Available online at www.sciencedirect.com

ScienceDirect

Biochimie Open 6 (2018) 17–23

http://www.journals.elsevier.com/biochimie-open

Research paper

Glycan binding profile of a fucolectin-related protein (FRP) encoded by the SP2159 gene of *Streptococcus pneumoniae*

Albert M. Wu [a,*], Tanuja Singh [a], Yung Liang Chen [b], Kimberly M. Anderson [c], Su Chen Li [c], Yu Teh Li [c]

[a] *Glyco-Immunochemistry Research Laboratory, Institute of Biomedical Sciences, College of Medicine, Chang-Gung University, Kwei-san, Tao-yuan, 333, Taiwan*
[b] *Graduate Institute of Medical Laboratory Science and Biotechnology, Yuanpei University of Medical Technology, Hsinchu City 300, Taiwan*
[c] *Department of Biochemistry and Molecular Biology, Tulane University, School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112, USA*

Received 27 August 2017; accepted 17 December 2017
Available online 23 December 2017

Abstract

The recombinant fucolectin-related protein (FRP) of unknown function, encoded by the SP2159 gene of *Streptococcus pneumoniae,* was expressed in *E. coli*. In this study, its glycan-recognition epitopes and their binding potencies were examined by enzyme-linked lectinosorbent and inhibition assays. The results indicate that FRP reacted strongly with human blood group **ABH** and L-Fucα1→2-active glycotopes and in their polyvalent (super) forms. When expressed by mass relative potency, the binding affinities of FRP to poly-L-Fucα1→glycotopes were about 5.0×10^5 folds higher than that of the mono-L-Fucα1→glycotope form. This unique binding property of FRP can be used as a special tool to differentiate complex forms of L-Fucα1→2 and other forms of glycotopes.

Keywords: Fucolectin-related protein; Glycotopes; Polyvalency; Glycan recognition epitopes

1. Introduction

Streptococcus pneumoniae commonly found in human nose and throat is the major organism to cause pneumonia, septicemia, meningitis and otitis media [1]. These diseases result in millions of deaths worldwide each year. Virulence of *S. pneumoniae* is associated with capsular polysaccharides, cell wall, and/or membrane components. For example, the encapsulated smooth strain of *S. pneumoniae* has been shown to kill mice in DNA transformation studies that resulted in the discovery of hereditary material [2]. *S. pneumoniae* is known to produce a hemolysin that causes incomplete hemolysis (α-hemolysis) and to express bacterial surface lectins that facilitate adhesion to epithelial cells of the respiratory tract [3].

Through our studies of the blood group A and B-cleaving endo-β-galactosidase (E-ABase) of *Clostridium perfringens,* we have shown that E-ABase shares 34% sequence identity with a fucolectin-related protein (FRP) of unknown function encoded by the SP2159 gene of *S. pneumoniae* ATCC BAA-334 [4,5]. The recombinant FRP encoded by SP2159 gene and expressed in *E. coli* contains 1038 residues of amino acids with a molecular mass of 117.74 kDa [4]. In order to decipher the biological function of this FRP, its binding profile was analyzed using our established enzyme-linked lectinosorbent assay (ELLSA) and inhibition assay [6] using a series of mammalian monomeric glycotopes and their natural polyvalent forms. Our results showed that: (i) FRP bound avidly with human blood group **A**, **B**, **H,** and L-Fucα1-active glycans; (ii) L-Fucα1→2 related glycotopes and their polyvalent (super) forms are the two essential recognition elements. The high density of L-Fucα1→2 related glycotopes, expressed as mass relative potency, were about 5.0×10^5 folds more potent

* Corresponding author. Glyco-Immunochemistry Research Laboratory, Institute of Biomedical sciences, College of Medicine, Chang-Gung University, No.259, Wenhua 1st Rd., Guishan Dist., Taoyuan City, 33302, Taiwan.
E-mail address: amwu@mail.cgu.edu.tw (A.M. Wu).

https://doi.org/10.1016/j.biopen.2017.12.002

than their monomeric L-Fucα1→ glycotope; (iii) the recognition site of FRP should be a combination of a cavity shape as the major site to hold the most active L-Fucα1→2 residue and a broad groove shape as subsite for adopting the other part of the glycotope for enhancement, such as L-Fucα-1→2Galβ1→4(L-Fucα1→3)Glc glycotope (**LDFT**); (iv) hydrophobicity is important for both the α− and β− anomers of L-Fuc-complex; (v) by comparing the FRP lectin with the other two L-Fucα1→2 specific lectins, *Pseudomonus aeruginosa*-II Lectin (PA-IIL) [7] and *Anguilla anguilla* agglutinin (AAA) [8], the unique potency of FRP recognition factors are defined. In this report, we have upgraded the classical concept of lectin-glycan interactions from the mono- or oligo-glycotope level to a complex polyvalent (super)-glycotopes level. The unique binding property of FRP can be used as a special tool to distinguish the complex forms of L-Fucα1→2 and other forms of glycotopes.

