcine tissue due to its presence of α-galactosyl end groups which make porcine tissue incompatible for transplantation into humans (*11*). Interestingly, our lab also showed that the GS-I $B_4$ isolectin bound to stimulated but not to resident murine macrophages indicating the presence of α-galactosyl end groups in stimulated macrophages (*12*).

An investigation into the isolation, characterization, and biological properties of the lima bean lectin proved to be extremely profitable. This lectin, which recognizes α-D-*N*-acetylgalactosaminyl-end groups specifically agglutinates human type A erythrocytes.

We isolated the lectin on immobilized blood group type A substance (*13*). The lectin is composed of two active species of molecular weight of 247,000 and 125,000 with identical amino acid compositions and subunits of 31 Da. Lima bean lectin does not contain methionine but possesses two half-cysteine residues per 31,000 molecular weight subunits (*13*). One of the sulfhydryl-containing subunits is free, the other being involved in a disulfide linkage between two subunits. The free sulfhydryl group was shown to be essential for carbohydrate binding activity (*14*). A peptide containing this sulfhydryl (SH) group was isolated by proteolytic digestion and characterized (*15*). Maximum homology was found with a sequence in concanavalin A and other legume lectins. The presence of one carbohydrate binding site per subunit was shown by equilibrium dialysis (*16*). A second seminal finding was the presence of a specific, high affinity binding site for adenine on the lima bean lectin molecule (*17*, *18*). We also identified the presence of adenine binding sites in other legume lectins (*Dolichos biflorus, Phaseolus vulgarus*, and soybean) (*18*). This high affinity adenine binding site on the lima bean lectin was labeled with the photoaffinity probe 8-azidoadenine (*19*) and the peptide containing the label isolated following digestion with trypsin and shown to possess extensive homology to adenine binding sites in other legume lectins (*19*). The adenine binding site was also studied by electron spin resonance (ESR) using a spin-labeled probe (*20*).

The search for lectins that bind sialic acid proved to be very rewarding. These 9- carbon acidic sugars are omnipresent in the animal world. They are typically found at the termini of *N*- and *O*-linked glycans. We discovered several of these lectins which are now used routinely by scientists world-wide. The first was isolated from elderberry bark (*Sambucus nigra*) which recognizes the Neu5Aca2-6Gal/GalNAc sequence (*21*). In its immobilized form this lectin recognizes and binds sialylated glycoproteins and glycopeptides and proved useful for the fractionation of sialylated oligosaccharides glycopeptides and glycoproteins (*22*). A second sialic acid binding lectin was isolated form the fruting body of the polypore mushroom *Polyporus squamosus* (*23*). It recognizes the sequence Neu5Ac α2-6Gal β1-4Glc/GlcNAc which occurs ubiquitously in glycoproteins and glycolipids. It has found wide application as a histochemical stain for identification of this epitope (*24*). This lectin has been crystallized and its X-ray structure, combined with its sialylated oligosaccharide, determined (*25*). The sialic acid binding lectin from the slug, *Limax flavus*, has also been studied. It recognizes terminal sialic acid groups (*26*). The lectin was cloned in our laboratory; therefore, its amino acid sequence is known (*27*).

We later conducted studies on the banana lectin. We carried out an in-depth study of its carbohydrate binding specificity and

found it to exhibit some extraordinary properties. Besides its known property of recognizing terminal, nonreducing α-mannosyl/glucosyl groups we determined it to bind to *internal* α-1,3-mannosyl/glucosyl groups and also to reducing terminal β-1,3-glucosyl units as occur in laminarin, the polysaccharide from the brown alga *Laminaria digitata* (*28*, *29*). The lectin precipitated with elsinan, a linear polysaccharide containing internal α-1,3-glucosyl linkages and nigeran which contains, on the average, alternating α-1,3- and α-1,4-glucosidic bonds. This is the first instance of a mannose/glucose-binding lectin reacting with *internal* α-1,3-linked glucosyl group (*28*). The X-ray structure of the lectin was determined with a laminaribiose molecules in its binding sites (*30*). The structure has a B-prism-I fold similar to other family members, but differs from them in its mode of sugar binding. The presence of a second, previously unreported sugar binding site, was also identified.

