Family 18 Chitolectins: Comparison of MGP40 and GP39

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ABSTRACT

Glycosidases and lectins both bind sugars, but only the glycosidases are catalytic. The glycosidases occur among 90 evolved protein families. Family 18 is one of the two familes of chitinases (EC 3,2.1.14). Interestingly, lectins are also in this evolutionary group of Family 18 glycosidase proteins. Proteins belonging to the enzymatically inactive class ("chitolectins") have a highly similar binding site to the catalytic Family 18 enzymes. One major exception is a glutamic acid which acts as the essential acid/base residue for chitin cleavage is replaced with leucine or glutamine. We present our comparison of the recently obtained structures of two Family 18 chitolectins, MGP40 (Mohanty, Singh *et al.*, 2003) and GP39 (Fusetti, Pijning *et al.*, 2003; Houston, Anneliese *et al.*, 2003).

INTRODUCTION

Chitinases (EC 3.2.1.14) hydrolyze chitin β(1→4) linkages in order to make available the large quantities of carbon and nitrogen elements potentially trapped in the biosphere as insoluble GlcNAc (Keyhani & Roseman, 1999). Henrissat and Davies (Henrissat & Davies, 2000) have classified glycosyl hydrolases into 91 evolutionary groups based on their homologous amino acid sequences. These enzyme families are further organized into clans that have common three-dimensional structures, but not necessarily common primary sequences. Glycosidase Family 18 and 19 encompass the chitin hydrolases. Of these, Family 18 proteins belong to two distinct classes. One class of Family 18 proteins (chitinases) are active and they bind and hydrolyze oligosaccharides, whereas the other class of proteins ("chitolectins") are highly similar, but are inactive. The chitolectins bind but do not hydrolyze oligosaccharides. Through natural evolution the chitolectin members of Family 18 proteins (Bakkers, Kijne *et al.*, 1999; Bleau, Massicotte *et al.*, 1999) have lost their hydrolysis potential because the catalytic acid/base glutamic acid in the substrate groove has been substituted by non-active amino acids (e.g. Leu).

Currently, the physiological role of these non-enzymatic proteins has not been elucidated. The best evidence based on their unique times of physiological expression indicates that these proteins are involved in tissue remodeling. Most likely due to their structures they function via binding oligosaccharides. Since they all have a signal peptide, they are likely secreted whereby they can interact with extracellular matrix proteoglycans or cell surface sugars in glycolipids or glycoproteins. These proteins would therefore not function in a stand-alone mode, but instead

would function either as direct cofactors during molecular signaling processes or help to present oligosaccharide targets to other proteins.

MGP40

Recently, an x-ray structure for one member (MGP40) of the chitolectin class of proteins was determined (Mohanty, Singh *et al.*, 2003). The structure is consistent with the $(\beta/\alpha)_8$ barrel topology of the Family 18 glycosidase proteins (Bortone, Monzingo *et al.*, 2002; Fusetti, Von Moeller *et al.*, 2002; Hollis, Monzingo *et al.*, 2000; Houston, Anneliese *et al.*, 2003; Houston, Eggleston *et al.*, 2002; Houston, Shiomi *et al.*, 2002; Matsumoto, 1999; Mohanty, Singh *et al.*, 2003; Orikoshi, Baba *et al.*, 2003; Perrakis, Tews *et al.*, 1994; Prag, 2000; Rao, 1999; Rao, 1995; Sun, Chang *et al.*, 2001; Terwisscha van Scheltinga, 1995; Terwisscha van Scheltinga, 1994; Vaaje-Kolstad, Vasella *et al.*, 2003; Van Aalten, Komander *et al.*, 2001; Van Roey, Rao *et al.*, 1994; Varela, LIera *et al.*, 2002; Waddling, Plummer *et al.*, 2000; Watanabe, Ishibashi *et al.*, 2001). MGP40 is an Asn-linked glycoprotein itself. Mohanty et al. in this study propose that the single disaccharide that is covalently linked to the protein (at Asn39) forms hydrogen bonds with Arg84 and Ile40, influencing the backbone conformation of loop Val75-Phe85, which in turn alters the disposition of Trp78. Since Trp78 is an essential member of the binding site, its altered positioning leads to constriction of the binding site, thereby leading to the inability of the MGP40 to bind sugar. Therefore, they proposed that oligosaccharides cannot bind to the putative binding site. Finally, the authors point out that Arg84, which is hydrogen-bonded to the covalent sugar, is conserved in this particular class of chitolectins. The active chitinases in the Family 18 group which bind and cleave sugars possess a Pro in place of Arg84, which in turn leads to inability of hydrogen bonding to the covalent linked sugar. Subsequently, there is a "relaxed" backbone conformation of the Val75-Phe85 loop in the Family 18 chitinases and thereby no constriction of the binding site.

