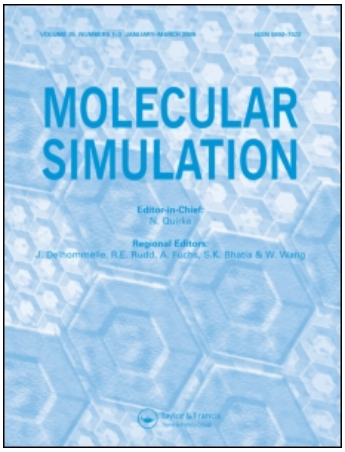

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Antibody-ligand docking: insights into peptide-carbohydrate mimicry

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Antibody–ligand docking: insights into peptide–carbohydrate mimicry

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Despite the enormous clinical importance for xenotransplantation, very little is known about the 3D structural basis for natural antibody recognition of the major carbohydrate xenoantigen, its derivatives and their peptide counterparts. Fundamentally, understanding the nature of peptide–carbohydrate cross-reactivity is necessary to allow a rational design of useful inhibitors. To satisfy this need, we have initiated a project to investigate the structural aspects of antibody recognition of carbohydrate xenoantigens and their peptide mimics by molecular docking. We aim to analyse critical ligand–protein interactions with a focus on identifying the degree of structural carbohydrate mimicry exhibited by peptide ligands. In this paper, we present docking simulations of complexes between a prototypical xenoreactive monoclonal antibody and two ligands: the major carbohydrate xenoantigen, terminal galactose-α(1,3)-galactose epitope [Galα(1,3)Gal] and a peptide inhibitor Galpep1 (DAHWESWL).

Keywords: xenotransplantation; antibody; carbohydrate; peptide; docking; dock

1. Introduction

In transplantation, as in transfusion therapy, the use of ABO-blood group incompatible organs practically always leads to organ rejection. This rejection is mediated by the recipient's antibodies (anti-A and/or anti-B agglutinins) recognising specific carbohydrate antigens on the cell surface of donor organs. Inhibition of these antibodies may allow successful engraftment for ABO-incompatible transplantations [1].

Major successes in clinical transplantation and a relative shortage of human donor organs has lead to the development of the concept of animal organ transplantation, called xenotransplantation [2,3]. However, the implementation of this approach is complicated by the presence, in the sera of all humans, of natural or preformed xenoreactive antibodies. They recognise the major carbohydrate xenoantigen—terminal galactose-α(1,3)-galactose epitope [Galα(1,3)-Gal], expressed on cells of lower mammals and prominent on the surface of porcine organs [4]. These antibodies, henceforth referred to as anti-Gal antibodies, initiate hyperacute rejection of xenografts.

Removal or neutralisation of anti-Gal antibodies has been achieved with the use of synthetic oligosaccharides, carrying the Galα(1,3)Gal epitope [5]. However, this approach was not entirely successful and resulted in an eventual return of the antibodies and slow development of partial graft injury. Also, using oligosaccharides for anti-Gal neutralisation proved to be problematic due to their low potency and stability and synthetic difficulties [6]. These problems identified a need for alternative agents, such as peptides, which could functionally and/or structurally substitute Galα(1,3)Gal [7]. Such peptides could be characterised as glycomimetics, i.e. "non-carbohydrate compounds that are molecular mimics of carbohydrates" [8]. Successful identification of such ligands may allow antibody inhibition to be used for preventing hyperacute xenograft rejection and potentiate the use of xenotransplantation for treatment of patients with organ failure.

