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GLYCOPINION MINI-REVIEW

Lectin domains in the toxin of *Bordetella pertussis*: selectin mimicry linked to microbial pathogenesis

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The pathogenesis of many infectious diseases is critically determined by prokaryotic lectins which enable differential recognition and activation of targeted eukaryotic cells. Some bacterial adhesins mimic and co-opt eukaryotic cell-cell adhesion motifs. This is illustrated by the toxin of *Bordetella pertussis*. Pertussis toxin mediates intoxication of eukaryotic cells by elevation of cAMP and it serves as an adhesin binding the bacteria to ciliated cells and respiratory macrophages. These activities are mediated by the lectin-like properties of the binding oligomer of the toxin. A comparison of pertussis toxin and the selectins involved in leukocyte trafficking indicates that these prokaryotic and eukaryotic C-type lectins share some element of primary sequence similarity, three dimensional structure, and biological activities. Such mimicry suggests a link between eukaryotic cell-cell adhesion motifs and microbial pathogenesis.

Keywords: selectin, adherence, fibronectin, microbial pathogenesis, toxin, pertussis

Introduction

Targeting of bacteria or their toxic components to specific cells during infection is accomplished by prokaryotic proteins which frequently recognize eukaryotic carbohydrates. For example, bacterial adhesins are cell-surface bound lectins mediating colonization of sequential sites during the progression of infection. Bacterial toxins exert their toxic activity after recognizing the preferred target cell by its carbohydrate surface profile. Recently, it has become appreciated that the ligation of a eukaryotic glyconjugate by a bacterial lectin, be it an adhesin or a toxin binding subunit, does not just serve to anchor the bacteria, but rather leads to significant cross talk between the engaged partners. The results of this interaction is the stimulation of the eukaryotic cell to undergo a response which further aids the progression of the bacteria along the course of infection [1]. From this point of view, significant aspects of the signs and symptoms of infectious diseases can be reduced at the molecular level to the biology of prokaryotic lectins. This also implies that, by necessity, the development of therapeutics is driven towards an understanding of these lectins.

At first glance, it would seem reasonable that bacteria might solve the task of targeted carbohydrate recognition by building lectins in the image and likeness of eukaryotic lectins that normally guide the trafficking of host cells through the carbohydrate-based address system. However, the great diversity of structures of both bacterial fimbriae and the binding subunits of most toxins indicates the contrary: bacterial lectins are often structurally unique and quite distinct from the eukaryotic lectins which bind to the same targets. This discussion will focus on an exception which illustrates that mimicry between prokaryotic and eukaryotic lectins occurs and can provide a unique insight into important biological systems.

Bordetella pertussis and whooping cough

Bordetella pertussis causes the exclusively human disease whooping cough [2]. After entering the host, the bacteria adhere to ciliated cells on the mucosal surface of the respiratory tract. Over the next 7–14 days, the microorganisms multiply *in situ*. This leads to a catarrhal stage in which the patient experiences symptoms of a respiratory infection, such as rise in body temperaure and mild coughing. One or two weeks later the clinical pictures changes due to the release of pertussis toxin (PT), adenylate cyclase toxin, dermonecrotic toxin, tracheal cytotoxin and other factors capable of causing systemic reactions. The resulting symptoms include severe coughing, profound leuokcytosis and loss of weight. This paroxysmal phase has a duration of 6–8

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weeks. Bacteria can be isolated from respiratory secretions during the first 2 weeks after the onset of paroxysmal coughing. Thereafter they can be found within macrophages, a sheltered compartment which may promote continued colonization of the respiratory tract after the destruction of the ciliated cells. Carbohydrates seem to play an important role in the progression of whooping cough by providing attachment sites for *B. pertussis* on both ciliated respiratory epithelial cells and macrophages and by determining the susceptibility of a given host cell to the toxic effects of PT. Thus, the initial targeting of bacteria to the lung and subsequent toxic effects are determined by the biology of the lectins of *B. pertussis*.

