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Structure and Ligand-induced Conformational Change of the 39-kDa Glycoprotein from Human Articular Chondrocytes*

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The 39-kDa 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 remodeling and defense against pathogens. Similar to other Chi-lectins, HCGP39 promotes the growth of connective tissue cells. The ability of HCGP39 to activate cytoplasmic signaling 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 GlcNAc$_8$ 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 that binds chitin-like oligosaccharide ligands and possibly plays a role in innate responses to chitinous pathogens, such as fungi and nematodes.

The 39-kDa human cartilage glycoprotein (HCGP39)[1] 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 remodeling 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 are 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 tumor 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 and the extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase signaling 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 that they consist of a $(\beta\alpha)_8$ barrel with in some cases an extra α/β domain inserted in one of the barrel loops (14–18). The chitinases have a cleft lined with solvent-exposed aromatic residues, where they bind their substrates, oligomers of *N*-acetylglucosamine (14, 15, 19–21). The glutamate and aspartate at the end of the conserved D*XX*D*X*D*X*E sequence motif are essential for catalysis (19, 21, 22, 23), 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 characterization 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 D*XX*D*X*D*X*E motif and are therefore inactive; they are secretory proteins and thought to be involved in tissue remodeling 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 that 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 struc-

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The atomic coordinates and structure factors (code 1HJX and 1HJW) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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[1] The abbreviations used are: HCGP39, the 39-kDa human cartilage glycoprotein; HCHT, human macrophage chitinase; Chi-lectin, chitinase-like lectin; IDGF, imaginal disc growth factor.

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TABLE I
Details of data collection and structure refinement

The values in parentheses are for the highest resolution shell. The 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 noncrystallographic symmetry present in $P2_12_12_1$ (4 molecules/a.s.u.) has become crystallographic translational symmetry in $I2_12_12_1$ (2 molecules/a.s.u.), with similar unit cell dimensions.

Data set	HCGP39	HCGP39+GlcNAc$_8$
Space group	$P2_12_12_1$	$I2_12_12_1$
Cell dimensions (Å)	a = 109.56	a = 109.49
	b = 122.05	b = 123.63
	c = 136.10	c = 136.22
Resolution range (Å)	15-1.85 (1.92-1.85)	25-2.30 (2.38-2.30)
No. of observed reflections	681418 (66944)	295880 (15858)
No. of Unique reflections	153614 (15110)	39388 (3694)
Redundancy	4.4 (4.4)	7.5 (4.3)
$I/\sigma I$	9.5 (3.7)	10.7 (2.3)
Completeness (%)	99.0 (98.4)	96.2 (91.1)
R_{merge}	0.076 (0.519)	0.068 (0.624)
Monomera/a.s.u.[a]	4	2
R_{cryst}, R_{free}	0.193, 0.206	0.206, 0.254
r.m.s.d.[b] from ideal geometry		
Bonds (Å)	0.016	0.015
Angles (°)	1.6	1.6
B factor r.m.s.d. (Å^2) (bonded main chain)	1.15	1.11
B protein (Å^2)	19.5, 29.1, 27.5, 31.9	49.7, 66.7
B ligand (Å^2)		69.9, 75.1
B_{wilson} (Å^2)	25.1	64.8

[a] a.s.u., asymmetric unit.
[b] r.m.s.d., root mean square deviation.

tural 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 GlcNAc$_8$ 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.

MATERIALS AND METHODS

Structure Solution—HCGP39 was purified from human chondrocytes as described previously (1); however, heparin-Sepharose affinity chromatography was used as a 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 crystallization, the protein solution was thawed and concentrated to 7 mg/ml. Crystals were grown using the sitting drop vapor 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 dithiothreitol). The crystals grew as bars with maximum dimensions of 0.1 × 0.1 × 0.4 mm after approximately 1 week. A complex with GlcNAc$_8$ was obtained by co-crystallization in the same conditions, in the presence of 3 mg/ml of the ligand. All of the crystals were cryoprotected in 30% glycerol, 70% mother liquor prior to freezing in a cryostream. The data were collected on beamlines ID14-4 (ESRF, Grenoble, France) and X11 (DESY, Hamburg, Germany) 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 of the four molecules in the asymmetric unit were found, and 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 macrocycles noncrystallographic symmetry restraints and map averaging were used. The statistics of the final model are shown in Table I.

