Crenomytilus grayanus Lectin, CGL, Could Be Developed into a Potential Biosensor for Cancer Diagnosis

CGL halts the growth of a tumor cell through recognizing that globotriose, Gb3, exists abundantly on a cancer cell surface.

Lectins are proteins that bind carbohydrates and are observable in various species, such as animals, plants and bacteria. As lectins are capable of recognizing the specific functional group of a carbohydrate on a surface of potential pathogens, they play vital roles in the innate immune response. Regarding carbohydrate binding, lectins apply varied ways, including hydrogen bonds, van der Waals forces, hydrophobic interactions and metal coordination, to bind their target sugar moieties.

According to the various combinations of lectin-carbohydrate binding, lectins have been classified into many superfamilies, including galectins, C-type, I-type, P-type, pentraxins and so on.[1] In this report, a novel lectin, from sea mollusk *Crynomytilus grayanus*, was purified and characterized. The *C. grayanus* lectin (CGL) is proposed to belong to the galactose-binding lectin family because of its great galactosamine (GalNAc) and galactose (Gal) specificity.[2] The protein sequence, however, is not similar to that of galectins or other lectins except MytiLec (88% sequence identity). Structure modelling shows that CGL has a common β-trefoil fold that is found in cytokins, agglutinins and actin-cross-linking proteins. From an evolutionary point of view, these proteins have varied ligand-binding preferences and biological functions. To study CGL in detail, a research team led by Shih-Hsiung Wu (Academia Sinica and National Taiwan University) thus solved the structure of CGL in apo form and in a complex with various ligands, including galactose, galacosamine and globotriose, using Taiwanese beamline **SP 12B2** at SPring-8.[3]

Based on the overall structure, CGL has a typical β-trefoil fold consisting of 13 β-strands and three α-helixes. **Figure 1(a)** reveals that CGL can fold into three subdomains, each of which possesses one carbohydrate-binding site. To investigate that the homodimer form does not result from crystal packing, size-exclusion-chromatography multi-angle light scattering (SEC-MALS) and analytical ultracentrifugation analysis with a sedimentation velocity mode (SV-AUC) were performed; both experimental data clearly indicate that CGL has a dimeric form in solution.

To elucidate the ligand-binding mode, the authors subsequently solved the structures of two complexes,