Seven different molecular structures with abilities to bind to human cells have been identified for *B. pertussis* [3]. Two important adhesins are lectins: PT and filamentous haemagglutinin (FHA). PT and FHA contain multiple carbohydrate recognition domains with the ability to discriminate between cells from different species as well as between distinct target cells such as ciliated respiratory epithelium and macrophages [4, 5]. The presence of both adhesins is crucial for the development of whooping cough. We will review the function and structure of PT with special reference to the cell recognition properties of its carbohydrate binding domains.

Structure, function and binding characteristics of PT

PT is a major virulence factor of *B. pertussis*. The toxin is hexameric with a classical A-B structure [6]. The A protomer is composed of a single subunit (S1) with a molecular weight of 26 kDa. The pentameric B oligomer is composed of four different subunits: S2 (22 kDa), S3 (22 kDa), two S4 (12 kDa) and S5 (11 kDa). Binding of PT to carbohydrate residues in glycoproteins and glycolipids is mediated by the S2 and S3 subunits as evidenced by the ability of purified subunits to bind to glycoconjugates and by antibodies to these subunits to block target cell recognition by the toxin [7–9]. When the B oligomer engages a sialylated glycoprotein, subunit S1 ADP ribosylates G_i-proteins leading to increased cAMP and toxicity to eukaryotic cells [10]. However, the B oligomer is also biologically active on its own. The B oligomer mediates binding of bacteria to eukaryotic cells and is mitogenic for T cells [4, 11, 12]. The receptor carbohydrates bound in these latter instances appear to be multiple, including specific recogition of lactosylceramide and gangliosides [7, 12, 13].

Studies on the binding properties of the assembled B oligomer have indicated recognition of at least two different classes of carbohydrate ligands: α2-6 linked sialic acid and lactosamine-containing glycoconjugates [7, 9, 10, 14]. For each target cell thus far examined, it appears that there exist multiple receptors for the B oligomer suggesting that a given carbohydrate target is recognized on several glycoconjugate backbones. Unlike other bacterial toxins, carbohydrate recognition is preserved as the subunits of the B oligomer are dissociated into S2S4 or S3S4 dimers or S2 and S3 monomers. Observations regarding S3 consistently identify sialic acid as the carbohydrate ligand in model complex carbohydrates as well as in gangliosides prepared from eukaryotic cells [7, 13]. Furthermore, free sialic acid is a strong inhibitor of S3-dependent bacterial binding to macrophages [11]. S2 is 80% homologous to S3 in amino acid sequence but the binding pattern of S2 seems to be more complicated. Sialic acid can be bound in model complex carbohydrates, but S2 also binds lactosylceramide and non-sialylated glycolipids prepared from ciliated cells [7, 15]. Thus, PT can be viewed as an elaborate lectin with two related but distinguishable binding units which confer complex binding specificities.

Given the heterogeneity of carbohydrate ligands bound by the B oligomer, it is not surprising that the lectin domains have been difficult to localize (Table 1). Regardless of the target cell or the technique used to indicate lectin activity of a region of the B oligomer (i.e. antibody inhibition studies, peptide analogues), the amino terminal regions of S2 and S3 have been consistently implicated as the lectin domains. By comparing the amino acid sequences of S2 and S3 with known lectins, two possible lectin motifs have been identified which are situated in tandem [7, 15] (Table 1). The far amino terminal region extending from residues 18–23 (alpha helix 1) has features of the wheat germ agglutinin (WGA) lectin pocket (residues 62–67) [15].

Table 1. Residues in PT S2 and S3 which affect carbohydrate recognition.

*Region**	*Description*	*Effect of mutation*	*Reference*
18–23	Resembles WGA	Loss of all lectin activity	15, 18
39–48	Resembles α2 helix of selectins	Switch of lectin specificity Lactose vs sialic acid	7
Asn 93 Asn 105		Loss of all lectin activity	17

* Residues are numbered according to S2 sequence.

