The Role of Gc Protein Oligosaccharide Structure as a Risk Factor for COPD

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Abstract. *Background: The risk of chronic obstructive pulmonary disease (COPD) is related to Gc protein allele type, such as Gc*1F, Gc*1S, Gc*2. It has been reported that Gc*1F increased COPD risk, while Gc*2 suppressed the risk. Thus, the allele type of Gc protein is an important factor in COPD. These Gc proteins differ in sugar composition at Thr418 or Thr420. In this study, features of the sugar structure of modeled Gc proteins were investigated. Materials and Methods: Gc protein (Gc1F, Gc1S, Gc2) models were constructed based on X-ray data of vitamin D binding protein (ID=1J7E) using InsightII-Discover with the Homology module, and the molecular orbital (MO) parameters [e.g., dipole moment, solvation free energy (dGW)] of the oligosaccharide were analyzed. Results: The MO parameter of the sugar moiety was different for each Gc protein model. In β-1,4 bond models, the dipole moment of Gc2 protein was larger (56.6 debye) than Gc1 type (Gc1F: 21.9, Gc1S: 29.8 debye) protein, and it was directed towards the intermolecular space. The Gc2 oligosaccharide region was the most hydrophobic (dGW=–999.4 KJ) among the Gc proteins analyzed in this study. The electrostatic potential (ESP) field of β-1,4 type Gc2 protein was similarly distributed to β-1,4 linked Gc1-type proteins (Gc1F, Gc1S). In the β-1,3 type Gc protein models, the results of these parameters (i.e., dipole moment, dGW and ESP) were similar to those of β-1,4 type models. Conclusion: The relationship between COPD risk and the features of the sugar structure in Gc proteins was examined, and it appeared that the active factors (i.e., dipole moment, dGW) might be risk factors for COPD, but passive factors (i.e., ESP) did not affect COPD risk. The bond type (β-1,4 or β-1,3) between galactose and N-acetylgalactosamine did not affect the molecular features.*

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Key Words: Gc protein, Gc protein-derived macrophage activating factor (GcMAF), *N*-acetylgalactosamine.

Chronic obstructive pulmonary disease (COPD) is characterized by expiratory airflow obstruction and hyperinflation due to inflammation of peripheral airways and loss of lung elastic recoil. Tobacco smoking is the major risk factor for COPD, however only 15% of smokers develop clinically relevant airflow obstruction (1). This suggests that genetic factors are likely to have a role in the determination of individual susceptibility to COPD. Polymorphism of several candidate genes has been examined in relationship to COPD development (2). One of the candidates is the gene encoding the group-specific component of serum globulin (Gc-globulin), called Gc-protein, a vitamin D-binding protein. Gc-protein is a multifunctional (polymorphic) 55 kDa protein (3), and its functions include being a macrophage-activating factor (MAF) precursor (4) and co-work with phagocytic cells (5). These functions suggest a role for Gc-protein in chronic inflammation in the lung.

There are three common polymorphisms in structures of Gc-protein which are encoded by one of the three co-dominant alleles of the Gc-globulin gene, Gc*1F, Gc*1S and Gc*2, and >124 variant alleles (6). At least three types of homo dimer protein (Gc1F-1F, Gc1S-1S, Gc2-2) and three hetero dimers (Gc1F-1S, Gc2-1F, Gc2-1S) are reported (7, 8). The major phenotypes of Gc1F, Gc1S and Gc2 differ in only four amino acids (152, 311, 416 and 420) (7, 8). Recently, it has been reported that there was an increased proportion of Gc*1F homozygotes in the patients with COPD compared with the healthy smokers, while Gc*2 homozygotes suppressed COPD risk in smokers (9, 10). The C-terminal of Gc protein (domain 3) has a single glycosylation site at amino acid Thr418 or Thr420. The carbohydrate structures have been investigated by analysis