GP39

Very recently x-ray crystal structures of another Family 18 chitolectin (GP39) was report independently by two different groups (Fusetti, Pijning *et al.*, 2003; Houston, Anneliese *et al.*, 2003). These structures are also consistent with the $(\beta/\alpha)_8$ barrel topology of the Family 18 proteins. The GP39 protein structures also show a disaccharide covalently linked to Asn39 of GP39. However, more importantly these structures depict in addition an oligosaccharide bound in the active site. Different crystal structures of this protein show complexes with di, tri, tetra,

penta and hexasaccharide bound in the active site. Based on these studies and in contrast to the suggestions made by Mohanty et al it appears that the chitolectin group of proteins from Family 18 can bind chitin oligosaccharides. We compare the sequence and the structure of these two Family 18 proteins to justify this point. We also propose a mechanism for the interaction between the chitolectin and oligosaccharides.

RESULTS & DISCUSSION

Comparison of MGP40 and GP39

Primary Sequence Comparison

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HumGP39[81]   YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISNDHIDTWEWNDVTLYGMLN
MGP40  [60]   YKLICYYTSWSQYREGDGSCFPDAIDPFLCTHIIYSFANISNNEIDTWEWNDVTLYDTLN
HumCH1 [81]   AKLVCYFTNWAQYRQGEARFLPKDLDPSLCTHLIYAFAGMTNHQLSTTEWNDETLYQEFN
               **:**:*.*:***:*:.  :*. :*  ****:**:**.::*.::.* **** ***  :*

HumGP39[141]  TLKNRNPNLKTLLSVGGWNFGSQRFSKIASNTQSRRTFIKSVPPFLRTHGFDGLDLAWLY
MGP40  [120]  TLKNRNPKLKTLLSVGGWNFGPERFSKIASKTQSRRTFIKSVPPFLRTHGFDGLDLAWLY
HumCH1 [141]  GLKKMNPKLKTLLAIGGWNFGTQKFTDMVATANNRQTFVNSAIRFLRKYSFDGLDLDWEY
               **: **:*****::******.::*:.:.:.::.*:**::*.  ***.:.****** * *

HumGP39[195]  PGRR-----DKQHFTTLIKEMKAEFIKEAQP-GKKQLLLSAALSAGKVTIDSSYDIAKIS
MGP40  [174]  PGRR-----DKRHLTGLVKEMKAEFAREAQA-GTERLLLSAAVSAGKIAIDRGYDIAQIS
HumCH1 [201]  PGSQGSPAVDKERFTTLVQDLANAFQQEAQTSGKERLLLSAAVPAGQTYVDAGYEVDKIA
               ** :     **.::* *::::   * :***. *.::******:.**:  :* .*:: :*:

