

Monte Carlo Docking Study for the Role of Glycosidic Residues in Determining the Human 2G12 Antibody-Binding Specificity with Series of Manno-Disaccharides

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Viral attachment to host cell surfaces is the primary step in transcriptional replication and pathogenesis.¹ Thus, an understanding of chemical nature of the structures involved in the attachment, can lead to new methods for preventing pathogenic infections.² If the attachment of the virus to the host cell surface involves a receptor-mediated process, it should be inhibited by substances that block this interaction. Recent experimental study done by Calarese *et al.*³ showed that crystal structure of human antibody protein 2G12 neutralized a broad range of human immunodeficiency virus type 1 (HIV-1) by binding a cluster of carbohydrates on the surface of gp120 envelope glycoprotein. They found mannopyranose-containing sugar material may produce an especially potent antiadhesive agent. This mannopyranose (Man) is a biologically important natural monosaccharide that has an axial hydroxyl group at the C₂-position. They suggested extracellular Man_nGlcNac₂ glycan chain bound to the recognition pocket of 2G12 antibody proteins, in which only terminal α -Man-1,2- α -Man moiety could be accommodated in the binding pocket. This is a primary step for antigen-antibody binding for the process of HIV neutralization. Interesting binding specificity for the α -Man-1,2- α -Man moiety was dominated by the hydrogen bond forming geometry of the ligand-receptor complex. Inhibition study with IC₅₀ value revealed the α -Man-1,2- α -Man inhibits binding of 2G12 to gp120 better than the other manno-disaccharides such as α -Man-1,3- α -Man, α -Man-1,4- α -Man, or α -Man-1,6- α -Man by more than 10 times.³

In the present study, we performed Monte Carlo (MC) docking simulations of the series of manno-disaccharides with receptor 2G12 protein to obtain molecular basis on the binding specificity of α -Man-1,2- α -Man compared with the other α -Man-1,3- α -Man, α -Man-1,4- α -Man, or α -Man-1,6- α -Man. This docking study from a LigandFit program⁴ showed that binding of manno-disaccharides to the receptor protein was specifically regulated by kinds of glycosidic linkages, whereas a binding strength was dominated by non-reducing mannose part of the series of disaccharides. The experimentally observed inhibitory effect of α -Man-1,2- α -

Man was investigated by the present MC docking study in terms of the highest Dock Score of α -Man-1,2- α -Man. The present computational aspects on the binding specificity of the α -Man-1,2- α -Man may be used for a research field of structure based design for sugar-type bioactive materials.

Binding orientation of α -Man-1,2- α -Man within binding pocket. Predicting the correct binding mode of ligand molecule within binding pocket of receptor is a critical step when the docking simulation is used for structure based drug design.⁴ Thus we examined docked poses for the α -Man-1,2- α -Man from the 150,000 trial of MC docking simulation. Evaluated 200 docked poses of α -Man-1,2- α -Man determined by LigandFit could be clustered into two major (**a**, **b**) and three minor (**c**, **d**, **e**) binding modes. Snapshots for two major clusters were represented in the Figure 1. The clustering was performed with five different poses showing the highest docking scores on the basis of RMSD criterion. Each cluster was grouped, whose RMSDs were less than 1.7 Å vs the each top-ranked docked pose. Each cluster ranked 85 poses for cluster **a**, 78 poses for cluster **b**, 19 poses for cluster **c**, 10 poses for cluster **d**, and 8 poses for cluster **e**, respectively. All five clusters showed consensus binding orientation of the ligand faced to the binding pocket of 2G12 protein. Non-reducing end moiety of the α -Man-1,2- α -Man lodged in the binding pocket and remained reducing end residue was exposed to the surface of 2G12 protein. This result means there are kinds of specific interaction between manno-disaccharides and receptor 2G12 protein.

Effect of number of MC trials and applied force fields. To prove reliability of the present docking study, we compared the Dock Scores of each top-ranked pose of disaccharides calculated with different force fields or MC trial conditions. With LigandFit program, combinations with two different energy functions, Dreiding and CFF produced similar results, as shown in Figure 2. An α -Man-1,2- α -Man ranked highest Dock Score compared to those of α -Man-1,3- α -Man, α -Man-1,4- α -Man, or α -Man-1,6- α -Man in the docking results of both force fields. Moreover, all the results were not readily affected by the number of MC trials. Top

