Structure and ligand-induced conformational change of the 39 kD glycoprotein from human articular chondrocytes

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Abstract

The 39 kD human cartilage glycoprotein (HCGP39), a member of a novel family of chitinase-like lectins (Chi-lectins), is overexpressed in articular chondrocytes and certain cancers. Proposed functions of this protein include a role in connective tissue remodelling and defense against pathogens. Similar to other Chi-lectins, HCGP39 promotes the growth of connective tissue cells. The ability of HCGP39 to activate cytoplasmic signalling pathways suggests the presence of a ligand for this protein at the cell surface. There is currently no information regarding the identity of any physiological or pathological ligands of the Chi-lectins or the nature of the protein-ligand interaction. Here, we show that HCGP39 is able to bind chitooligosaccharides with micromolar affinity. Crystal structures of the native protein and a complex with GlcNAc₆ show that the ligand is bound in identical fashion to family 18 chitinases. However, unlike the chitinases, binding of the oligosaccharide ligand to HCGP39 induces a large conformational change. Thus, HCGP39 could be a lectin which binds chitin-like oligosaccharide ligands, and possibly plays a role in innate responses to chitinous pathogens, such as fungi and nematodes.
Introduction

The 39 kD human cartilage glycoprotein (HCGP39) is a secreted lectin, initially identified in articular chondrocytes [1] and synovial cells [2] and subsequently in macrophages [3], smooth muscle cells [4] and a variety of others. The protein is overexpressed in many pathological conditions involving extensive connective tissue remodelling or increased deposition of connective tissue components, such as in arthritic cartilage [1] and inflamed or hyperplastic synovium [1, 5] and fibrotic liver [6]. Increased serum levels of HCGP39 have been reported for certain types of breast and colon cancer [7, 8] and is related to poor prognosis [8]. A similar correlation has been found in patients with malignant gliomas [9], where serum levels may be indicative of the tumour burden. In patients with rheumatoid or osteoarthritis, serum HCGP39 levels show some positive correlation with disease progression, and decrease following treatment suggesting that the protein is involved in the disease process [10]. Recently, it was shown that HCGP39 is a potent growth factor, inducing cell proliferation through activation of protein kinase B (PKB) and the ERK1/2 MAP kinase signalling pathways [11]. Similar activity has been shown for an orthologous protein from guinea-pig [12] and a homolog in Drosophila [13].

HCGP39 is homologous (53% sequence identity) to the human macrophage chitinase (HCHT) and other family 18 chitinases. Structural analyses of these enzymes have shown they consist of a (βα)₈ barrel with in some cases an extra α/β domain inserted in one of the barrel loops [14, 15, 16, 17, 18]. The chitinases have a cleft lined with solvent exposed aromatic residues, where they bind their substrates, oligomers of N-acetyl glucosamine [14, 15, 19, 20, 21]. The glutamate and aspartate at the end of the conserved DxxDxDxE sequence motif are essential for catalysis [22, 23, 19, 21], and these residues are absent in HCGP39. HCGP39 does not possess chitinase
activity, but, as expected from sequence conservation, is able to bind chitin [24]. Therefore, the protein can be defined as a chitinase-like lectin (Chi-lectin).