2. Materials and methods

2.1. Lectin preparation and biotinylation

Isolation of the *Streptococcus pneumoniae* SP2159 gene encoding FRP and the expression of FRP in *E. coli* were based on the procedures described previously [4]. Biotinylation of FRP was carried out according to the method of *Duk* et al. [6].

2.2. Super glycotopes containing glycoproteins and polysaccharide

ABH and L-Fucα1→2 blood group active glycoproteins and the sialic acid-containing glycoproteins were prepared from human ovarian cyst fluid [9–13]. The glycan chains of the cyst gps consist of multiple bioactive saccharide branches attached via *O*-glycosidic linkages at their reducing ends to serine or threonine residues of the polypeptide backbone [11,12].

Blood group precursor gps (P-1) from cyst gps were prepared by mild acid hydrolysis at pH 1.5, 100 °C for 2 h and/or by Smith degradation to remove most of the blood group key sugars (L-Fuc-α-1→2, GalNAc-α-1→3 and Gal-α-1→3) [14–17].

Mammalian salivary glycoproteins from ovine (OSM), bovine (BSM), porcine (PSM) submandibular/glands and rat sublingual gland (RSL), were purified according to the method of Tettamanti and Pigman [18] and Herp et al. [19]. Their asialo products were prepared by mild acid hydrolysis at pH 2.0, 80 °C for 90 min and dialyzed against water to remove the released sialic acid.

Glycophorin and asialo-glycophorin were provided by Duk et al. [6]. The **Tn**-type glycophorin (GalNAc-α-1-Ser/Thr) was prepared from asialo-glycophorin by periodate oxidation and mild acid hydrolysis (Smith degradation) [6].

Pneumococcus type XIV polysaccharide was a gift from the late B. Lindberg [20] through the late E. A. Kabat [14]. The Human α_1-acid gp, poly-2,8-*N*-acetylneuraminic acid capsular polysaccharide from *Escherichia coli* and mannan from *Saccharomyces cerevisiae* were purchased from Sigma (St. Louis, MO, USA).

Fig. 1 shows several FRP active glycotopes of blood active glycoproteins prepared from human ovarian cyst fluid.

2.3. Monosaccharides and oligosaccharides

Monosaccharides and oligosaccharides were purchased from Sigma Chemical Company (St. Louis, MO, USA) and Dextra (Berkshire, UK).

2.4. Lectin-enzyme binding assay on microtiter plate

The lectin-enzyme binding assay was performed according to the procedures of Duk et al. [6]. The volume of each reagent applied to the plate was 50 μl/well, and all incubations were performed at room temperature (20 °C) except for coating at 4 °C. The reagents, if not indicated otherwise, were diluted with Tris-buffered saline (TBS, 0.05 M Tris, 0.15 M NaCl, pH 7.35) in 0.05% Tween 20 (TBS-T buffer). The TBS-T buffer was also used for washing the plate between changing the reagents. For examining the effect of Ca^{+2}, Mg^{+2} and EDTA on the FRP binding, 2 mM of Ca^{+2}, Mg^{+2} and EDTA were added to the 1st testing well and followed by sequential twofold dilution. The well without adding Ca^{+2}, Mg^{+2} or EDTA served as a control.

For inhibition studies, the twofold serially diluted inhibitor was mixed with an equal volume of solutions containing a fixed amount of FRP. The reference lectin inhibitor was also diluted in the same manner. After incubation at room temperature for 1 h, the binding of FRP to the plate coated with an ovarian cyst gp that contained blood group **H** L-Fuc-α-1→2Gal and L-Fuc-α-1→3/4GlcNAc glycotopes was determined as described above [6]. The inhibitory activity was estimated from the inhibition curve and is expressed as the amount of inhibitor (ng/well for glycoprotein or nmole/well for small saccharides) giving 50% inhibition of the FRP binding as compared to that without the inhibitor.

3. Results

3.1. FRP-glycan interaction

The binding intensities of FRP with various glycans, according to the interaction profiles obtained, are shown in Fig. 2. Among the glycoproteins tested, FRP reacted best with all human blood group **A**, **B**, **H** L-Fucα1→2-active glycoproteins. Less than 0.6 ng each of Ovarian Cyst Medon, Ovarian Cyst Beach Phenol Insoluble, or Ovarian Cyst JS Phenol Insoluble was required to interact with 10 ng of FRP lectin to yield an absorbance (A_{405}) value of 1.5 within 4 h. While Ovalian Cyst Medon and Ovarian Cyst JS 1st Smith Degraded were not binding FRP. Other blood group precursor glycoproteins and polysaccharides exhibited very weak inhibition and gave A_{405} values less than 0.2. These weak inhibitors were both the sialyl- and asialyl-glycoproteins of HOC 350, human α_1-acid glycoprotein, human glycophorin, OSM, PSM and asialofetuin. To prove that the different affinities between FRP and glycoproteins were not due to the