Despite the enormous clinical importance for xenotransplantation, very little is known about the 3D structural basis for natural antibody recognition of the major carbohydrate xenoantigen, its derivatives, and their peptide counterparts [9]. Fundamentally, understanding of the nature of peptide–carbohydrate cross-reactivity is necessary to allow a rational design of useful inhibitors. To satisfy this need, we have initiated a project to investigate the structural aspects of antibody recognition of carbohydrate xenoantigens and their peptide mimics by molecular docking. Specifically, we are interested in exploring binding preferences of a panel of monoclonal IgG and IgM anti-Gal antibodies [10,11] with respect to Galα(1,3)Gal, its glucose and *N*-acetylglucosamine derivatives, and peptide inhibitors. We aim to analyse critical ligand–protein interactions with a focus on identifying the degree of structural carbohydrate mimicry

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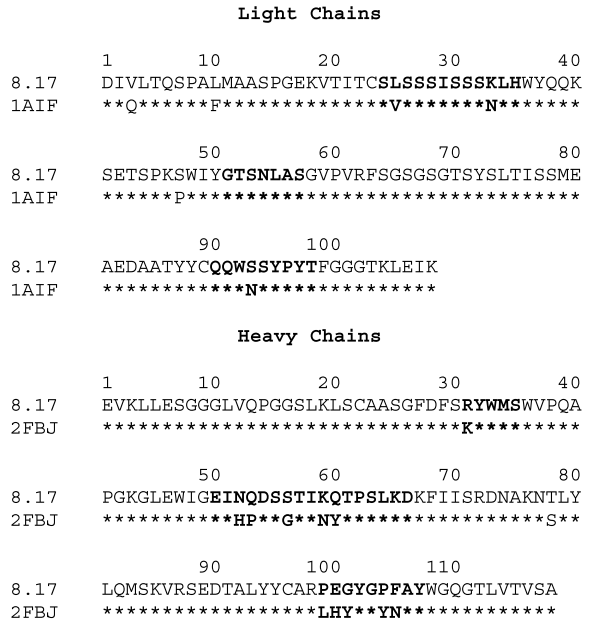

Figure 1. Sequences of the 8.17 variable domains aligned with the crystal structure templates (PDB codes 1AIF and 2FBJ), used for homology modelling. Amino acid identities are shown as asterisks (*) and CDRs are in bold face. Residues are numbered sequentially.

exhibited by peptide ligands. In this paper, we present docking simulations of complexes between Galα(1,3)Gal and a peptide inhibitor with a prototypical anti-Gal monoclonal antibody, 8.17.

Table 1. Antibody 8.17 residues involved in intermolecular interactions in docked complexes (top 10 ranked poses).

	Hydrogen bonding		vdW interactions	
	Galpep1	Galα(1,3)Gal	Galpep1	Galα(1,3)Gal
Arg 31H	6	–	8	–
Tyr 32H	3	–	10	1
Trp 33H	–	3	12	10
Glu 50H	–	–	–	2
Asn 52H	–	–	1	–
Gln 53H	–	–	4	–
Thr 57H	–	–	1	–
Lys 59H	4	6	1	–
Glu 100H	–	1	7	4
Gly 101H	–	–	15	7
Tyr 102H	2	9	9	5
Gly 103H	–	2	5	–
Lys 33L	–	13	1	3
Trp 91L	–	–	3	–
Trp 92L	–	1	7	8
Ser 93L	–	1	–	1
Ser 94L	–	–	1	–
Tyr 95L	1	–	2	–
Tyr 97L	1	8	–	–

2. Methods

2.1 Homology modelling

The hybridoma that produces anti-Gal monoclonal antibody 8.17 IgM was generated in Gal knockout mice [11]. From this hybridoma, the genes that encode variable (V) regions of the antibody heavy (H) and light (L) chains were cloned and sequenced [10]. Homology modelling of the fragment-variable (Fv) portion of the monoclonal antibody 8.17 was performed with the Homology module of the Insight II program, version 98.0 (Accelrys Software Inc.) following procedures previously developed by us for polyreactive antibodies [12]. Translated amino acid sequences of the 8.17 VL and VH domains were used to search the Protein Data Bank (PDB) for crystal structures suitable for generating template-based homology models (Figure 1). The 8.17 VL domain shared 94% sequence identity with the 409.5.3 anti-idiotype Fab (PDB code 1AIF), which was resolved at 2.9 Å [13]. The 8.17 VH shared 88% sequence identity with the galactan-binding J539 Fab (PDB code 2FBJ), which was resolved at 2.6 Å [14]. There were no gaps in the alignments of the two crystal structure templates with the 8.17 VL and VH domains. Furthermore, the complementarity determining regions (CDR) of the templates had the same canonical structures as predicted for the 8.17 antibody. There are no canonical structures for the CDR3 of the heavy chain, but this region of J539 was predicted to have similar base and loop structures as the 8.17 antibody [15]. Since, the J539 antibody shares substantial identity with other anti-Gal monoclonal antibodies under investigation by our group (data not shown), the 2FBJ was used as a reference structure for generating the relative orientations of the VL and VH domains in the 8.17 Fv homology model.

