GAG positioning on IL-1RI; A mechanism regulated by dual effect of glycosylation

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Abstract

IL-1RI is the signaling receptor for the IL-1 family of cytokines that are involved in establishment of the innate and acquired immune systems. Glycosylated extracellular (EC) domain of the IL-1RI binds to agonist such as IL-1β or antagonist ligands and the accessory protein to form the functional signaling complex. Dynamics and ligand binding of the IL-1RI is influenced by presence of the glycosaminoglycans (GAGs) of the EC matrix. Here a combination of molecular dockings and molecular dynamics simulations of the unglycosylated, partially N-glycosylated and fully N-glycosylated IL-1RI EC domain in the apo, GAG-bound and IL-1β-bound states were carried out to explain the co-occurring dynamical effect of receptor’s glycosylation and GAGs. It was shown that the IL-1RI adopts two types of “extended” and “locked” conformations in its dynamical pattern, and glycosylation maintains the receptor in the latter form. Maintaining the receptor in the locked conformation disfavors IL-1β binding by burying its two binding site on the IL-1RI EC domain. Glycosylation disfavors GAG binding to the extended IL-1RI EC domain by sterically limiting the GAGs degrees of freedom in targeting its binding site, while it favors GAG binding to the locked IL-1RI by favorable packing interactions.

Key words: glycosaminoglycan, glycosylation, IL-1RI, molecular dynamics simulation

Introduction

Interleukin 1 family of proteins is the key player in establishing inflammatory and immune responses (Arend et al. 2008; Sims and Smith 2010). Among the 11 known family members, interleukin 1β (IL-1β) is involved in the acute inflammatory and autoimmune conditions resulting in the activation of mitogen-activated protein kinase and kappa-light-chain-enhancer of activated B cells in the cytosol (Bowie and O’Neill 2000; Krumm et al. 2014). Thus, inhibition of IL-1β is of great pharmacological importance (Braddock and Quinn 2004). IL-1β activates its target cells by binding to the IL-1 transmembrane receptor type 1 (IL-1RI) with high affinity to form the signaling complex (Figure 1A) (O’Neill 2008; Wang et al. 2010). The complex will obtain its biological function upon the recruitment of another transmembrane receptor known as the IL-1 receptor accessory protein (IL-1RAcP) (Wang et al. 2010). Resolved crystal structure of IL-1β is composed of 12 β-strands forming a tetrahedron (Priestle et al. 1989) resembling the structure of heparin-binding growth factors (Murzin et al. 1992) (Figure 1A). IL-1β binds to IL-1RI extracellular (EC) domain that is composed of three immunoglobulin-like subdomains (D1, DII, and DIII) (Wang et al. 2010) via several connecting loops (Vigers et al. 2000; Wang et al. 2010; Thomas et al. 2012) (Figure 1A). Biochemical studies have suggested that upon the immune responses, IL-1β is retained on the target cells by interacting with the glycosaminoglycans (GAGs) of the EC matrix (Ramsden and Rider 1992). Due to the fact that IL-1β could only trigger the cellular signals while it is in complex with its signaling receptor (IL-1RI), it is crucial to first elucidate the dynamics of IL-1RI in the GAG-bound and IL-1β-bound states. As the GAGs of the EC matrix that are bound to the receptor would play the most critical role in the IL-1β-IL-1RI binding mechanism and activation of the subsequent immune response.
GAGs are anionic polysaccharides essential for several biological processes (Samsonov and Pisabarro 2016). Most noticeable GAGs binding to IL-1β includes hyaluronic acid, heparin, and dermatan sulfate (Gogly et al. 1998; Ramsden and Rider 1992). However, the mechanism of GAG binding to IL-1β and IL-1RI is mainly unclear (Figure 1B).

Furthermore, it is known that the EC domain of IL-1RI is glycosylated in vivo (Vigers et al. 2000; Thomas et al. 2012; Krumm et al. 2014). Glycosylation is a posttranslational modification that occurs in several transmembrane receptors (Mariño et al. 2010; Azimzadeh Irani 2018) and has shown to be essential for their proper folding, arrangement within the cell membrane, and ligand binding (Azimzadeh Irani et al. 2017; Kaszuba et al. 2015). The process is the attachment of oligosaccharides to nitrogen atom of asparagine/arginine sidechain or to hydroxyl oxygen of serine/threonine/tyrosine of proteins by the oligosaccharyl transferase in the endoplasmic reticulum (Kornfeld and Kornfeld 1985; Shental-Bechor and Levy 2008; Mariño et al. 2010). Crystallographic studies have suggested important structural role for the glycans in maintaining the interactions with IL-1RI in the fully glycosylated system is not surprising as six oligosaccharides are attached to different subdomains of the receptor (Fig S.20).