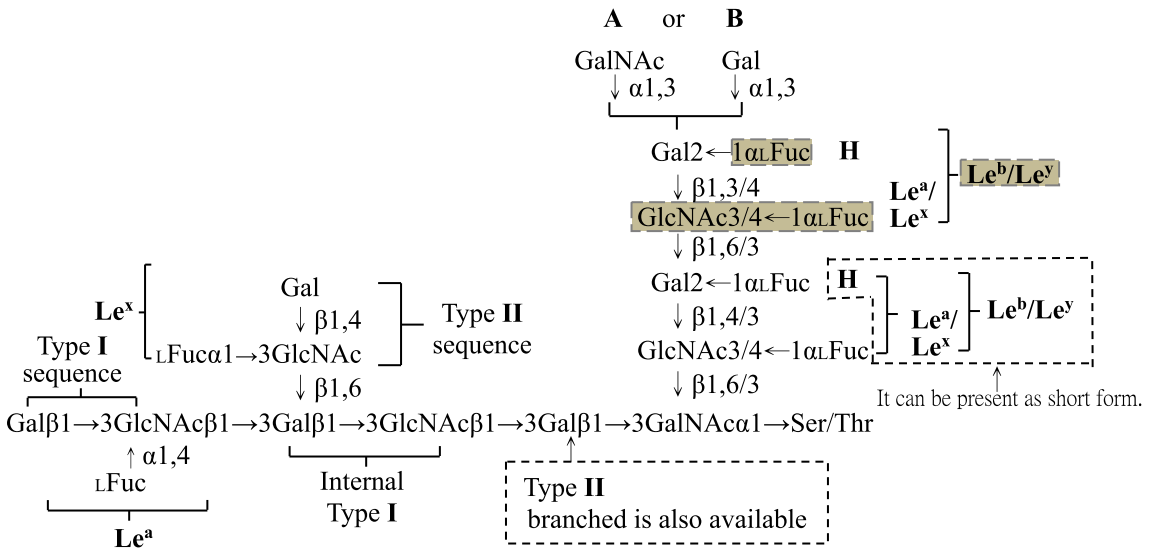

Fig. 1. **Proposed FRP active glycotopes(shaded) of blood active glycoproteins prepared from human ovarian cyst fluid** [11. 12]. As shown in Fig. 2 and 3, CystJS phenol insoluble (H, Le^b/Le^y) Cyst Mcdon (A_h and Le^y), Cyst 14 phenol insoluble (A_h/Le^y) and Cyst Beach phenol insoluble (B_h/Le^y) are rich in glycotopes (shaded) for FRP binding. The "$_h$" in $\mathbf{A}_h$ and $\mathbf{B}_h$ indicating in active crypto H(Lfucα1→2).

variation in the plate adsorption, the binding intensity of each glycoprotein was further examined by an inhibitory assay described in Section 3.2.

3.2. Inhibition of FRP-glycan binding by various polyvalent glycotopes (super glycotopes or glycoproteins)

Fig. 3 shows the inhibitory potencies of various inhibitor glycoproteins toward the binding between FRP and Ovarian Cyst JS Phenol Insoluble glycoprotein that contained human blood group **H** L-Fucα1→2Gal and L-Fucα1→3/4GalNAc-active glycotopes. Among the inhibitor glycoproteins tested, the Cyst JS Phenol Insoluble glycoprotein was the most potent inhibitor. It was 5.0×10^5 times more active than L-Fuc monosaccharide and 6.3×10^5 more active than the inhibition by **H** L-Fucα1→2Gal disaccharide. The FRP-glycan interaction was also strongly inhibited by Cyst 14 Phenol Insoluble that contained GlcNAcα1→3(L-Fucα1→2)-Gal and Cyst Beach Phenol Insoluble that contained Galα1→3(L-Fucα1→2)-Gal. All other glycans, such as hog gastric mucins and asialo HOC 350, tested in the amount from 278 ng to 5.6 × 103 ng, showed only about 10^3-fold weak inhibitory activity and did not reach 50% inhibition. These results indicated that, in mammalian glycans, a high density of the polyvalent form of L-Fucα1→, as well as human blood group **H** L-Fuc-α-1→2Gal glycotope, is the determinant for effective binding by FRP.

