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JB Reflections and Perspectives

Toshiaki Osawa†: biochemistry of lectins and their applications in immunochemistry and cellular biology

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†Professor Toshiaki Osawa, a prominent scientist who contributed to the establishment of lectinology, passed away on April 1st, 2010. His scientific achievements have had a great deal of influence in the fields of biochemistry, immunology, glycobiology and cell biology.

Lectins are proteins that agglutinate cells and exhibit an antibody like, sugar-binding specificity. Professor Toshiaki Osawa has discovered, purified and characterized many plant lectins that display diverse biological activities. Using lectins as biochemical tools, he developed methods to determine the biochemical structures of glycoprotein glycans that react with lectins; separated and characterized glycoproteins and cell populations; analysed the mechanisms by which lectins activate cells; and characterized several cytokines produced by immune cells stimulated by lectins. The studies on lectins, the field he took strong leadership, developed into an essential hub of the biology of multicellular organisms.

Keywords: lectin/lymphocyte/mitogen/sugar structure/T-cell hybridoma.

From carbohydrate chemistry to lectin biochemistry

Professor Osawa was born on 10 November 1930 in Maebashi, Gunma prefecture. He joined the laboratory of Shichiro Akiya in the Faculty of Pharmaceutical Sciences at the University of Tokyo, where he obtained his B.S. in 1953. Professor Akiya influenced Prof. Osawa's interest in biochemistry and carbohydrate chemistry and, when Prof. Akiya retired and moved to the Faculty of Medicine at Tokyo Medical and Dental University, Dr Osawa also moved to continue his work as a research assistant. After two further years of research, he was awarded his PhD by the University of Tokyo in 1960. From 1962 to 1964, he worked as a research fellow in Prof. Roger W. Jeanloz's laboratory at Massachusetts General Hospital affiliated with Harvard Medical School in Boston. During his time at Prof. Jeanloz's laboratory, he undertook the chemical synthesis of muramic acid and its derivatives, including the disaccharides of *N*-acetylmuramic acid and *N*-acetylglucosamine; work that provided the basis for the structural determination of bacterial cell wall peptideglycans (*1*, *2*). Subsequently, he returned to the School of Medicine at Tokyo Medical and Dental University as an Associate Professor and, in 1967, he joined the Faculty of Pharmaceutical Sciences at the University of Tokyo as an Associated Professor. In 1971, he was appointed as Professor directing the Division of Chemical Toxicology and Immunochemistry. His interests covered a wide variety of research fields including biochemistry, immunochemistry and tumour biology. During his tenure at the University of Tokyo, more than 100 graduate students obtained PhD degrees under his supervision. He also served as Dean to the Graduate School of Pharmaceutical Sciences of the University of Tokyo from 1989 to 1990, as the President of the Japanese Biochemical Society from 1986 to 1987 and as President of the Japanese Society for Carbohydrate Research from 1988 to 1990. After his retirement from the University of Tokyo, he served as Head of the Yakult Central Institute from 1991 to 2001, and President of the Tokyo University of Pharmaceutical Sciences from 2003 to 2007.

Biochemistry of plant lectins

When he first joined Prof. Akiya's laboratory in the Faculty of Pharmaceutical Sciences at the University of Tokyo, Prof. Osawa began the chemical synthesis of amino sugar derivatives (*3*, *4*). He continued to study synthetic carbohydrate chemistry for more than 10 years, during the period he also began his intensive study on plant lectins using three novel approaches: (i) purification of lectin molecules using biochemical techniques; (ii) determination of the sugar structures required for lectin interactions using many synthetic sugar derivatives; and (iii) determination of the distinct native ligand(s) displayed on erythrocytes by measuring the interaction between glycoproteins purified from erythrocytes and plant lectins. The first approach led to the development of affinity chromatography using a sugar-immobilized column for the purification of lectins. Using these techniques, Prof. Osawa's group purified lectins from more than 50 plants and characterized them extensively (*5*–*9*). His knowledge on chemical sugar synthesis proved to be an advantage for the second approach and paved foundations for the idea that oligosaccharide structures were essential for interactions with lectins. Professor Osawa's group identified glycoproteins present on the surfaces of erythrocytes (*10*–*13*), lymphocytes (*14*–*19*) and platelets (*20*–*23*). The knowledge was essential to further

Fig. 1 Professor Toshiaki Osawa.