Sequence analysis and biochemical characterisation have revealed the presence of several other Chi-lectins in humans and other eukaryotes. Chitinase 3-like 2 (YKL-39 [25], UniGene Cluster Hs.154138) is widely expressed, with a predominance in lymphoid tissues and cells. High expression levels of this family member were reported in osteoarthritic cartilage 2 [26]. Ym1 is a murine protein expressed by macrophages upon infection with the nematodal parasite Trichinella spiralis [27] and acts as a chemotactic agent for eosinophils [28]. Imaginal Disc Growth Factors (IDGFs) are a family of four Chi-lectins from Drosophila melanogaster with mitogenic activity [13]. Oviductin is a glycoprotein secreted by the oviduct [29]. These Chi-lectins share several properties: they show homology to family 18 chitinases, but lack key residues from the DxxDxDxE motif and are therefore inactive, they are secretory proteins, and thought to be involved in tissue remodelling and/or immune responses. However, the physiological ligands for these proteins have not been defined. Ym1 has been shown to interact with GlcN oligomers, yet the physiological relevance of this finding is not clear [27]. Although the native structures of Ym1 and IDGF2 have been solved [30, 31], and suggest binding of carbohydrate ligands would be possible, there is currently no structure of a Chi-lectin in complex with a ligand. The identity of the cellular receptors mediating the biological effects reported for YM-1 and HCGP39 are currently not known. Detailed structural information on the protein-ligand interactions would allow us to characterize the physiological processes affected by the Chi-lectins more clearly.

Here, we describe the high-resolution crystal structure of HCGP39, and show that it, unlike the Ym1 and IDGF2 structures, possesses a binding cleft conserved in structure and sequence with
HCHT. We demonstrate that HCGP39 interacts with chitooligosaccharides with high affinity. A crystal structure of a complex with GlcNAc₈ shows how this lectin is able to bind chitooligosaccharides, and suggests that the binding cleft is compatible with the binding of longer chitin fragments or other polymeric carbohydrates.
Methods

Structure solution

HCGP39 was purified from human chondrocytes as described previously [1], however, heparin-sepharose affinity chromatography was used as final step, following ion-exchange on DEAE sepharose and gel filtration. The protein was frozen in the buffer of the final purification step (20 mM HEPES, 0.5 M NaCl, pH 7.2) at a concentration of 1.8 mg/ml. For crystallisation, the protein solution was thawed, and concentrated to 7 mg/ml. Crystals were grown using the sitting drop vapour diffusion method, using equal volumes of protein and mother liquor (0.2 M ammonium sulfate, 0.1 M sodium citrate pH 4.6, 25% PEG 4000, 100 mM DTT). Crystals grew as bars with maximum dimensions of 0.1 x 0.1 x 0.4 mm after approximately one week. A complex with GlcNAc₈ was obtained by co-crystallisation in the same conditions, in the presence of 3 mg/ml of the ligand. All crystals were cryoprotected in 30% glycerol / 70% mother liquor prior to freezing in a cryostream. Data were collected on beamlines ID14-4 (ESRF, Grenoble) and X11 (DESY, Hamburg) and processed using the HKL package [32] (Table I). The native structure was solved by molecular replacement with AMoRe [33], using the human chitinase structure [18] as a search model against 8-4 Å data. The positions and orientations of three out of the four molecules in the asymmetric unit were found, the fourth monomer was located by a real-space search with FFFEAR [34], using the human chitinase structure as a search model. After rigid body refinement in CNS [35] using 15-3.5 Å data, the R-factor was 0.46. This was followed by model building in O [36] and further refinement in CNS, including several rounds of simulated annealing. A final round of refinement was performed with REFMAC [37], including a description of anisotropy with TLS [38]. During the initial macrocy-
icles non-crystallographic symmetry restraints and map averaging were used. Statistics of the final model are shown in Table I.

For the complex with GlcNAc, refinement was started from the native HCGP39 structure, using CNS rigid body refinement. The protein model was built using iterative cycles of O and CNS. The ligand was only included until well defined by unbiased (i.e. before inclusion of any ligand) $|F_o| - |F_c|$, $\phi_{calc}$ maps (shown in Fig. 3A). Structural comparisons described here were performed with the first monomers in the coordinate files, some of the loops of the further monomers being less well defined, and the overall backbone B-factors higher. This is true in particular for the complex with GlcNAc, where the first monomer has a $<\text{B}>$ of 49.7 Å², and for the second monomer $<\text{B}> = 66.7$ Å². This is also reflected in relatively high Wilson B-factors for the complex, compared to those of the native structure (Table I).