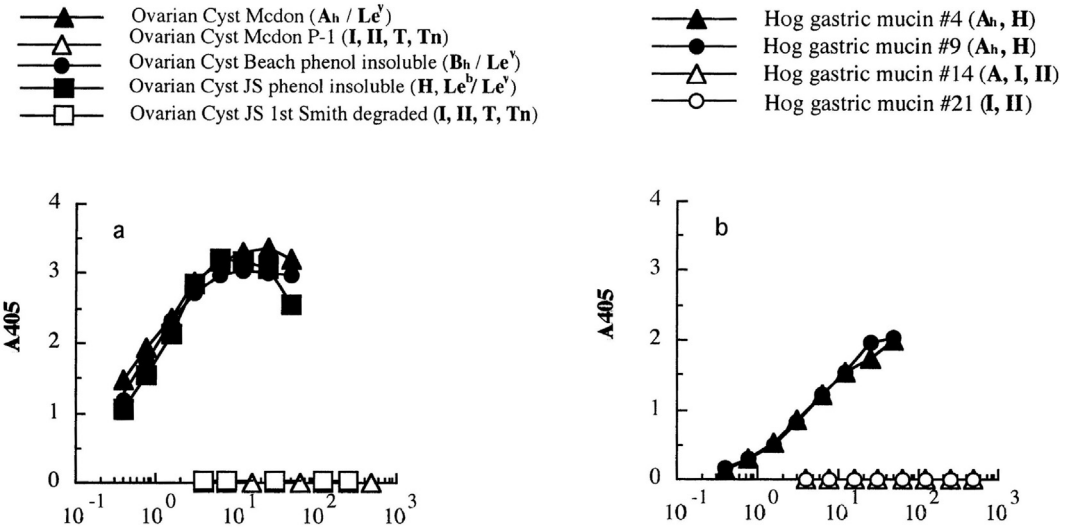

Fig. 2. **The ELLSA of FRP binding affinities with various glycoproteins**. The notations in the parentheses indicate the active glycotopes that are present in the glycans of the glycoproteins. Their glycotope structures are shown in Fig. 1. Ten μg of the glycoprotein under the test was used per well in a total volume of 50 μl. The detailed method is described in Method Section 2.4.

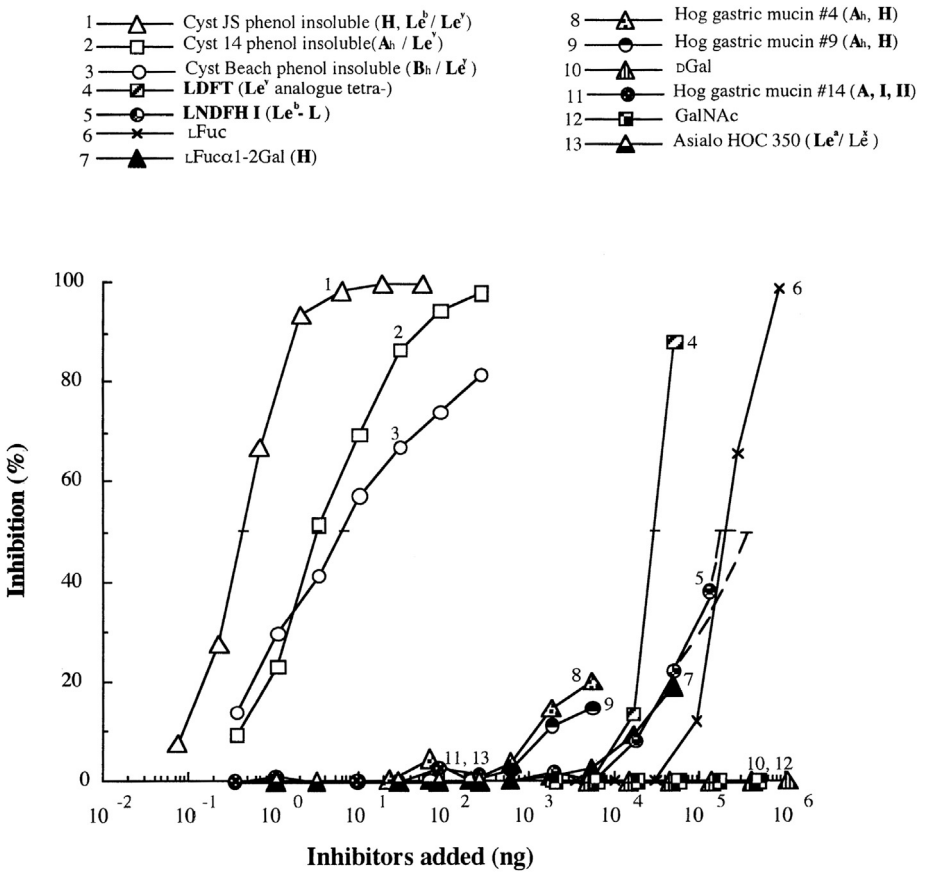

Fig. 3. **The inhibitory activities of various polyvalent glycotopes toward the binding of FRP to human blood group Fucα1→active ovarian cyst JS phenol insoluble glycoproteins.** The notations in the parentheses indicate the blood group active glycotopes that are present in the glycoproteins. The structure feature of each notation is shown in Fig. 1. Cyst Tighe phenol insoluble (10 μg) was coated on each well. The inhibitor under the test was twofold serially diluted and mixed with a fixed amount of FRP in the solution. The detailed method is in Method Section 2.4.