explore the biological effects of lectins to these cells. Lectins specific for human ABH(O)-type erythrocytes and other blood groups led to nearly the first report on structures of glycoprotein carbohydrate chains on human erythrocytes with blood group epitopes. In early days of lectin studies, lectins were classified into glucose/mannose-binding lectins, galactose/*N*-acetylgalactosamine-binding lectins, fucose-binding lectins, *N*-acetylglucosamine-binding lectins and sialic acid-binding lectins, based on their degree of inhibition by haptenic monosaccharides, so-called Makela's classification (*24*). However, even in the early 1970's, Prof. Osawa's group demonstrated that lectins recognize larger sugar sequences expressed on cell surfaces by showing that lectins interact with a wide variety of oligosaccharides derived from glycoproteins and cells (*11*, *25*). Based on these results, the group initiated and established methods to determine oligosaccharide structures using sequential lectin affinity chromatography with small amounts of tritium-labelled oligosaccharides (*26*). Professor Osawa's innovative ideas and passion have continuously led the field to apply lectins to characterize glycoprotein glycans with medical and biological significance, which became a cutting edge of the current glycomics technology.

Molecular immunology inspired by the property of lectins

Peter Nowell discovered that human peripheral blood lymphocytes undergo mitosis when extracts of *Phaseolus vulgaris* seed were added to the culture (*27*). It was believed at the time that such blastogenesis was induced by the binding of a lectin in the extract. However, little was known on the lymphocyte surface molecules recognized by this lectin, the mechanism of signal transduction leading to mitosis, and cellular and molecular basis of the immune response. As described above, lectins bind surfaces of B cells and T cells, whose phenomenon lead to the identification of B- and T-cell mitogens (*28*, *29*). Lectins specific for B- and T cells transduce signals for cell activation and growth. A variety of B and T cells with distinct antigen specificities are widely stimulated by B- and T-cell mitogens, enabling analysis of the mechanisms responsible for the activation of these cells without the need for specific antigens. Though plant lectins may not be native ligands for lymphocyte receptors, their use opened the door to research on receptor-mediated activation of these cells. Using lectin-activated lymphocytes, Prof. Osawa found that an increase in membrane fluidity triggered the stimulation of the cells (*30–33*), which was intimately associated with the Rho small G-protein and phospholipase A2 (*34–36*).

Lectins bind specifically to various sugar sequences and, based on the different developmental or functional stages, several different oligosaccharide structures are thought to be expressed on the cell surface. By using lectins with different sugar-binding specificities, Prof. Osawa succeeded in separating and enriching several T cell subsets, including helper T cells, killer T cells, natural killer cells and lymphokine-activated killer cells (*37–42*). This separation of lymphocyte subsets improved understanding of the complex nature of the immune system, including cell–cell communication and cytokine networks.

Because the distinct sugar structures displayed on cell surfaces directly correlate with different developmental and functional stages *in vivo*, they are sometimes utilized as targets for a specific function. It is known that activated macrophages, which are responsible for cell-mediated cytotoxicity, selectively interact with cancer cells. Professor Osawa showed that this interaction was dependent upon the sugar structure of the cancer cells, and the receptor for cancer cells, which was named macrophage lectin, was purified and characterized (*43*, *44*). Subsequent studies demonstrated that this receptor was a C-type lectin specific for the oncogenic Tn sugar antigens expressed on gastrointestinal mucins (*45*). The production of specific monoclonal antibodies against macrophage lectin, the cloning of macrophage lectin cDNA, and functional analysis of this lectin have been undertaken by Prof. Irimura's group (*46–48*).

Application of lectins to cancer biology

Professor Osawa also discovered several plant lectins that interact specifically with tumour cells. He was one of the first biochemists to analyse modifications to the sugar chains on the surface of tumour cells using specific lectins. This led to the application of lectin-conjugated toxins in tumour therapy (*49*, *50*).

He also attempted to purify several pro-inflammatory cytokines secreted by lectin-activated lymphocytes for application in cancer therapy (*51*, *52*). To obtain large amounts of these cytokines, he established a separation method for T-cell hybridomas (*53*, *54*) secreting lymphotoxin, macrophage activating factor and macrophage migration inhibitory factor by fusing lectin-activated T cells with myeloma cells (*55–61*), and performed further cDNA cloning encoding these cytokines from these hybridomas (*62*, *63*).