2.2 Docking

Ligand structures, carbohydrate Galα(1,3)Gal and peptide Galpep1 (DAHWESWL) [7], were generated in Sybyl, version 6.9.1 (Tripos, Inc.). Automated docking was carried out with the program DOCK, version 4.0 (UCSF) [16]. The molecular surface of the protein binding sites, designated by solvent-exposed CDR amino acids as well as several framework residues, was generated using the program MS. This surface served as input for the SPHGEN module of DOCK, which produced overlapping spheres to fill the binding cavity of the antibody. Force field scoring grids were created by the GRID module of DOCK. Automated docking was performed with: (i) incremental construction approach for conformational sampling; (ii) uniform-sampling for orientational search; and (iii) electrostatic and van der Waals (vdW) interactions within the AMBER force field for scoring and ranking. Large rigid segments of a ligand (greater than five heavy atoms) were chosen as anchors and, during each iteration of the

docking search, the orientation sampling was initially limited to these segments. Then the remaining segments were re-attached in a number of possible arrangements controlled by the docking parameters. All docked ligand poses were minimised on-the-fly (i.e. prior to scoring and ranking) to 0.1 kcal/mol, with the maximum number of iterations set to 100. Additional geometry optimisation was performed for the highest scoring pose of each ligand. Here the energy convergence criterion was 0.01 kcal/mol, with a maximum number of iterations set to 1000. Program settings used were the same as described previously [17].

For each complex, a total of 100 poses were saved and clustered based on structural similarity (rmsd $\leq$ 2 Å). The most populated cluster was taken to represent a docking solution. The top 10 clusters were analysed with respect to their intermolecular interactions using the program LigPlot [18]. Hydrogen bonding and vdW contacts were tallied and a "count" was generated for each binding site residue (Table 1). Such "count" reflects how many times a given residue makes a particular type of contact to a ligand residue (carbohydrate monomer or amino acid, respectively). The detailed description of this approach to the interpretation of docking results is described elsewhere [17].

3. Results

3.1 Conformational aspects of antibody–carbohydrate docking

Investigation of carbohydrate binding to antibodies requires an appreciation of the conformations accessible to these carbohydrates in solution. It is not unreasonable to expect that an antibody generated against a particular carbohydrate will recognise its lowest energy and/or most populated conformation in solution. Conversely, it is possible that energetically less favourable conformations, which may be much less populated in solution, could be selected upon receptor binding. It has been demonstrated, using Lewis antigens binding to antibodies and lectins as an example, that both these outcomes are achievable [19]. The docking of carbohydrate ligands presented here was developed with both these possibilities in mind.

The docking approach, previously employed by us for peptides [17,20–22], was adapted for carbohydrate ligands. A comparative analysis of several docking approaches was carried out. Specifically, the tactics to handle sugar flexibility was compared: (i) by means of pre-generated conformer libraries using random or systematic variation of torsions or experimentally observed solution conformations [23]; (ii) during docking using either conformational search or incremental construction of the ligands. The benchmarking of the suitability of the above approaches for antibody–carbohydrate docking indicated that building sugar conformation during docking via the incremental construction of the carbohydrate ligands gives the best computational performance (in terms of the potential energy space sampled) and produces the lowest energy pose. Furthermore, the distribution of glycosidic torsion angles in the docked structures was found to agree with experimentally observed values [24,25] and independent modelling calculations [26].