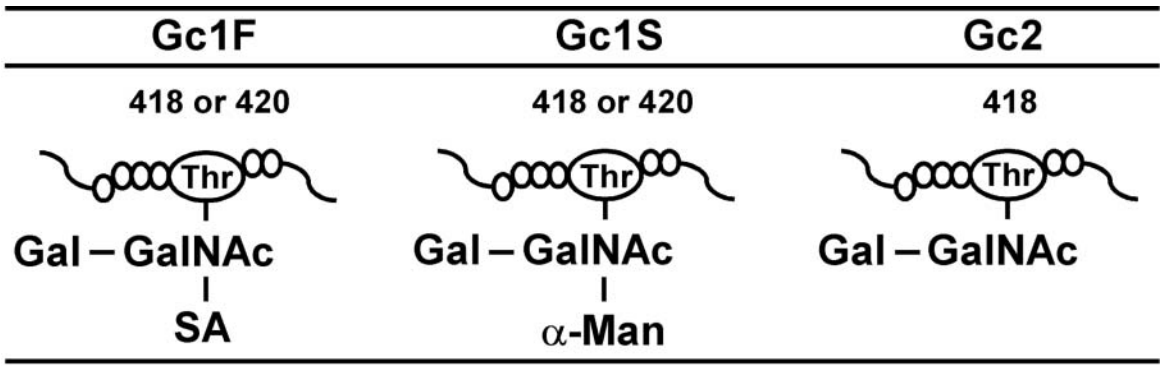

Figure 1. *Sugar moiety composition of Gc proteins. Gal: β-galactose, GalNAc: α-N-acetylgalactosamine, SA: sialic acid, α-Man: α-mannose.*

of the products following treatment with some glycolytic enzymes (4, 11, 12). Gc1-type protein has a branched trisaccharide with *N*-acetylgalactosamine (GalNAc) attached to the core protein and a galactose molecule, and a sialic acid (Gc1F) or mannose molecule (Gc1S). Gc2 has a single glycosylated chain with a core GalNAc conjugated to a terminal galactose molecule (Figure 1). These Gc proteins are the precursors of macrophage activating factor (MAF). Gc protein-derived MAF (GcMAF) synthesized by β-galactosidase (from B-cells) and sialidase (from T-cells), retained the GalNAc molecule at Thr418 or Thr420 (13).

As shown in Figure 1, the glycosylation pattern was different between Gc-proteins, and these structural polymorphisms seem to be involved in the COPD risk. In this study, Gc-protein models were constructed using Insight II-Discover and their structural features were analyzed. The dipole moment (the parameter for reactivity to an external factor), electrostatic potential and solvation free energy (index of hydrophobicity) were determined for an oligosaccharide portion, including a 6 amino acid sequence (416 ~ 421).

Materials and Methods

Reagents. All reagents were of guaranteed grade and used without further purification. β-Galactosidase from bovine testes (Code No. G4142) and neuraminidase (Code No. N2876) were from Sigma-Aldrich Japan Co. (Tokyo, Japan). β-Galactosidase from jack bean was from Seikagaku Co. (Tokyo, Japan).

GcMAF preparation. One μL of Gc protein (5 μg/mL) was diluted 1:50 with 10 mM sodium phosphate buffer (pH 6.0) and treated with 1 μL of sialidase (10 U/mL) and 1 μL of β-galactosidase (jack bean: 10 U/mL) or 5 μL of β-galactosidase (bovine testes: 1 U/mL) at 37˚C overnight. Reaction was stopped on ice.

Macrophage. Resident mouse peritoneal macrophages were collected and centrifuged at 1,000 rpm for 10 min at 4˚C. Macrophages ($5x10^5$ cells/well) were suspended in RPMI-1640 medium and transferred in 24-well plates, each well containing a coverslip. Cells were incubated at 37˚C in a 5% CO_2 incubator for 1 h. After washing with medium to remove non-adherent cells, the adherent macrophages were treated with GcMAF for 3 h, and then used for the phagocytosis assay as described previously (14).