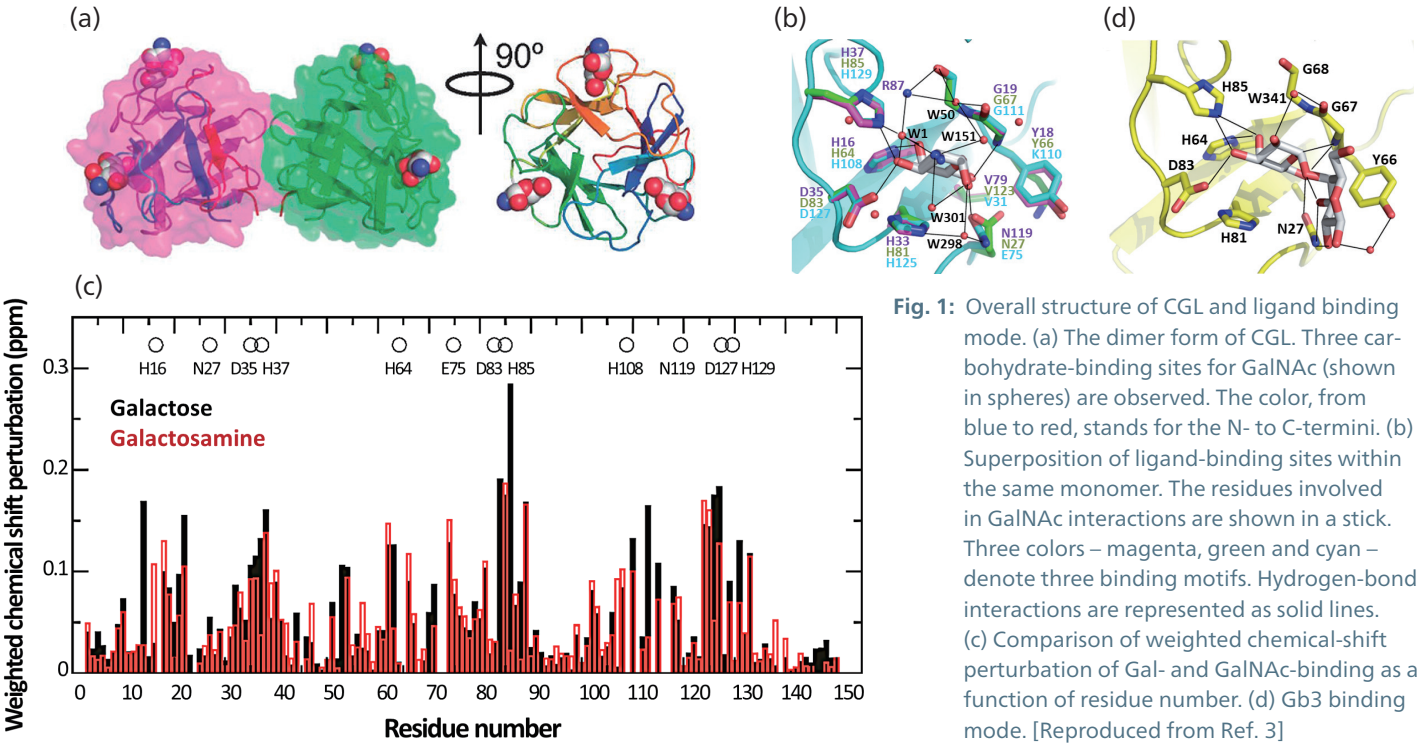

Fig. 1: Overall structure of CGL and ligand binding mode. (a) The dimer form of CGL. Three carbohydrate-binding sites for GalNAc (shown in spheres) are observed. The color, from blue to red, stands for the N- to C-termini. (b) Superposition of ligand-binding sites within the same monomer. The residues involved in GalNAc interactions are shown in a stick. Three colors – magenta, green and cyan – denote three binding motifs. Hydrogen-bond interactions are represented as solid lines. (c) Comparison of weighted chemical-shift perturbation of Gal- and GalNAc-binding as a function of residue number. (d) Gb3 binding mode. [Reproduced from Ref. 3]

CGL-Gal (1.56 Å) and CGL-GalNAc (1.70 Å). Based on the CGL-GalNAc structure, three GalNAc-binding sites were discovered in each CGL monomer; their superposition within the same monomer shows that all these sites utilize almost the same amino acids to bind ligands. Several water molecules are involved in the water-mediated ligand interaction (**Fig. 1(b)**). Similar binding modes were found in the CGL-Gal structure. To elucidate the carbohydrate-binding events, a NMR titration experiment was conducted. Consistent with the crystallographic conditions, the concentration between ligands (Gal and GalNAc) and CGL was 5 mM versus 0.1 mM. On the basis of the chemical-shift perturbation result, two conclusions were that the residues involved in ligand binding were observed to undergo significant motion, and that the overall binding modes and binding sites of both carbohydrates are the same because of the similar patterns of chemical shifts (**Fig. 1(c)**). On comparison of NMR and crystallographic data, these findings are mutually consistent.

According to previous work, MytiLec recognizes an important sugar, globotriose (Gb3), that exists abundantly on the surface of a cancer cell. Interestingly, CGL shares 88% sequence identity with MytiLec, which means that CGL might have the ability to bind the Gb3. The high-resolution CGL-Gb3 structure proves the hypothesis: the binding mode is primarily through the terminal galactose moiety (**Fig. 1(d)**). The authors subsequently selected MCF7, the breast cancer cell line with abundant Gb3 on the cell surface, to test whether CGL could inhibit the cell viability of MCF7. A detachment of MCF7 cells was certainly observed upon addition of CGL (**Figs. 2(a) and 2(b)**). A 3D fluorescence-imaging reconstruction with fluorescently labelled CGL shows that CGL almost bound on the surface of MCF7 cells (**Figs. 2(c)-2(e)**). A cytotoxicity assay indicated that CGL has a dose-dependent anti-cancer activity (*e.g.* 33% cell death with 200 μg/mL CGL) (**Fig. 2(f)**). At this point, a lectin-based biosensor could be developed for cancer diagnosis. (Reported by Chun-Hsiang Huang)