One of the very few human type B blood group-specific lectins was isolated from the mushroom *Marasmius oreades*. Our laboratory purified it to homogeneity, cloned the gene for its expression, and studied its carbohydrate binding specificity (*31*, *32*). The protein is a homodimer with two binding sites (*31*). It has exclusive specificity for Gal α-1,3 Gal—containing epitopes (*31*). This disaccharide sequence, present in porcine tissues, poses a barrier to its use in animal-to-human organ transplantation. The X-ray crystal structure of the lectin, complexed with the linear trisaccharide Gal α-1,3 Gal β-1,4GlcNAc, was determined at 2.4 Å resolution (*33*). The structure adopts a ricinB/B-trefoil fold at its N-terminus while the C-terminal domain serves as a dimerization interface.

A lectin with an unusual specificity for Gal α-1,4-Gal units was isolated from the mushroom *Lyopyllum decastes* (*34*). It is a homodimer composed of noncovalently associated monomers of molecular mass 10,276 Da (*34*). Interestingly, it does not show significant homology to any known protein sequence. The Gal α-1,4-Gal disaccharide sequence is relatively rare in humans but is present in the bacteria *Shigella dysenteriae* and *E. coli* 0157:H7, both of which are responsible for fatal food-borne illnesses in humans.

A complete chapter in the work in my laboratory encompasses the lectin present in *Amaranthus caudatus* seeds. High in its content of nutritious proteins, the seeds were used by ancients in South America as an important component of their diet. Isolated on a Synsorb-T affinity column, the lectin is a homodimer with a molecular mass of 33 kDa with two binding sites per dimer (*35*). It is not glycosylated and requires no metal ions for binding activity. The lectin has a unique binding site directed against the T-antigen (Gal β1,3GalNAc). Interestingly, the T-antigen is expressed in carcinomas of the breast, pancreas, lung, and colon and can be detected readily by the *A. caudatus* lectin. The lectin was crystallized and its X-ray crystallographic structure bound to the T-antigen determined (*36*). Numerous contacts are observed between both the Gal and GalNAc moieties of the T-antigen. A critical feature of the carbohydrate ligand is the orientation of the C4 hydroxyl of GalNAc; this group must be axial as opposed to equatorial for optimal lectin binding (*36*).

A major group of lectins studied by our laboratory are found in bulbs from the plant family *Amaryllidacea*e. These include those from the spring flowers snowdrop (*Galanthus nivalis*), daffodil (*Narcissus pseudonarcissus*), and amaryllis (*Hippeastrum hybr.).* They are all α-D-mannose-specific lectins which do not recognize α-D-glucose. All these lectins occur as dimers. Although they all contain cysteine residues the dimers are not linked by disulfide bonds. A prime example is the snowdrop, the first in this group of mannose-specific lectins studied (*37*). It is a tetrameric protein composed of identical 12.5 kDa subunits. The lectin protein has been sequenced and cloned and its X-ray crystallographic structure determined (*38*). Not surprisingly, its amino acid sequence did not reveal any significant homology with several mannose/glucose binding lectins (Con A, pea, lentil). However, bulb lectins belonging to the same family as snowdrop (*Amaryllidaceae*), daffodil and amaryllis, showed high amino acid similarity among each other (approximately 80–89%).

Most recently, my laboratory has been studying the carbohydrate molecules which decorate the surface of human embryonic stem cells. Employing a panel of lectins and anti-carbohydrate antibodies the carbohydrates that are present on day 12 of human embryonic stem cells (hESC) differentiation as embryoid bodies was determined (*39*). Among the carbohydrate epitopes we identified were both terminal and internally linked α-D-mannopyranosyl groups, poly-*N*-acetyllactosaminyl chains and both α-2,3- and α-2,6-linked *N*-acetylneuraminyl acid and β-D-galactosyl groups. However, no terminal nonreducing α-D-galactosyl, *N*-acetyl-β-D-glucosaminyl nor *N*-acetyl-α-D-galactosaminyl groups were found by this approach. After 28 days increases in GalNAc, the T-antigen (Gal β1,3GalNAc) and difucosylated LacNAc were observed (*40*).

So, I feel as if I've been pretty fortunate to have had the opportunity to work in and supervise a laboratory for close to 50 years with the thrill of discovery and to work with a group of outstanding students and colleagues, and happily, it continues to this day.

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