HumGP39[255]  QHLDFISIMTYDFHGAWRGTTGHHSPLFRGQEDASPDRFSNTDYAVGYMLRLGAPASKLV
MGP40  [234]  RHLDFISLLTYDFHGAWRQTVGHHSPLFRGQEDASSDRFSNADYAVSYMLRLGAPANKLV
HumCH1 [261]  QNLDFVNLMAYDFHGSWEKVTGHNSPLYKRQEESGAAASLNVDAAVQQWLQKGTPASKLI
               ::***:.:::*****:*. ..**:***:: **::..    *.* **   *: *:**.**:

HumGP39[314]  MGIPTFGRSFTLASS-ETGVGAPISGPGIPGRFTKEAGTLAYYEICDFLRGATVHRTLGQ
MGP40  [293]  MGIPTFGRSFTLASS-KTDVGAPISGPGIPGRFTKEKGILAYYEICDFLHGATTHRFRDQ
HumCH1 [320]  LGMPTYGRSFTLASSSDTRVGAPATGSGTPGPFTKEGGMLAYYEVCSWK-GATKQRIQDQ
               :*:**:********* .* **** :*.* ** **** * *****:*.:  *** :*  .*

HumGP39[374]  QVPYATKGNQWVGYDDQESVKSKVQYLKDRQLAGAMVWALDLDDFQGSFCGQDLRFPLTN
MGP40  [353]  QVPYATKGNQWVAYDDQESVKNKARYLKNRQLAGAMVWALDLDDFRGTFCGQNLTFPLTS
HumCH1 [379]  KVPYIFRDNQWVGFDDVESFKTKVSYLKQKGLGGAMVWALDLDDFAGFSCNQG-RYPLIQ
               :***  :.****.:** **.*.*. ***:: *.************ *  *.*.  :** .

HumGP39       AIKDALAAT [383]
MGP40         AVKDVLAEV [362]
HumCH1        TLRQELS   [386]
               :::: *:
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Figure 1. Sequence alignment for Human GP39 (362 residues); Goat MGP40 (362 residues); and Human Chitotriosidase (366 residues) is shown. *Identity*(*), 181 residues, 49.05%; *Strongly similar*(:), 78 residues, 21.14%; *Weakly similar*(.), 41 residues, 11.11%; *Different* 69 residues, 18.70%.

The primary sequences of MGP40 and GP39 are highly identical (83% identity) (see **Figure 1**). We found that since Arg84 is conserved between the two proteins, it is not the cause for the lack of oligosaccharide binding in MGP40 as proposed by Mohanty et al. (Mohanty, Singh *et al.*, 2003). Hence we compared the aromatic residues in both proteins, focusing on the TRP and TYR residues present in the floor of the binding groove that are likely to interact strongly with the sugar molecules. These residues also are important for chitin oligosaccharide binding by the active Family 18 chitinases. The interactions between the protein aromatic residues and the sugars in a hexasaccharide are listed in **Table 1**.

Table 1. Aromatic residues interacting with the oligosaccharides.

Sugar	*-6*	*-5*	*-4*	*-3*	*-2*	*-1*	*+1*	*+2*	*+3*
GP39	*W71*	*Y34*	-	*W31*	-	*W352*	*W99*	-	*W212*
MGP40	*W50*	*Y13*	-	*W10*	-	*W331*	*W78*	-	*W191*
Chi1	*W71*	*Y34*	-	*W31*	-	*W358*	*W99*	-	*W218*

All of the aromatic residues listed in Table 1 that define the binding groove are conserved between MGP40 and GP39, and even the human chitinase, chitotriosidase. Having ruled out Arg84 and the composition of binding groove aromatics as the cause for a lack of observed oligosaccharide binding by MGP40, we mapped out the full binding region of GP39 by identifying all residues within 4.5 Å of the sugar residues in the hexasaccharide-bound GP39 structure (PDB code: 1HJW), wherein the hexasaccharide binds from –4 to +2 subsites. The identified residues in GP39 were compared to the equivalent residues in MGP40. The residues are highly identical between the proteins near the binding site with only three differences observed between the two proteins (**Table 2**).

