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Allostery in C-type lectins

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Highlights

Abstract:

C-type lectins are the largest and most diverse family of mammalian carbohydrate binding proteins. They share a common protein fold, which provides the unifying basis for calcium mediated carbohydrate recognition. Their involvement in a multitude of biological functions is remarkable. Here, we review the variety of tasks these lectins are involved in alongside with the structural demands on the overall protein architecture. Subtle changes of the protein structure are implemented to cope with such diverse functional requirements. The presence of a high level of structural dynamics over a broad palette of time scales is paired with the presence of secondary binding sites and allosteric coordination of remote sites and renders this lectin fold a highly adaptable scaffold.

Introduction

Carbohydrates cover every living cell. The dense fur composed of complex, branched structures is exposed to the exterior of a mammalian cell and determines processes such as cellular homing, differentiation and cell-cell communication [1-3]. Many protein families have convergently evolved to recognize carbohydrates based on their stereochemistry, glycosidic bonds and composition [4]. C-type lectins receptors (CLR) resemble the largest and most diverse family of mammalian lectins. Their unifying ability to recognize carbohydrates via a Ca^{2+} ion as a co-factor gave them their name [2,4], with only a few exceptions of CLRs recognizing their ligands in absence of Ca^{2+} [5].

Many CLRs are found in the immune system being involved in cellular adhesion, self-/non-self recognition and glycoprotein turnover [1-3]. Intracellular signaling is initiated either through receptor clustering or by recognition of monovalent ligands – a mechanism not well understood [3]. For example, dendritic cell-specific intracellular adhesion molecule 3 grabbing non-integrin (DC-SIGN) can elicit monosaccharide specific cell signaling [6]. While mannose-containing ligands lead to a release of proinflammatory cytokines, fucose-containing ligands suppress the pro-inflammatory response [7]. These effects on a cellular level cannot be explained by our insights into the molecular recognition [8,9]. Overall, the role of CLRs in key processes of the

immune system has sparked interest in their role as therapeutic targets for adjuvants, induction of the innate immunity or antitumor response as well as targets for anti-inflammatory or anti-microbial drugs [1,10].

The C-type lectin domain fold

All CLRs share the C-type lectin domain (CTLD) [5,11-13]. The looped structure with N- and C-terminal antiparallel β- sheets is connected by two flanking α-helices and a three-stranded antiparallel β-sheet in the hydrophobic center of the domain harboring a conserved 'WIGL' motif [5,11]. A maximum of four Ca^{2+} sites have evolved with three being located in upper loop, with Ca^{2+}-2 site being the carbohydrate site (Figure 1). Two pairs of highly conserved disulfide bridges stabilize the domain. Overall, this fold can undergo high sequence variability and is hence well-suited for immune cell receptors coevolving with pathogens [14]. Here, we will focus on those CLRs utilizing the conserved Ca2+ site for carbohydrate recognition.

Structure and function of the long loop

An evolutionary and structurally variable long loop embeds the canonical Ca^{2+}-2 site (Figure 1). In this loop the essential residues for Ca^{2+} coordination comprising the EPN or QPD motif for mannose/fucose/glucose or galactose specificity, respectively. Moreover, the 'WND' motif couples the Ca^{2+} cage to a central tryptophan of the hydrophobic core stabilizing the fold [12]. The Ca^{2+} ligates two vicinal hydroxyl groups of the monosaccharide ligand [5] and the proline of the EPN/QPD restricts the loop conformation to a closed state in the presence of Ca^{2+} [12]. Only a few structures are available in both apo and holo state, such as tetranectin [15], MBP-C and MBP-A [16]. In absence of Ca^{2+}, the proline returns to the trans isomer, a slow process that was proposed to function as a conformational switch preventing ligands from rebinding [15-19]. For mincle and MCL, the long loop is pushed close to the Ca^{2+}-2 site, in contrast to DC-SIGNR [20]. This loop flexibility suggests high entropic costs upon carbohydrate recognition and together with the enthalpic penalties arising from desolvation of the carbohydrate site, low ligand affinities arise [21].

