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Theoretical analysis of binding specificity of influenza viral hemagglutinin to avian and human receptors based on the fragment molecular orbital method

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Abbreviations: HA, hemagglutinin; RBS, Receptor Binding Site; FMO, fragment molecular orbital; HF, Hartree-Fock; MP2, Moeller-Plesset second-order perturbation; IFIE, inter-fragment interaction energy; PDB, Protein Data Bank

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Abstract

The hemagglutinin (HA) protein of the influenza virus binds to the host cell receptor in the early stage of viral infection. A change in binding specificity from avian α2-3 to human α2-6 receptor is essential for optimal human-to-human transmission and pandemics. Therefore, it is important to reveal the key factors governing the binding affinity of HA-receptor complex at the molecular level for the understanding and prediction of influenza pandemics. In this work, on the basis of ab initio fragment molecular orbital (FMO) method, we have carried out the interaction energy analysis of HA-receptor complexes to quantitatively elucidate the binding specificity of HAs to avian and human receptors. To discuss the binding property of influenza HA comprehensively, a number of HAs from human H1, swine H1, avian H3 and avian H5 viruses were analyzed. We performed detailed investigations about the interaction patterns of complexes of various HAs and receptor analogues, and revealed that intra-molecular interactions between conserved residues in HA play an important role for HA-receptor binding. These results may provide a hint to understand the role of conserved acidic residues at the receptor binding site which are destabilized by the electrostatic repulsion with sialic acid. The calculated binding energies and interaction patterns between receptor and HAs are consistent with the binding specificities of each HA and thus explain the receptor binding mechanism. The calculated results in the present analysis have provided a number of viewpoints regarding the models for the HA-receptor binding specificity associated with mutated residues. Examples include the role of Glu190 and Gln226 for the binding specificity of H5 HA. Since H5 HA has not yet been adapted to human receptor and the mechanism of the specificity change is unknown, this result is helpful for the prediction of the change in receptor specificity associated with forthcoming possible pandemics.

1. Introduction

Over the past century, emergence of epidemic influenza has been serious threat to human health. The origin of human influenza viruses is thought to be avian influenza virus because all the subtypes are found in avian host. Newly adapted avian influenza virus to human host or reassortant virus could be pandemic because we have no immunity for it, as shown in our history such as 1918 (H1N1), 1957 (H2N2) and 1968 (H3N2) pandemics. Recently, the first H5 avian influenza virus infected patient was reported and emergence of new pandemic influenza is alerted (The World Health Organization Web site, 2006).

Influenza virus binds to receptors on the host cell surface by hemagglutinin (HA) protein. The HA exists as a homotrimer on the viral surface and each monomer is composed of two subunits, HA1 and HA2 (Fig. 1A). The receptor binding site (RBS) is located at membrane-distal tip of each monomer. Three secondary structure elements, Helix190 (residues 190-198), Loop130 (residues 135-138) and Loop220 (residues 221-228), and conserved residues Tyr98, Trp153 and His183 (H3 HA numbering) are involved in the RBS (Fig. 1B). Receptors contain glycans with terminal sialic acids and HAs primarily recognize the terminal part. Because the rich linkage type of sialic acid to vicinal galactose on the targeted cell differs according to species (α2-3 or α2-6), HA acquires the binding specificity for the linkage type on the host. Human influenza viruses preferentially recognize α2-6 (human receptor), whereas avian influenza viruses prefer α2-3 (avian receptor). Hence, it is believed that the change of receptor binding preference of avian HA is the critical first step of adaptation to human host (Suzuki et al., 2000).

In the case of H1, H2 and H3 subtypes, it was shown that as few as two mutations at the RBS are responsible for the adaptation to human host by genetic and mutational studies (Connor et al., 1994; Glaser et al., 2005; Naeve et al., 1984; Rogers and D'Souza, 1989). The substitutions Gln226Leu and Gly228Ser switch the binding specificity of H2 and H3 HAs from avian to human receptor, whereas the substitutions Glu190Asp and Gly225Asp change the binding specificity of H1 HA. To understand the structural basis of the receptor specificity of HAs, X-ray crystal structures of HA / receptor analogue complexes have been determined (Eisen et al., 1997; Gamblin et al., 2004; Ha et al., 2001, 2003; Stevens et al., 2006). By the structural studies, a number of interaction sites involved in the receptor binding have been revealed and several suggestions have been made as to the mechanism of how HAs recognize the different linkages and change the receptor specificity with only two residue substitutions. It was, however, difficult to

evaluate inter- and intra-molecular interaction energies of HA-receptor complexes quantitatively, and thus the roles of each residue in the receptor binding are not well understood.

Recently, several computational studies of influenza HA were performed to reveal the mechanism of HA-receptor binding. Li and Wang (2006) have investigated H5 avian HA and addressed the important residues for the receptor binding qualitatively by using semiempirical methods. Their assessment of important residues for receptor binding was consistent with experimental results. In ab initio theoretical studies (Sawada et al., 2006, 2007), binding energies and interaction patterns between receptors and H3 HAs were investigated quantitatively. The calculations, performed at Hartree-Fock (HF) level with the minimal STO-3G basis set, were mainly focused on the electrostatic and hydrogen bond interactions. Thus the van der Waals dispersion interaction, an effective energetic factor in hydrophobic interaction, was not taken appropriately into account. In consequence, the interactions between some conserved residues and receptors were not well evaluated.

Here, we perform ab initio theoretical studies on the binding specificity of HAs (H3 avian HA, H1 human HA, H1 swine HA and H5 avian HA) to avian and human receptors. We report the interaction patterns of receptor binding domain of HAs estimated by ab initio fragment molecular orbital (FMO) calculations at MP2/6-31G level, taking account of electron correlation effects.

----------Figure 1----------

2. Computational methods

The molecular mechanics calculations on the basis of classical force fields have often been employed for the analysis of ligand binding of biomolecular systems, but their computational accuracies remain to be assessed. In contrast, ab initio calculations such as molecular orbital method evaluate various physical quantities with high accuracy. However, applicability of the conventional molecular orbital method has been limited to small molecules because huge computational costs are required. The fragment molecular orbital (FMO) method proposed by Kitaura et al. overcomes this problem of size limitation (Fedorov and Kitaura, 2007; Kitaura et al., 1999). This method can deal with the proteins with hundreds of amino acid residues including polarization effects, and can evaluate energies of the system with high accuracy. The binding energy of HA

and receptor is then evaluated as

$$\Delta E = E_{complex} - (E_{HA} + E_{receptor}) \quad (1)$$

where E_{HA}, $E_{receptor}$ and $E_{complex}$ refer to the energies of HA, receptor and their complex, respectively.

In addition, inter-fragment interaction energy (IFIE) (Ito et al., 2007; Fukuzawa et al., 2006a, 2006b) obtained in the FMO calculations provides useful information for specifying important residues. The IFIE value is defined by the following expression:

$$\Delta E_{IJ} = (E'_{IJ} - E'_{I} - E'_{J}) + Tr(\Delta P_{IJ} V_{IJ}) \quad (2)$$

where ΔP_{IJ} is a difference density matrix, V_{IJ} is an environmental electrostatic potential for fragment dimer IJ from other fragments, and E'_{I} and E'_{IJ} are energies of fragment monomer I and dimer IJ without environmental electrostatic potential, respectively. These values ΔE_{IJ} then represent interaction energies of a ligand with an amino acid residue or between amino acid residues because each amino acid is assigned as a single fragment. The IFIEs were calculated in this study to analyze the interaction pattern and to estimate the contributions of each residue to binding. In order to address the change of IFIEs between fragment I belonging to HA and fragment J on the receptor binding, it is also convenient to introduce

$$\Delta\Delta E_{IJ} = \Delta E_{IJ}(\text{HA-receptor complex}) - \Delta E_{IJ}(\text{uncomplexed HA}) \quad (3)$$

and their summation over the fragments J ($\neq$I),

$$\Delta\Delta E_{I}^{total} = \sum_{J} \Delta\Delta E_{IJ} \quad (4)$$

The latter then refers to the contribution of each fragment I to the binding affinity between HA and receptor. It is noted here that $\Delta\Delta E_{IJ} = \Delta E_{IJ}$(HA-receptor complex) when the fragment J belongs to the receptor.