3.2 Structural validation of antibody–carbohydrate docking

Our carbohydrate docking procedure was carefully validated using the experimental structures of antibody–carbohydrate complexes (PDB reference codes: 1M7D [27], 1MFA [28], 1MFD [29,30], 1S3K [31] and 1UZ8 [32]). High resolution structures were chosen to have short 2–4 residue carbohydrates with the level of flexibility similar to those under investigation here (i.e. no 1,6 connectivity and no exo-cyclic chain, as in sialic acids). A range of binding site types (grooves and cavities) and sizes was covered. The small binding cavities (as exemplified by 1MFD [29,30]) were found to be best for reproducing the carbohydrate ligand binding poses, observed by experiment, followed by groove-shaped binding sites (as exemplified by 1M7D [27]), which also provided close prediction of experimentally observed binding modes. Large binding site cavities (as exemplified by 1UZ8 [32]) allowed generation of "true" binding modes within the sampled docking output. However, the correctly predicted structures were commonly ranked lower on the hit list.

The representative overlay of the experimental and docked structures for an antibody with a small binding pocket is shown in Figure 2. The most critical interactions observed in the crystal structure, as analysed by the program LigPlot [18], are reproduced in the docked complex. Three of the four hydrogen bonds are predicted correctly, as are two of the three nonspecific hydrophobic contacts. Minor differences between the two structures are due to slight shifts in the positions of the ligand atoms. The positions and orientations of the ligand residues are very similar in the experimental and docked structures. Interactions not reproduced in the docked structure are compensated by interactions with closely located protein residues.

3.3 Analysis of protein–ligand interactions in docked complexes of anti-Gal antibodies

3.3.1 Docking of the carbohydrate ligand

Following docking of the Galα(1,3)Gal ligand into the 8.17 antibody binding site, the top 100 poses were compared for structural similarity and 38 clusters were produced. Figure 3 (top) illustrates the top 10 clusters. The top two clusters encompass 34 of the saved poses, indicating a significant preference for these poses. Two main binding modes adopted by Galα(1,3)Gal involve, as proposed [9], an

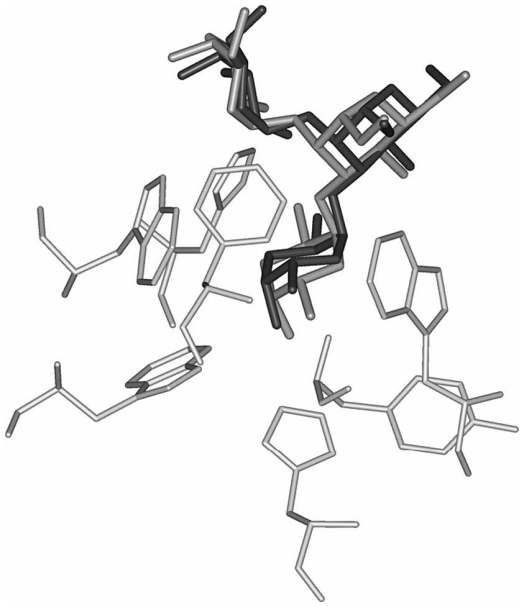

Figure 2. Docked antibody complex with a trisaccharide ligand. Crystal structure (PDB code 1MFD [29,30]), dark grey; docked structure, light grey; protein residues surrounding the ligand, white.

