This Week's Citation Classic®

Lotan R, Skutelsky E, Danon D & Sharon N. The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). *J. Biol. Chem.* 250:8518-23, 1975.

[Dept. Biophysics and Sect. Biological Ultrastructure, Weizmann Inst. Science, Rehovoth, Israel]

This paper describes the first purification and characterization of an anti-T lectin from peanut. The use of affinity chromatography on an immobilized galactopyranosylamine derivative to obtain a homogeneous protein by a single-step procedure is described along with some physicochemical properties, hemagglutinating activity, and carbohydrate- and glycoprotein-binding specificity of the pure lectin. [The *SCI*® indicates that this paper has been cited in over 520 publications.]

The Purification of Peanut Lectin

Reuben Lotan
Department of Tumor Biology
M.D. Anderson Cancer Center
University of Texas
Houston, TX 77030

March 26, 1990

In the early 1970s, lectins attracted a great deal of attention, following the demonstration that these saccharide-binding proteins are useful probes for cell surface glycoproteins that could detect changes that occurred after malignant transformation.[1,2] I joined the laboratory of Nathan Sharon and Halina Lis as a PhD student in 1971 to study these interesting proteins.

In 1973, towards the end of my doctoral research on soybean agglutinin (SBA) and wheat germ agglutinin (WGA), we investigated together with Yehuda Marikovsky and David Danon, both from the Section of Biological Ultrastructure at the Weizmann Institute, the binding and distribution of ferritin-SBA on the surface of young and "old" red blood cells (RBC). Our aim was to determine whether the decrease in cell surface sialic acid, which occurs during senescence of RBC *in vivo*, is accompanied by an exposure of galactose (Gal) and/or N-acetylgalactosamine (GalNAc) residues, which usually occupy a penultimate position to sialic acid on oligosaccharide chains of cell surface glycoproteins. Unexpectedly, we found that "old" RBC possessed fewer SBA receptors on their surface than young RBC, suggesting that the decrease in sialic acid content during aging of these cells is not accompanied by unmasking of Gal and/or GalNAc residues. Since SBA has a higher affinity for GalNAc than for Gal, it was not clear from the above study which of these sugars is present in lower amounts on the "old" cells as compared to the young ones.

Ehud Skutelsky, from the Section of Biological Ultrastructure, suggested that a lectin from peanut might be more suitable for our studies because a crude peanut extract had been reported to agglutinate only sialidase-treated RBC and to exhibit anti-T specificity (Galβ1-3GalNAc).[3,4] This information convinced me that it would be worthwhile to purify the lectin from peanut and to use it in the study of RBC aging. Although at the time my mentor, Sharon, was skeptical about the advantage of purifying yet another lectin, he did not object that I give it a try. I was routinely using ε-aminocaproyl-β-D-galactopyranosylamine bound covalently to Sepharose for the purification by affinity chromatography of SBA, and it seemed likely that this matrix would be useful also for the isolation of peanut lectin.

Indeed, I was successful in obtaining in a single step a homogeneous protein, peanut agglutinin (PNA), which I then characterized. Independently, the same lectin was isolated by the group of Toshiaki Osawa.[5] I found out about their results when Osawa and I were hanging our posters on two sides of the same board at the Third International Symposium on Glycoconjugates, in Brighton, England, in 1975.

Subsequently, in a collaboration with Skutelsky and Danon, we found that RBC senescence does not involve unmasking of galactose residues despite the loss of cell surface sialyl residues.[6] These results indicated that the use of sialidase-treated RBC, which do bind PNA, for studies of RBC clearance from the circulation *in vivo* is not justified.

PNA proved to be a lectin with many applications. Yair Reisner (then a fellow PhD student) and Sharon demonstrated that PNA selectively agglutinates immature thymocytes and developed a method for the isolation of this subpopulation, and, subsequently, of subpopulations of bone marrow cells. These methods have paved the way for the use of lectins for the isolation of bone marrow cells capable of being transplanted into immunocompromised recipients without causing the lethal graft versus host disease.[7,8] In addition, PNA is extensively employed for the immunohistochemical analysis of Galβ1-3GalNAc-containing glycoconjugates in a variety of normal and pathological specimens including carcinomas.[9]

1. **Sharon N & Lis H.** Lectins: cell agglutinating and sugar-specific proteins. *Science* 177:949-59, 1972. (Cited 1,340 times.) [See also: **Sharon N & Lis H.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology*. Philadelphia: ISI Press, 1986. p. 57.]
2. **Nicolson G L.** The interactions of lectins with animal cell surfaces. *Int. Rev. Cytol.* 39:89-190, 1974. (Cited 840 times.) [See also: **Nicolson G L.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology*. Philadelphia: ISI Press, 1986. p. 59.]
3. **Bird G W G.** Anti-T in peanuts. *Vox Sang.* 9:748-9, 1964. (Cited 150 times.)
4. **Uhlenbruck G, Pardoe G I & Bird G W G.** On the specificity of lectins with a broad agglutination spectrum. *Z. Immunitaetsforsch.* 138:423-33, 1969. (Cited 165 times.)
5. **Terao T, Irimura T & Osawa T.** Purification and characterization of a hemagglutinin from Arachis hypogaea. *Hoppe Seyler's Z. Physiol. Chem.* 356:1685-93, 1975. (Cited 65 times.)
6. **Skutelsky E, Lotan R, Sharon N & Danon D.** Distribution of the T-antigen on erythroid cell surfaces. Studies with peanut agglutinin, an anti-T specific lectin. *Biochim. Biophys. Acta* 467:165-74, 1977. (Cited 40 times.)
7. **Reisner Y, Linker-Israeli M & Sharon N.** Separation of mouse thymocytes into two subpopulations by the use of peanut agglutinin. *Cell Immunol.* 25:129-34, 1976. (Cited 485 times.) [See also: **Reisner Y.** Citation Classic. *Current Contents/Life Sciences* 30(44):19, 2 November 1987.]
8. **Reisner Y, Itzicovitch L, Meshorer A & Sharon N.** Hemopoietic stem cell transplantation using mouse bone-marrow and spleen cells fractionated by lectins. *Proc. Nat. Acad. Sci. USA* 75:2933-6, 1978. (Cited 145 times.)
9. **Damjanov I.** Biology of disease: lectin cytochemistry and histochemistry. *Lab. Invest.* 57:5-20, 1987. (Cited 15 times.)

1A25