The coordinates used in this study were prepared from crystallographic structural data obtained from Protein Data Bank (PDB). The structures employed in the calculations and its experimental binding specificities are listed in Table 1 (Eisen et al., 1997; Gamblin et al., 2004; Ha et al., 2001, 2003; Matrosovich et al., 2000; Rogers and D'Souza, 1989; Stevens et al., 2006). Stereo view of each HA complexed with avian and

receptor are shown in Fig. 2. We optimized the location of unlocated oxygen atoms and hydrogen atoms of the complexes by molecular mechanics energy calculations based on the MMFF force field and cut out 82 residues of receptor binding domain of the optimized complex for the use in FMO calculations. Molecular mechanics and dynamics calculations were carried out by using the Molecular Operating Environment (MOE) software (Chemical Computing Group Inc.).

Fragmentation of the complexes for the FMO calculations was performed as follows (Fig. 3): Sialic acid was devided into two fragments and sugar was treated as a single fragment. Each amino acid residue at the receptor binding site (RBS) was assigned as a single fragment except cysteine pair forming S-S bond which was treated as a single fragment. Ab initio FMO calculations with the MP2/6-31G method were carried out by using the ABINIT-MP program (Mochizuki et al., 2004a, 2004b; Nakano et al., 2000, 2002).

----------Figures 2, 3----------

3. Results

3.1. Avian H3

For avian H3 / avian receptor complex, three kinds of sialic acid-galactose receptors registered in PDB were employed (PDB ID: 1MQM). Two of the receptors, R1 and R2, are of Sia1-Gal2-GlcNAc3 type, and the other, R3, is of Sia1-Gal2 type (numbering is according to the registered order in PDB file). We have found for the three structures that the interaction distances (see Figs. 1 and 2) between avian receptor and conserved residues vary significantly. Figure 4a illustrates the interaction distances between avian receptor and conserved residues at the RBS of avian H3, suggesting significant differences in the receptor binding energy among them. Because H3 avian HA contacts with only Sia1-Gal2 part and GlcNAc3 is exposed to solvent, we used the Sia1-Gal2 part for the FMO calculation. The receptor binding energy ΔE was then calculated to be -342.8 kcal/mol, -308.3 kcal/mol and -352.9 kcal/mol for R1, R2 and R3, respectively. The contributions of each residue to the receptor binding estimated from $\Delta\Delta E_I^{total}$ are illustrated in Figure 4b for R1, R2 and R3. As shown in the figure, the variations in the binding energy associated with some residues such as Ser137, Ala138, His183, Glu190 and Gln226 are remarkable. In particular, $\Delta\Delta E_I^{total}$ for Glu190 and Gln226 take both

positive and negative values, indicating the sensitivity to the structure.

Employing R3 as a stable binding structure, we analyzed $\Delta\Delta E_I^{total}$ for all the residues in HA, where the binding energy was divided into the intra-molecular (intra-HA) and intermolecular (HA-receptor) contributions. As shown in Fig. 4c, $\Delta\Delta E_I^{total}$'s for conserved residues such as Tyr98, Ala138, Trp153, His183, Glu190, Leu194, Gln226 and Gly228 were found to take totally negative values, thus stabilizing the receptor binding. In the case of Glu190, the intermolecular interaction with the receptor was destabilized, while the intra-molecular interaction was stabilized more strongly so that the total $\Delta\Delta E_I^{total}$ became negative. When $\Delta\Delta E_I^{total}$ for Glu190 was decomposed into each contribution from the fragment J, the contributions from Tyr98, Trp153 and His 183 to the stabilized binding were found to be significant, as illustrated in Fig. 4d. These results indicate that intra-molecular (intra-HA) interactions between conserved residues play an important role for HA-receptor binding.

As for avian H3 / human receptor complex, we performed the FMO calculations for two kinds of receptors, R1 and R2, both of which are of Sia1-Gal2 type (PDB ID:1MQN). Inter-atomic distances in the two structures differ significantly between each other, as in the case of avian H3 / avian receptor complex. Figure 4e illustrates the interaction distances between human receptor and residues at the RBS of avian H3, showing substantial structural variations between R1 and R2. The calculated binding energies ΔE's were then found to be -271.1 kcal/mol and -292.4 kcal/mol for R1 and R2, respectively. Even taking account of the fluctuations between the two structures, these magnitudes of binding energies are much smaller than those for avian H3 / avian receptor, explaining the binding preference for the latter. (The calculated ΔE values in the present study are listed in Table 2.) Comparing $\Delta\Delta E_I^{total}$ between R1 and R2 (Fig. 4f), we have found that there are significant differences for Tyr98, Ser136, Ser137, His183, Glu190 and Gln226. In particular, $\Delta\Delta E_I^{total}$ for Gln226 changes the sign between R1 and R2. The contribution to the receptor binding associated with Glu190 is also very sensitive to the structure. It is observed in Fig. 4f that R1 shows less advantageous interactions with Tyr98, Loop130 and Gln226 as compared to R2, while the latter shows much larger repulsive interaction with Glu190. This trade-off seen in H3 HA / human receptor complexes thus suggests the importance to cope with the repulsive interaction with Glu190. Figure 4g shows that repulsive interaction of Glu190 with carboxyl group of sialic acid overcomes the advantageous inter- and intra-molecular interactions and destabilizes the receptor binding.

Figure 4h illustrates a comparison of $\Delta\Delta E_I^{total}$ between avian H3 / avian receptor (R3) and avian H3 / human receptor (R2). As seen in the figure, the IFIEs associated with

Tyr98, Ser136, Ser137, Ala138, His183, Glu190 and Gln226 differ significantly between the two complexes, suggesting that these residues would play essential roles for the binding specificity of avian H3 to avian receptor. In particular, Glu190 shows a very significant difference between avian and human receptors, indicating the importance for the binding specificity.

The human receptor is complexed with avian H3 at more distant location than the avian receptor. This shift in the binding location of human receptor has been ascribed to the avoidance of disadvantageous interactions between hydrophobic groups in the human receptor and the polar Gln226 (Ha et al., 2003), while the avian receptor can form hydrogen bonding with Gln226. However, the present quantitative analysis has shown that the human receptor loses favorable interactions with Loop130 and His183 due to this shift, whose magnitudes seem to be greater than the gain associated with Gln226 (vide infra).

----------Figure 4----------

3.2. Human H1

We have six X-ray crystal structures, R1 – R6, for human H1 / avian receptor (Sia1-Gal2-GlcNAc3) complex (PDB ID:1RVX). Since all the structures are seen to be similar regarding the location of receptor, we employed R1 as a representative structure for the IFIE analysis. Because the GlcNAc3 part is exposed to water solvent and has no contact with HA, we used the Sia1-Gal2 part for the FMO calculation. Through the calculations, we have found that Glu190 interacts repulsively with the avian receptor and makes the receptor binding unstable even taking account of the change of intra-molecular (intra-HA) interactions (Fig. 5a). This result is somewhat strange in the light of the experimental fact of mutations that Glu190 is indispensable for the avian receptor binding. The calculated binding energy of human H1 and avian receptor R1 was -293.3 kcal/mol.