For the complex with GlcNAc$_8$, 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. 3*A*). 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 being higher. This is true in particular for the complex with GlcNAc$_8$, where the first monomer has a $\langle B \rangle$ of 49.7 Å^2, and for the second monomer $\langle B \rangle$ = 66.7 Å^2. This is also reflected in relatively high Wilson B factors for the complex, compared with those of the native structure (Table I).

Analysis of Ligand Binding—Binding of chitooligosaccharides to HCGP39 was analyzed 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 to 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$_4$. 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 dithiothreitol, and 150 mM NaCl to an initial volume of 0.485 ml. After preincubating 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 of the 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 nonlinear 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 GlcNAc$_6$ was analyzed in an identical fashion, except that the final concentration range of the ligand was 0–71.4 μM.

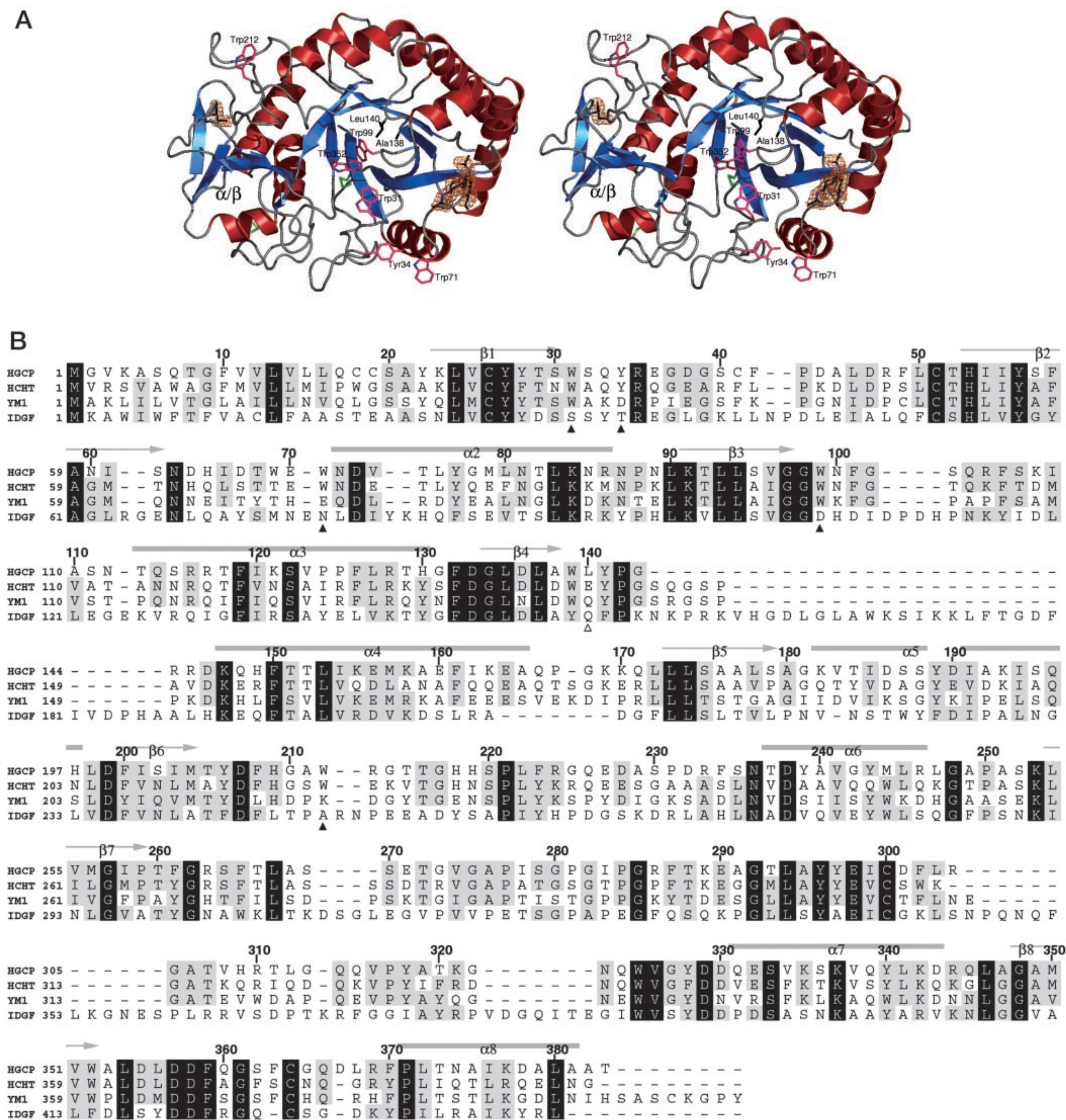

FIG. 1. **Overall structure and sequence alignment.** *A*, stereo image of HCGP39. The native structure of HCGP39 is shown as a *colored ribbon* (helices, *red*; strands, *blue*) in stereo. Ile[311] and the $GlcNAc_2$ *N*-linked glycan on Asn[60] are shown as stick models with *black carbons*, together with the final $2F_o - F_c, \phi_{calc}$ map (*orange*) contoured at 1.0 σ. The side chains of Leu[140] and Ala[138] 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*, and similar residues are indicated by *gray 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*.

RESULTS AND DISCUSSION

Overall Structure—The structure of the 39-kDa 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. 1*A* and Table I). The structure reveals the standard $(\beta/\alpha)_8$ barrel, as expected from the high sequence identity (53%) with the human macrophage chitinase (Fig. 1*B*, *HCHT*, and Ref. 18). Similar to many other chitinase structures solved to date, an extra α/β domain is inserted between strands β7 and α7, which gives the active site a groove-like character (15–17) (Fig. 1*B*). Two disulfide bridges, conserved with HCHT, are observed. Like most family 18 chitinases, three conserved *cis*-peptides are present (positions 57 and 58, positions 140 and 141, and