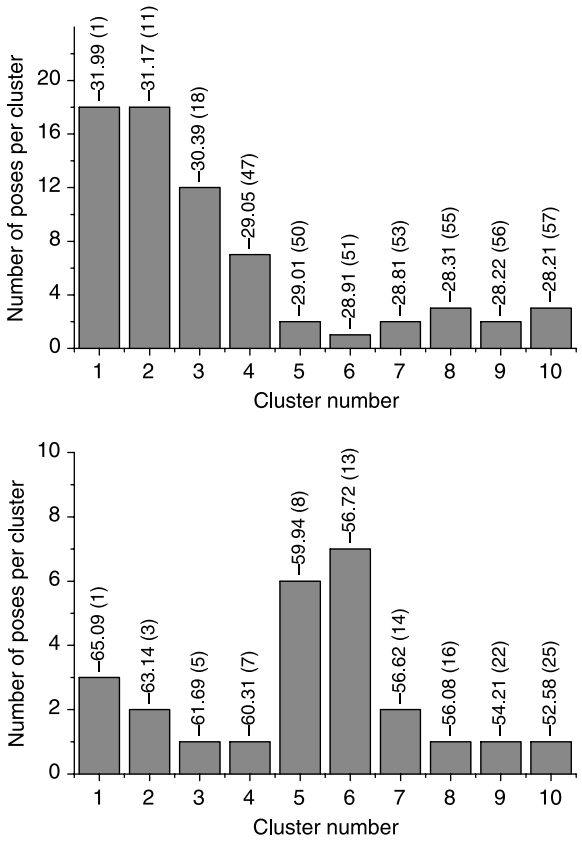

Figure 3. Column plots for the top 10 clusters for the antibody complex with the ligands: Galα(1,3)Gal (top) and Galpep1 (bottom). Values above columns correspond to DOCK energy scores (kcal/mol). Value in parenthesis corresponds to the lowest pose number, representative of a given cluster.

anchoring mechanism. Either the terminal α-galactose or a down-stream β-galactose residue penetrate deeper into the binding site cavity, formed by CDR loops (excluding LCDR2). Amino acids forming the floor and lower walls of the cavity are Lys 33 and Trp 92 of the light chain and Trp 33, Lys 59, Glu 100, Gly 101 and Tyr 102 of the heavy chain. The binding mode, where the terminal α-galactose penetrates deepest into the cavity (Figure 4 (a), (c)), is lower in energy (by 0.8 kcal/mol) and is representative of the top 10 docked poses (Figure 3 (top)). Also as hypothesised [9], the aromatic residues in the structurally conserved positions in the binding pocket are important for carbohydrate recognition (Figure 4 (a), (c)).

Analysis of intermolecular interactions in the top 10 docked complexes (Table 1, Figure 5) showed that the aromatic amino acids (in particular, Trp 92L, Tyr 32H, Trp 33H and Tyr 102H) display a variety of stabilising vdW interactions with the hydrophobic faces of the carbohydrate residues and an array of specific hydrogen bonding interactions important for selecting a particular pose. Interestingly, the phenylalanine residues present at the conserved positions in the binding pocket were also expected to be critical for carbohydrate binding [9]. However, these residues do not contribute significantly to the ligand recognition in our docking simulations. This could be due to the capability of tyrosine and tryptophan residues to participate in both hydrogen bonding and vdW interactions, whereas phenylalanine is limited to vdW interactions only. Other residues, prominent in their binding to the carbohydrate ligand (i.e. observed in four or more docked complexes out of ten analysed) include Lys (33L and 59L), Glu 100H and Gly 101H.

3.3.2 Docking of the peptide ligand

The top 100 docked poses for the antibody 8.17 and the Galpep1 ligand (DAHWESWL) produced 85 clusters when compared for structural similarity. The top 10 clusters are shown in Figure 3 (bottom). Clusters five and six are the most populated: six and seven poses, respectively. Two predominant binding modes are: burying a peptide residue in the binding site cavity formed by the amino acids of five CDR loops (described above) or wedging an anchoring peptide residue between Glu 100H (HCDR3) and Trp 33H (HCDR1). These two residues are located on the upper wall of the cavity, and their opposing positions (Figure 5) provide an excellent opportunity for sandwiching a ligand side-chain. The preferred peptide residues acting as anchors are Trp4 (wedged) and Trp7 (buried). In the most populated docked solution