This report features the work of Shih-Hsiung Wu and his co-workers published in J. Am. Chem. Soc. ***138****, 4787 (2016).*

SP 12B2 BM – Protein X-ray Crystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Sciences

| References |

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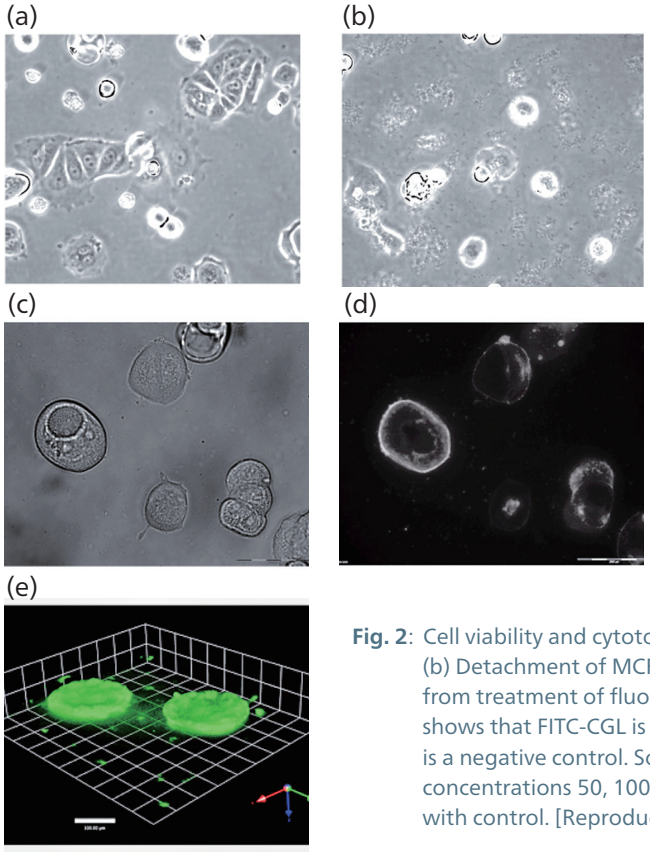

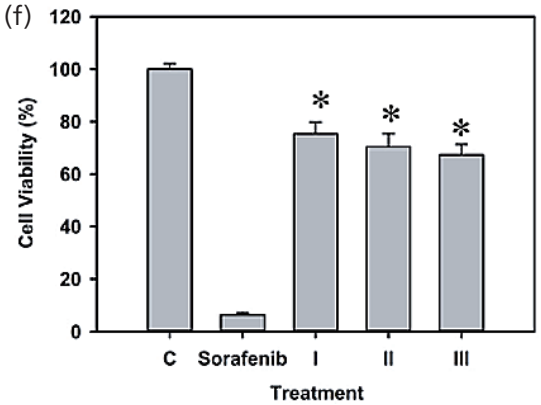

Fig. 2: Cell viability and cytotoxicity assays. (a) MCF7 adhere on the plate without treatment of CGL. (b) Detachment of MCF7 after CGL adding. (c) Light microscopy image of MCF7 is produced from treatment of fluorescein isothiocyanate (FITC)-CGL. (d)-(e) Fluorescence image of MCF7 shows that FITC-CGL is distributed primarily on the surface of MCF7. (f) Cell viability assays. C is a negative control. Sorafenib, an anti-cancer drug, is a positive control. I, II and III indicate concentrations 50, 100 and 200 μg/mL, respectively, to treat CGL. *$P < 0.05$ for comparison with control. [Reproduced from Ref. 3]