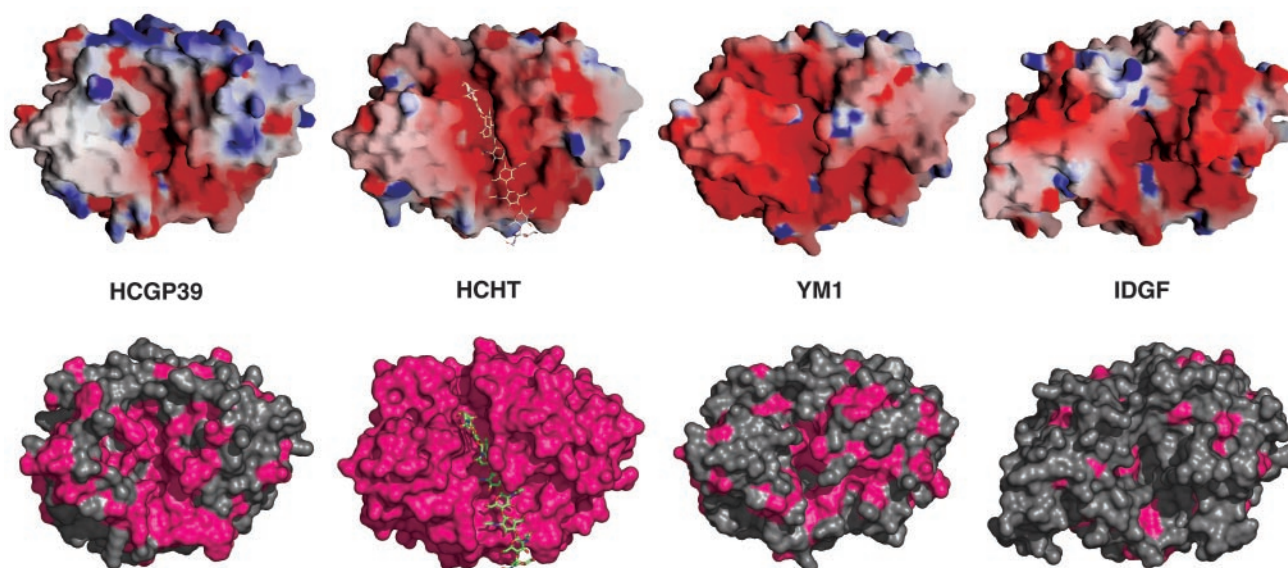

FIG. 2. **Structural comparison of the Chi-lectins.** The molecular surfaces calculated from the crystal structures of HCGP39, the human macrophage chitinase (*HCHT*) (18), Ym1 (30), and IDGF-2 (31), are compared for two properties. The *top panel* shows electrostatic surface potential, calculated with GRASP (47) (*red*, < -7.5 kiloteslas; *blue*, $> +7.5$ kiloteslas). The *bottom panel* shows sequence conservation, compared with the HCHT structure (*magenta*, conserved; *gray*, nonconserved). For HCHT, a model of $GlcNAc_9$, described previously (18), is also shown as a stick model.

positions 352 and 353). An ordered $GlcNAc_2$ *N*-linked glycan is attached to Asn[63], the single predicted glycosylation site. Sequence data bases suggest polymorphism on residue 311; the electron density maps identify this residue as Ile rather than Thr.

Family 18 chitinases contain a sequence motif D*XX*D*X*D*X*E, which lies on strand β4 (Fig. 1*B*). Structural analysis has shown that the glutamate is the catalytic acid, which protonates the glycosidic bond (14–17, 19, 20). The neighboring 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 (19, 42, 43). Mutation of either of these residues significantly impairs catalysis (19, 21, 22, 23). In HCGP39, these residues correspond to Leu[140] and Ala[138] (Fig. 1). HCGP39 possesses no chitinase activity; in addition it has been shown that mutation of Glu[140] (the catalytic acid) to Leu in HCHT renders the enzyme catalytically inactive (24). 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. 1*B*) and the nature of the residues lining the HCGP39 putative binding cleft (Fig. 1*A*). Similar to HCHT (18) and most of the other chitinase structures (15–17), this cleft is lined with solvent-exposed aromatic residues (Fig. 1*A*). Structural studies on chitinases have shown that these residues are important for interacting with the hydrophobic faces of the pyranose rings in the chitooligosaccharides $GlcNAc_5$ and $GlcNAc_8$ (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 Chi-lectins have a range of roles and are thought to interact with a variety of carbohydrate ligands (4, 13, 27–29). Structural information has recently become available for Ym1, a Chi-lectin isolated from mice infected with the nematodal pathogen *T. spiralis* (27, 30), and IDGF2, a Chi-lectin with growth factor properties from *D. melanogaster* (13, 31). Similar to HCGP39, these proteins have mutations in the family 18 chitinase D*XX*D*X*D*X*E 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 (18, 31) (Fig. 1). Analysis of the solvent-accessible surface in terms of electrostatic potential and sequence conservation further demonstrates this (Fig. 2). Compared with 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 conserved and more negatively charged (Fig. 2). The putative ligand binding site of IDGF2 has a pocket character, and almost no residues are conserved, compared with HCHT (Fig. 2). There is no biochemical evidence for IDGF-binding carbohydrate (13), and crystallographic soaking studies with chitoologisaccharides 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 Chitooligosaccharides—Although the structures of three Chi-lectins are now known (HCGP39, Ym1 (30), and 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 describe here support this, we attempted to crystallize complexes of HCGP39 and oligosaccharides. Co-crystallization trials in the presence of $GlcNAc_8$ 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 chitooligosaccharide (Fig. 3*A*). Statistics of the refinement are shown in Table I.