As for the human H1 / human receptor (Sia1-Gal2-GlcNAc3) complex, we also have six X-ray structures, R1 – R6 (PDB ID:1RVZ). The location of the receptors is very similar. We have thus employed R1 as a representative structure for the IFIE analysis. Because GlcNAc3 part have no contact with HA as in the avian receptor case, we used the Sia1-Gal2 part for the FMO calculation. The binding energy of human H1 and human receptor was then estimated to be -335.9 kcal/mol by the FMO calculation,

which is much lower (more stable) than that for avian receptor. The calculated $\Delta\Delta E_I^{total}$ value for Gln226 of human receptor complex is lower (more stable) than that of avian receptor complex (Fig. 5c). This result explains the experimental results indicating that H1 avian HA acquires the binding ability for human receptor with retaining Gln226, in contrast to H2 and H3 HAs (Glarser et al., 2005; Rogers et al., 1989). It was found in mutation experiments that Asp225 is important for the human H1 / human receptor binding (Glaser et al., 2005). However, as seen in Fig. 5b, our IFIE analysis has shown that Asp225 has essentially no contribution to the receptor binding, since the intermolecular (HA-receptor) interaction gives unfavorable contribution compensating the favorable contribution due to the intra-molecular (intra-HA) interaction.

----------Figure 5----------

3.3. Swine H1

H1 swine HA is known to bind to human receptor more strongly than to avian receptor (Rogers and D'Souza, 1989). We have first performed the FMO calculation for swine H1 / avian receptor complex, where the structure of receptor analogue is available for Sia1 (PDB ID:1RVT). The Gal part could not be observed in X-ray experiments and this observation probably reflects the low affinity of the HA for avian receptor (Gamblin et al., 2004). The result for the total IFIE, $\Delta\Delta E_I^{total}$, is illustrated in Fig. 6a. The binding energy ΔE was calculated to be -363.3 kcal/mol.

As for the swine H1 / human receptor complex, we have three X-ray crystal structures for receptor analogues, two for Sia1-Gal2 and one for Sia1-Gal2-GlcNAc3-Gal4-Glc5 (PDB ID:1RVO). Since the GlcNAc3-Gal4 interacts with the residues Asp190 and Ser193, we have employed the latter for the FMO analysis. The calculated result for $\Delta\Delta E_I^{total}$ is shown in Fig. 6b. As seen in the figure, Asp190 makes a favorable contribution to the receptor binding both by inter- and intra-molecular interactions. The calculated binding energy ΔE was found to be -390.5 kcal/mol, which is lower than that for the avian receptor.

Figure 6c compares the calculated results for $\Delta\Delta E_I^{total}$ between avian and human receptors. It is observed in the figure that the calculated values of $\Delta\Delta E_I^{total}$ are substantially different between them for Thr136, Asp190, Lys222 and Gln226. Sum of $\Delta\Delta E_I^{total}$ of the residues at the RBS of avian and human complexes are -190.6 kcal/mol and -280.9 kcal/mol, respectively. Although Asp190 and Gln226 significantly interact with Gal2 of human receptor, the structure of Gal2 is missing for the avian receptor

possibly due to the weak interaction with the swine H1 (Gamblin et al, 2004). We performed the IFIE analysis concerning the Sia1 to compare the interaction patterns of common part for both the receptors. The calculated pattern of $\Delta\Delta E_I^{total}$ for the avian receptor complex, in which only Sia1 is visible, shows stronger interaction generally than that of human receptor complex in contrast with the interaction patterns of full receptors (Fig. 6d). Sum of $\Delta\Delta E_I^{total}$ of the residues in the RBS of avian and human receptor complexes are -190.6 kcal/mol and -158.2 kcal/mol, respectively. The $\Delta\Delta E_I^{total}$ for Asp190 in the case of human receptor takes a negative value (Fig. 6c), showing a favorable contribution to the receptor binding. This result can explain the experimental result that the mutation Glu190Asp in H1 avian HA converts the binding specificity from avian receptor to human receptor (Glaser et al., 2005). The calculated binding energies of -363.3 kcal/mol and -390.5 kcal/mol for the avian and human receptors, respectively, are consistent with the experimental result that swine H1 have higher binding affinity for human receptor than for avian receptor (Rogers and D'Souza, 1989).

----------Figure 6----------

3.4. Avian H5

For avian H5 / avian receptor complex, we have an X-ray crystal data for a receptor analogue, Sia1-Gal2-GlcNAc3 (PDB ID:1JSN). Because GlcNAc3 part has no contact with HA, we used the Sia1-Gal2 part for the FMO calculation. Through the calculation, we have found that $\Delta\Delta E_I^{total}$ for Glu190 takes negative values both for inter- and intra-molecular interactions (Fig. 7a). The binding energy ΔE was calculated to be -299.2 kcal/mol. As shown in Fig. 7a, $\Delta\Delta E_I^{total}$ for conserved residues such as Tyr98, Ala138, Trp153, His183, Glu190, Leu194, Gln226 and Gly228 were found to take totally negative values, thus stabilizing the receptor binding.

As for avian H5 / human receptor complex, we have an X-ray crystal data for the receptor analogue with Sia1 (PDB ID:1JSO). The Gal part could not be observed in X-ray experiments and this observation probably reflects the low affinity of the HA for human receptor (Ha et al., 2001). Through the IFIE analysis, we have found that $\Delta\Delta E_I^{total}$ for Glu190 takes a positive value, in which the positive intermolecular interaction is greater in magnitude than the negative intra-molecular interaction (Fig. 7b). The comparison of $\Delta\Delta E_I^{total}$ between the avian and human receptors is shown in (Fig. 7c). The sum of $\Delta\Delta E_I^{total}$ of the residues in the RBS of avian and human receptor

complexes are -198.3 kcal/mol and -194.7 kcal/mol, respectively. It is seen in the figure that the signs of interactions are opposite between them for Glu190 and Gln226. These residues are thus considered to play important roles for the binding specificity of receptors. These results seem to be consistent with the recent experimental results of mutation analysis and glycan microarray analysis that each substitution Glu190Asp and Gln226Leu decreases the binding strength of H5 HA for avian receptor (Stevens et al., 2006). Interaction patterns of Sia1, which have approximately the same structure in both avian and human receptors, are compared between avian and human (Fig. 7d). In contrast to the interaction patterns of full receptors, the interactions of human receptor with HA are stronger than those of avian receptor at almost all residues. The sum of $\Delta\Delta E_I^{total}$ of the residues in the RBS of avian and human receptor complexes are then -174.5 kcal/mol and -194.7 kcal/mol, respectively. The calculated binding energy for the avian H5 / human receptor was -283.9 kcal/mol, which is higher (more positive) than that for avian receptor. This result is thus consistent with the experimental fact that avian H5 has a higher binding specificity to avian receptor than to human receptor.

----------Figure 7----------

4. Discussion

4.1. Variations of Complex Structure and Interaction Pattern

The X-ray crystal structural study of avian H3 complexed with α2-3 (avian) and α2-6 (human) receptor analogues showed that the α2-6 linkage sialic acid shifts slightly upward and its potential interaction distances with avian H3 are longer than in the α2-3 linkage (Ha et al., 2003). Nonpolar atoms of α2-6 sialoside were moved away from polar Gln226, while 9-hydroxyl of α2-3 sialoside interacts with Gln226, suggesting that the location shift of human receptor may alleviate the unfavorable interactions. These results are consistent with the lower affinity of the avian HAs for α2-6 receptors and with the binding preference of mutant Gln226Leu for α2-6 linkage. We calculated the interaction energies between each fragment of receptors and the residues in the receptor binding domain of HA to describe the binding properties and elucidate the receptor binding mechanism. Two α2-6 and three α2-3 receptor analogues registered in the PDB coordinate files were employed in the FMO calculations for the complexes to compare the binding properties.