Analysis of Ligand binding

Binding of chitooligosaccharides to HCGP39 was analysed using the intrinsic tryptophan fluorescence of the protein and ligand-induced changes of the solvent environment of tryptophan residues [39]. Fluorescence measurements were carried out with a Perkin-Elmer LS50B fluorescence spectrophotometer equipped with Flu-Sys software (version 1.02, [40]) and a thermostatted cuvette holder at 25 °C. Emission spectra were recorded from 310-460 nm upon excitation at 295 nm. Both the excitation and emission slits were opened to 5 nm and the spectra were recorded at a scan speed of 25 nm/min. Initial binding experiments were performed with GlcNAc. HCGP39 (5.9 μl of a 42.5 μM solution) was added to a 0.5 ml quartz cuvette containing 25 mM Tris-HCl pH 7.4, 1 mM DTT and 150 mM NaCl to an initial volume of 0.485 ml. After pre-incubating the solution for 10
min. at 25 °C within the cuvette holder, 2.5 μl aliquots of various GlcNAc$_4$ stock solutions were added to the mixture (to a final volume of 0.5 ml after six additions) and the emission spectrum was recorded after each addition following mixing and a 5 min. incubation. All spectra were corrected for the background emission signal from both the buffer and the unbound ligand. The concentrations of the ligand were corrected for dilution effects, making the final concentration range 0-1.55 μM. Both the fluorescence of the macromolecule and the background fluorescence of the ligand were linear over the concentration range used in this study. The fluorescence intensity from a solution containing 0.1 μM GlcNAc$_4$ and 0.5 μM HCGP39 remained constant up to 3 h after the initial mixing, indicating a stable and rapidly formed equilibrium complex. The equilibrium dissociation constant could be obtained from fitting the fluorescence intensity data to the following single site binding equation using non-linear regression analysis [41]: 

$$ F - F_o = (F_b - F_o) * (L_o / (K_d + L_o)) $$

where $F$ and $F_o$ refer to the fluorescence intensity in the presence and absence of GlcNAc$_4$ respectively, $F_b$ refers to maximum fluorescence signal of the HCGP39:GlcNAc$_4$ complex at saturation, $L_o$ is the initial [GlcNAc$_4$] and $K_d$ is the equilibrium dissociation constant. The above protocol was also performed using 0.75 μM of HCGP39 and gave a similar $K_d$ value (data not shown). Binding of GlucNAc6 was analysed in an identical fashion, except that the final concentration range of the ligand was 0 - 71.4 μM.
Results & Discussion

Overall structure

The structure of the 39 kD human cartilage glycoprotein (HCGP39) was solved by molecular replacement and refined against 1.85 Å synchrotron data (Table I), to a final R-factor (R_free) of 0.183 (0.206), with good geometry (Fig. 1A, Table I). The structure reveals the standard ($\beta/\alpha$)$_8$ barrel, as expected from the high sequence identity (53%) with the human macrophage chitinase (HCHT, Fig. 1B, [18]). Similar to many other chitinase structures solved to date, an extra $\alpha/\beta$ domain is inserted between strand $\beta7$ and $\alpha7$, which gives the active site a groove-like character [15, 17, 16] (Fig. 1B). Two disulfide bridges, conserved with HCHT, are observed. Like most family 18 chitinases, three conserved cis-peptides are present (57-58, 140-141 and 352-353). An ordered GlcNAc$_2$ N-linked glycan is attached to Asn63, the single predicted glycosylation site. Sequence databases suggest polymorphism on residue 311, the electron density maps identify this residue as Ile rather than Thr.