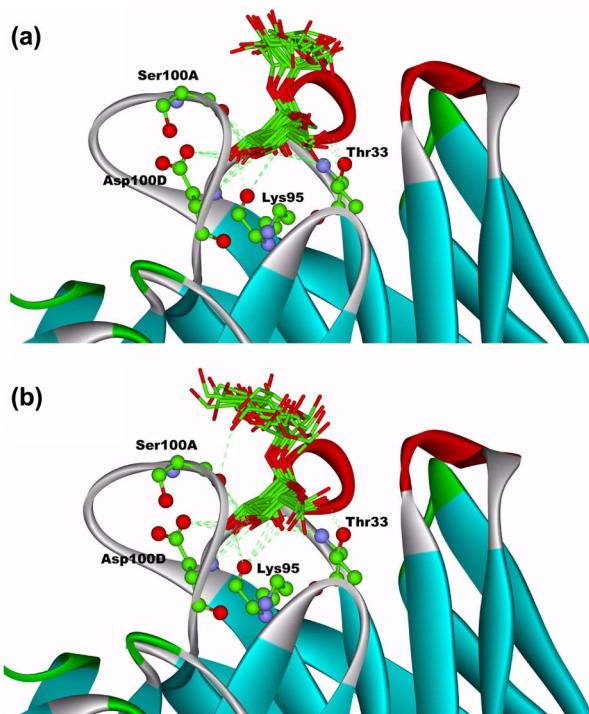


Figure 1. Snapshots of docked poses for α -Man-1,2- α -Man. The 200 poses produced by LigandFit were assorted into five different clusters. Only two major (a, b) clusters were shown to avoid redundancy.

ranked Dock Score for each pose was nearly identical between different MC trials (Figure 2). That means the

present docking study does not suffer from convergence problem. After 150,000 trials of MC docking with CFF, α -Man-1,2- α -Man ranked the highest Dock Score with receptor 2G12 protein, in which the value was 97.37. Other α -Man-1,4- α -Man, α -Man-1,3- α -Man, and α -Man-1,6- α -Man showed the Dock Scores of 89.02, 86.21, and 87.08, respectively. These scores are comparable with the IC₅₀ values from the experiments performed by Calarese *et al.*,³ in which the inhibitory effect was only found in the case of α -Man-1,2- α -Man. Other α -Man-1,4- α -Man, α -Man-1,3- α -Man and α -Man-1,6- α -Man did not show any inhibitory effect. The MC result shows the highest Dock Score only for the inhibitory manno-disaccharide, α -Man-1,2- α -Man, related with the experimental³ IC₅₀ analysis. This comparison indicates that the present docking approach finely explained the experimental result on the molecular basis.

Detailed binding geometry and binding specificity. To obtain detailed information of binding mode for each manno-disaccharide with the receptor protein, we analyzed hydrogen bond between ligands and receptor protein. In the crystal structure, nine hydrogen bonds were observed between α -Man-1,2- α -Man and binding pocket of 2G12 protein. In which six hydrogen bonds were coupled between hydroxyl groups directly attached to the non-reducing mannopyranosic ring and specific amino acids of binding pocket. Each 2,3,4-OH group of mannose residue was linked with Thr33N, Lys95O, Asp100DO, Ser100AO, Asp100DN of the binding site residues by hydrogen bond. This strong hydrogen bond cluster may provide binding capability of