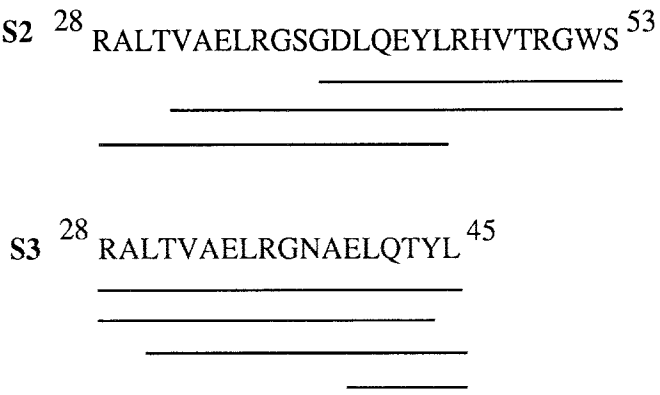

Figure 1. Peptides derived from the α2 helices of S2 and S3 which inhibit leukocyte adherence. Active peptides are indicated by the underlined residues.

Mutation of the WGA-like region of S2 abolishes all carbohydrate recognition. This region is followed at residues 39–48 by a second α helix that is similar to the α2 helix of the C-type selectins [7]. In the selectins, this helix is involved in interactions between the lectin and EGF domains that can modulate lectin binding activity [16]. Substitution of the PT residues Tyr^{44}, Leu^{45}, His^{47}, Arg^{50} or Gly^{51} in the α2 helix or swap mutations of this region of S2 (Thr^{31}-Ile^{54}) and S3 (Thr^{31}-Thr^{61}) interchanges lactosylceramide and ganglioside binding and concomitantly recognition of macrophages versus ciliated cells [7]. In addition to the WGA-like and selectin-like regions, the far carboxyterminus must be considered in a model of the carbohydrate binding domain since mutations of Asn93 or Asn105 of S2 or Lys105 of S3 abolish carbohydrate recognition [7, 17].

The recent solution of the crystal structure of a dimeric form of PT supports the contention that the amino termini of S2 and S3 form a composite of a plant and animal lectin arranged in tandem [18]. Residues 18–23 of S2 superimpose in three dimensions on residues 62–67 of WGA. This segment in S2 is followed by an α helix (residues 39–48) and four β sheets that superimpose on the core of the C-type lectin mannose binding protein. The crystal structure also indicates that the C termini of S2 and S3 lie close to the amino termini, explaining the ability of mutations in the carboxy-terminal β sheets 5 and 6 to strongly affect ligand binding [7, 17]. The actual carbohydrate binding site is yet to be visualized. The α2 helices of S2 and S3 are exposed on the basolateral surfaces of the subunits while the WGA-like site is on the vertical surface facing the other subunits of the assembled B oligomer. Both sites are sufficiently exposed to participate potentially in carbohydrate recognition of a target cell. The binding of a carbohydrate to S2 or S3 appears to be a complex process influenced by several spatially distinct regions. The definitive points of contact between the sugar and the PTS2 and S3 lectins remains to be defined.

Selectin-like features of subunits S2 and S3: a hypothesis

Based on primary amino acid sequence similarity, it has been proposed that S2 and S3 of PT have features of C-type lectins [7]. Biological activities of S2 and S3 consistent with those of selectins were then demonstrated *in vitro* and *in vivo* [19–21]. S2 exhibits a strong, dose-dependent, inhibition of neutrophil binding to P-selectin coated surfaces while S3 was shown to be more effective in blocking binding to surfaces coated with E-selectic [20]. Synthetic peptides containing amino acid sequences from the selectin-like regions of S2 and S3 inhibit neutrophil adherence to human endothelial cells. Peptides containing S2 sequences 28–45, 32–53, 39–53 and amino acids 28–45 from S3 were all potent inhibitors of leukocyte binding (Fig 1). Finally, the 28–45 sequence of both S2 and S3 subunits prevent inflammation *in vivo* [21]. When injected intravenously into rabbits with meningitis, these 18 amino acid peptides attenuated leukocyte recruitment to the cerebrospinal fluid and prevented blood brain barrier injury [22]. These effects are critically dependent on the peptide sequence. These findings strongly suggested that S2 and S3 mimic the selectins and predicted that some similarity might be expected at the level of protein structure.