Family 18 chitinases contain a sequence motif DxxDxDxE, which lies on strand $\beta4$ (Fig. 1B). Structural analysis has shown that the glutamate is the catalytic acid, which protonates the glycosidic bond [14, 15, 16, 17, 19, 20]. The neighbouring aspartate plays a key role in orienting the N-acetyl group of the -1 sugar for nucleophilic attack on the anomeric carbon, and stabilizes the subsequently formed oxazolinium ion intermediate [42, 43, 19]. Mutation of either of these residues significantly impairs catalysis [22, 23, 19, 21]. In HCGP39, these residues correspond to Leu140 and Ala138 (Fig. 1). HCGP39 possesses no chitinase activity, in addition it has been shown that mutation of Glu140 (the catalytic acid) to Leu in HCHT renders the enzyme catalytically inactive.
However, both HCGP39 and this HCHT mutant are able to bind chitin particles with high affinity [24]. The reason for this is apparent from sequence comparison (Fig. 1B) and the nature of the residues lining the HCGP39 putative binding cleft (Fig. 1A). Similar to HCHT [18] and most of the other chitinase structures [15, 16, 17], this cleft is lined with solvent exposed aromatic residues (Fig. 1A). Structural studies on chitinases have shown that these residues are important for interacting with the hydrophobic faces of the pyranose rings in the chitooligosaccharides GlcNAc₅ and GlcNAc₈ [16, 20]. Thus, the HCGP39 structure supports the observation that this protein is able to interact with chitin.

Comparison with other Chi-lectins

Several proteins with high sequence homology to family 18 chitinases, but lacking catalytic activity, have been reported. These chitinase-like lectins (Chi-lectins) have a range of roles and are thought to interact with a variety of carbohydrate ligands [27, 13, 4, 29, 28]. Structural information has recently become available for Ym1, a Chi-lectin isolated from mice infected with the nematodal pathogen *Trichinella spiralis* [27, 30], and Imaginal Disc Growth Factor 2 (IDGF2), a Chi-lectin with growth factor properties from *Drosophila melanogaster* [13, 31]. Similar to HCGP39, these proteins have mutations in the family 18 chitinase DxxDxDxE motif (Fig. 1). Structural comparison with active chitinases has shown that these Chi-lectins lack several residues that are important for binding chitin, most notably some of the solvent exposed aromatics [31, 18] (Fig. 1). Analysis of the solvent accessible surface in terms of electrostatic potential and sequence conservation further demonstrates this (Fig. 2). Compared to HCHT, HCGP39 has a similarly shaped, conserved binding cleft, with a similar charge. Ym1 does not show a well-defined cleft, and it is poorly con-
served and more negatively charged (Fig. 2). IDGF2’s putative ligand binding site has a pocket character and almost no residues are conserved, compared to HCHT (Fig. 2). There is no biochemical evidence for IDGF binding carbohydrate [13], and crystallographic soaking studies with chitoooligosaccharides have been unsuccessful [31], which is not surprising considering the poorly conserved pocket. For Ym1, binding of short GlcN oligomers has been demonstrated, which is in agreement with the nature and shape of its putative ligand binding site [27]. For HCGP39, however, the structural data are compatible with a possible function as a chitin-binding lectin.

Complexes with chitoooligosaccharides

Although the structures of three Chi-lectins are now known (HCGP39, Ym1 [30], IDGF2 [31]), so far there are no structural data on how these proteins bind carbohydrate ligands. Because the native HCGP39 structure is compatible with a chitin binding function, and the biochemical data described here support this, we attempted to crystallise complexes of HCGP39 and oligosaccharides. Co-crystallization trials in the presence of GlcNAc₈ yielded crystals under similar conditions as for the native crystals. Diffraction data were collected to 2.3 Å, and the structure of the complex solved by molecular replacement. Initial refinement of the protein yielded unbiased $|F_o| - |F_c|$, $\phi_{calc}$ maps that allowed the construction of models for the chitoooligosaccharide (Fig. 3A). Statistics of the refinement are shown in Table I.