By comparing the interaction patterns of the receptor-HA complexes, it was found that the differences in interaction patterns between human receptors were significant. However, even taking account of the fluctuations between the structures, the magnitudes of interaction energies of human receptor are much smaller than those of avian receptor: Examples include Tyr98, Ser137, His183, Glu190 and Gln226 residues (Fig. 4). Thus, these results explain the binding specificity of the H3 avian HA for avian receptor. Many of the amino acid residues in the RBS mainly interact with sialic acid part, whereas Glu190 and Gln226 interact with both sialic acid and galactose parts. Because the position of galactose differs according to the linkage type (α2-3 or α2-6), we supposed the two residues Gln190 and Gln226 are involved in receptor binding specificity. The difference in Glu190 residue is mainly due to the weaker interaction with galactose part. The galactose of α2-3 complex tilts toward Helix190 and interacts with Glu190 (by -15 kcal/mol), whereas that of α2-6 complex is exposed to solvent and has a weaker interaction with Glu190 (by -2 kcal/mol). In consequence, the weaker interaction in the latter was overcome by the repulsive interaction between the two acids. These results are consistent with the mutational study indicating that mutant Glu190Ala human H3 shows a slightly higher binding affinity to human receptor (Martin et al., 1997), galactose of which has no contact with 9-hydroxyl group (Eisen et al., 1997).

There are a lot of X-ray crystal structures for H3 HA complexed with avian or human receptor and their interaction patterns vary significantly. In particular, the interactions with Gln226 are found to be very sensitive to the location relative to receptor. The sensitivity of Gln226 is consistent with the correlation between the loss of the binding ability to avian receptor and the substitution Ala138Ser in H1 subtype (Rogers and D'Souza, 1989), because Ser138 is situated near Gln226 and the location of Gln226 would change due to the interaction with Ser138.

As mentioned above, the shift of receptor is thought to alleviate the unfavorable interactions between Gln226 and unpolar group of galactose with α2-6. Further in this study, it was shown that repulsive interaction with Glu190 is involved in the shift. However, the loss of binding energy by the shift is estimated as more than 30 kcal/mol and its magnitude differs according to receptors, suggesting the existence of some other factors besides the disadvantageous interactions.

Sialic acid parts of shorter oligosaccharide interact with the RBS of HA more strongly than those of longer ones, as observed in swine H1 and avian H5 complexes (Figs. 6d and 6d). Analogous phenomena were also observed in H3 HA-avian receptor complexes as well. These results suggest that the shift of sialic acid is partly ascribable

to an optimization of total interaction energy between receptor and HA through setting the position of oligosaccharide to interact with RBS with keeping the structural stress moderate. Our data may explain that α2-6 linkage sialic acid in the avian H5 / human receptor complex, which has only Sia1, shows no shift as observed in the avian H3 / human receptor complex (Ha et al., 2001, 2003), although both of the two have the unfavorable interactions.

4.2. Roles of Conserved Residues

It is known that Tyr98, Trp153 and His183 are conserved through all HAs (Skehel et al., 2000). In our FMO analysis, Tyr98, Trp153 and His183 have been found to interact with receptor and stabilize the receptor binding. In avian viruses, Tyr98, Ala138, Trp153, His183, Glu190, Leu194, Gln226 and Gly228 residues are conserved (Matrosovich et al., 1997). We found the interaction energies of these residues in H3 and H5 avian HA / avian complexes evaluated by the IFIE analysis take totally negative values, thus stabilizing the receptor binding. In the previous theoretical study (Sawada et al., 2006), the interaction of hydrophobic residue Trp153 with receptor was not well evaluated (nearly zero interaction). Our results are consistent with the experimentally supported importance of conserved hydrophobic residues for the receptor binding.

As compared with other conserved residues, hydrophobic residues Trp153 and Leu194 show relatively weak interactions with receptor. These facts suggest that the contributions by conserved hydrophobic residues such as Trp153 and Leu194 to the receptor binding would be mainly associated with the hydrophobic interactions via water solvent and/or the role for keeping the shape of RBS. In the present FMO analysis, the hydrophobic residues relevant to the binding interact predominantly with the sialic acid part common in the avian and human receptors, and therefore would give a minor contribution to the correlation between the IFIE and the binding specificity.

Gln226 has attracted much attention concerning the binding to avian receptor because avian H3 HAs change binding specificity from human to avian receptor by the substitution of Leu226Gln (Rogers et al., 1983) and Gln positioned at 226 is conserved in all the avian HAs (Matrosovich et al., 1997). Our data showed that the interactions of avian receptor with Gln226 are stronger than that of human receptor in H1 human, H3 avian and H5 avian HAs in contrast to the previous ab initio calculation (Sawada et al., 2006). Our results can explain the experimental results that Gln226 is important for avian receptor binding. In contrast to H2 and H3 human HAs, it has been remarked

that H1 human HA can bind to human receptor even with Gln226 since a hydrogen bond is formed between Gal2 and Lys222 due to the location of Gln226 that does not cause steric hindrance (Gamblin et al., 2004). However, as seen in Figs. 4a and 5b, our calculations have demonstrated that Gln226 energetically contributes to the stabilization of the binding to human receptor, especially in the case of swine H1 (by about -25 kcal/mol). These results indicate that Gln226 play a positive role for the stabilization of complex in addition to somewhat negative role regarding the steric hindrance and thus explain the experimental results that H1 HA acquires binding ability for human receptor with retaining Gln226.

Glu190 has been found to be conserved in avian HAs, but not in human HAs (Matrosovich et al., 1997). Therefore, it was suggested that Glu190 is important for the binding specificity to avian receptor, which is consistent with the mutant experiments that Glu190Ala increases the binding strength of human H3 to human receptor (Martin et al., 1997) and Asp190Glu causes H1 HA to change the binding specificity from human to avian receptor (Glaser et al., 2005). The present study has also demonstrated that the interaction patterns for Glu190 differ significantly between avian and human receptor in avian H3 and H5. These findings support the importance of interactions associated with Glu190 for avian receptor specificity. The important roles played by Glu190 such as the hydrogen bondings with 9-OH and Gal2 of receptor, the electrostatic repulsion with charged sialic acid, and the change in the interaction energy inside the HA would bring about the significant differences in the interaction patterns between avian and human receptors mainly due to its location relative to the receptor.

The calculated positive $\Delta\Delta E_I^{total}$ value in H1 human HA for Glu190 with avian receptor and for Asp225 with human receptor seem to be inconsistent with experimental facts indicating that these residues contribute to receptor binding (Glaser et al., 2005; Stevens et al., 2006). We supposed the reason for this inconsistency would be ascribed to the neglect of hydration waters in the FMO calculations, which could stabilize the receptor binding through the formation of hydrogen bond and the screening of repulsive Coulombic interactions (Ishikawa et al., 2006; Komeiji et al., 2007).