Results

Decreased flexibility of the IL-1RI EC domain upon glycosylation

In the rest of the text and figures the unglycosylated, partially glycosylated, and fully glycosylated EC domain of the IL-1RI will be referred to as IL-1RI, gIL-1RI, and fgIL-1RI, respectively. Time-dependent and average overall root-mean-squared deviation (RMSD) shows that the IL-1RI is at least 2 Å less flexible upon glycosylation in the second half of the simulation times (Figure 2A and C). Among the two glycosylated forms of the receptor, fully glycosylated one is the least flexible (Figure 2A and C). Subdomain-wise RMSD of the IL-1RI three subdomains shows that the decrease in the flexibility of the receptor is mainly due to the less flexible dynamics of DI and DIII after glycosylation (Supplementary data, Figure S1). The partially glycosylated IL-1RI shows to be less flexible mainly by its DI-reduced RMSD values (Supplementary data, Figure S1). This was expected as the only oligosaccharide in the partially glycosylated model was attached to DI and it acts as a shield that covers DI throughout the simulations time (Supplementary data, Figure S2). Whereas, the fully glycosylated receptor presented reduced RMSD values in both DI and DIII (Supplementary data, Figure S1). The noticeable decrease of flexibility in the fully glycosylated system is not surprising as six oligosaccharides are attached to different subdomains of the receptor (Fig S.20).

All simulations and average root-mean-squared fluctuations (RMSF) plots of the Ca atoms are also supportive of the gIL-1RI and fgIL-1RI decreased fluctuations (Figures 2B and D). Similar to the RMSD plots, although all the three subdomains present lower fluctuations in the glycosylated simulations, the most dramatic decreases were observed in DI and DIII for the fully glycosylated receptor and in DI for the partially glycosylated one (Figures 2B and D).
GAG-glycosylation regulate IL-1RI functions

Fig. 2. Decreased flexibility of gIL-1RI. (A) Backbone RMSD plots of IL-1RI, gIL-1RI, and fgIL-1RI calculated from all replicate simulations of each system are shown in blue, red, and green, respectively. (B) Cα atoms RMSF plots of IL-1RI, gIL-1RI, and fgIL-1RI are shown in blue, red, and green, respectively. DI, DII, and DIII domains are specified with red, blue, and yellow transparent boxes, respectively. (C) Average backbone RMSD plots of IL-1RI, gIL-1RI, and fgIL-1RI calculated from all replicate simulations of each system are shown in blue, red, and green, respectively. Standard error values of each plot are shown with transparent lines. (D) Average Cα atoms RMSF plots of IL-1RI, gIL-1RI, and fgIL-1RI are shown in blue, red, and green, respectively. DI, DII, and DIII domains are specified with red, blue, and yellow transparent boxes, respectively. Standard error values of each plot are shown with transparent lines.

gIL-1RI reduced flexibility leads to rigid body motion of DIII toward DI–DII and acquirement of the “locked” conformation

It is known that in addition to the typical extended conformation (Vigers et al. 1997; Wang et al. 2010; Thomas et al. 2012), IL-1RI adopts a compact conformation that could be a regulating mechanism for its signaling (Vigers et al. 2000). All the simulations presented here were initiated from the extended conformation (IL-1RI–IL-1β complex, PDB 4DEP).

Interestingly, the gIL-1RI and fgIL-1RI show a decrease in DI and DIII RMSD values calculated from the simulation times (Supplementary data, Figure S1). Visualization of the MD trajectories showed that the decrease in the DI–DIII flexibility upon glycosylation leads to the upward motion of DIII toward DI and DII (Supplementary data, Figures S3–S5). This motion leads to the conformational change of the receptor from “extended” to “locked”. Superimposition of the final structure of each simulation onto the starting structure and the reported RMSD values (Supplementary data, Figures S3–S5) indicates that although the unglycosylated system is overall more flexible with higher RMSD values, yet this higher flexibility does not necessarily lead to the extended to locked conformational change.