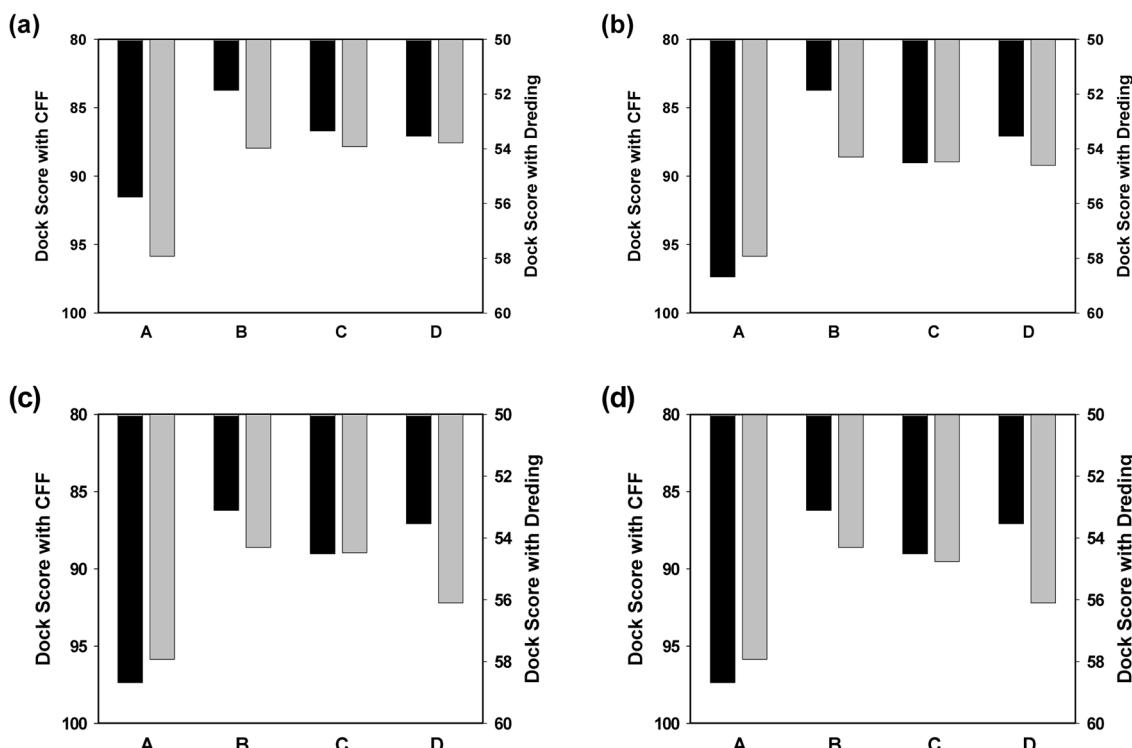


Figure 2. Comparison of different MC trials in ranking the 4-different disaccharides docked in 2G12 protein. The docking was performed with CFF (black) or Dreiding (gray) force field, and the top-scored poses using the Dock Score function in the LigandFit module of DS were selected.

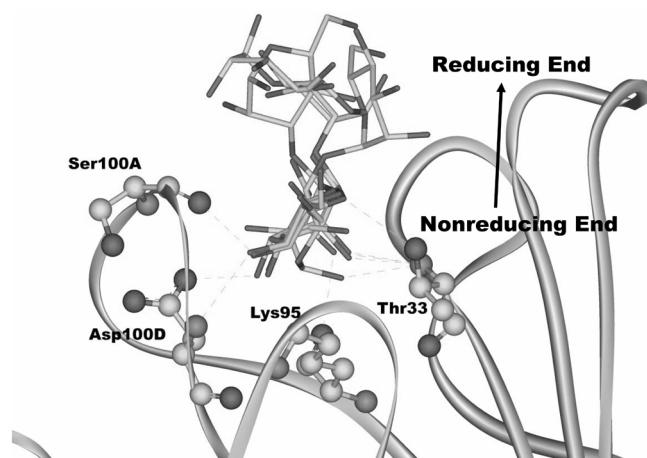


Figure 3. Front and back view for the docked poses of each disaccharide with the amino acid residues in the binding pocket of the receptor protein. The docked poses of α -Man-1,2- α -Man, α -Man-1,3- α -Man, α -Man-1,4- α -Man, and α -Man-1,6- α -Man were overlaid with each other. Selected residues in the pockets mediating hydrogen bond interactions with the ligands are labeled and the interactions shown in green.

mannose residue with the receptor 2G12 protein. Figure 3 is a superimposed picture of docked poses for each manno-disaccharide hydrogen bonded to the specific amino acid residues of receptor protein. All four manno-disaccharides interacted with receptor protein by the non-reducing end moiety. These non-reducing end interactions were observed to be super-imposable between four different manno-disaccharides showing little differences. However, remaining reducing end moieties could not be overlaid in their binding geometry.

That result means the above amino acids cluster of binding pocket may recognize the shape of single mannose residue but not entire manno-disaccharides. So binding capability of manno-disaccharides are dominated by non-reducing end interaction but binding specificity is determined by the kind of glycosidic linkage between non-reducing and reducing end moiety. Strong binding affinity of the α -Man-1,2- α -Man can be explained by specific geometry governed by α -1,2-glycosidic linkage between non-reducing and reducing end of mannopyranosic residues as shown in Figure 3. We

think α -1,2-linked manno-disaccharide drives their geometry to fit binding pocket of receptor protein without instability or fluctuation. This stable docked conformation of α -Man-1,2- α -Man induced by specific glycosidic linkage might make it has inhibitory effect on the binding of 2G12 protein to gp120 of HIV. The experimentally observed inhibitory effect of α -Man-1,2- α -Man was confirmed by the present docking study in terms of the highest Dock Score of α -Man-1,2- α -Man.