Structural biology of lectins

The molecular structure of lectins was also one of the early interests of Prof. Osawa. During the late 1980s, the structural analysis of lectins, based on protein sequencing and cDNA cloning, rapidly advanced and the relationship between the structure of plant lectins and their sugar-binding specificity was resolved at the amino acid level. He also characterized the amino acid sequences and cDNAs of plant lectins (*64*, *65*). Subsequently, small sugar-binding peptides, which also determine lectin specificity, were identified from various leguminous lectins (*66*, *67*).

Perspectives

Lectins: from tools for biochemical analyses to targets for biological research

Because several plant lectins specifically bind to and aggregate receptors on the surface of erythrocytes, lymphocytes and tumour cells via sugar chains, it is possible to study the receptor-mediated activation of these cells by lectins. As discussed above, the initial purification of receptors carrying specific functions was performed using lectin affinity chromatography, which was a fundamental approach used in biochemistry. Lectins were first identified as proteins that aggregate cells via cell-surface sugar moieties (*68*), and more recent research proposes that lectins are sugar-binding proteins that have no enzymatic activity. Before 1980s, many lectins were purified from plants and widely used as tools for biochemical research. However, the underlining biological functions of lectins in plants were not studied vigorously. The first lectin identified in animals was hepatic lectin, which participates in the clearance of asialoglycoproteins from serum (*69*). The hepatic lectin played a pivotal role in homeostasis of not only plasma proteins but also blood cells, which has awakened many researchers' interest in the function of animal lectins. Other lectins, now classified as C-type lectins and galectins, were successively purified from several mammalian tissues or cells and extensively characterized (*70*). Recently, many kinds of lectins, including M-type, L-type, P-type, I-type, R-type and F-type lectins are identified from prokaryote, fungi, plants, invertebrate and vertebrate. Furthermore, many genes encoding putative lectin domains are also found based on bioinformatic analyses. These indicate that lectin-like molecules are widely distributed in animals and involved in many physiological phenomena, which provided an insight into the diverse lectin function in nature.

There are several key differences between plant and animal lectins. First, animal lectins consist of several domain structures together with a lectin domain. Receptors having a lectin domain usually contain an endocytosis motif, an immunoreceptor tyrosine-based activation motif (ITAM), or an immunoreceptor tyrosine-based inhibitory motif (ITIM), in the cytoplasmic domain. Additional domains may also participate in the regulatory function of lectins and the transduction of lectin receptor-mediated signalling via interaction with other proteins. Second, animal lectins bind sugars weakly compared with plant lectins; the K_a values of plant lectin–sugar interactions range from 10^6 to $10^7\,M^{-1}$, while animal lectin–sugar interactions are around $10^4\,M^{-1}$. Furthermore, the preparation of large amounts of plant lectins is quite easy, whereas animal lectins are not abundantly expressed and cannot be detected without using specific antibodies. Third, animal lectin activity is dramatically affected by certain physiological conditions, such as clustering, association with other proteins, pH and calcium concentration. These regulatory mechanisms are sometimes closely related to the biological role of lectins.

Several problems must be overcome to enable us to study animal lectins. The first is detection of the weak binding ability of lectins to their sugar ligands. This may be achieved by tetramerization of lectin molecules by introducing enzymatic biotinylation tag (*71*). Expression of lectins on cell surface of cultured mammalian cells as membrane-bound chimeric proteins also useful in detecting weak sugar-binding activity (*72*). The second is to identify native extra/intracellular ligands of lectins. This may be achieved by using a variety of native oligosaccharides along with frontal affinity chromatography on a lectin-immobilized column (*73*). Peptide portion also might participate in recognition of ligands for animal lectins. The third is to identify the mechanism responsible for regulating lectin-mediated recognition. Experiments incorporating proteomic techniques, such as mass spectroscopy with matrix-assisted laser desorption ionization (MALDI) and electron spray ionization (ESI), may help to identify small amounts of associated protein. Quantitative analysis of the weak interactions between proteins and sugars using surface plasmon resonance (SPR) or evanescent waves may also provide insights into these regulatory mechanisms (*74*). These approaches enable us to understand biological significance of sugar recognition processes by identification of their native sugar ligands and the regulation mechanisms of sugar-binding activity in cells, tissues or organs.

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Conflict of interest
None declared.

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