The complex with GlcNAc₈ reveals the binding site for this chitoooligosaccharide. The molecule covers subsites -4 to +2, in similar conformation and location as observed previously for chitinase-chitoooligosaccharide complexes [16, 20, 18] (Fig. 3A). Poor density corresponding for possible further sugars in the -5 and -6 subsites is present, but could not be unambiguously interpreted,
and was therefore not used for model building. GlcNAc\textsubscript{8} is the longest commercially available chitooligosaccharide, but is unfortunately supplied at only 70\% purity. It is possible that the density for the ligand represents a mixture of GlcNAc\textsubscript{8} and shorter chitooligosaccharides, and that the -5 and -6 subsites are therefore partially occupied. If the ChiB-GlcNAc\textsubscript{5} complex is superposed onto the HCGP39-GlcNAc\textsubscript{8} complex, the largest positional difference between equivalent atoms is 1.4 Å (for the +2 sugar). Similar to the complexes observed previously, the -1 sugar is in the boat conformation. However, the N-acetyl group which is positioned for nucleophilic attack on the anomeric carbon in the chitinase B-GlcNAc\textsubscript{5} complex [19] is pointing down towards the core of the (βα)\textsubscript{8} in the HCGP39-GlcNAc\textsubscript{8} complex (Fig. 3A). This is due to the absence of an aspartate at position 142 (alanine in HCGP39) which generates a small cavity, allowing the N-acetyl to assume a more relaxed conformation. Similarly, Leu140 is not able to hydrogen bond the glycosidic oxygen between the -1 and +1 subsites, as seen for the catalytic glutamate in the ChiB-GlcNAc\textsubscript{5} complex [19]. Apart from the different residues Ala138 and Leu140, key hydrogen bonds with the -1 sugar are conserved (Trp99, Asn100, Asp207, Arg263). Two conserved aromatics, Trp352 and Trp99 stack with the sugars in the -1 and +1 subsites respectively. The conserved Trp31 stacks with the -3 sugar. Hydrogen bonds are formed from the -3 N-acetyl group to Asn100 and from the -4 O6 hydroxyl to Glu70 (Fig. 3A). In addition, the cleft binding cleft appears to continue beyond +2 and -4 subsites (Fig. 3A), suggesting that it may also support binding of polymeric chitin, in line with the observed binding to chitin particles [24].
Ligand-induced conformational change

Previous experiments have shown that HCGP39 and the IDGFs act as a growth factors [13, 12, 11]. It is possible that ligand binding, and a possible associated conformational change, modulates this growth factor function. Comparison of the native HCGP39 structure to the complex with GlcNAc₈ shows there is such a conformational change, and of a magnitude not previously observed in other lectins and family 18 chitinases (Fig. 3B,C). In the native structure, Trp212 is not lining the binding cleft, even though this residue is conserved in many other chitinases where it has been shown to form part of the +2 binding site [19, 20, 18] (Fig. 1B). Instead, it, and the associated β6α6 loop, has unfolded onto the surface of the protein (Figs. 1A,3B,C). In parallel, His209, which in the HCHT structure is buried behind Trp212 [18], lies in the binding cleft and is positioned in a space normally occupied by the +2/+3 sugars [19, 20]. The HCGP39 complex with GlcNAc₈, however, shows that Trp212 and His209 occupy positions more similar to those previously observed in chitinase structures, with Trp212 lining the cleft, and His209 behind it (Fig. 3B,C). In addition, Arg213, a residue unique to HCGP39, has moved from a solvent exposed position to lie behind Trp212 where it forms a buried salt bridge with Asp232 (Fig. 3B), an interaction not observed in other chitinases or Chi-lectins. On the other side of the cleft, Trp99 has rotated 180° around χ₁, stacking its indole ring with the +1 sugar (Fig. 3B,C). Associated with this is a shift of Asn100 towards the ligand. Thus, there are significant ligand-induced conformational changes with side chain positional shifts of up to 11.8 Å (for Trp212) and shifts in Cα positions of up to 8.0 Å (for Trp212). These conformational changes are not induced by differing crystal contacts, as the native and ligand-bound structures crystallise in essentially the same crystal form, with similar unit cell dimensions (see legend Table I). Thus, in particular His209 seems to act as a sugar-sensing residue,
a lever which is pushed inwards upon occupation of the +2 pocket, allowing Trp212/Arg213 to take up positions in the binding cleft. The surface properties of the protein in this area are significantly altered upon ligand binding: a hydrophobic tryptophan lying on the surface of the protein is moved into the cleft and the polar histidine/arginine removed from the solvent. It is currently not known if and how ligand binding would affect the signalling properties of HCGP39. It is possible that the observed conformational change could be involved in the transduction of the ligand binding event to HCGP39-interacting partners, but further studies are needed to confirm this.

