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This Week's Citation Classic®

Allen A K, Neuberger A & Sharon N. The purification, composition and specificity of wheat-germ agglutinin. *Biochemical J.* 131:155-62, 1973.
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The paper describes the purification of the lectin from wheat germ and the resolution of three isolectins. On analysis it was shown to be very cystine-rich. A wide range of mono- and oligosaccharides were tested as inhibitors, and from these experiments a proposal was made for a binding site made up of three or four subsites with differing specificities. [The *SCI*® indicates that this paper has been cited in over 340 publications.]

Wheat-Germ Agglutinin—A Proposed Structure of the Binding Site

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In 1971 I was working as a postdoc in the department of Albert Neuberger at St. Mary's Hospital Medical School in a well-appointed laboratory, which rather surprisingly was a conversion from a multistory stables of the Great Western Railway.

I was investigating the specificities of lysozymes from various sources and to that end had synthesized a variety of derivatives of N-acetylglucosamine.[1] Nathan Sharon from the Weizmann Institute in Israel was spending a sabbatical in the department as a Royal Society Visiting Professor and was preparing a review on lectins that was subsequently published in *Science*[2] and became a *Citation Classic* in 1982. Since he was one of the earliest lectinologists, he was able to infect us with his enthusiasm, and he suggested that we investigate the effect of inhibitors on wheat-germ agglutinin (WGA), which was already known to bind to N-acetylglucosamine polymers.

It was obvious to me that the published preparations were not yielding a pure protein, so I purified it from wheat germ by conventional ion-exchange and gel chromatography, finding in the process that it consisted of at least three isolectins. WGA was very rich in cystine and therefore very different from the legume lectins concanavalin A and soybean agglutinin, which lacked this amino acid.

Neuberger's department had been devoted to various aspects of sugar chemistry and biochemistry for many years, and there was therefore a very useful range of derivatives and oligosaccharides that could be tested as inhibitors in addition to those that I had already prepared.[1] Initially, we confirmed that the monosaccharide specificity was for N-acetylglucosamine, and then we looked at the action of the methyl ethers of the sugar. Substitution of the hydroxyl groups at C-6 or C-4 gave effective inhibitors, but substitution at C-3 with a methyl group meant that it was a poor inhibitor. From this we concluded that as far as the monosaccharide binding site was concerned, the acetamido at C-2, the orientation of the hydroxyl at C-4, and an unsubstituted hydroxyl at C-3 were necessary.

When we looked at the oligosaccharides as inhibitors, it was apparent that polymers of N-acetylglucosamine were much better inhibitors than the monosaccharide, the trisaccharide being 3,000 times better. It was also apparent that a tetrasaccharide from bacterial cell walls with alternating N-acetylglucosamines would also bind strongly. We then realized that we were looking at a similar situation to that which was known to be the case for the binding site of hen egg-white lysozyme. We therefore proposed that the binding site was a cleft in the molecule rather than a pocket and that there were three or four subsites with differing specificities such that an alternating polymer of N-acetylglucosamine would bind as well as a homopolymer.

Although our paper has been cited for the purification, analysis, and isolectin studies, most of the citations concern the detailed assessment that we made of the specificity of the binding site and to some extent the propositions that we made regarding the binding site.

The lectin has been sequenced and shown to have 171 amino acids in a fourfold partially conserved repeat, and in a series of papers C.S. Wright and coworkers[3] have shown from X-ray crystallographic analysis that each lectin molecule has two clefflike binding sites. A useful review has been written by I.J. Goldstein and R.D. Poretz.[4]

Subsequently, we have all continued with "lectinology"—I think that this is Sharon's fifth *Citation Classic* on lectins. Neuberger and I have worked since on a variety of lectins, but we have made a particular study of a lectin from potato tubers[5] with a similar specificity to WGA. It seems to have a similar binding site, and we have evidence for sequence homology with the site of WGA. However, other domains of the lectin are very different, containing glycosylated hydroxyproline residues.[6]

1. **Allen A K, Davies R C, Neuberger A & Morgan D M L.** The separation of methyl ethers of methyl 2-acetamido-2-deoxy-D-glucopyranosides on a strongly basic ion-exchange resin. *Carbohyd. Res.* 51:149-55, 1976. (Cited 5 times.)
2. **Sharon N & Lis H.** Lectins: cell-agglutinating and sugar-specific proteins. *Science* 177:949-5, 1972. (Cited 1,335 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. Reprinted from: *Current Contents/Life Sciences* 25(21):20, 24 May 1982.]
3. **Wright C S.** Refinement of the crystal structures of wheat-germ agglutinin isolectin 2 at 1.8Å resolution. *J. Mol. Biol.* 195:501-29, 1987. (Cited 15 times.)
4. **Goldstein I J & Poretz R D.** Isolation, physiochemical characterization and carbohydrate-binding specificity of lectins. (Liener I E, Sharon N & Goldstein I J, eds.) *The lectins—properties, functions and applications in biology and medicine.* Orlando, FL: Academic Press, 1986. p. 33-247. (Cited 110 times.)
5. **Allen A K & Neuberger A.** The purification and properties of the lectin from potato tubers—a hydroxyproline-containing glycoprotein. *Biochemical J.* 135:307-14, 1973. (Cited 165 times.)
6. **van Holst G-J, Martin S R, Allen A K, Ashford D, Desai N N & Neuberger A.** Protein conformation of potato (*Solanum tuberosum*) lectin determined by circular dichroism. *Biochemical J.* 233:731-6, 1986. (Cited 5 times.)

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