Ca^{2+} sites 1 and 3 located on the opposite side of the long loop and can influence primary Ca^{2+}-2 site (Onizuka et al., 2012; Furukawa et al., 2013). CLRs with accessory binding sites show a higher order Ca^{2+} dependence and sharper transitions between active and inactive states. The human hepatic asialoglycoprotein receptor (ASGPR) experiences a high allosteric cooperativity between the three Ca^{2+} sites [18,22]. Similarly, the macrophage galactose receptor (MGL) has a Ca^{2+} affinity of 0.21 mM with third order dose dependency [23]. Rearrangements of the primary carbohydrate site in absence of secondary Ca2+ site occupancy are observed for mincle. Under limiting Ca^{2+} concentration this mechanism prevents carbohydrate recognition [2,20,24,25]. For CLR lacking secondary Ca^{2+} sites, basic amino acids are located in those positions [26-28].

The affinity for Ca^{2+} is pH sensitive. Since many mammalian CLRs are endocytic, the environment of the endo-lysosomal pathway determines cargo release [2,16,22]. Such pH dependency of glycan recognition has been reported for instance for DC-SIGN, DC-SIGNR and Langerin [9,29]. However, even at low pH but saturating Ca^{2+} conditions, the carbohydrate affinity might be unaffected [30]. Interestingly, while the murine SIGNR1, 2, 3 and 7 have the expected pH dependency, SIGNR8 increases Ca^{2+} affinity at low endosomal pH by an unknown mechanism [31]. For non-endocytic CLRs, such as MBP-A, a lower pH susceptibility is observed by shifting the half maximum binding to a pH around 5 suggesting the pH sensitivity is encoded in the CRD fold [32].

The role of the neck domain

Another layer of complexity in understanding CLR structure and function is provided by their oligomerize state. Coiled-coil interactions of the stalk region promote the formation of homotrimers [23,29,33,34], homodimers [35], homotetramers [36], or even heterodimers [20]. The spatial presentation of the CRDs by the extracellular domain (ECD) overcomes the lack of monovalent affinity and is also important for the carbohydrate recognition. This has functional consequence: for some neck domain oligomers this process is pH dependent, such as for DC-SIGN [37]. This likely aids to the endosomal cargo release by decreasing the avidity. Additionally, the coiled-coil structure may transmit information upon extracellular ligand recognition to the cellular inside [36,38]. Monovalent ligands can induce cellular signaling via DC-SIGN [6,7]. Moreover, antibodies against the DC-SIGN CRD favor clathrin-mediated uptake of the cargo, while anti-neck domain antibodies promote a non-clathrin mediated route [39].

Structural plasticity of the neck is also observed once ligands are bound [40]: The length of DC-SIGN shrinks by 5 nm upon ligand binding. This is in line with observations for the mannose-binding protein [33] and DC-SIGNR [41]. Finally, there is dynamic interaction between the CLR oligomers. Super resolution nanoscopy revealed the formation of DC-SIGN nanoclusters (7.5 ± 2.7 tetramers per cluster), a property determined by the neck [42]. This nanocluster formation has again functional consequences: truncating the neck domain favors endocytosis of micron-sized zymosan particles over much smaller particles of virus size (40 nm).