Molecular modeling. The Homology models of the Gc-proteins (Gc1F, Gc1S, Gc2) were based on the crystal structure of vitamin D-binding protein (1J7E) using InsightII-Discover with the Homology module (Accelrys Inc., USA) and minimized under consistent valence forcefield (CVFF) as described previously (15). The sugar moieties were conjugated to the modeled core protein at Thr418, and optimized using Discover.

Semiemprical molecular orbital calculation and electrostatic potential. The z-matrix data at the oligosaccharide portion and near six amino acids (416 ~ 421) was extracted from the modeled protein data, and molecular orbital (MO) calculation was performed with a PM3 Hamiltonian using MOPAC (Fujitsu Limited, Japan). The stable and transient structures were initially built with general parameters of bond length, bond angles and dihedral angle, and refined with the eigen-vector following (EF) optimization method (16). The electrostatic potential fields were calculated using a Delphi module with insightII-Discover (Accelrys Inc., USA). The –1.0 kT/e contour was displayed as gray mesh and the +1.0 kT/e contour was displayed as black mesh.

Results

Carbohydrate structure of Gc1F. In order to determine the bond type between galactose (Gal) and *N*-acetylgalactosamine (Figure 1), β-galactosidase from jack beans, which hydrolyzes the Gal β-1,3 linkage much more slowly than other linkages (17), and β-galactosidase from bovine testes, which hydrolyzes both β-1,4 and β-1,3 linkages (18), were used to modify the Gc1F protein. GcMAF, which was obtained from the Gc1F-1F phenotype by jack bean β-galactosidase treatment, increased the ingestion index (case C in Figure 2). There was no significant difference in the ingestion index using bovine testes β-galactosidase (case D). These results show that the Gc1F-1F phenotype has an oligosaccharide with Gal β-1,4 linkage.

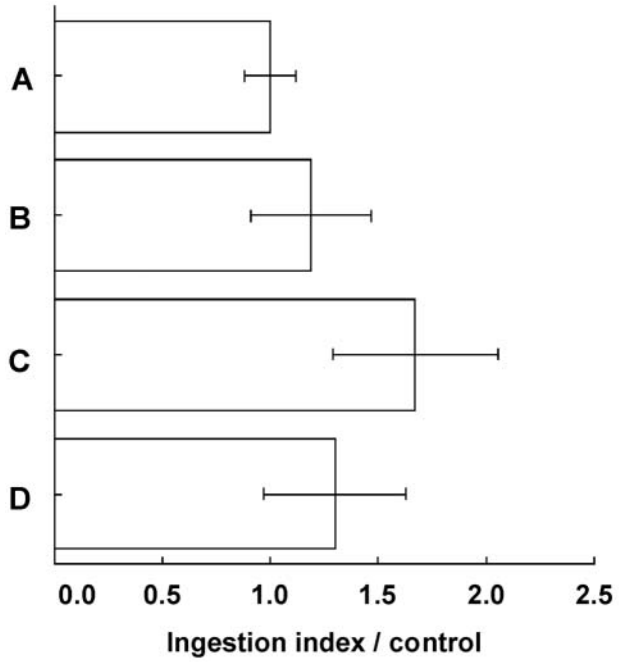

Figure 2. *Effect of β-galactosidase on macrophage ingestion. Gc1F-1F was modified by various enzymes, and macrophage ingestion activity was measured. A: control (without Gc1F-1F). B: Gc1F-1F (10 pg/ml), C: Gc1F-1F (10 pg/ml) + β-galactosidase (jack bean) + sialidase, D: Gc1F-1F (10 pg/ml) + β-galactosidase (bovine testes) + sialidase. The results are the mean (±SE) of three determinations.*

Table I. *Allele and genotype frequency in COPD patients and healthy smoker control subjects*.*

Variables	Patients with COPD n=103 (%)		Control n=88 (%)	
Allele				
Gc*1F	120	(58)	87	(49)
Gc*1S	46	(22)	47	(27)
Gc*2	40	(19)	42	(24)
Genotype				
1F-1F	33	(32)	15	(17)
1F-1S	29	(28)	27	(31)
1F-2	25	(24)	30	(34)
1S-1S	3	(3)	5	(6)
1S-2	11	(11)	10	(11)
2-2	2	(2)	1	(1)

*Data summarized from reference 9.