Here it was shown that the locked conformation is more frequent in the dynamical patterns of gIL-1RI (three out of six simulations) and fgIL-1RI (two out of three simulations) compared to the IL-1RI (one out of six simulations) (Supplementary data, Figures S3–S5). The above observation shows that the fully N-glycosylated IL-1RI presents a similar but more pronounced dynamical pattern of the partially glycosylated one. By presenting a noticeably less flexible RMSD, RMSF values (Figure 1) and by adopting the locked conformations in two out of three simulations. One should note that in the simulations presented here, the IL-1RI does sample the extended and locked conformations in both unglycosylated and glycosylated simulations (Supplementary data, Figures S3–S5). Thus, the locked conformation is not a specific feature of the gIL-1RI/fgIL-1RI dynamics and the extended–locked conformational change seems to be a robust mechanism for the receptor’s regulation in vivo.

In order to characterize the observed conformational change upon glycosylation with respect to the “extended to locked” transition, the distance between the center of masses of DI and DIII was calculated (Figure 3). Comparison of the calculated distance values from the simulations with the DI–DIII distance in the locked crystal structure shows a clear shift toward the locked conformation upon
glycosylation (Figure 3). This shift occurs at a higher rate (after 10 ns) and with a higher frequency in the fully glycosylated simulations (Figure 3). The decrease in the DI–DIII distance is driven by the appearance of novel motions within DI–DIII upon glycosylation (Figure 4). Dynamics cross correlation map (DCCM) of the three systems shows that the most noticeable alteration in the partially glycosylated system is the anticorrelated motions occurring between residues “135–145, 175–185” of DI and DIII (Figure 4; Supplementary data, Figure S6). Residues 135–145 form a loop and a short β-strand in the midst of DI. The abovementioned loop is located directly downstream of the IL-1β binding site I on the receptor (Krumm et al. 2014). The strong anticorrelated motion of these residues would disturb the IL-1β binding to the receptor upon glycosylation and could decrease the gIL-1RI–IL-1β binding affinity. The other set of anticorrelated motion occurs in a tone-downed fashion between residues “45–65” of DI and “225–235, 275–285” of DIII (Figure 4; Supplementary data, Figure S6). Residues “45–65” form a long surface loop and two short antiparallel β-strands in the N-terminal segment of DI. Movement of this long loop toward DIII is expected as no energy will be required to disturb the protein fold in this region (Supplementary data, Figure S6). Residues “175–185, 225–235” and “275–285” are all β-strands that move toward each other in a hinge motion manner upon glycosylation. Stability of the secondary structures in both IL-1RI and gIL-1RI shows that the extended to locked conformational change is a rigid body motion between DI–DII and DIII (Supplementary data, Figure S8 and S9).

The fully glycosylated receptor shows a set of more systematic anticorrelated motions between DI and DIII (Figure 4; Supplementary data, Figure S7). These motions occur between residues “135–165” of DI and “235–245, 265–275 and 295–305” of DIII (Figure 4; Supplementary data, Figure S7). Residues “135–165” contain two long loops that connect three β-strands in the midst of DI (Supplementary data, Figure S7). The not strictly ordered conformation of these loops facilitates the rigid body motion between DI and DIII. While residues “235–245” and “295–305” are two sets of β-strands and residues “265–275” forms a loop in DIII (Supplementary data, Figure S7), all of these regions are involved in interleukin binding site II on the receptor (Wang et al. 2010; Thomas et al. 2012). Thus, the anticorrelated motion that is induced by the glycan moieties would disfavor interleukin binding to the fully glycosylated IL-1RI. Similar to the partially glycosylated and unglycosylated systems, the stability of the secondary structures in the fully glycosylated receptor supports the rigid body motion mechanism between DI and DIII (Supplementary data, Figure S8 and S9).