The role of secondary sites

Carbohydrate ligands for CLRs are usually part of oligo- or polysaccharides. The specificity usually arises from the presence of secondary carbohydrate recognition sites in close proximity of the primary site, which generates an extended recognition interface. For instance, selectins do not show measurable affinity for monosaccharide ligands and rely on the presence of such secondary sites [43]. This is a general principle found for many convergent lectins [4]. These secondary sites show a higher sequence variability between family members, as exemplified for the human and murine Langerin [44]. Other examples are E-selectin, DC-SIGN, MGL, DCIR, and BDCA-2 [8,27,28,45,46]. For mincle, a CLR that recognizes glycolipids, the specificity for the acyl chains is generated by an induced secondary site highlighting the structural plasticity of the CLR fold [20,24,25]. Crystals grown at low pH lack the accessory Ca^{2+} and reveal a hydrophobic grove allowing for glycolipid ligand binding: a glutamate that forms the Ca^{2+} cage is shifted, suggesting a coupling of the two sites in the capture and release mechanism [25].

Unexpectedly, secondary sites in CLRs distal from the primary site have been reported. In SIGN-R1 a structurally separate, Ca^{2+} independent binding site for repetitive microbial polysaccharides and dextran sulfate on the opposite side of the CRD was discovered [47]. Similar findings have been observed for Langerin (see below) [48]. On the other hand, this is an effect previously observed for other lectins, such as the heat-labile enterotoxin from E. coli for which NMR experiments demonstrated remote secondary blood group binding sites affecting the primary carbohydrate site [49]. The relevance of these secondary sites cannot be underestimated for their biological role in determining the fine carbohydrate specificity, but also for drug design focusing on lectins [10,50,51]. Secondary sites offer the potential to target challenging, hydrophilic primary sites not amendable to traditional medicinal chemistry [50-52].

Figure 1: CLR fold, Ca2+ site names, central proline, … disulfide bridges highlight, show evolutionary conservation?,

Example 1: selectins

Selectins are a subfamily of CLRs consisting of P-, E-, L-selectin. They are homing receptors involved in cell adhesion and leukocyte trafficking and are conserved between species. These type I transmembrane proteins carry a CRD directly connected to an epidermal growth factor (EGF-)-like domain. The minimal binding motif of P- and E-selectin is sialyl LewisX binding to the canonical carbohydrate binding site, while a secondary site binds sulfated tyrosines generating specificity and affinity for PSGL1, its natural glycoprotein ligand. This is supported by a crystal structure of E-selectin with sialyl LewisX originated from soaking experiments [45]. Moreover, a PSGL-1 glycopeptide fragment bound to P-selectin induced an extended conformation suggesting a conformational coupling of the secondary and the primary sites [45]. However, a co-crystal structure of a four domain fragment comprising the CRD, the EGF and two additional SCR domains in presence of sialyl LewisX shows the extended conformation with conformational stretching of E-selectin important for catch-bond mediated leukocyte recruitment [53]. Strikingly, in the new ligated structure the long loop changes its conformation, in particular Gln85 is relocated by about 10Å. Consequently, the low affinity structure of E-selectin, present in solution, recognizes the carbohydrate [45,53]. This recognition allows the transition to a high affinity, extended state, already in the absence of flow conditions, suggesting a two-state model [53]. This data is supported by ligand triggered allostery enabling long loop closure [54]. Taken together, the CRD cannot be considered isolated from other domains and undergoes allosterically mediated rearrangements to fulfil its role in cellular adhesion. From a molecular perspective it is remarkable that a small molecular ligand in the absence of flow conditions is able to promote such an extended structural transition [53,54].

Figure 2: Show the selectin hypothesis

Example 2: langerin

Langerin is an endocytic, type II transmembrane receptor found on Langerhans cells of the skin [55]. The extracellular α-helical neck region of this homotrimer is pH-insensitive and the CRD harbors a single Ca^{2+} imbedded in an EPN motif [29]. Despite the preference for mannose and fucose, sulfated galactose is also recognized, highlighting the role of secondary sites [56]. Since langerin is a recycling receptor, extracellular uptake and endosomal release must be tightly coupled. Endosomal pH lowers the Ca^{2+}-affinity and promotes cargo release [30,55]. However, the finding that the Ca^{2+}-affinity is pH sensitive is surprising, because Ca^{2+} is not solvent-exposed and there are no protonizable residues in its vicinity. Thus, there must be a pH sensor in some other region of Langerin which allosterically communicates with carbohydrate binding site.