The solution of the crystal structure of PT revealed that the α2 helix and the next four β sheets in the core of S2 and S3 resembled a C-type lectin, mannose binding protein [18]. To explore possible structural similarities between the selectins and PT, a comparison of the newly published crystal structures of PT and E-selectin was undertaken (Fig. 2). A striking resemblance of structure is evident in the cores of these two proteins as shown by the ability to superimpose the α2 helix and the next four β sheets in the S3 and E-selectin crystal structures (Fig. 2, regions red and purple). The composition of the α2 helix has a profound influence on ligand binding by S2 and S3. Comparing this region of PT with the selectins, this helix is positioned on the basilar face of each of the proteins and the primary sequence of residues 19–52 of S2 and S3 resembles region 15–46 of the selectins (Fig. 3). The residues exposed to the outer surface of the helix are conserved between S3 and E-selectin: glutamine, tyrosine and isoleucine (Fig. 4). There are, however, differences in the microenvironment of each residue as illustrated by the different positioning of the tyrosine ring relative to the helical axis. Site specific mutation of S3 tyrosine 44 abolishes carbohydrate recognition, while substitution at neighbouring sites does not [7]. Similarly, substitution of sites neighbouring the corresponding tyrosine in E-selectin does not affect ligand binding [16], but mutation of any of the three conserved residues of the helix has not been done. This α2 helix lies in the region of contact between the lectin and EGF domains of E-selectin (Fig. 2). Taken together with the observation that the EGF domain profoundly affects carbohydrate ligation by the topographically distant lectin domain of selectins, it is

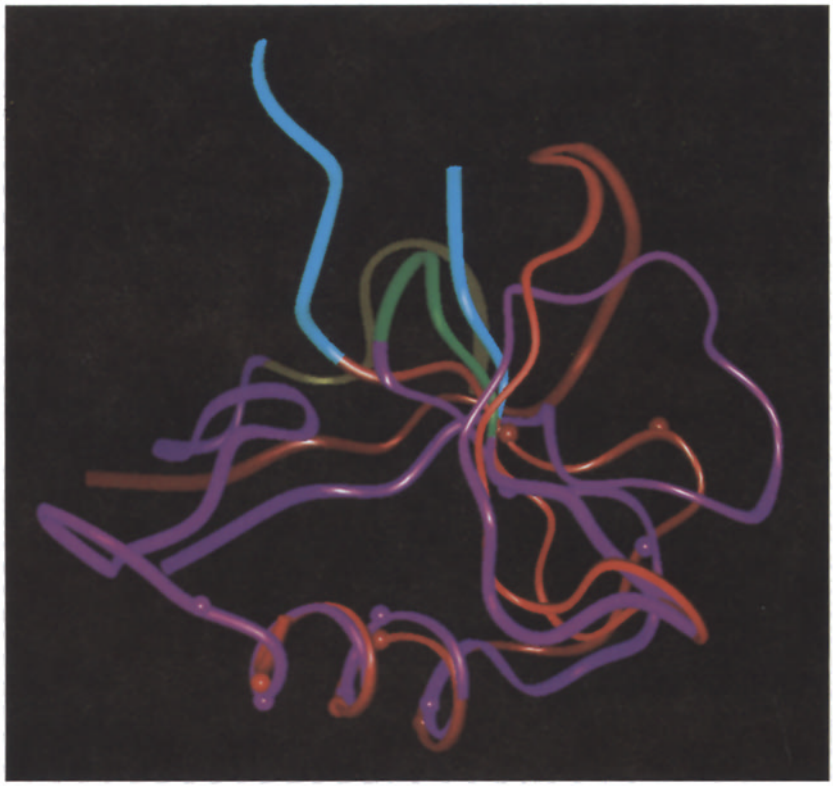

Figure 2.