3.3. Inhibition of FRP-glycan binding by mono- and oligosaccharides

In order to further probe glycotopes in FRP molecule, various known mammalian structural units and monosaccharide derivatives were used to test the inhibition toward the binding of FRP and the human blood group **H** L-Fucα1→2Gal- and L-Fucα1→ 3/4GlcNAc-active Ovarian Cyst JS Phenol Insoluble glycoprotein. Table 1 lists the inhibitory potencies as Molar Relative Potencies for various simple glycans.

Among the monosaccharides and their derivatives, *p*-nitrophenyl-β-L-Fuc (Table 1 Sample 1) was the most potent inhibitor, which was twice more active than *p*-nitrophenyl-α-L-Fuc (Sample 2) and 69.1 times more active than the free L-Fuc (Sample 8). The β-anomer of L-Fuc was preferred over

Table 1
The inhibitory potencies of oligo saccharides and monosaccharides toward the binding of FRP and Ovarian Cyst JS Phenol Insoluble glycoprotein.

Sample No.	Sample name	Molar Relative Potency	
		$_{L}$Fuc as 1.0	$_{L}$Fucα1→2Gal as 1.0
1	p-Nitrophenyl-β-$_{L}$Fuc	69.1	91.1
2	p-Nitrophenyl-α-$_{L}$Fuc	34.5	45.6
3	Methyl-β-$_{L}$Fuc	21.9	29.2
4	$_{L}$Fucα1→2Galβ1→4[$_{L}$Fucα1→3]Glc (**LDFT, Le^{y}**)	5.7	8.2
5	Methyl-α-$_{L}$Fuc	4.4	5.8
6	$_{L}$Gal	4.4	5.8
7	$_{L}$Fucα1→2Galβ1→3[$_{L}$Fucα1→4]GlcNAcβ1→3Gal β1→4Glc (**LNDFH I, Le^{b}-L**)	1.8	2.6
8	$_{L}$Fuc	1.0	1.3
9	$_{L}$Fucα1→2Gal (**H**)	0.7	1.0
10	$_{D}$Ara	0.09	0.12
11	$_{D}$GalNAc	0	0

the α-anomer in both *p*-nitrophenyl and methyl glycosides (Samples 1 versus 2; 3 versus 5). Moreover, both α- and β-derivative of p-nitrophenyl-L-Fuc were more active than the methyl-α- and β-analogue (Samples 1 and 2 versus 3 and 5). This indicated the presence of probable apolar nature of FRP protein around the vicinity of the saccharide-binding site.

Among the oligosaccharides tested, the glucose containing oligosaccharide L-Fucα1→2-Galβ-1→4(L-Fucα1→3) Glc (Sample 4 **LDFT**) was the best inhibitor. It was about three times as active as Fucα1→2Galβ1→3(Fucα1→4) GlcNAcβ1→ 3Galβ1→4Glc] (Sample 7. **LNDFH I)** and 8 times more active than **H** disaccharide (L-Fucα1→2Gal) (Sample 9).

With the same pentose structure, D-Ara (Sample 10) was 12.5 times less potent than L-Fuc. No inhibition of FRP binding was observed with the following monosaccharides: D-Man (2.7×10^4 nmol), L-Rhamnose (1.5×10^4 nmol), D-Fuc (2.3×10^4 nmol), D-Gal (2.2×10^4 nmol), methyl α-L-Rhamnoside (1.4×10^4 nmol), D-Glc (2.1×10^4 nmol), and D-GalNAc (7.4×10^3 nmol).

3.4. Effect of Ca^{2+}, Mg^{2+} and EDTA on the reactivity of FRP in the binding assay

Metal ions such as 2 mM of Ca^{2+} or Mg^{2+} or ion chelator with 2 mM EDTA in a final volume of 50 μl Tris-buffered saline containing 10 ng FRP did not affect the binding activity of FRP-glycan interaction. This indicates that the lectin does not require metal ions for binding.

4. Discussion

Prior to encounter with FRP, we had cloned and studied an endo-β-galactosidase, E-ABase from *Cl. Perfringens* [4], that could efficiently cleave off the terminal trisaccharide GlcNAcα1-3[Fucα1-2]-Gal from human blood group A glycoproteins and Galα1-3[Fucα1-2]-Gal from group B glycoproteins. The FRP encoded by *S. pneumoniae* SP2159 gene called our attention because, after our survey of the gene bank, SP2159 gene showed a 34% identity with the gene of E-ABase. While FRP was listed as "function unknown", we suspected that FRP might be a related endo-β-galactosidase of *S. pneumoniae*. However, we found that the recombinant FRP did not have any glycosidase activity. Our current analysis on the glycan binding activity of FRP clearly showed that FRP is a L-Fucα1-recognizing lectin that has high affinity toward human blood group **A**, **B**, and **H** determinants and cell surface glycoproteins. This specific affinity of FRP toward numerous cell surface glycans may provide an effective tool for *S. pneumoniae* to adhere onto the cell surfaces and gain entrances to infect human and other animals.