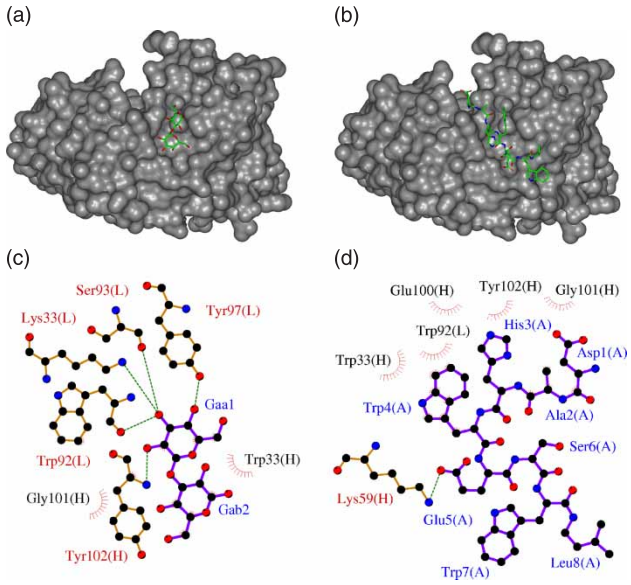

Figure 4. Docked solutions of monoclonal antibody 8.17 complexes with the carbohydrate Galα(1,3)Gal ((a) and (c)) and the Galpep1 peptide DAHWESWL ((b) and (d)). (a,b) Views of the 3D structures. The solid surface represents a solvent-accessible surface with a probe radius of 1.4 Å. Ligand atoms: carbon, green; oxygen, red; nitrogen, blue. Hydrogen atoms omitted for clarity. (c,d) Schematic 2D plots of intermolecular interactions in the docked structures. Non-bonded interactions: vdW, red spokes; hydrogen bonds, dashed green lines. Covalent bonds: ligand, purple; protein, brown. Protein: side-chains are shown only for residues, to which a ligand is hydrogen-bonded; a red single spoked arcs show residues involved only in vdW contact(s) with a ligand. Atoms: carbon, black; oxygen, red; nitrogen, blue. The plots were created with the program LigPlot [18]. Chain identifiers: L, light chain of the antibody 8.17; H, heavy chain of the antibody 8.17; A, peptide Galpep1.

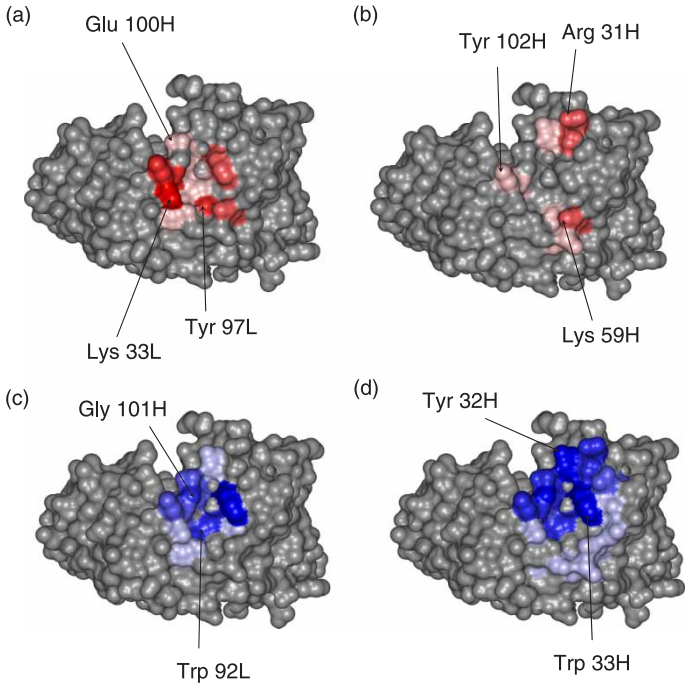

Figure 5. Views of the antibody 8.17 binding site, mapped with respect to its residues involved in intermolecular interactions with the carbohydrate Galα(1,3)Gal ((a) and (c)) and the Galpep1 peptide DAHWESWL ((b) and (d)). All views represent the same orientation of the protein. Residues involved in hydrogen bonding contacts are shown in red ((a) and (b)); residues participating in vdW interactions are shown in blue ((c) and (d)). Colour depth signifies the number of contacts a particular residue makes with the residues of the carbohydrate and peptide ligands.