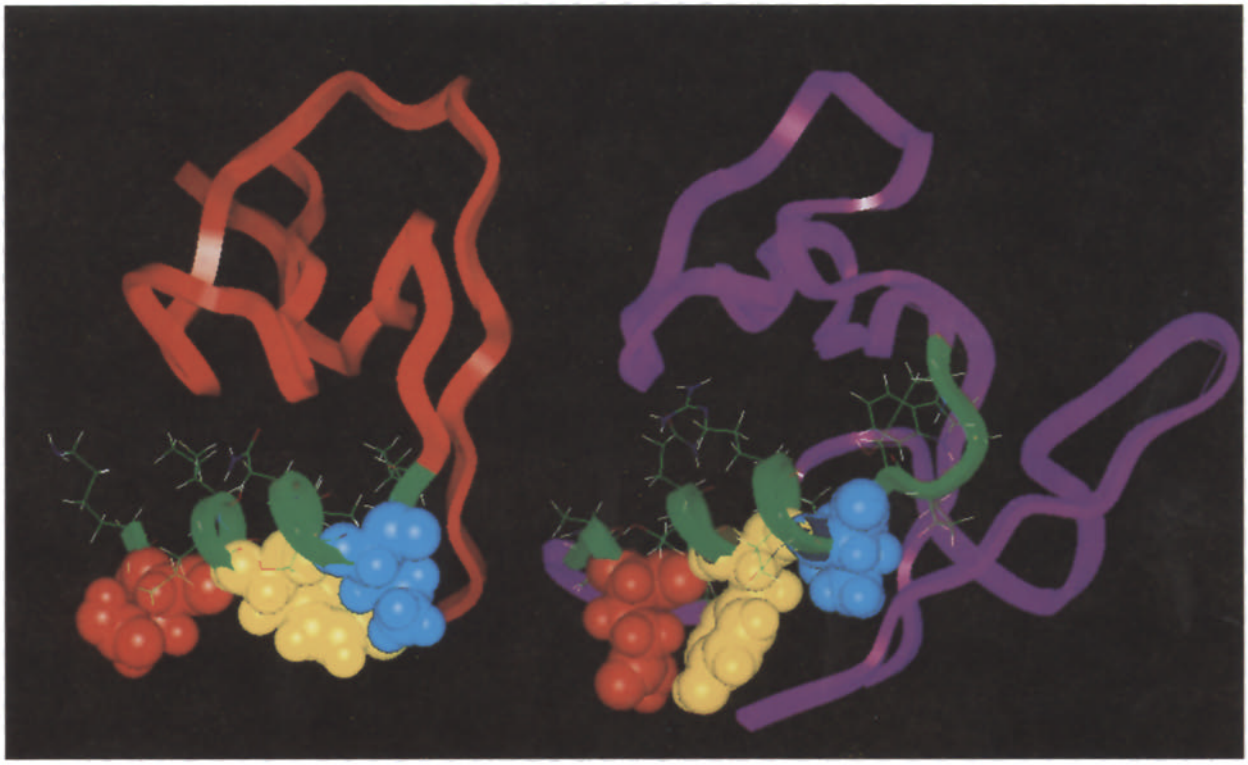

Figure 4.