Over the past few decades, fucose-binding lectins in various living organisms from plants (UEA-I of *Ulex europaeu*) [21], animals (*Anguilla anguilla* agglutinin from eel) [8,22], to micro-organisms *Pseudomonas aeruginosa* PA-IIL [7,23] and *Aleuria aurantia* AAL [24], have been reported. Owing to their L-Fucα1→ specificity, these lectins are widely used as reagents in hematological and glyco-biological studies [25–28]. The investigations on human blood group determinants [9–11] showed that L-Fucα1-glycotopes in human blood group chemistry are very complex. Factors that can affect a specific blood group determinant to exhibit different binding potencies toward specific lectins are: (i) The density of the L-Fucα1→glycotopes in the saccharide moiety; (ii) the location of the L-Fucα1→glycotopes, whether it is at the terminal or at the penultimate location of the saccharide chain; and (iii) the L-Fucα1→glycotopes linking to the next saccharide, such as Gal, Glc or GlcNAc. In order to be used as a tool for biomedical and immunochemical studies, their recognition patterns should be established. There has been no report to compare the reactivity of all known lectins to bind various glycoproteins or oligosaccharides under a set of standard condition. In this study, we analyzed the binding potencies of FRP toward a collection of glycoproteins, oligosaccharides and mono-saccharides under the same conditions as that performed for assaying Anguilla anguilla agglutinin (AAA) [8]. Therefore, we can make a direct comparison of the lectin potencies between FRP and AAA. Although both FRP and AAA lectins recognize L-Fucα1→2Gal-glycotopes, AAA had the highest potency toward the binding of hog gastric mucin #4 and #9 glycoproteins [8] and FRP had the highest affinity toward the binding of ovarian cyst glycoproteins as shown in Fig. 2 (See Fig. 1 for the structures of **A/Le^y, B/Le^y, and A**). Unlike the ovarian cyst glycoproteins, the hog gastric mucins #4 and #9 showed much weaker binding affinity to FRP (Fig. 2). These results suggest that FRP can recognize and interact much stronger than AAA toward the polyvalent form of L-Fucα1→2Gal-glycotopes. This property of FRP can be used as a special reagent to detect the presence of the polyvalent glycotopes.

Among the oligosaccharide ligands tested for the inhibition of the binding between FRP and ovarian cyst phenol insoluble glycoprotein, the gluco-analogue of the **LDFT, Le^y** hapten [Fucα1→2Galβ1→4(Fucα1→3) Glc] (Table 1, Sample 4) was the best inhibitor. This oligo-saccharide having poly L-Fuc-α→2-Gal glycotope exhibited 5.7 times higher inhibitory potency (molar relative potency) than the monosaccharide L-Fuc (Table 1, Sample 8) and 8 times higher inhibitory potency than the monomeric L-Fucα1→glycotope (Table 1, Sample 9). These results suggest that FRP from *S. pneumoniae* efficiently interacted with and bound to the polyvalent L-Fucα1→glycotopes on the saccharide moieties of mammalian cell surface glycoproteins.

The binding profile of FRP is similar to those of two other L-Fuc specific lectins, *Anguilla anguilla* agglutinin (AAA) [8,19] and *Pseudomonas aeruginosa*-II Lectin (PA-IIL) [7], but the roles of polyvalent enhancement to these lectins are very different [7,8,28]. FRP resembles AAA and PA-IIL in its affinity toward L-Fucα1→ related blood group active glycoproteins. However, FRP showed unique features that are different from PA-IIL and AAA lectins: (i) The affinity enhancement by polyvalent glycotopes for FRP can be up to 5.0×10^5 times over the monomeric L-Fucα1→ 2Gal, while that for PA-IIL [7] and AAA [8] showed only 4 and 1.2×10^2 times over

monomeric L-Fucα-1→2Gal, respectively; (ii) The PA-IIL had an affinity to high density D-Man (Mannan) [7] and demonstrated a dual carbohydrate-recognition system, while FRP showed no interaction with any of the non-L-Fucα1→ containing glycoproteins (**II**, **T/Tn** mammalian structures and *N*-glycans such as Mannan) [7]. Although the FRP has been listed as a protein of "unknown function", it displayed a very effective binding activity toward the polyvalent L-Fucα1→glycotopes.