A major challenge in elucidating allosteric networks is that often the two functional states at the active site are not realized by two distinct static structures but rather by a shift of the conformational ensemble at the active site [57]. This is particularly true for Langerin in which the two states correspond to lower and higher Ca^{2+}-affinity, where both states are in principle capable of binding Ca^{2+}. We analyzed the Ca^{2+} affinity under varying pH using NMR spectroscopy [30]. Changes in the local conformational distribution around a nucleus due to Ca^{2+} binding were detected as perturbations in its chemical shift [58]. Firstly, we found that, in contrast to other CLRs [19], the proline of the long loop of langerin is in cis 75% even in the absence of Ca^{2+}. This eliminated the possibility that the cis/trans-isomerization acts as a pH sensor. However, even amino acids distal

from the primary site were affected by Ca^{2+} binding. We quantified this transfer of information from the Ca^{2+} binding to these distal amino acids using molecular dynamics simulations and mutual information (MI) analysis. MI measures to which extent the conformation of an internal coordinate q_i influences the distribution of conformations in another coordinate q_j by comparing the actual joint probability distribution $p(q_i, q_j)$ to the hypothetical joint probability distribution of two fully independent coordinates $p(q_i)p(q_j)$. Both distributions can be determined from molecular dynamics simulations, which makes MI analysis a powerful tool for the computational elucidation of allosteric mechanisms [59,60].

This combined approach of protein NMR, side-directed mutagenesis and molecular dynamics simulation, revealed a conserved, allosteric network of communicating amino acid side chains located distal from the neck region. The MI-network mirrors the CLR-fold of Langerin. In particular, the long loop and the short loop appear as tightly connected clusters in the MI network. In the simulations, these two regions are in contact via a number of transient hydrogen bonds. Yet, from the MI analysis one has to conclude that little information is transmitted across these hydrogen bonds. The only exception is the connection between histidine 294 in the long and lysine 257 in the short loop. Indeed, we could identify the hydrogen bond between the side chains of these two residues, which is broken if H294 is protonated at around pH 5.5, as the critical pH-sensor. This is corroborated by the fact the pH-sensitivity of the Ca^{2+} affinity is drastically decreased in the H294A mutant.

Overall it was surprising to find that perturbing the network increased Ca2+ affinity, which suggests that the architecture of the CRD originally provided higher affinities and to adjust for endosomal release at defined pH and Ca2+, such allosteric modulation was implemented [30]. Interestingly, this network is also addressed in the absence of Ca2+, when heparin fragments are recognized likely by a secondary site [48,61]. The availability of such secondary sites then allowed us to identify the first allosteric inhibitor of langerin [50]

Figure 3: Show our work on langerin

Example 3: DC-SIGN

DC-SIGN is a homotetrameric, endocytic CLR expressed by dendritic cells and macrophages. It harbors three Ca2+ binding sites and of which the canonical site promotes the recognition of high mannose glycans as well as fucosylated oligosaccharides as found in Lewis type antigens. Recently, similar to Langerin, also galactose was shown to bind to DC-SIGN despite its EPN motif [62]. This CLR is involved in pathogen recognition via carbohydrate antigens such HIV [63] and has sparked interest in this receptor for entry inhibition [48,64]. Many reports focus on carbohydrate- analogs using the core monosaccharide as an anchor, growing it into adjacent secondary sites. In an elegant approach, using phage display, primary site binding mannose was used to anchor a secondary site-binding peptide yielding high specificity for this CLR [65]. However, only a limited number of drug-like DC-SIGN inhibitors in the low micromolar affinity regime [51,66,67]. Since the identified chemotypes had no similarity to carbohydrates and no structural information was available, the inhibition mechanism remained unclear. We recently screened a fragment library using several orthogonal methods against DC-SIGN and identified five secondary sites that potentially influence the primary site following an allosteric mechanism [51]. One of these secondary sites was recently verified [68]. Taken together, allosteric modulation of DC-SIGN might also be a suitable mode of inhibition of this protein target, steering our attention away from the challenging primary site [51,67,69].