4.3. Interactions mediated by water molecules

In this FMO analysis, we have shown that the calculated ΔE values are basically consistent with the receptor binding preference of each HA. However, it was also found

that there are some inconsistencies in inter-subtype comparison of ΔE (Table 2.). Although H5 avian HA has binding specificity for avian receptor, the IFIE analysis has shown that many residues in the RBS of H5 avian receptor complex have weaker interaction with avian receptor than that of human receptor complex. Consequently, the difference in the calculated ΔE values between avian and human receptor complexes is relatively small (about 15 kcal/mol) which seems to be not enough to account for the binding specificity for avian receptor. These results suggest that the avian receptor shifts upward to set out the interaction between oligosaccharide and RBS, as in the case of H1 swine HA. In a recent glycan microarray analysis, it was shown that oligosaccharide length essential for the binding differs by HAs (Stevens et al., 2006). Some HAs bind to Sia1-Gal2 receptor and others do not. Considering that there is a case that the GlcNAc part, which has no contact with the RBS, is essential for the receptor binding, the interaction between GlcNAc and the RBS mediated by unspecified water molecules may be present. It was also found that the binding energy of H1 swine HA / avian complex is lower (more attractive) than that of H1 human HA / avian complex, although the former has a lower preference for avian receptor. By X-ray crystal structure study of H1 human HA / avian receptor complex, it was found that crystal water molecules mediate the interaction between HA and the avian receptor (Gamblin et al., 2004). These water molecules may play an important role for the binding. In this calculation, we neglected water molecules and GlcNAc part of receptor saccharides which has no contact with HA. We speculate that some inconsistencies have partly resulted from the absence of water molecules that could mediate the hydrogen bonding between HA and receptor.

4.4. Roles of Acidic Residues

The receptors have a sialic acid with negative charge at the terminal. Therefore main parts of residues important for the receptor binding would be basic or polar ones. However, there are conserved acidic residues such as Glu190, Asp190 and Asp225 in the RBS, which have been confirmed to contribute to the receptor binding by mutant experiments (Glaser et al., 2005; Martin et al., 1997; Stevens et al., 2006). An interesting question is then concerned with how these acidic residues that repulsively interact with the receptor could contribute to the stabilization of binding. X-ray crystal structure data have shown that the formation of hydrogen bonding between acidic residues and receptor is essential for the stabilization of receptor binding. Our FMO

analysis concerning $\Delta\Delta E_I^{total}$, however, has shown that the interactions between the acidic residues and sialic acid part of receptor would make the binding unstable, while the inter-residue (intra-HA) interactions are significantly stabilized by the binding with all the conserved acidic residues. This finding suggests that the changes in the intra-HA interactions in addition to those in the residue-receptor interactions play an important role when considering the contributions by acidic residues to the receptor binding.

We have observed in the FMO-IFIE analysis that the residues stabilized by the interactions with acidic residues on the binding are widely distributed over the HA structures employed in the calculations. We thus need to perform the electronic structure calculations including the residues apart from the RBS as well in the case that these long-range molecular interactions are also important for the binding. Such findings could not be obtained by either X-ray crystal structure analysis or the MO analysis focusing only on the residues locally around the RBS.

5. Conclusions

In this work we have carried out the IFIE analysis of HA-receptor complexes on the basis of ab initio FMO-MP2 method with inclusion of electron correlation effects associated with the van der Waals dispersion interactions. To discuss the binding property of influenza HA comprehensively, a number of HAs from human H1, swine H1, avian H3 and avianH5 subtypes were investigated. The calculated results obtained in the present analysis have provided a number of viewpoints regarding the models for the binding specificity between HA and receptor associated with mutated residues. Examples include the roles of Glu190 for the binding specificity of H3 HA to avian and human receptors and the roles of Gln226 for the binding of H1 HA to human receptor. It has also been shown that Glu190 and Gln226 would play important roles for the binding specificity of H5 HA. Since H5 HA has not yet been adapted to human receptor and the mechanism of the specificity change from avian to human receptors is unknown, this result is helpful for the in silico prediction of the change in receptor specificity associated with forthcoming possible pandemics.

We performed detailed investigations about the interaction patterns of complexes of avian H3 and receptor analogues, in which the importance of location shift of α2-6 linkaged sialic acid associated with relevant receptor size was suggested. Without knowing the interaction of galactose and GlcNAc with RBS of HA, probably mediated by

water molecules, it would be difficult to comprehensively elucidate the receptor binding specificity and the mechanism of adaptation to novel hosts. In addition to the intermolecular interactions between HA and receptor, unexpected contributions to the receptor binding through the change of intra-molecular interactions between Glu190 and other residues have been suggested. These results may provide a hint to understand the role of conserved acidic residues in the RBS which are destabilized by the electrostatic repulsion with sialic acid. There are several acidic residues in HAs that are conserved and are shown to be important for the receptor binding through mutation experiments. Toward the understanding of the roles of acidic residues, we have elucidated that all the important acidic residues, in spite of their repulsive interactions with receptor, make a contribution to the receptor binding by stabilizing the intra-molecular (intra-HA) interactions. This finding, in contrast to a common sense in structural biology that ligand-protein interaction plays a major role for the binding, would provide a novel viewpoint emphasizing the importance of the change in intra-molecular interactions.

In summary, in terms of ab initio FMO analysis, we have established a computational method to quantitatively predict the binding affinity of HAs to avian and human receptors (see Table 2). Through the IFIE analysis based on the FMO calculations, we could also specify and characterize important residues which would play an essential role in the binding specificity between HA and receptor.

Acknowledgements

We would like to thank Prof. Kuniyoshi Ebina, Dr. Mika Ito, Ikuo Kurisaki and Takatoshi Fujita for useful discussions. This work was partially supported by CREST, Japan Science and Technology Agency.

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Figure legends

Fig. 1. Structure of 1963-H3 avian HA (PDB ID:1MQM). (A) Overview of the H3 avian HA trimer, depicted as a ribbon representation. Monomers 2 and 3 are colored in silver and the featured monomer1 is colored according to its subunits; HA1 is colored in yellow and HA2 is colored in magenta. The receptor binding site is mainly composed of three components; 190-Helix, 220-Loop and 130-Loop. (B) Receptor binding domain of the HA used in the present calculation (82 residues). Some amino acid residues important for receptor binding are depicted. Possible hydrogen bonds are represented by dotted lines. Atomic distances used to evaluate the variation of complex structure are defined as follows; hydrogen bonds of Tyr98 with 8-hydroxyl (**a**), Ser136 and Ser137 with the carboxylate (**b**)(**c**), and His183 and Glu190 with 9-hydroxyl (**d**)(**e**). These five hydrogen bonds are represented by green dotted lines.

Fig. 2. Interactions of human H1, swine HA, avian H3, avian H5 HAs with avian receptor (left; a, c, e, g) and with human receptor (right; b, d, f, h). Possible hydrogen bonds are represented by broken lines. (a) H1 human HA with avian receptor and (b) H1 human HA with human receptor (PDB ID: 1RVX and 1RVZ). (c) H1 swine HA with avian receptor and (d) H1 swine HA with human receptor (PDB ID: 1RVT and 1RVO). (e) H3 avian HA with avian receptor and (f) H3 avian HA with human receptor (PDB ID: 1MQM and 1MQN). (g) H5 avian HA with avian receptor and (h) H5 avian HA with human receptor (PDB ID: 1JSN and 1JSO).

Fig. 3. Fragmentation of molecules for FMO calculation. Saccharides of (a) α2-3 and (b) α2-6 receptor analogues. (c) Hemagglutinin protein. Sia, Gal and GlcNAc refer to sialic acid, galactose and N-acetylglucosamine, respectively.

Fig. 4. Structural variation and interaction patterns of H3 avian HA-receptor complexes. Atomic distances between HA and receptor were measured; (a) Avian receptor and (e) human receptor. The atoms of which inter-atomic distances were determined are shown in Fig. 1B. $\Delta\Delta E_I^{total}$ of each amino acid residue in the receptor binding site of (b) three avian receptor complexes and (f) two human receptor complexes are compared. $\Delta\Delta E_I^{total}$ of (c) avian (R3) and (g) human (R2) receptor complexes are divided into inter and intra-molecular interactions. (d) $\Delta\Delta E_I^{total}$ for Glu190 was decomposed into each contribution from the fragment J. (h) Comparison of $\Delta\Delta E_I^{total}$ of each amino acid residue between avian and human receptor complexes.