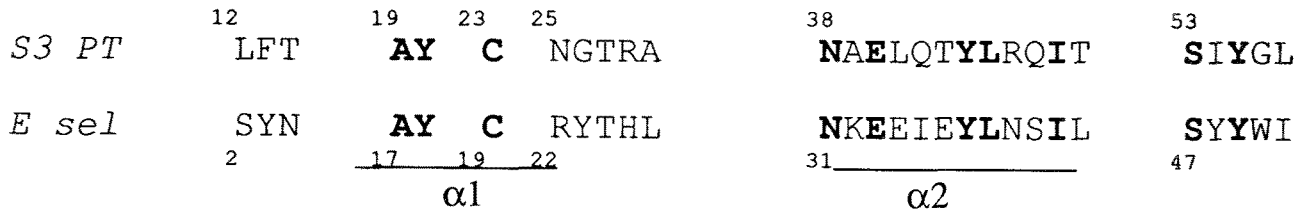

Figure 3. Structurally equivalent residues in the amino termini of S3 of PT and E selectin (E sel) as determined by Fig. 2. Identical residues are indicated in bold; α helical structure predicted for both proteins is underlined.

reasonable to suggest that the α2 helix on the basilar face of S2 and S3 could modulate ligand binding by acting as a point of influential contact with the incoming surface of a eukaryotic cell.

Both PT and the selectins bind sialylated fucosylated polylactosamines. The putative lectin site in the selectins is an extended surface loop (Fig. 2, blue) which is drastically truncated in S2 and S3 (Fig. 2, green). One interpretation of this difference is that the two molecules bind ligand completely differently. This same loop in the selectins binds calcium, a necessary co-factor for lectin activity. The fact that calcium is not required for S2 or S3 to bind ligand is consistent with the absence of the extended loop. An alternative interpretation is also plausible. It is possible that the extended loop of the WGA site (Fig. 2, yellow) might substitute for the missing large selectin-like loop even though it is positioned more posteriorly. This would explain the influence of mutations in the WGA loop on ligand binding and would suggest that the superior surface of S2, S3 and the selectins would present a common point of contact with the carbohydrate. A final resolution of the relationship of the selectin and toxin ligand binding pockets awaits the solution of crystal structures with bound ligand.

In summary, PT is a composite lectin mimicking a plant lectin, perhaps at the carbohydrate binding pocket, and a eukaryotic selectin as the lectin core structure. Mimicry is sufficient to engender biological interference between prokaryotic and eukaryotic versions of this selectin motif, raising the possibility of medically relevant therapeutic strategies utilizing bacterial variants of host selectins.

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Figure 2. Schematic pictures of the crystal structures of PT S3 and E-selectin. Superimposed ribbon representations of S3 (purple) and E-selectin (red) created by the Biosym Insight II Homology program. The coordinates for E-selectin residues 32–57 and 89–120 were provided by Dr B Graves as described [16]. The coordinates for S2/S3 were not made available and therefore the structure for S3 residues 10–90 was derived from the primary sequence overlaid on to the coordinates of mannose binding protein (MBP) according to the similarity described for the crystal structures of MBP and PT S3 [18]. The α2 helix lies on the anterior basolateral surface in this orientation. Regions of similarity beginning at the α2 helix and extending through the next four β sheets are shown in purple (S3) and red (E-selectin). Amino acid residues which are identical between the two sequences are shown as neighbouring spheres and are indicated in bold in Fig. 3. The calcium/carbohydrate binding site of E-selectin is a large loop indicated in blue. The small loop at this position in S3 (residues 49–52) is shown in green. The WGA-like region at the amino terminus of S2/S3 is depicted in yellow on the posterior vertical face of the S3 protein, a surface facing towards the other subunits of the assembled B oligomer.

Figure 4. Comparison of the topography of the basilar face of the α2 helix (green) of S3 (purple) and E-selectin (red). The orientation is similar to Fig. 2. The conserved glutamine (red), tyrosine (yellow), and isoleucine (blue) residues which form the outward facing surface of the α2 helix of both lectins are shown as space filling models. This surface is presented to the incoming eukaryotic cell in the case of S3 and interact with the EGF domain in the case of E-selectin.

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