(cluster six), Trp4 is wedged between Glu 100H and Trp 33H (Figure 4(b), (d)). Such "end-on insertion" and "wedging" mechanisms were previously described by us for a human IgM monoclonal antibody Mez along with a variety of accommodating binding modes, which are adopted by large peptide ligands when docked into relatively small binding sites [17,20]. In the case of Galpep1 docking into the relatively small carbohydrate-tailored binding site of 8.17, this binding site, similar to Mez, can not accommodate the whole of the peptide ligand. The anchor (Trp4) and the surrounding residues fill the cavity, whereas the rest of the peptide "spills over" and the complex is stabilised by vdW and hydrogen bonding interactions with the outlying residues, mainly Arg 31H and Tyr 32H (Figure 4(b), (d)).

The peptide demonstrates lower population counts per cluster and a greater overall number of clusters, compared to the carbohydrate ligand (Figure 3). This indicates a higher degree of flexibility and a greater ability of the peptide to adopt to the binding site of the antibody. This is not unexpected, given the size of the peptide. However, this observation is significant from the point of view of possible carbohydrate mimicry exhibited by this peptide. Namely, while the peptide displays more binding modes than the carbohydrate, does it contact the same residues in the binding site?

Both Glu 100H and Trp 33H are significant for vdW binding to the peptide, when alternative binding modes (top 10 ranked docked complexes) are considered (Table 1, Figure 5). Other aromatic residues involved in peptide recognition include tyrosines (95L, 97L, 32H, 102H) and tryptophans (91L, 92L). Interestingly, similar to carbohydrate binding, the antibody 8.17 utilises both hydrogen bonding and vdW interactions for residues Tyr 95L and Tyr 102H. Other residues, prominent in their binding to the peptide ligand (i.e. observed in four or more docked complexes out of ten analysed) include Arg 31H, Gln 53H, Lys 59H, Gly 101H and Gly 103H.

4. Discussion

Using peptides as ligands/inhibitors for carbohydrate-binding xenoreactive antibodies is one of the approaches to tackle the xenograft rejection problem. Several investigations have been carried out to design peptide inhibitors for anti-Gal antibodies [7,33–35]. We have established that a synthetic peptide Galpep1 (DAHW-ESWL) could specifically block the binding of human natural IgG antibodies to pig cells [7]. It has been also shown that Galpep1 can inhibit the binding of purified human anti-Gal IgG antibodies to Galα(1,3)Gal-BSA (bovine serum albumin) [36].

In all previous studies, related proteins, such as IB4 lectin [7,33,34] or anti-B blood group monoclonal antibody [35], but not anti-Gal xenoreactive antibodies were used for peptide design. Furthermore, no consideration was given to the 3D structural basis for natural antibody recognition of the major carbohydrate xenoantigen, its derivatives, and their peptide counterparts. Fundamentally, a structural understanding of the nature of peptide–carbohydrate cross-reactivity is necessary to allow a rational design of useful inhibitors, and this understanding is necessarily based on structural considerations in antibody–ligand complexes.

The nature of peptide–carbohydrate cross-reactivity could be dissected when one analyses intermolecular interactions in protein-ligand complexes. On one hand, it could be due to peptide interactions with macromolecular targets, similar to those exhibited by respective carbohydrates—"structural mimicry". Alternatively, peptides may bind to carbohydrate-specific proteins simply by offering greater complementarity to their binding sites. This type of binding, reminiscent of protein-mimicking mimotopes [37], is due to non-specific binding ("functional mimicry" of Vyas *et al.* [38]). Here we analyse the interactions of a prototypical carbohydrate-specific monoclonal antibody with carbohydrate and peptide ligands with a view to delineate the nature of their cross-reactivity.

In order to systematically rationalise carbohydrate–peptide cross-reactivity, it is also crucial to distinguish between the ligand- and receptor-based contributions to the probable mimicry. Kieber-Emmons *et al.* [39] suggested that the peptide–carbohydrate mimicry stems from peptide functionalities replicating chemical interactions of the mimicked carbohydrates. Such type of structural mimicry is exemplified by the complex of porcine pancreatic α-amylase with a carbohydrate substrate-mimicking inhibitor [40]. On the other hand, the concept of structural mimicry could be interpreted in terms of a protein receptor. In this interpretation, the structural mimicry is thought to originate from the binding site functionalities engaging in the same type of interactions with the mimicking peptide ligand as with the mimicked carbohydrate [41]. In this paper, we focus on the latter aspect.