Table 2. Differences between MGP40 and GP39 in the binding subsites.

GP39	**Interacting Sugar**	**MGP40**
T163	+2	A
M183	-1, +1	L
T272	-2	G

Of those three amino acids T163 and T272 are ~4 Å away from the +2 and the –2 sites respectively, and M183 is ~3 Å away from the –1 and +1 sugars. Hence the contribution from their interactions with the sugar to the overall interaction is very minimal. The majority of the favorable interaction between the protein and sugar is due to the interaction between sugar rings and the aromatic residues at –6, -5, -3, -1 +1 and +3 (**Table 1**).

Structural Comparison: 78-85 Loop

Mohanty et al. proposed that the W78-F85 loop in MGP40 is altered due to the hydrogen bond between Arg84 and the covalently linked sugar at Asn39. Hence, we compared the backbone configuration of the W78-F85 loop in MGP40 with the equivalent residues in GP39. Upon superimposing the protein structures (PDB codes: 1LJY and 1HWX) we observed that Arg84 is in identical position in both proteins. Interestingly, we did not observe the proposed hydrogen bond between Arg84 and the covalent sugar in MGP40. The sugar is ~4 Å away from Arg84. The sequence comparison of the W78-F85 (MGP40) loop is shown below.

MGP40 (78)WNFGPERF(85)
GP39 (99)WNFGSQRF(107)

The backbone conformation of W78 – F85 is very similar between GP39 and MGP40 (**Figure 2**). The major difference observed in this loop is at position 82 where a Pro is present in MGP 40 and a Ser in GP39. This variation could potentially be the cause of slightly different backbone positioning observed in the two proteins. We also observed that Trp78/99 sidechains are in different positions in the two proteins. The slight difference in the backbone positioning should not contribute to this difference in their sidechains. We observed that the GP39 structures, when crystallized without any sugars (PDB code: 1HJX and 1NWR), have the W99 sidechain conformation similar to that of W78 in MGP40. Thus it appears that W78/99 at the +1 position is the "gate" of the binding site. It constricts the binding site in absence of sugar and swings out to open up the binding site in the presence of sugar (**Figure 2**).