Table II. *Molecular parameters of the Gc-protein oligosaccharide region.*

	Dipole (debye)	dGW (KJ)
1,4-bond model		
Gc1F	21.93	–748.2
Gc1S	29.78	–669.9
Gc2	56.60	–999.4
1,3-bond model		
Gc1F	30.52	–773.1
Gc1S	29.48	–530.8
Gc2	40.20	–921.0
GcMAF	1.68	–346.7

Gc protein type for COPD risk factor. Gc-protein allele and genotype frequency in 103 COPD patients and 88 healthy smokers are summarized in Table I. The allele frequency in the COPD patients was Gc*1F (58%) >> Gc*1S (22%) > Gc*2 (19%). Gc1F including the genotypes 1F-1F: 32%, 1F-1S: 28% and 1F-2: 24%, increased the risk for COPD, while the Gc2 type (2%) was associated with decreased risk of COPD (9). The relationship between allele type and COPD risk has been similarly reported (10).

Structural feature at oligosaccharide region. The dipole moments of the Gc-proteins (Gc1F, Gc1S and Gc2) are shown in Figure 3. The β-1,4 linked Gc models displayed different dipole directions (A), and their intensities were 21.93 (Gc1F), 29.78 (Gc1S) and 56.60 debye (Gc2) (Table II). The dipole direction of the β-1,3 linked models was similar to the β-1,4 linked Gc proteins (Figure 3B). GcMAF was weak, 1.68 debye, and the direction was similar to the Gc2 type protein (Figure 3C). In the β-1,4 linked Gc models, the Gc2 type was more hydrophobic (dGW = –999.4 KJ) than Gc1 types (Gc1F: –748.2, Gc1S: –669.9 KJ). The order of hydrophobicity in the β-1,3 linked Gc models, Gc2 (–921.0 KJ) was higher than Gc1 types (Gc1F: –773.1, Gc1S: –530.8 KJ) (Table II). It was interesting that the hydrophobicity of GcMAF was low, and the value was –346.7 KJ.

The dipole directions of the β-1,4 linked Gc proteins are shown on the Gc1F monomer structure (Figure 4, upper panel), and the Gc2 moment was directed toward the outer area of the molecule. In the dimer structure (lower panel in Figure 4), the Gc2 moment was directed toward free space and interacted easily with external factors (*e.g.,* COPD-involved chemical compounds), while the moments of Gc1F and Gc1S were directed to another amino acid chain (B chain) and had difficulty interacting with external molecules. GcMAF had a weak dipole moment (1.68 debye) but its direction was advantageous for interaction with other compounds.