The complex with $GlcNAc_8$ reveals the binding site for this chitooligosaccharide. The molecule covers subsites −4 to +2, in similar conformation and location as observed previously for chitinase-chitooligosaccharide complexes (16, 18, 20) (Fig. 3*A*). Poor density corresponding for possible further sugars in the −5 and −6 subsites is present but could not be unambiguously

FIG. 3. *A*, electron density maps. The binding sites for the HCGP39 complex with $GlcNAc_8$ (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 $GlcNAc_8$. The solvent-exposed aromatics toward the nonreducing 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 $GlcNAc_6$ 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 *gray ribbon*, with key side chains shown as sticks with *black carbons* and label names starting with the *letter a*. For conformational changes of >1.0 Å, the backbone of the HCGP39-$GlcNAc_8$ 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 the *letter h*. The oligosaccharide is shown with *orange carbons*.

interpreted and was therefore not used for model building. $GlcNAc_8$ 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 $GlcNAc_8$ and shorter chitooligosaccharides and that the −5 and −6 subsites are therefore partially occupied. If the ChiB-$GlcNAc_5$ complex is superposed onto the HCGP39-$GlcNAc_8$ complex, the largest positional difference between equivalent at-

oms 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-Glc-NAc_5 complex (19), is pointing down toward the core of the $(\beta\alpha)_8$ in the HCGP39-$GlcNAc_8$ complex (Fig. 3*A*). 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, Leu^{140} is not able to hydrogen bond the glycosidic oxygen between the −1 and +1 subsites, as seen for the catalytic glutamate in the ChiB-$GlcNAc_5$ complex (19). Apart from the different residues Ala^{138} and Leu^{140}, key hydrogen bonds with the −1 sugar are conserved (Trp^{99}, Asn^{100}, Asp^{207}, and Arg^{263}). Two conserved aromatics, Trp^{352} and Trp^{99}, stack with the sugars in the −1 and +1 subsites, respectively. The conserved Trp^{31} stacks with the −3 sugar. Hydrogen bonds are formed from the −3 *N*-acetyl group to Asn^{100} and from the −4 O_6 hydroxyl to Glu^{70} (Fig. 3*A*). In addition, the cleft binding cleft appears to continue beyond +2 and −4 subsites (Fig. 3*A*), 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 (11–13). It is possible that ligand binding and a possible associated conformational change modulate this growth factor function. Comparison of the native HCGP39 structure to the complex with $GlcNAc_8$ shows there is such a conformational change and of a magnitude not previously observed in other lectins and family 18 chitinases (Fig. 3, *B* and *C*). In the native structure, Trp^{212} 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 (18–20) (Fig. 1*B*). Instead, it, and the associated $\beta_6\alpha_6$ loop, has unfolded onto the surface of the protein (Figs. 1*A* and 3, *B* and *C*). In parallel, His^{209}, which in the HCHT structure is buried behind Trp^{212} (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 $GlcNAc_8$, however, shows that Trp^{212} and His^{209} occupy positions more similar to those previously observed in chitinase structures, with Trp^{212} lining the cleft and His^{209} behind it (Fig. 3, *B* and *C*). In addition, Arg^{213}, a residue unique to HCGP39, has moved from a solvent-exposed position to lie behind Trp^{212} where it forms a buried salt bridge with Asp^{232} (Fig. 3*B*), an interaction not observed in other chitinases or Chi-lectins. On the other side of the cleft, Trp^{99} has rotated 180° around χ_1, stacking its indole ring with the +1 sugar (Fig. 3, *B* and *C*). Associated with this is a shift of Asn^{100} toward the ligand. Thus, there are significant ligand-induced conformational changes with side chain positional shifts of up to 11.8 Å (for Trp^{212}) and shifts in Cα positions of up to 8.0 Å (for Trp^{212}). These conformational changes are not induced by differing crystal contacts, because the native and ligand-bound structures crystallize in essentially the same crystal form with similar unit cell dimensions (see legend Table I). Thus, in particular His^{209} seems to act as a sugar-sensing residue, a lever that is pushed inwards upon occupation of the +2 pocket, allowing Trp^{212}-Arg^{213} 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 is removed from the solvent. It is currently not known whether and how ligand binding would affect the signaling 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 with 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).