The observed ligand-induced conformational changes in HCGP39 are unusual compared to the chitinase structures solved previously (including the closely related human macrophage chitinase), in which ligand binding only results in minor adjustments of side chains lining the binding cleft [14, 19, 20, 21, 44, 45].

Biochemical evidence for the interaction of HCGP39 with chitooligosaccharides

To obtain an accurate estimate of the binding affinity of GlcNAc oligomers for HCGP39 the equilibrium binding was analysed fluorometrically, using the intrinsic tryptophan fluorescence of the protein (Fig. 4). Addition of GlcNAc₆ (Fig. 4) or GlcNAc₄ to a 0.5 μM solution of HCGP39 resulted in a concentration-dependent increase in the fluorescence intensity between 310 and 460 nm, with no shift in the λ_max (panel A) indicating a rearrangement of the solvent environment of one or more tryptophan residues upon interaction with the ligand. The fluorescence data fitted well to a model assuming a binary interaction, showing saturable binding (panel B) with a dissociation constant of 331 +/- 5 μM for GlcNAc₄ and 6.7 +/- 0.7 μM for GlcNAc₆. The decrease in K_d values
with increasing length of the oligosaccharides suggests a tighter interaction within the carbohydrate binding site. These values are consistent with the trends observed for the binding of 4MU-GlcNAc derivatives to HCHT (Krupa et al., manuscript in preparation), suggesting that the non-conservative Glu140Leu and Asp138Ala substitutions do not significantly affect the binding of chitin oligomers to HCGP39.

**Concluding remarks**

The data presented here suggests that oligomeric chitin could be a physiological ligand for the Chi-lectin HCGP39, although binding of other carbohydrate polymers cannot be excluded. The conservation and tight interactions of the aromatic residues in the binding cleft as seen in the complex with GlcNAc$_8$ further support such a role. Chitooligosaccharides bind with $\mu$M affinity, and binding induces a significant conformational change. Further testing of the residues involved will be possible upon expression and and mutagenesis of HCGP39 in a recombinant form, as it has so far only been possible to obtain the protein by purification directly from chondrocytes.

Chitin is a structural component of fungal and nematodal human pathogens. It is possible that HCGP39 acts as a chitin sensor, switching on innate defenses, helping to direct macrophages to the site of invasion and to regulate the inflammatory response as a consequence of infection. This is similar to the proposed role of another Chi-lectin, eosinophil chemotactic cytokine ECF-L, which is thought to direct components of the immune system to the site of nematode infections [28]. Our results may aid interpretation of genomic data on the Chi-lectin family in a structural context, most notably as this represents the first example of a protein from this family interacting with its carbohydrate ligand.
Acknowledgements

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Table I

Details of data collection & structure refinement. Values between brackets are for the highest resolution shell. Crystals were cryo-cooled to 100 K for data collection. All measured data were included in structure refinement. The packing in these crystal forms is essentially the same - the translational non-crystallographic symmetry present in P2₁₂₁₂₁ (4 molecules / a.s.u.) has become crystallographic translational symmetry in I₂₂₂₂ (2 molecules / a.s.u.), with similar unit cell dimensions.