Glycosylation disfavors GAG binding to the extended IL-1RI while supporting the locked IL-1RI binding to GAG by favorable packing interactions

Docking results of the IL-1RI–GAG and gIL-1RI–GAG show that the GAG-binding site is mainly located within the positively charged pocket formed by DI and DII (Figure 5; Supplementary data, Figure S10) as predicted in this study earlier (see Methods section). Dynamics and binding affinity of the IL-1RI/gIL-1RI in the GAG-bound state were examined by performing a series of 50 ns MD simula-
GAG-glycosylation regulate IL-1RI functions

Fig. 5. GAG docking to the gIL-1RI. Most populated binding modes with smallest RMSD obtained by DBSCAN clustering are shown with violet, purple, and green sticks. The oligosaccharide glycan attached to the receptor and the IL-1RI are shown with atom name sticks and gray surface, respectively. (A) shows GAG-binding modes to the minimized glycosylated IL-1RI model. (B–F) present GAG-binding modes to the gIL-1RI conformations obtained after 10, 20, 30, 40, and 50 ns of MD simulation.

Fig. 6. Backbone RMSD plots of IL-1RI and gIL-1RI are shown in blue and red, respectively. The values were calculated from the GAG-bound to IL-1RI and gIL-1RI simulations.

tions (Supplementary data, Table SIII) using the GAG–IL-1RI and GAG–gIL-1RI docking outputs (Supplementary data, Figures S11 and S12). These GAG–IL-1RI and GAG–gIL-1RI conformations were all selected from the top five most populated clusters of each system. The selection criteria were to choose the most distinct binding modes within the top five clusters in order to enhance the conformational sampling (Supplementary data, Figures S11 and S12).

Backbone RMSD of the IL-1RI and gIL-1RI from the GAG-bound simulations shows similar results to the apo simulations (Figure 6). Decreased flexibility of the GAG-bound gIL-1RI system supports the same observations from the apo gIL-1RI receptor that presents less flexible dynamics upon glycosylation. The average RMSD of the receptor is ~5 Å lower in the glycosylated receptor. Similar to the apo simulations, the most dramatic decrease occurs in the DI that is shielded by the attached oligosaccharide glycan (Supplementary data, Figure S13). The role of glycosylation in the GAG binding to IL-1RI was examined by calculating the GAG–IL-1RI/gIL-1RI binding-free energies from the simulations (Figure 7). Among the IL-1RI–GAG simulations, binding is most favorable when the receptor is in the extended unglycosylated state (ΔG = −22, −20, and −18 kcal/mol). In this state, the GAG is properly coordinated in the positively charged pocket (Figure 7, ΔG = −22 kcal/mol). Positioning the GAG on the surface of DI that is shielded with the glycan moiety in the gIL-1RI is the least favorable binding mode (Figure 7, ΔG = −0.1 kcal/mol), suggesting that the GAG binding to the IL-1RI does not interfere in glycosylation and its structural effects on the receptor functioning. GAG binding to the locked IL-1RI is shown to be the second least favorable binding mode (Figure 7, ΔG = −5 kcal/mol) due to the spatial barrier formed by DIII toward the positively charged GAG-binding pocket (Figure 7). In the glycosylated systems the gIL-1RI–GAG-binding energies are in general less favorable compared to the unglycosylated ones (Figure 7, ΔG = −15, −17, and −19 kcal/mol), suggesting that glycosylation does not support GAG binding to the IL-1RI. Mainly because the presence of the flexible oligosaccharide glycan that is attached to the proximity of the positively charged pocket limits the GAG’s spatial degrees of freedom in binding to the pocket.

However, glycosylation interestingly plays an opposite role in the GAG binding to the locked IL-1R conformation. Dynamics of the glycosylated receptor show that the GAG binding is more favorable to the locked gIL-1RI compared to the locked IL-1RI (Figure 7, ΔG = −5 and −17 kcal/mol). The energetic components of the calculated binding-free energies show a significant increase in the
Van der Waals (VDW) energy of the locked gIL-1RI–GAG binding compare to all other extended and locked glycosylated and unglycosylated systems (Supplementary data, Figure S13). A close look at the energetic components shows that VDW energy is the main driving force in the GAG binding to both IL-1RI and gIL-1RI (Supplementary data, Figure S13). Specifically there is a dramatic increase in the VDW energy of the locked gIL-1RI–GAG binding (−65 kcal/mol) compare to the extended gIL-1RI–GAG (−41 kcal/mol) and the locked IL-1RI–GAG complexes (−30 kcal/mol), suggesting that the favorable packing interactions in the locked glycosylated receptor maintain the GAG in its binding pocket (Figure 7). It is worth mentioning again that the GAG–IL-1RI binding is indeed most favorable in the extended unglycosylated state. Where the absence of the oligosaccharide glycan allows the GAG to target its binding pocket, bind to it and remain in it. However, while the receptor is in the locked conformation (it was shown in the previous sections that locked is the dominant conformation in the gIL-1RI dynamics), glycosylation supports the GAG binding by establishing favorable packing interactions. One should note that there is a threshold for the supportive role of glycosylation in the GAG binding to the IL-1RI with respect to the receptor compaction (Figure 7, ΔG = 19 kcal/mol). Meaning, if the locked receptor adopts a highly compact configuration, the electrostatics repulsion is so high (Supplementary data, Figure S14, ΔE = 333 kcal/mol) that cannot be overcome by the favorable packing interactions.