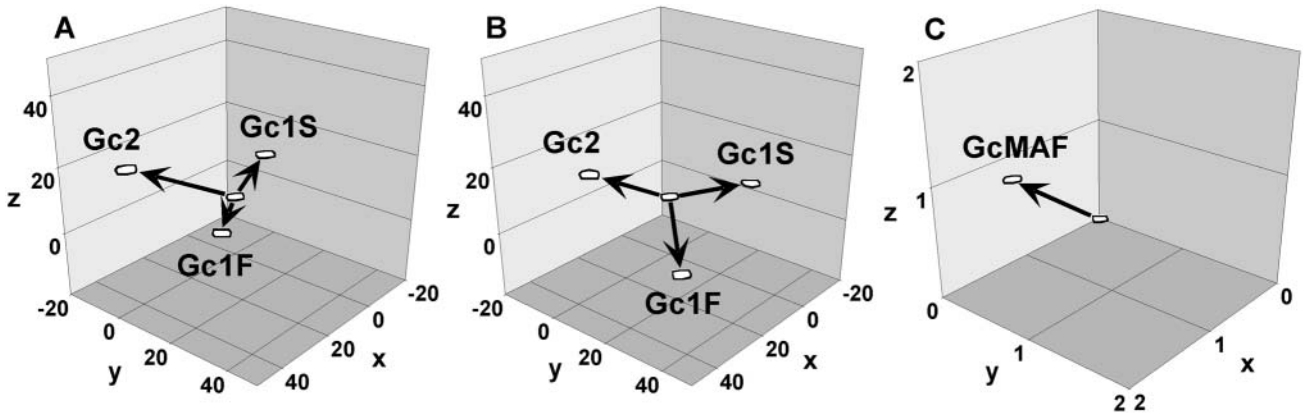

Figure 3. *Dipole moment of Gc proteins and GcMAF. The direction and strength of the dipole moment (arrow) of each Gc protein and GcMAF are shown. A: β-1,4 linked Gc1F, Gc1S and Gc2 models. B: β-1,3 linked Gc1F, Gc1S and Gc2 models. C: GcMAF.*

Electrostatic potential of Gc protein. The electrostatic potential field of the β-1,4 linked Gc1F protein monomer is shown in Figure 5, and the core protein was substantially occupied with a plus field (black). A small minus field was distributed near the root of the oligosaccharide region (gray). The other β-1,4 linked Gc-proteins (Gc1S and Gc2), the β-1,3 linked Gc-proteins (Gc1F, Gc1S, Gc2) and GcMAF displayed similar electrostatic potential field distribution, as well as β-1,4 linked Gc1F (data not shown). In the dimer molecule, these field patterns were similar to those of the monomers (data not shown).

Discussion

Cigarette smoking is a major risk factor for chronic obstructive pulmonary disease (COPD). The relationship between the COPD risk and Gc-protein type (allele or genotype) had been reported, and the Gc2 type suppressed COPD risk while the Gc1F increased the risk (9, 10) (Table I). In the β-1,4 bond Gc2 protein models, the dipole moments of both monomers and dimers was directed toward the outer area, which should be advantageous for interaction with COPD-involved external factors. In the dimer state, the dipole direction of Gc1F and Gc1S was towards an other amino acid chain (*e.g.,* B-chain), and would make interaction with external factors difficult. The intensity of the Gc2 moment was 56.6 debye, higher than the Gc1F (21.9 debye) and Gc1S (29.8 debye), so Gc2 should be more reactive. The hydrophobicity of the Gc2 oligosaccharide region was higher (dGW = –999.4 KJ) than the Gc1 type (Gc1F and Gc1S), which might enable it to scavenge environmental hydrophobic compounds. The affinity of the Gc2 oligosaccharide region to the cell membrane is higher than the Gc1 type sugar moiety. Although the hydrophobicity of GcMAF in the oligosaccharide region was very low (–346.7 KJ), the dipole moment in the dimer was directed towards the outer area as with the Gc2 protein, reactivity towards external chemical compounds would be more likely than with the Gc1 type protein. Thus, GcMAF seems to have a role (physiological function) which is different from other Gc proteins brought about by oligosaccharide modification, and may be concerned with homeostasis through the immunity system.

In the β-1,3 bond Gc models, the Gc2 oligosaccharide region displayed useful molecular parameters (*i.e.,* dipole moment, dGW) for interaction with external factors (chemical compounds) as in the β-1,4 bond-involved models. From these results, it was thought that the bond position of the Gal-GalNAC portion was not significant as a COPD risk-factor. *In vivo*, the oligosaccharide structure is made up of various combinations (*e.g.,* β-1,4 bond, β-1,3 bond), and further investigation is required to elucidate whether or not there is a relationship between reactivity and the bond position.