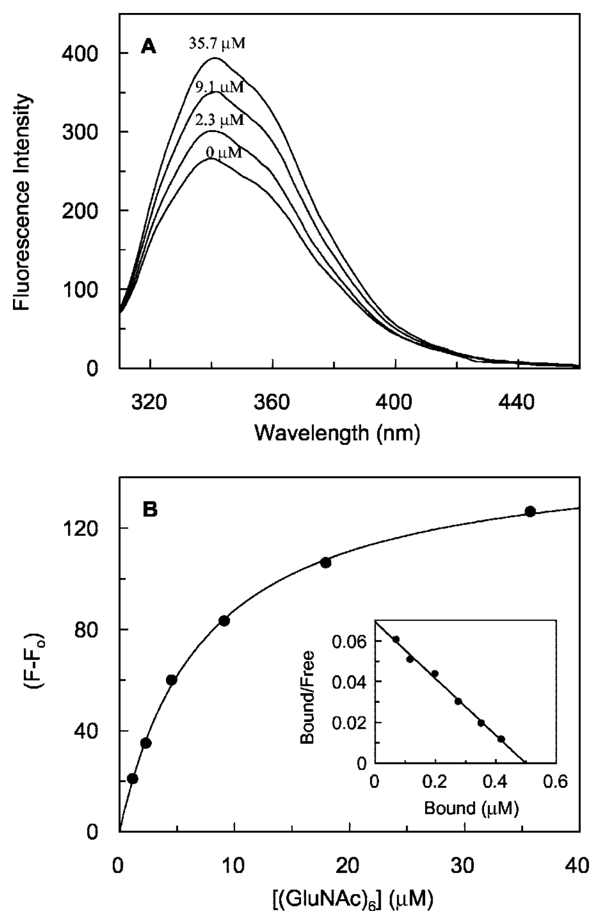

FIG. 4. **Effect of $GlcNAc_6$ on the intrinsic fluorescence of HCGP39.** Increasing amounts of $GlcNAc_6$ were added to 0.5 μM of HCGP39 in 25 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, and 150 mM NaCl, and the emission spectra were recorded from 310–460 nm upon excitation at 295 nm. *A* shows the emission spectra of HCGP39 in the presence of increasing amounts of GlcNAc. No shift in λ_{max} from 340 nm was observed. *B* shows the binding curve determined for $GlcNAc_6$. Relative fluorescence intensity data ($F - F_o$) at 340 nm were fitted to a binary interaction model (see "Materials and Methods"). The Scatchard plot inset illustrates the 1:1 stoichiometry of binding between $GlcNAc_6$ and HCGP39.

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 analyzed fluorometrically, using the intrinsic tryptophan fluorescence of the protein (Fig. 4). The addition of $GlcNAc_6$ (Fig. 4) or $GlcNAc_4$ 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} (Fig. 4*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 (Fig. 4*B*) with a dissociation constant of 331 ± 5 μM for $GlcNAc_4$ and 6.7 ± 0.7 μM for $GlcNAc_6$. The decrease in K_d values with the 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,[2] suggesting that the non-conservative E140L and D138A substitutions do not significantly affect the binding of chitin oligomers to HCGP39.

Concluding Remarks—The data presented here suggest 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 μM affinity, and binding induces a significant conformational change. Further testing of the residues involved will be possible upon expression and mutagenesis of HCGP39 in a recombinant form, because 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 because this represents the first example of a protein from this family interacting with its carbohydrate ligand.

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