IL-1β binding stabilizes the receptor and glycosylation could indirectly regulate the IL-1β–gIL-1RI binding affinity

IL-1β-bound simulations show that IL-1RI is significantly stabilized upon ligand binding (Supplementary data, Figure S15A). Comparing the overall RMSD plots of IL-1RI/gIL-1RI/gfIL-1RI in the apo and GAG-bound states (Figures 1 and 6) to the IL-1β-bound simulations plots shows the dramatic 10 Å decrease in the scale of these plots. This is expected due to the formation of several interactions between IL-1β and the receptor in its two binding sites (I and II) (Krumm et al. 2014). IL-1β binding to the IL-1RI via sites I and II maintains the receptor conformation in the extended form through all the unglycosylated, partially glycosylated, and fully glycosylated simulations time. Furthermore, backbone RMSD plots of the IL-1β-bound to IL-1RI, gIL-1RI, and fgIL-1RI simulations are in agreement with the previous observations showing that the receptor is less flexible upon glycosylation (Supplementary data, Figure S15A). Although the decreased flexibility is not as significant (overall ∼2 Å) and as persistent as the apo and GAG-bound glycosylated receptor simulations. This is again related to the overall less flexible dynamics of the complex that is induced by the ligand binding. To examine the ligand dynamics upon glycosylation of the receptor, IL-1β backbone RMSD plots were calculated from all simulations (Supplementary data, Figure S15B). It was shown that IL-1β presents a similar dynamical pattern in all of the IL-1RI/gIL-1RI/gfIL-1RI-bound simulations except for one of the glycosylated receptor simulations. In this simulation, the cytosine’s flexibility has slightly (0.5 Å) increased in the last 60 ns of the time (Supplementary data, Figure S15B). To back up this observation, the binding-free energies of IL-1β to IL-1RI, gIL-1RI, and fgIL-1RI were calculated from the simulations.

Due to the highly stabilized dynamics of the IL-1β-bound simulations, binding-free energy and calculation of its energetic components was performed over the last 50 ns of each simulation (Supplementary data, Figures S16 and S17) (see Methods section for details).

The average of all calculations for the unglycosylated and partially glycosylated systems shows that the binding-free energy is slightly more favorable after glycosylation (Supplementary data, Figure S16).

The difference between the two average values of the unglycosylated and partially glycosylated is 2 kcal/mol (ΔG of IL-1β–IL-1RI = −110; ΔG of IL-1β–gIL-1RI = −112), and the standard error in both calculations is 11. By subtracting the standard error from the difference between the two averages, it could be explained that the binding-free energies are in the same range with and without glycosylation. The binding-free energy of 1β–IL to the fully glycosylated receptors also falls within the same range (ΔG of IL-1β–fgIL-
IβRI = −101). Hence, glycosylation does not directly favor or disfavor IL-1β binding to IβRI. However, it could indirectly disfavor IL-1β binding by supporting the extended to locked conformational change as explained before. As the IL-1β could only bind to the extended conformation of the receptor.

**Discussion**

IL-1 family is nearly the most important cytokines involved in the innate and acquired immune systems (Boraschi et al. 2018; Dinarello 2018; Mantovani et al. 2018). By including 11 members, 10 receptors (Boraschi et al. 2018), various up and down regulatory mechanisms, and signaling features (Sims et al. 1988), IL-1 family lays out an utterly complex query in molecular and structural biology (Finzel et al. 1989; Priestle et al. 1989; Schreuder et al. 1997; Vigers et al. 1997, 2000; Sims and Smith 2010; Liu et al. 2013). Activation of the IL-1-dependent pathways in the target cell is triggered by agonist IL-1α/IL-1β binding (Boraschi et al. 2018) to the EC portion of the only known signaling receptor, IβRI (Sims et al. 1988; Krumm et al. 2014).