In all Gc proteins studied, the electrostatic potential field was distributed in a similar manner with most of the core protein occupied by the plus potential and a slight negative charge near the root of the oligosaccharide region. From these results, it was considered that a static (passive) factor, such as electrostatic potential, was not concerned with the reaction between the Gc proteins and COPD-related external factors. Positive (active) structural factors (*i.e.,* dipole moment, dGW) were considered to be related to the interaction with COPD-related factors (*e.g.,* tobacco-involved chemical substance). In the dimer state, the electrostatic field did not change according to the oligosaccharide structure (data not shown), and the dipole moment in the oligosaccharide region may play an important role in biological action *in vivo*. The GcMAF monomer and dimer indicated the same electrostatic potential as Gc proteins, and structural factors in the macrophage-activating ability of GcMAF are now being examined.

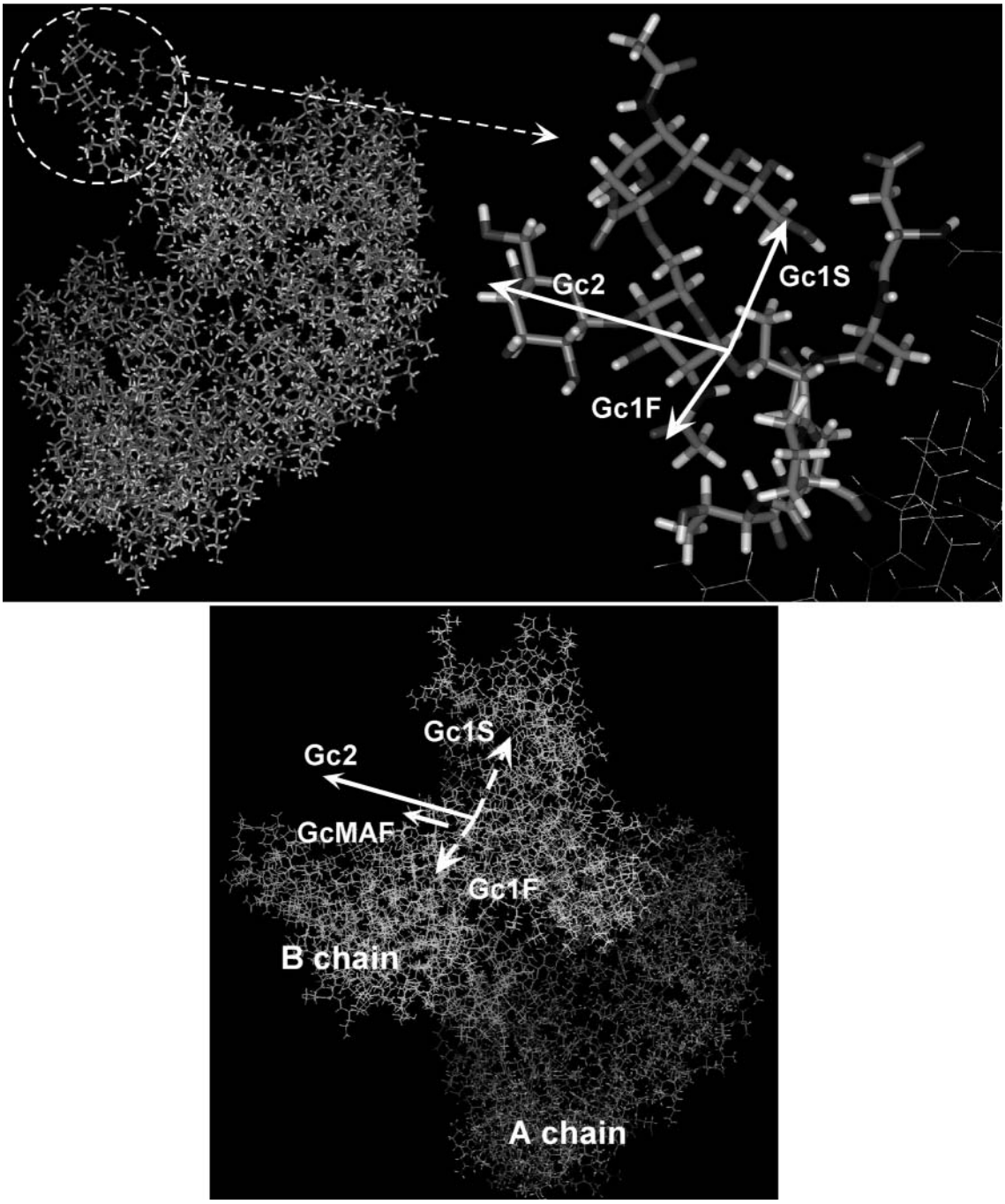

Figure 4. *Dipole feature of the Gc protein molecule. The upper panel shows the monomer state of Gc1F protein and an enlarged view of the sugar moiety. The monomer model structures of each of the Gc