During the last decade, the L-Fucα1→specific lectins, also called F-type lectins, have been studied from various aspects: The crystal complex of AAA with fucose showed that L-Fucα1→ recognition site formed specific structure "fold" that provided the structural determinants of oligosaccharide specificity involving in innate immunity [29]; identification and bioinformatics analysis of the fucose gene cluster of *S. pneumoniae*, strains 100993, R6 and type 4 for their promoter systems in DNA [30]; the structural analysis of blood group antigen recognition by *S. pneumoniae* TIGR4 [31]; the fucose lectin domain analysis of *S. mitis* 3LEI and *S. pneumoniae* 2J1S [32]; and the affinities for blood group antigens among microbial F-type lectins from *Cyanobium* sp. PCC 7001, *Myxococcus hansupus* and *Leucothrix mucor* [33]. Although the bacterium *S. pneumoniae* ATCC BAA-334 that we used in our study was not a member in the above cited studies, the general observations about the F-type lectins are: (i) The structural fold of F-type lectins from many organisms are similar to AAA. For example, two proteins of *S. pneumoniae* TIGR4 (Entrez entries SP2159 and NP346573) have domains that are homologous to AAA. In addition, *S. pneumoniae* Entrez entry NP 346573 has a tandem of three AAA-like recognition domains [29]; (ii) the genomes organization of the fucose operon is conserved in all three publicly available *S. pneumoniae* genome sequences of strains 100993, R6 and type 4 [30]; (iii) The *S. pneumoniae* fucose utilization operon includes a gene of SpGH98 glycohydrolase family. One of the three fucose recognition module is likely to be associated with the role of SpGH98 glycohydrolase catalytic activity. The functions of the other two tandem fucose binding modules are still unknown [31]; and (iv) the bacterial fucose recognition domains generally presented as a single copy, with the exception of three sequence clusters in *S. mitis*, *S. pnemoniae* and *Myxococcus* sp. [32]. Since the genome organization of the same species of organism are largely conserved except some minor variations, we can expect the fucose recognition domains of *S. pneumoniae* ATCC BAA-334, that we used, would be similar to the *S. pneumoniae* NP346573 whose gene fold had been tested. However, *S. pneumoniae* is unable to survive in L- or D-fucose as the sole carbon source in a semi-refined medium, the functions of fucose recognition domains of *S. pneumoniae* remain to be elucidated [30].

Acknowledgements

This work was supported by Grants from the Chang-Gung Medical Research Project (CMRP No. 180482 and 170443), Kwei-san, Tao-yuan, Taiwan, the National Science Council (NSC 100-2320-B-182-027 and 100-2320-B-182-028), Taipei, Taiwan.

References

[1] O.T. Avery, C.M. MacLeod, M. McCarty, Studies on the chemical nature of the substance inducing transformation of pneumococcal types, J. Exp. Med. 98 (1944) 451–460.
[2] S. Alvarez, J. Guarderas, C.G. Shell, S. Holtsclaw-Berk, S.L. Berk, Nosocomial pneumococcal bacteremia, Arch of Int Med 146 (1986) 1509–1512.
[3] M. Shani-Sekler, S. Lifshitz, I. Hillel, R. Dagan, N. Grossman, F. Fleminger, Y. Mizrachi-Brauner, Initial steps in *Streptococcus pneumoniae* interaction with and pathogenicity to the host, Adv. Exp. Med. Biol. 479 (2000) 61–71.
[4] K.M. Anderson, H. Ashida, K. Maskos, A. Dell, S.C. Li, Y.T. Li, A clostridial endo-β-galactosidase that cleaves both blood group A and B glycotopes: the first member of a new glycoside hydrolase family GH98, J. Biol. Chem. 280 (2005) 7720–7728.
[5] H. Tettelin, et al., Complete genome sequence of a virulent isolate of streptococcus pneumoniae, Science 293 (2001) 498–506.
[6] M. Duk, E. Lisowska, J.H. Wu, A.M. Wu, The biotin/avidin-mediated microtiter plate lectin assay with the use of chemically modified glycoprotein ligand, Anal. Biochem. 221 (1994) 266–272.
[7] A.M. Wu, Y.P. Gong, C.C. Li, N. Gilboa-Garber, Duality of the carbohydrate-recognition system of *Pseudomonas aeruginosa*-II lectin (PA-IIL), FEBS Lett. 584 (2010) 2371–2375.
[8] A.M. Wu, J.H. Wu, T. Singh, J.H. Liu, A. Herp, Lectinochemical studies on the affinity of *Anguilla anguilla* agglutinin for mammalian glycotopes, Life Sci. 75 (2004) 1085–1103.
[9] E.A. Kabat, Blood Group Substances: Their Chemistry and Immunochemistry, Academic Press, New York, 1956, pp. 135–139.
[10] K.O. Lloyd, E.A. Kabat, Immunochemical studies on blood groups. 41. Proposed structures for the carbohydrate portion of A, B, H, Lewis^{a} and Lewis^{b} substances, Proc Natl Acad Sci USA 61 (1968) 1470–1477.
[11] A.M. Wu, Structural concepts of the blood group A, B, H, Le^{a}, Le^{b}, I and i active glycoproteins purified from human ovarian cyst fluid, Adv. Exp. Med. Biol. 228 (1988) 351–392.
[12] A.M. Wu, Human blood group ABH/Ii, Le$^{a,\ b,\ x,\ y}$ and sialyl Le$^{a,\ x}$ glycotopes, internal structures and immunochemical roles of human ovarian cyst glycoproteins. (HOC), Adv. Exp. Med. Biol. 705 (2011) 33–51.
[13] A. Pusztai, W.T.J. Morgan, Studies in immunochemistry. 18. the isolation and properties of a sialomucopolysaccharide possessing blood-group Le^{a} specificity and virus-receptor activity, Biochem. J. 78 (1961) 135–146.
[14] P.Z. Allen, E.A. Kabat, Immunochemical studies on blood groups XXII. immunochemical studies on the nondialyzable residue from partially hydrolyzed blood group A, B, O (H) substances (P_1 fraction), J. Immunol. 82 (1959) 340–357.
[15] E.A. Kabat, H. Baer, A.E. Bezer, V. Knaub, Immunochemical studies on blood groups. chemical changes associated with destruction of blood group activity and enhancement of the type XIV cross-reactivity by partial hydrolysis of hog and human blood group A, B and O substances, J. Exp. Med. 88 (1948) 43–57.
[16] S. Leskowitz, E.A. Kabat, Immunochemical studies on blood groups. XV. the effect of mild acid hydrolysis on the glucosamine and galactosamine in blood group substances, J. Am. Chem. Soc. 76 (1954) 5060–5065.
[17] A.M. Wu, E.A. Kabat, B. Nilsson, D.A. Zopf, F.G. Gruezo, J. Liao, Immunochemical studies on blood groups. purification and characterization of radioactive ^{3}H-reduced di- to hexasaccharides produced by alkaline beta-elimination- borohydride ^{3}H reduction of Smith degraded blood group a active glycoproteins, J. Biol. Chem. 259 (1984) 7178–7186.
[18] G. Tettamanti, W. Pigman, Purification and characterization of bovine and ovine submaxillary mucins, Arch. Biochem. Biophys. 124 (1968) 41–50.
[19] A. Herp, C. Borelli, A.M. Wu, Biochemistry and lectin binding properties of mammalian salivary mucous glycoproteins, Adv. Exp. Med. Biol. 228 (1988) 395–435.
[20] B. Lindberg, J. Lonngren, D.A. Powell, Structural studies on the specific type-14 pneumococcal polysaccharide, Carbohyd Res 58 (1977) 177–186.