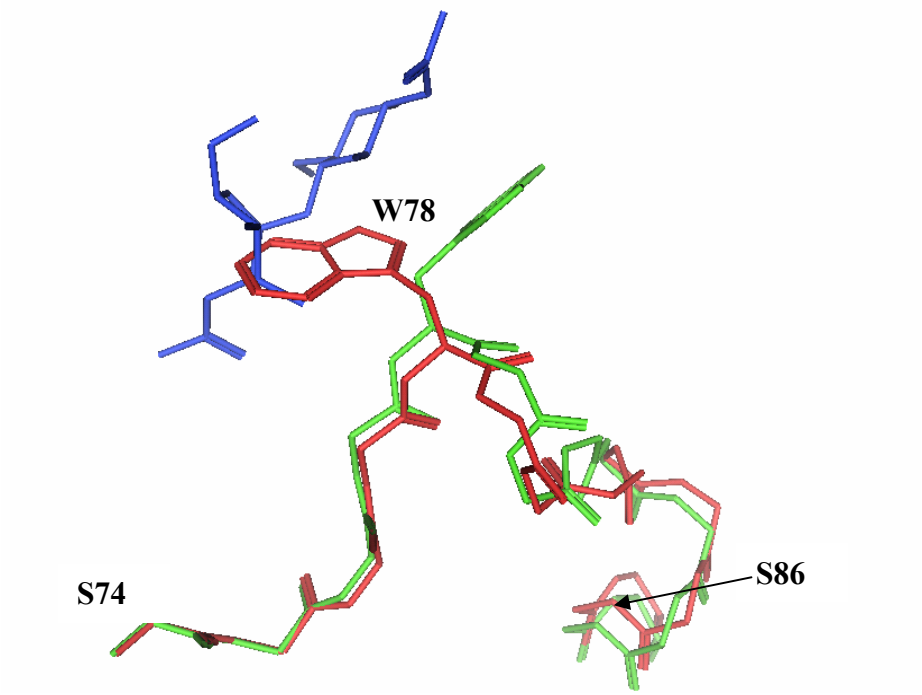

Figure 2: Backbone of the residues 74-86 of MGP40 (red) and equivalent residues 95-107 of GP39 (blue) are shown. Sidechains of W78 (MGP40) and W99 (GP39) are also shown. The –1 +1 sugars found in the GP39 structure are shown in blue. The proteins and sugar are depicted in stick configuration.

We believe that there are no potential causes for the lack of observed oligosaccharide binding in MGP40. It appears that the MGP40 in this study was not crystallized in appropriate conditions and hence did not have sugar in the binding site. Careful crystallization and x-ray structure determination should allow sugars in the binding site of MGP40.

Structural Comparison: +1 Binding Site

As mentioned above, the Trp at +1 position [W788 (MGP40) and W99 (GP39)] has two different conformations depending on the presence of the sugar. To further investigate the +1 binding site, we superimposed structures from different Family 18 proteins. We observe that the proteins with no sugar bound, MGP40 and two GP39 structures (pdb ids: 1LJY, 1NWR, 1HJX) (Fusetti, Pijning *et al.*, 2003; Houston, Anneliese *et al.*, 2003; Mohanty, Singh *et al.*, 2003) have the Trp in a different –

"pinching" orientation as opposed to the "stacking" orientation in structures with the sugar bound (**Figure 3**). As seen in the figure the Trp in the "pinching" orientation (red) pinches the –1 binding site, whereas Trp in the stacking configuration (green) stacks with the +1 sugar to provide the favorable hydrophobic interaction. Hence, we propose that the Trp at +1 position functions as the "gate" for this large binding site. Whether the "key" is just the knocking of an oligosaccharide on this "gate" or involves a more sophisticated system of conformational change is yet to be determined. Fusetti et al. point out the rotation of the side chain of the Trp to make this parallel stacking interaction. Furthermore, as mentioned by the authors, this differing orientation of the +1 Trp is not observed in chitotriosidase (pdb: 1LG1 and 1LG2) (Fusetti, Von Moeller *et al.*, 2002). Upon further investigation, we found that in Chitinase B (pdb: 1E15 and 1E6N) the +1 Trp has the same orientation in the apo- and holo form. Thus, there appears to be a clear distinction between the chitolectin and chitinase classes of Family 18 proteins; namely the +1 Trp. In chitolectins +1 Trp has distinct pinched and stacked conformations in the apo and the holo forms respectively, whereas in chitinases +1 Trp has the same orientation in the apo and the holo forms.