proteins are similar. Their dipole moments are shown together (arrows: Gc1F, Gc1S, Gc2). The lower panel shows the dimer state (A and B chain) of Gc1F-1F. The structures of Gc protein dimers and GcMAF are similar, and the dipole moments of the A chain are shown together (arrows: Gc1F, Gc1S, Gc2, GcMAF).*

It is clear that the Gc gene allele is concerned with differences in susceptibility to COPD. Since the structure of each Gc protein differs only near the sugar chain, the oligosaccharide structure and its neighboring structure should specify the susceptibility to COPD. We are now further analyzing the Gc type of COPD patients by examining the molecular design of Gc2 oligosaccharide-derived anti-COPD agents.

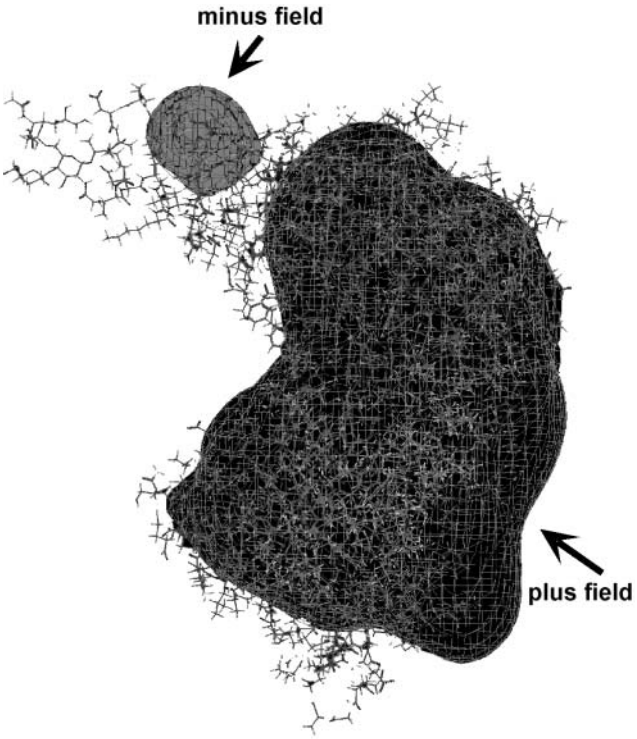

Figure 5. *Electrostatic potential field of the Gc1F model. The distribution of electrostatic potential fields are shown. The –1.0 kT/e contour is displayed as gray mesh and the +1.0 kT/e contour is displayed as black mesh.*

Acknowledgements

This work was supported, in part, by Grant-in-Aid for Scientific Research (15590098) from the Ministry of Education, Science, Sports and Culture, Japan.

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Received May 23, 2006
Revised July 28, 2006
Accepted August 22, 2006