A couple of mechanisms had been proposed for withholding the activity of IL-1 complexes in the cell. One is binding of the receptor antagonist IL-1Ra to the EC domain of the IβRI that results in the lack of necessary conformational changes for recruitment of the accessory protein (Vigers et al. 1994; Schreuder et al. 1997). The other is high-affinity binding of the receptor antagonists to the IβRII that acts as a decoy receptor (Colotta et al. 1993; Garlanda et al. 2013; Boraschi et al. 2018) due to its very short IC segment and loss of the TIR domain (Peters et al. 2013). Another proposed downregulating mechanism is interference of the IL-1R8 in the IL-1/IL-1RI binding to the accessory protein via EC and IC contacts (Wald et al. 2003; Garlanda et al. 2004; Qin et al. 2005; Bulek et al. 2009). Thus, the only upregulating mechanism of the IL-1/IL-1RI binding bears great importance in both inhibition and activation of the immune responses. Several crystal structures have characterized the EC domain of the IβRI and its agonist ligand, IL-1β (Vigers et al. 1997; Adamek et al. 2005; Wang et al. 2010). Furthermore, previous structural and molecular studies explained that receptor glycosylation and GAGs of the EC matrix play critical roles in proper functioning and ligand binding of the IL-1RI (Ramsden and Rider 1992; Wang et al. 2010), yet the atomistic details remained unclear. Here the most comprehensive computational study to the best of the authors’ knowledge is presented to explain the interplay between GAG-glycosylation in the IL-1RI dynamics and function. It was shown that glycosylation of the IL-1RI maintains the receptor in the compact locked conformation and hence disfavors GAG and IL-1β binding by burying the two binding sites on the IL-1RI EC domain. The extended to locked transition could be another downregulating mechanism that is mediated by glycosylation of the IL-1RI. Unraveling this atomistic mechanism was only possible by monitoring the dynamics of glycosylated receptor that was performed here for the first time to the best of the authors’ knowledge. It is worth mentioning that both glycosylated and unglycosylated simulations were initiated from the extended conformation, and yet the transition toward the locked conformation in the glycosylated receptor proves the downregulating role of glycosylation in the IL-1RI functioning. In the equilibrium between locked and extended conformation, it is impossible for the IL-1β to bind the locked conformation due to the complete occlusion of its binding site. Whereas GAG binding to the IβRI is regulated by a dual mechanism in the way that GAG binds both the extended and locked IβRI with less favorable binding-free energy in the latter form. The receptor is glycosylated in vivo, and it was shown that the receptor is maintained in the locked conformation upon glycosylation. Thus the locked conformation that is the dominant form of the receptor binds to GAG more favorably upon glycosylation. This more favorable binding is due to the stronger packing interactions close to the GAG-binding site on the receptor (Figure 5). The dual mechanism proposed here for the role of glycosylation in GAG binding to the IL-1RI illustrates that glycosylation does not favor GAG binding to the receptor in its essence. However, when the receptor adopts the locked conformation, glycosylation supports GAG binding by favorable packing interactions close to the GAG-binding site. These findings could shed light on the atomistic details of the two less-studied yet essential factors of the cellular biology that are protein glycosylation and GAGs of the EC matrix. Although each of these factors have been extensively studied experimentally (Cummings et al. 1983; Ramsden and Rider 1992; Takahashi et al. 2008; Liu et al. 2011; Koehler et al. 2017) and in the recent years computationally (Kaszuba et al. 2015; Samsonov and Pisabarro 2016; Azimzadeh Irani et al. 2017), the atomistic details of their simultaneous action had not been addressed before. Here the glycosylation-GAG interplay in dynamics and ligand binding of the IL-1RI that is one of the most important signaling receptors in the immune systems was explained at the atomic level. The proposed atomistic mechanism indicates the usefulness of computer simulations in describing the dynamical properties of biomolecules and initiates a novel path in developing anti-inflammatory drug design by considering the structural effects of the glycosylation and GAGs.