Concluding remarks

Taken together the molecular architecture of the CLRs encodes for a multitude of functionalities. Under the pressure of a co-evolving self-glycome and microbial polysaccharides a commonly shared central motif, the Ca2+ coordination, is responsible for weak carbohydrate recognition, while the underlying Ca2+ affinity is fine tuned to enable extracellular cargo binding and intracellular release. The low affinity of the primary site is overcome by multimerization of the CRD into receptor oligomers, an additional process that can be pH-dependent. Furthermore, the pH sensitivity of the CRD itself must be adjusted, while the general architecture of the Ca2+ cage is highly conserved. Recent insights into CLR structural biology have generated a picture of a dynamic scaffold with mobilities on various time scales, fine-tuned to accompany all of the above-mentioned tasks. Since such dynamics and the availability of secondary sites is often tightly coupled to receptor function, new ways for addressing these lectins as drug targets are opening up.

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Fig. 1

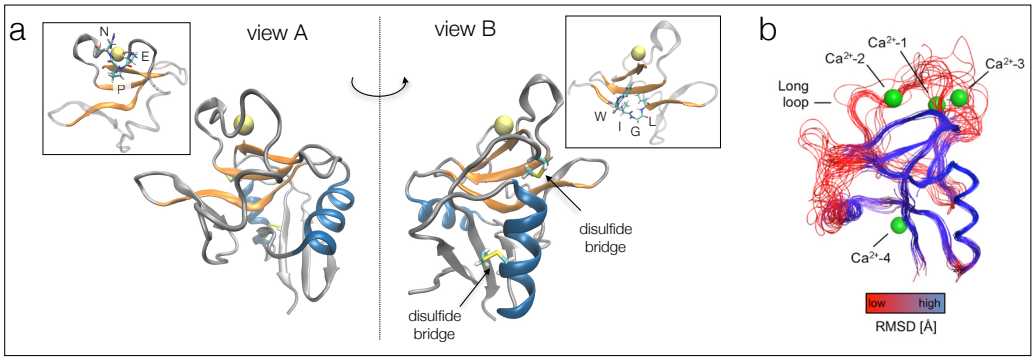

Fig. 2

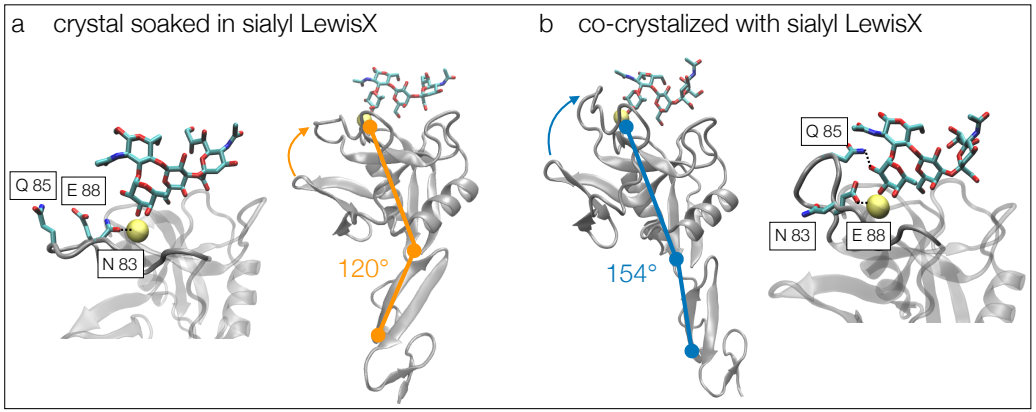

Fig. 3