The results presented here for the 8.17 anti-Gal monoclonal antibody recognition of its carbohydrate ligand Galα(1,3)Gal and its peptide counterpart Galpep1 (DAHW-ESWL) allow interesting inferences to be made. One structural aspect of antibody–ligand interaction relates to the mode of binding of the ligands. To address the issue of structural aspects of anti-Gal antibodies' recognition of their carbohydrate and peptide ligands, we have previously analysed general binding patterns that have been repeatedly identified in antibody complexes with small molecules (haptens), carbohydrate and peptide ligands [9]. This analysis was performed using the assumption that similar mechanisms will almost certainly mediate recognition of the major xenoantigen by natural antibodies. The docking simulations described here indicated that, as proposed [9], both ligands bind to the antibody 8.17 via an anchoring mechanism. Specifically, this involves an end-on insertion

of the terminal α-galactose residue and an aromatic side chain for the Galα(1,3)Gal and Galpep1, respectively.

Another aspect of the recognition relates to the participation of the antibody residues. There is a significant overlap in this antibody's recruitment of residues, involved in the recognition of its carbohydrate and peptide ligands (Table 1 and Figure 5). The most prominent intermolecular interactions involve hydrogen bonding contacts by Lys 59H and Tyr 102H and vdW contacts by Trp 33H, Glu 100H, Gly 101H, Tyr 102H and Trp 92L. Notably, the same residues, especially conserved aromatic amino acids such as Tyr 102H, participate in both hydrogen bonding and hydrophobic interactions. Significantly, such a dual role was noted for both the carbohydrate and peptide ligands. Generally speaking, participation in both types of interactions is not unexpected. However, it differs from the ligand binding preferences of the peptide-recognising human IgM antibody Mez [17,20]. When a large combinatorial peptide library was docked into the Mez binding site and the binding profile was scrutinised, it was found that Mez residues, participating in vdW interactions, were primarily located in the "floor" of the binding cavity while the hydrogen bonding residues were on the surrounding "walls". Furthermore, there was a clear division of roles: some residues participated in hydrogen bonding interactions (critical for orienting ligands and maintaining key elements in given positions), while others took part in stabilising hydrophobic interactions. Thus, both the locations and the roles of the protein residues were clearly separated.

Therefore, it can be suggested that ligand binding preferences are exercised differently by carbohydrate-specific antibodies, such as anti-Gal antibody 8.17, and peptide-recognising antibodies, such as Mez. Results obtained here and their comparison to the previous studies [17,20] indicate that the carbohydrate-recognising anti-Gal antibody 8.17 utilises similar structural elements for its binding to carbohydrate and peptide ligands.

5. Conclusions and future directions

The fundamental question of the nature of carbohydrate–peptide cross-reactivity is: is it achieved by (a) structural mimicry, (b) non-specific binding or (c) a combination of (a) and (b). Furthermore, if it is a combination of these possibilities, then to what extent does each contribute?

The results presented here suggest that, in case of xenoreactive antibodies, the peptide ligands mimic their carbohydrate counterparts by directly engaging protein residues involved in carbohydrate recognition. Further work is needed to analyse the intricacy of recognition from the "ligand's point of view". We are investigating which peptide functionalities are involved in the intermolecular interactions and how, if at all, they replicate chemical interactions of mimicked carbohydrate(s).

The present study is an example of how a comprehensive analysis of intermolecular interactions in a range of docked complexes (as opposed to a single top ranked docking solution) can be used to interrogate the ligand recognition preferences of a protein. We are extending this study to cover complexes of a panel of monoclonal IgG and IgM anti-Gal antibodies [10,11] with Galα(1,3)Gal, its glucose and *N*-acetylglucosamine derivatives, and peptide inhibitors. In our studies, we are considering peptides, for which the anti-Gal antibody binding and inhibition profiles have already been established experimentally [7,33–36].

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