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Figure legends

1. Overall structure and sequence alignment.

A: Stereo image of HCGP39. The native structure of HCGP39 is shown as a coloured ribbon (helices red, strands blue) in stereo. Ile311 and the GlcNAc2 N-linked glycan on Asn60 are shown as sticks models with black carbons, together with the final $2|F_o| - |F_c|, \theta_{cal}$ map (orange) contoured at 1.0 $\sigma$. The side chains of Leu140 and Ala138 are shown as black sticks. The two disulfide bonds are shown in green. Solvent-exposed aromatic residues lining the putative binding cleft and conserved with HCHT are shown as sticks with magenta carbons (see also B.)

B: Sequence alignment of HCGP39 and other Chi-lectins. Structure-based sequence alignment of HCGP39 (HCGP), Ym1, IDGF-2 and the human macrophage chitinase (HCHT). Conserved sequences are indicated by black boxes, similar residues by grey boxes. The secondary structure elements of the HCGP39 ($\beta/\alpha$)$_8$ barrel as indicated as calculated by DSSP [46]. Aromatic residues lining the binding cleft in HCGP39/HCHT are indicated by filled triangles. The position of the catalytic glutamate in HCHT is indicated by an open triangle.

2. Structural comparison of the Chi-lectins. The molecular surfaces calculated from the crystal structures of HCGP39, the human macrophage chitinase (HCHT) [18], Ym1 [30] and IDGF-2 [31], are compared for two properties. The top panel shows electrostatic surface potential, calculated with GRASP [47] (red: $<-7.5$ kT, blue: $>+7.5$ kT). The bottom panel shows sequence conservation, compared to the HCHT structure (magenta: conserved, grey:}

18
non conserved). For HCHT, a model of GlcNAc₉, described previously [18], is also shown as a sticks model.

3. **A: Electron density maps.** The binding sites for the HCGP39 complex with GlcNAc₈ (NAG8) is compared with the HCHT-allosamidin complex. Ligands are shown as sticks with orange carbon. An unbiased (i.e. before including any ligand model) \(|F_o| - |F_c|, \phi_{calc}\) map (magenta) is shown, contoured at 2.25 σ. Residues interacting with the ligand are shown as sticks with black carbons. Hydrogen bonds are shown as green dotted lines.

**B: Conformational changes in the binding site.**

Molecular surfaces are shown for apo-HCGP39 (APO) and the complex with GlcNAc₈ (NAG8). The solvent exposed aromatics towards the non-reducing end of the ligand are shown as sticks with purple carbons. The residues undergoing conformational changes upon ligand binding (99-100 and 209-213) are shown as sticks with green carbons. The structure for the GlcNAc₆ ligand observed in the density is shown as sticks with orange carbons.

**C: Conformational changes in the protein.**

The structure of apo-HCGP39 is shown in stereo as a grey ribbon, with key side chains shown as sticks with black carbons and label names starting with ‘a’. For conformational changes > 1.0 Å, the backbone of the HCGP39-GlcNAc₈ complex is shown in green. The same side chains are shown as for apo-HCGP39, but with carbons colored green, and label names starting with ‘h’. The oligosaccharide is shown with orange carbons.
4. **Effect of GlcNAc$_6$ on the intrinsic fluorescence of HCGP39.** Increasing amounts of GlcNAc$_6$ were added to 0.5 μM of HCGP39 in 25 mM Tris-HCl pH 7.4, 1 mM DTT and 150 mM NaCl and the emission spectra were recorded from 310-460 nm upon excitation at 295 nm. Panel A shows the emission spectra of HCGP39 in the presence of increasing amounts of GlcNAc$_6$. No shift in $\lambda_{max}$ from 340 nm was observed. Panel B shows the binding curve determined for GlcNAc$_6$. Relative fluorescence intensity data (F-F$_0$) at 340 nm were fitted to a binary interaction model (see Methods). The Scatchard plot inset illustrates the 1:1 stoichiometry of binding between GlcNAc$_6$ and HCGP39.
References


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