Fig. 5. Interaction patterns of H1 human HA-receptor complexes. The calculated $\Delta\Delta E_I^{total}$ values of each amino acid reside at the receptor binding site of (a) avian receptor complex and (b) human receptor complex are divided into inter and intra-molecular interactions. (c) Comparison of $\Delta\Delta E_I^{total}$ of each amino acid residue between avian and human receptor complexes.

Fig. 6. Interaction patterns of H1 swine HA-receptor complexes. The calculated $\Delta\Delta E_I^{total}$ values of each amino acid reside in the receptor binding site of (a) avian receptor complex and (b) human receptor complex are divided into inter and intra-molecular interactions. (c) Comparison of $\Delta\Delta E_I^{total}$ of each amino acid residue between avian and human receptor complexes (with full-receptor). (d) Comparison of $\Delta\Delta E_I^{total}$ of each amino acid residue in the receptor binding site between avian and human receptor complexes (with only Sia1).

Fig. 7. Interaction patterns of H5 avian HA-receptor complexes. The calculated $\Delta\Delta E_I^{total}$ values of each amino acid reside in the receptor binding site of (a) avian receptor complex and (b) human receptor complex are divided into inter and intra-molecular interactions. (c) Comparison of $\Delta\Delta E_I^{total}$ of each amino acid residue in the receptor binding site between avian and human receptor complexes (with full-receptor). (d) Comparison of $\Delta\Delta E_I^{total}$ of each amino acid residue in the RBS between avian and human receptor complexes (with only Sia1).

Tables

Table 1. HAs employed in the present study. Receptor binding preference, ordered receptor analogues in the X-ray crystal structure analysis and Protein Data Bank (PDB) ID are listed.

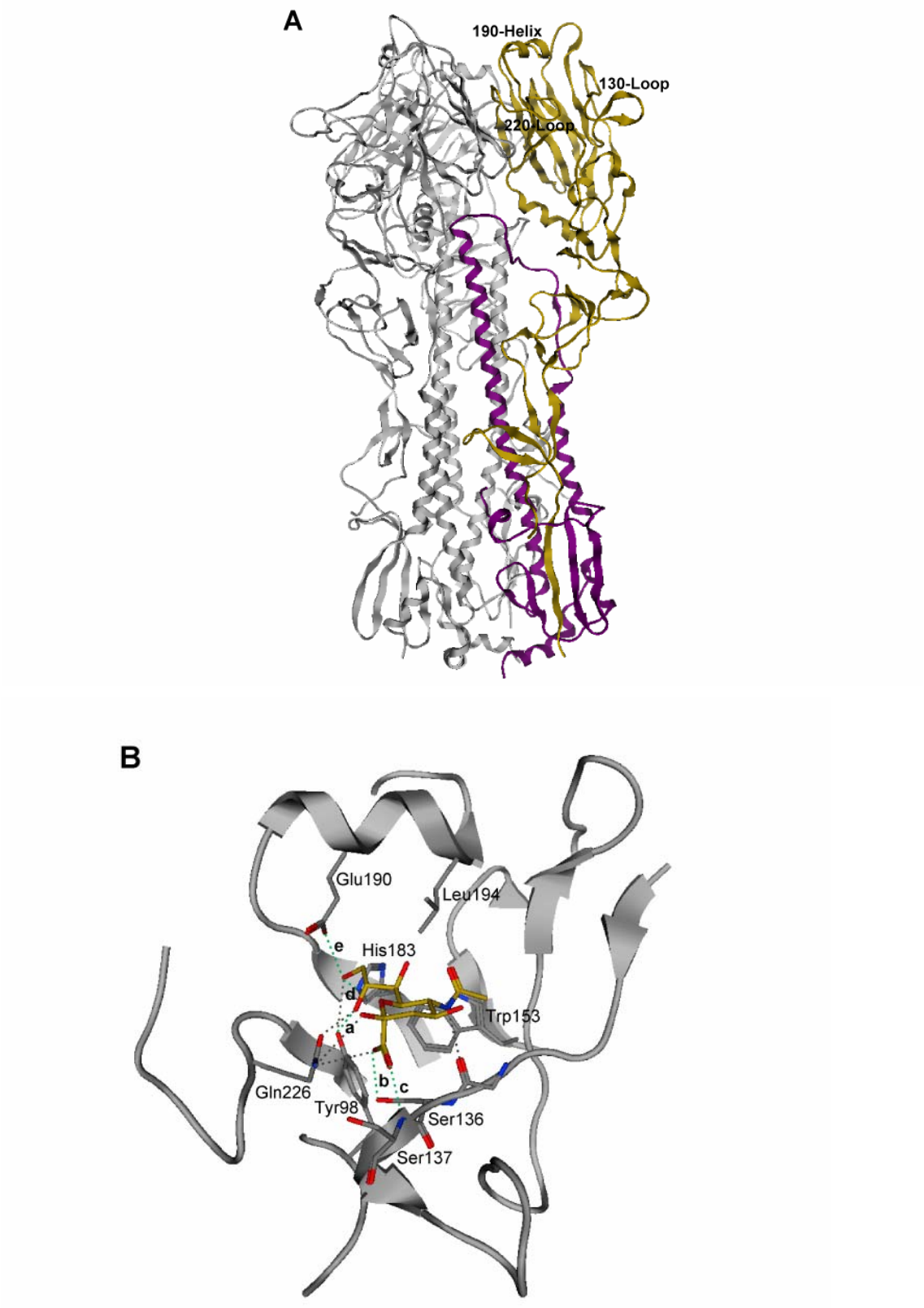
Subtype	Origin	Preference	Ordered receptor analogue	PDB ID
H1N1	human	α2-3, α2-6	α2-3 : Sia1-Gal2-GlcNAc3	1RVX
			α2-6 : Sia1-Gal2-GlcNAc3	1RVZ
H1N1	swine	α2-3 < α2-6	α2-3 : Sia1	1RVT
			α2-6 : Sia1-Gal2-GlcNAc3-Gal4-GlcNAc5	1RVO
H3N2	avian	α2-3	α2-3 : Sia1-Gal2-GlcNAc3	1MQM
			α2-6 : Sia1-Gal2	1MQN
H5N1	avian	α2-3	α2-3 : Sia1-Gal2-GlcNAc3	1JSN
			α2-6 : Sia1	1JSO

Table 2. The binding energies ΔE between HAs and receptors calculated by the FMO method at MP2/6-31G level. The receptor analogues used in the calculations differ among the complexes (see Sec. 3 for detailed descriptions). Energies are shown in units of kcal/mol.

Receptor	H3avianHA	H1humanHA	H1swineHA	H5avianHA
avian	-352.9	-293.3	-363.3	-299.2
human	-292.4	-335.9	-390.5	-283.9

Figures

Figure 1.