Additional analysis for a profile of hydrogen bonds between each manno-disaccharide and 2G12 protein was summarized in Table 1. Two hydrogen bond pairs between 3-OH and Asp100D and between 4-OH and Ser100A were revealed as the most important interaction from the distance analysis. Compared to crystal structure, docked pose of α -Man-1,2- α -Man showed the most similar hydrogen bond distance among all other docked pose of manno-disaccharides. Averaged hydrogen bond distances of α -Man-1,2- α -Man with the receptor was shorter than those of the other manno-disaccharides. That result demonstrates the α -Man-1,2- α -Man is more suitable ligand to 2G12 receptor protein as showing in the scoring function results. Moreover, standard deviation (SD) of the hydrogen bond distance between non-reducing end residue and binding pocket residue marked the lowest value of 0.16 in the α -Man-1,2- α -Man. Other Man-1,3- α -Man, α -Man-1,4- α -Man, and α -Man-1,6- α -Man showed the SD values of 0.18, 0.78, and 0.23, respectively. But the hydrogen bond between 6-hydroxyl and binding pocket could not be reproduced the distance of crystal structure in all docked poses of manno-disaccharides. This inaccuracy may be due to the lack of information for exo-cyclic effect⁶ in the CFF, which is critical to calculate conformation of carbohydrate. However, overall computational results were readily consistent with the previous experimental results on the specific binding of Man-1,2- α -Man. Moreover, the present docking study provided the suggestion on the molecular basis of binding specificity governed by the kinds of glycosidic linkages.

Computational Methods

Molecular docking simulations were performed with the

Table 1. Hydrogen bond distance (\AA) between ligand disaccharides and receptor 2G12 protein

Hydrogen-bonding pair	Man-1,2-Man (Crystal)	Man-1,2-Man	Man-1,3-Man	Man-1,4-Man	Man-1,6-Man
2O···Thr33N	2.803	2.778 ± 0.161	2.579 ± 0.123	3.112 ± 1.178	2.571 ± 0.143
2O···Lys95O	2.718	3.089 ± 0.251	3.259 ± 0.226	3.501 ± 0.745	3.259 ± 0.367
3O···Lys95O	3.136	3.381 ± 0.075	3.341 ± 0.265	3.290 ± 0.213	3.140 ± 0.176
3O···Asp100DO	2.616	2.651 ± 0.186	2.906 ± 0.140	2.950 ± 0.509	2.968 ± 0.232
4O···Ser100AO	2.811	2.703 ± 0.187	2.771 ± 0.209	3.017 ± 0.769	2.686 ± 0.174
4O···Asp100DN	3.035	3.094 ± 0.118	3.175 ± 0.123	3.528 ± 1.262	3.212 ± 0.304
6O···Gly93O	2.834	3.384 ± 0.603	3.562 ± 0.447	3.426 ± 0.598	3.883 ± 0.552
6O···Thr33O	2.747	4.093 ± 1.043	4.498 ± 0.892	4.681 ± 1.743	4.246 ± 1.016
3O···Ala31O	2.658	4.429 ± 1.128	4.281 ± 0.919	4.722 ± 1.653	5.864 ± 0.925
Average Value	2.823	3.291	3.372	3.581	3.543

Discovery Studio/LigandFit program (version 1.7, Accelrys Software Inc.) using a consistent force field (CFF) on a dual-Xeon workstation connected with molecular simulation grid (MGrid). The MGrid system supports collaborative and integrated research infrastructure for the molecular simulations. The receptor 2G12 protein structure was obtained from a crystallographic geometry of the Protein Data Bank (PDB id 1OP3). The series of manno-disaccharides was constructed and energy-minimized using the DS/Builder module. The binding site was reconstructed from the grid for the binding cavity of crystal structure of 2G12 antibody protein. The cavity construction task of the DS program was archived by employing a cubically shaped eraser to the each grid point. A series of four docking simulations for receptor 2G12 protein with α -Man-1,2- α -Man, α -Man-1,3- α -Man, α -Man-1,4- α -Man, or α -Man-1,6- α -Man, was performed on the reconstructed binding site of receptor protein. The docking of the disaccharides was carried out using the LigandFit module of DS. The docking process was assumed to be a 1:1 interaction between flexible ligands and rigid receptor protein during the MC runs.⁷ A CFF or Dreiding force field was used as an energy grid force field for docking and PLP1, PLP2, Jain, and PMF were used for scoring. A 13 Å cutoff was imposed on the calculation of non-bonded interactions, and distance-dependent dielectrics ($\epsilon=1r$) was used to mimic solvent screening during the conformational searches. The energy grid extension was settled to 8 Å and softened potential energy option was used. Conformational search of the Monte Carlo⁸ (MC) docking was performed, in which 2 Å of RMS threshold value was applied. An energy

tolerance⁹ of 10,000 kcal/mol was imposed to avoid significant overlap of van der Waals radii in the random search. Docked poses within of pre-existing clusters were discarded to avoid accepting similar poses. From these MC docking simulations, we obtained 200 docked poses for each manno-disaccharide with receptor protein. For the α -Man-1,2- α -Man, each docked pose was clustered by 1.7 Å of RMSD criterion.

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