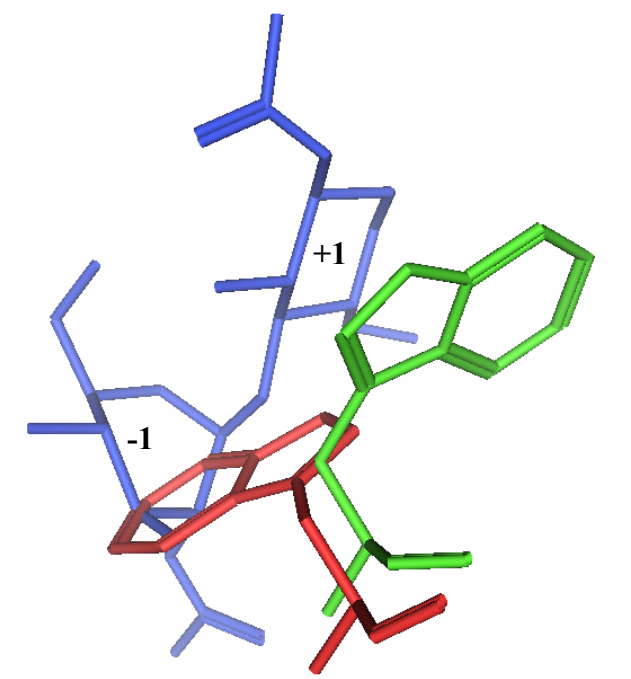

Figure 3: Trp99 of GP39 protein along with the binding sugars is shown in stick configuration. Trp99 of GP39 from a structure with no sugar bound (PDB code: 1HJX) is shown in red and with sugar bound (PDB code: 1HJW) is shown in green. The bound sugars at –1 and +1 positions are shown in blue. The proteins and sugar are shown in stick configuration.

Structural Comparison: +3 Binding Site, Conformation of Residues 209-213

Houston et al. proposed that W212 (+3 binding site) and the neighboring residues R213 and H209 in GP39 are in different orientation depending upon the presence of the oligosaccharide in the binding site. They propose that there is a ligand (oligosaccharide) induced conformational change in this region. The conformation of this region is different in their x-ray structures in the presence and absence of sugar. However, upon further investigation, we determined that in one of the four protein chains with no sugar (PDB code: 1HJX – chain B) the conformation of the 209-213 region was similar to one with the sugar bound. Furthermore, H209 and R213 in MGP40 (no sugar bound) are also in similar position to that of sugar-bound GP39. Finally, recently published x-ray structures of GP-39 (Fusetti, Pijning *et al.*, 2003) show no difference in the conformation of residues 209-213 in presence or absence of bound oligosaccharides. Thus, it would seem that binding of sugar likely does not influence the conformational change of H209, W212 and R213 in the GP39 structures as proposed by Houston et al.

Comparison of GP39 structures

As mentioned earlier, two groups have independently determined the structure of GP39 in the presence and absence of oligosaccharides. The tetrasaccharide and pentasaccharide have their sugars bound respectively in –2 to +2 and –3 to +2 configuration in both studies. Interestingly, hexasaccharide shows –4 to +2 binding in one study (Houston, Anneliese *et al.*, 2003) and –3 to +3 binding in the second study (Fusetti, Pijning *et al.*, 2003). W212 makes up the +3 binding subsite, and as described earlier it is similar between the two GP39 structures. Based on a) definite binding sites for tetrasaccharide (-2 to +2) and pentasaccharide (-3 to +2) and b) lack of a definite binding site for a hexasaccharide (-3 to +3 versus –4 to +2), it would appear that the energetic surface distal to the –3 and +2 subsites is shallow and not very well-defined. Fusetti et al. (Fusetti, Pijning *et al.*, 2003) also crystallized a disaccharide bound GP39. Interestingly, disaccharide appears to bind in the –5 -6 subsites rather than somewhere between -4 and +3 subsites. We further investigated the GP39 structures elucidated by Houston et al. We noticed that in tetrasaccharide-bound proteins there were also trisaccharides bound in the -5 –6 -7 subsites. Based on this information, we concur with Fusetti et al. that only larger (than tri) oligosaccharides bind in the traditional (-4 to +3) binding subsites, whereas the shorter oligosaccharides occupy the distal binding site.

CONCLUSION

In this study, we compare the structures of two Family 18 chitolectins, MGP40 and GP39. We propose that MGP40 is capable of binding oligosaccharides contrary to the authors' conclusion. We also show that the conformation of residues 209-213 is not altered upon ligand binding as proposed by the authors. Based on the two GP39 structures, we concur that there are two distinct binding sites; a distal site for trisaccharides and the main site for tetrasaccharides and larger. Most importantly, we propose that the Trp in the +1 position functions as a "gate" to the main binding site that is in the "pinched" conformation when the oligosaccharide is not bound and is in the "stacked" conformation while interacting with the oligosaccharide. This equivalent conserved residue in ChiA and ChiB, both active Family 18 chitinases, never shows a pinching conformation, but only an open one.

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