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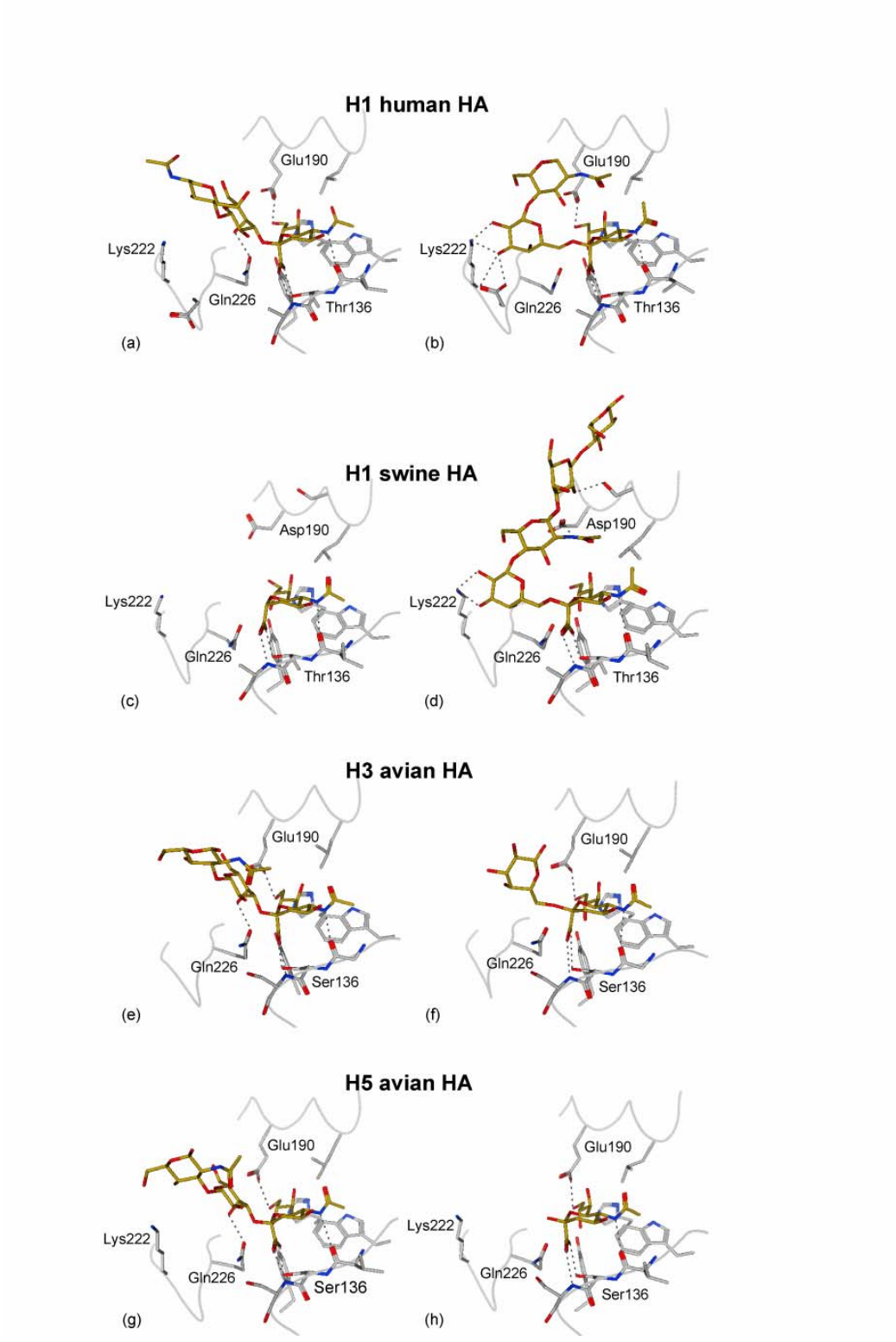
H1 human HA
Glu190
Lys222
Gln226
Thr136
(a)
Glu190
Lys222
Gln226
Thr136
(b)
H1 swine HA
Asp190
Lys222
Gln226
Thr136
(c)
Asp190
Lys222
Gln226
Thr136
(d)
H3 avian HA
Glu190
Gln226
Ser136
(e)
Glu190
Gln226
Ser136
(f)
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(g)
Glu190
Lys222
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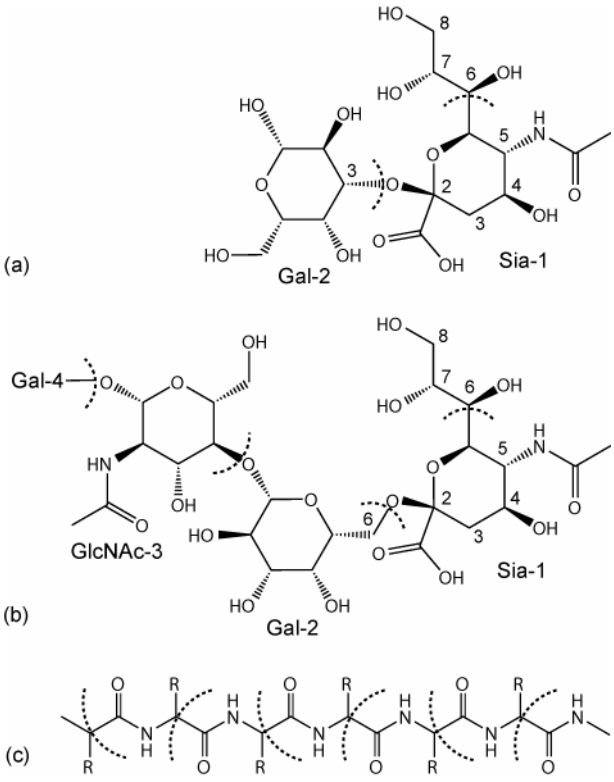
Ser136
(h)

Figure 3.