[21] V. Horejsi, Properties of *Ulex europaeus* II lectin isolated by affinity chromatography, Biochim. Biophys. Acta 577 (1979) 389–393.
[22] W.M. Watkins, W.T.J. Morgan, Neutralisation of the anti-H agglutinin in eel serum by simple sugars, Nature 169 (1952) 825–826.
[23] N. Gilboa-Garber, *Pseudomonas aeruginosa* lectins, Meth. Enzy. 83 (1982) 378–385.
[24] N. Kochibe, K. Furukawa, Purification and properties of a novel fucose-specific hemagglutinin of aleuria aurantia, Biochem 19 (1980) 2841–2846.
[25] W.J. Judd, The role of lectins in blood group serology, Crit. Rev. Clin. Lab Sci. 12 (1980) 171–214.
[26] I.E. Liener, N. Sharon, I.J. Goldstein, The Lectins, Academic Press, Orlando, 1986.
[27] A. Danguy, R. Kiss, J.L. Pasteels, Lectins in histochemistry. a survey, Biol. Struct. Morphog. 1 (1988) 93–106.
[28] M. Wagner, Light and electron microscopic lectin histochemistry using fluorochromes and ferritin as labels, Acta Histochem. Suppl. 36 (1988) 115–123.
[29] M.A. Bianchet, E.W. Odom, G.R. Vasta, L.M. Amzel, A novel fucose recognition fold involved in innate immunity, Nat. Struct. Biol. 9 (2002) 628–634.
[30] P.F. Chan, K.M. O'Dwyer, L.M. Palmer, J.D. Ambrad, K.A. Ingraham, C. So, M.A. Lonatto, S. Biswas, M. Rosenberg, D.J. Holmes, M. Zalacain, Characterization of a novel fucose-regulated promoter (P_{fcsk}) suitable for gene essentiality and antibacterial mode-of-action studies in Streptococcus pneumoniae, J. Bacteriol. 185 (2003) 2051–2058.
[31] A.B. Boraston, D. Wang, R.D. Bruke, Blood group antigen recognition by a *Streptococcus pneumoniae* virulence factor, J. Biol. Chem. 281 (2006) 35263–35271.
[32] R. Bishnoi, I. Khatri, S. Subramanian, T.N.C. Ramya, Prevalence of the F-type lectin domain, Glycobiology 25 (2015) 888–901.
[33] S. Mahajan, A. Khairnar, R. Bishnoi, T.N.C. Ramya, Microbial F-type lectin domains with affinity for blood group antigens, Biochem. Biophys. Res. Commun. 491 (2017) 708–713.