**Materials and methods**

**Construction of the glycosylated and unglycosylated IL-1RI models**

Chains D and E of the PDB 4DEP (Vigers et al. 1997) were selected as the starting structures of the IL-1β and unglycosylated IL-1RI, respectively. The two missing residues in chain E (Lys35 and Gly36) were built with Modeller in Discovery Studio 3.5 (Accelrys Software Inc. 2007), and the templates were taken from PDB 1G0Y (Vigers et al. 2000). As IL-1RI is not fully glycosylated in 4DEP PDB structure, GLYCAM server (Woods Group 2005-XXXX) GLYCAM Web, Complex Carbohydrate Research Center) was used to check all the exposed potential asparagines for N-linked glycosylation (Supplementary data, Table SI). To focus on the role of glycosylation on GAG binding to IβRI, the potential asparagines located in the predicted GAG-binding site (see the GAG docking to IβRI section below) and its proximity were considered for glycosylation. This would include the sites locate in DI and DII that are Asn83 and Asn176, respectively (Supplementary data, Table SI). It is mentioned in the literature that the glycans in the vicinity of the IL-1RAcp binding site (here Asn176) maintains the receptor-accessory protein interactions (Wang et al. 2010; Krumm et al. 2014). Thus for the purpose of this study, Asn83 was glycosylated with a common core (Supplementary data, Figure S20) (Woods Group 2005-XXXX) GLYCAM Web, Complex Carbohydrate Research Center). This model that was only glycosylated at Asn83 was mentioned as the “partially glycosylated” or gIL-1RI in all figures and text. The “fully glycosylated” or fIL-1RI model was glycosylated with the same type of glycan at all of the glycosylation sites including Asn83, Asn176, Asn217, Asn232, Asn246, and Asn280 (Supplementary data, Figure S20 and Table SI).
GAG docking to the glycosylated and unglycosylated IL-1RI

A hexamer of sulfated hyaluronan is a derivative of hyaluronan (Supplementary data, Figure S18 and Table SII) that is a common type of GAG binding to IL-1β (Ramsden and Rider 1992) was selected for docking to the unglycosylated and glycosylated forms of IL-1RI. This sulfated hyaluronan that is a GAG mimetic was used here to facilitate further in-silico attempts toward developing anti-inflammatory inhibitors targeting IL-1RI that are supported by the previous experimental studies (Barbucci et al. 1994; Hintze et al. 2012).

The GAG model was obtained and parameterized with the GLYCAM model (Woods Group (2005-XXXX) GLYCAM Web, Complex Carbohydrate Research Center) and the GLYCAM forcefield (Kirschner et al. 2008) for docking and simulations. To predict the plausible GAG-binding site electrostatic potential map of IL-1RI was calculated using the Adaptive Poisson–Boltzmann Solver (APBS) (Morris et al. 1998) within the PyMOL package (Figure 8). The most extensive positively charged region is a pocket formed by DI and DII of the receptor (Figure 8) and it is expected for the negatively charged GAG to bind to this pocket (Figure 8). Autodock 3.05 (Morris et al. 1998) was used to dock HA463 to unglycosylated and glycosylated IL-1RI, while the GAG molecule was treated flexible in the calculations (Supplementary data, Figure S19). The grid box was set to cover DI and DII (predicted GAG-binding sites) with enough distance from the surface residues for the GAG to explore the area completely. In this criterion IL-1RI is considered to be rigid. Although this is a well-accepted criterion for GAG docking to the proteins (Gehrke and Pisabarro 2015; Rother et al. 2016), it would not be efficient for the glycosylated IL-1RI as the glycan moieties are known to be very flexible (Azimzadeh Irani 2018). This issue was addressed by selecting various conformations of the glycosylated receptor and performs molecular docking for each conformation separately. These conformations were obtained from a 50 ns conventional MD on the glycosylated IL-1RI in which a conformation was selected in every 10 ns. The grid box dimensions for the unglycosylated IL-1RI and glycosylated IL-1RI were set to 126 ± 110 = 126 Å and 126 ± 126 = 126 Å, respectively, with spacing of 0.5 Å. One hundred independent runs of the Lamarckian genetic algorithm with an initial population size of 300 were carried out (Rother et al. 2016) and top 50 docking solutions were selected for clustering with DBSCAN algorithm (Ester et al. 1996).

MDs simulations

All simulations presented here were carried out with AMBER16 Molecular Dynamics package (Pearlman et al. 1995; Case et al. 2018). xleap module of AMBER package was used for creating parameters and topologies required for MD. Protein residues of IL-1RI and IL-1β were presented with AMBER ff14SB force field (Maier et al. 2015), Glycan units of the oligosaccharide attached to the IL-1RI and the GAG were presented with GLYCAM-06j force field (Kirschner et al. 2008). Apo, IL-1β-bound, and GAG-bound forms of glycosylated and unglycosylated IL-1RI were immersed in a box of TIP3P waters keeping at least 10 Å distance from the solute atoms to the edge of the box, then all systems were neutralized by adding counter ions. All systems were minimized in two cycles of 1500 and 6000 steps of steepest descent/conjugate gradient (Leach AR and Leach A 2001). The receptor and ligand were restrained in the first cycle to relax the waters and ions. The whole complex was then minimized in the second cycle with no restraint. Next, the systems were heated up to 300 K and equilibrated for 250 ps. Production runs were carried out for 50/100 ns in six/three replicates or single simulation under isothermal-isobaric (NPT) ensemble scheme (Supplementary data, Table SII). Constant pressure dynamics was carried out by using a weak coupling algorithm (Berendsen et al. 1984) with the reference pressure set to 1 bar and maintained with a relaxation time of 1 ps. The temperature was maintained at 300 K using Langevin dynamics (Pastor et al. 1988; Loncharich et al. 1992) with a collision frequency of 1 ps⁻¹ K⁻¹. For the GAGs in each GAG-bound simulation, pyranose rings were harmonically restrained (Rother et al. 2016) The nonbonded interactions (electrostatics and VDW) were computed with a 9 Å cut-off value and the particle mesh Ewald method was applied to simulate the long-range electrostatic interactions (Azimzadeh Irani et al. 2017).

Analyses of the MDs trajectories

RMSDs, RMSFs, and domain–domain distances were calculated with the cpptraj module of AMBER17 tools (Roe and Cheatham 2013; Case et al. 2018) over the 50/100 ns of the simulation time. RMSD and the distance between center of masses of DI and DII were calculated by considering the backbone atoms (Cα, C, and N). RMSF of each system was calculated for the Cα atoms. All trajectories were fitted to the starting conformation in order to present the conformational changes during the simulation time. DCCMs were calculated with Bio3D package (Grant et al. 2006; R Core Team 2013) for the Cα atoms of the apo glycosylated and apo unglycosylated IL-
Two mega trajectories were created by concatenating the six replicates of 50 ns MD for each system to perform the calculations. Although the glycans were not included in the calculations, their global effect is implicit in the dynamical pattern of the receptor. Secondary structures were calculated using the STRIDE method (Heinig and Frishman 2004). Free energies of the GAG and IL-1β binding to the IL-1RI in the glycosylated and unglycosylated forms were calculated with AMBERTOOLS17 MMPBSA.py script (Case et al. 2018) using the MMGBSA method (Feig and Brooks III 2004; Hou and others 2011; Homeyer and Gohlke 2012). The solvated single trajectory of the ligand–receptor was used for obtaining the coordinates of the ligand, receptor, and the complex (Sun et al. 2014) for each system as explained in detail before (Azimzadeh Irani et al. 2017). For the GAG-glycosylated IL-1RI and GAG-unglycosylated IL-1RI complexes binding-free energies were calculated from the last 10 ns of the simulation times. Using 10 conformations obtained in each 1 ns of this time frame the average values of all 10 calculations were reported. The IL-1β-bound complexes were treated differently as the whole receptor is significantly stabilized upon the IL-1β binding. In the way that the last 50 ns of each simulation was used for binding-free energy calculation using 200 frames for each calculation. All figures were made with VMD (Humphrey et al. 1996) and PyMOL (DeLano 2002).

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Conflict of interest statement

None declared.

Abbreviations

APBS, Adaptive Poisson–Boltzmann Solver; DCCM, Dynamics cross correlation map; EC, Extracellular; GAGs, Glycerosaminoglycans; IL-1RAcP, IL-1 receptor accessory protein; IL-1RI, IL-1 transmembrane receptor type I; IL-1β, Interleukin 1β; IC, Intracellular; MDs, Molecular dynamics; TIR, Toll-interleukin receptor

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