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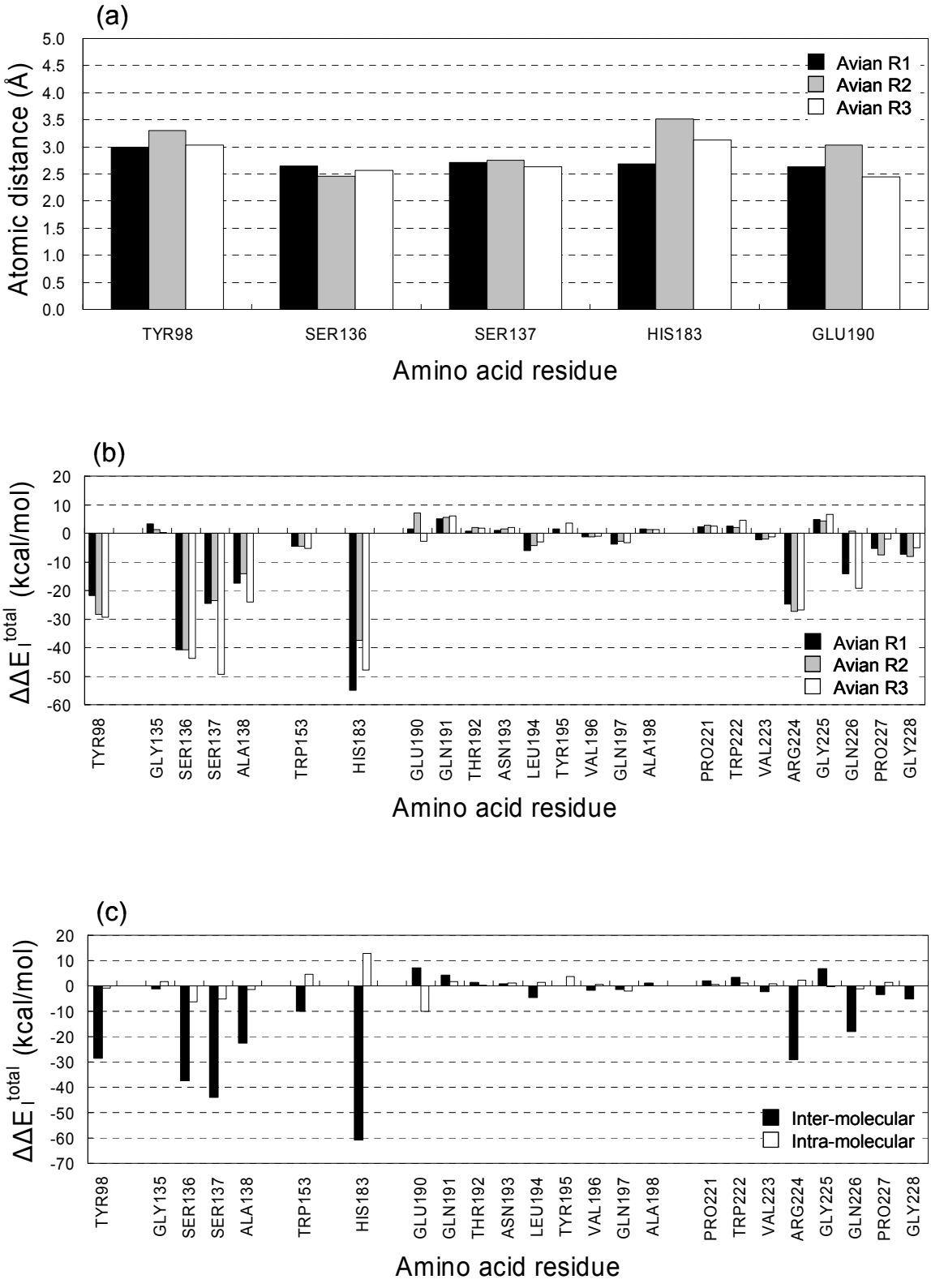
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0.5
0.0
Avian R1
Avian R2
Avian R3
TYR98
SER136
SER137
HIS183
GLU190
Amino acid residue
(b)
ΔΔE_I^total (kcal/mol)
20
10
0
-10
-20
-30
-40
-50
-60
Avian R1
Avian R2
Avian R3
TYR98
GLY135
SER136
SER137
ALA138
TRP153
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GLU190
GLN191
THR192
ASN193
LEU194
TYR195
VAL196
GLN197
ALA198
PRO221
TRP222
VAL223
ARG224
GLY225
GLN226
PRO227
GLY228
Amino acid residue
(c)
ΔΔE_I^total (kcal/mol)
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10
0
-10
-20
-30
-40
-50
-60
-70
Inter-molecular
Intra-molecular
TYR98
GLY135
SER136
SER137
ALA138
TRP153
HIS183
GLU190
GLN191
THR192
ASN193
LEU194
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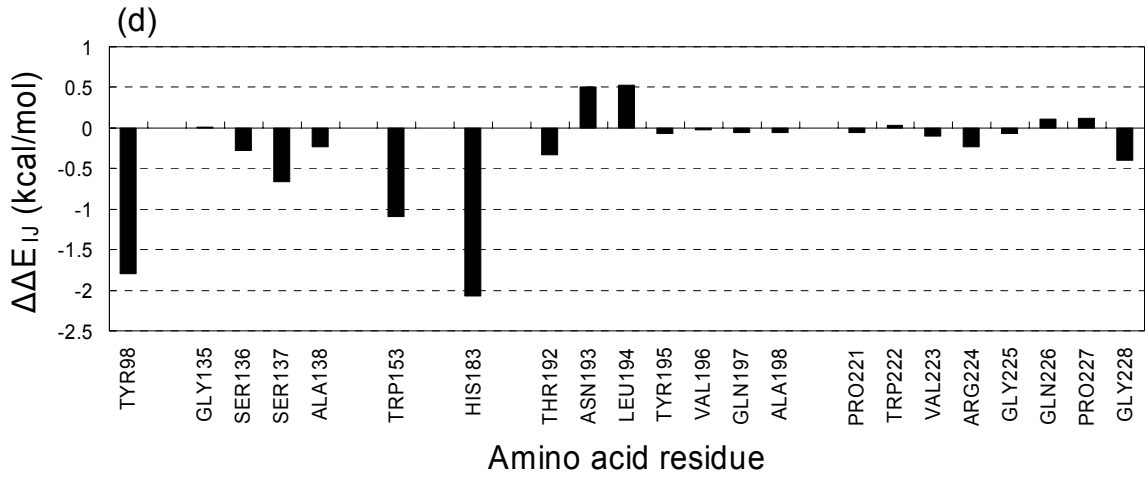
PRO227
GLY228
Amino acid residue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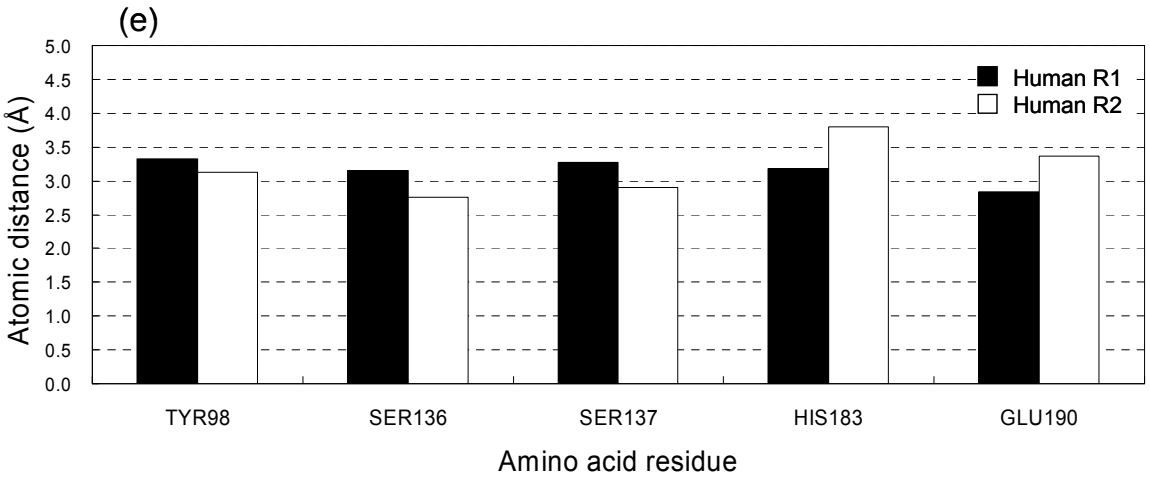
Amino acid residue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Human R2
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SER137
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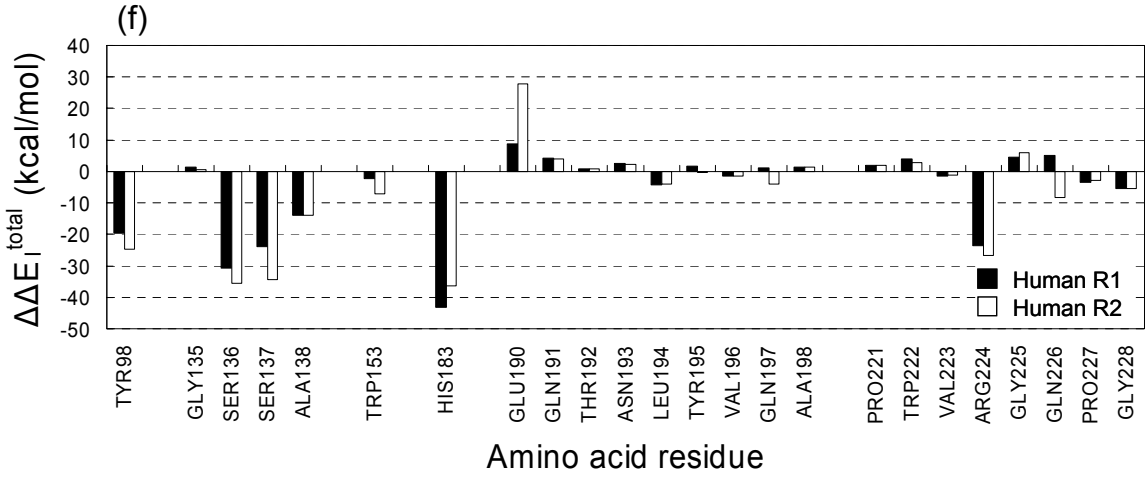
GLU190
Amino acid residue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-40
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Human R2
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GLY135
SER136
SER137
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TRP153
HIS183
GLU190
GLN191
THR192
ASN193
LEU194
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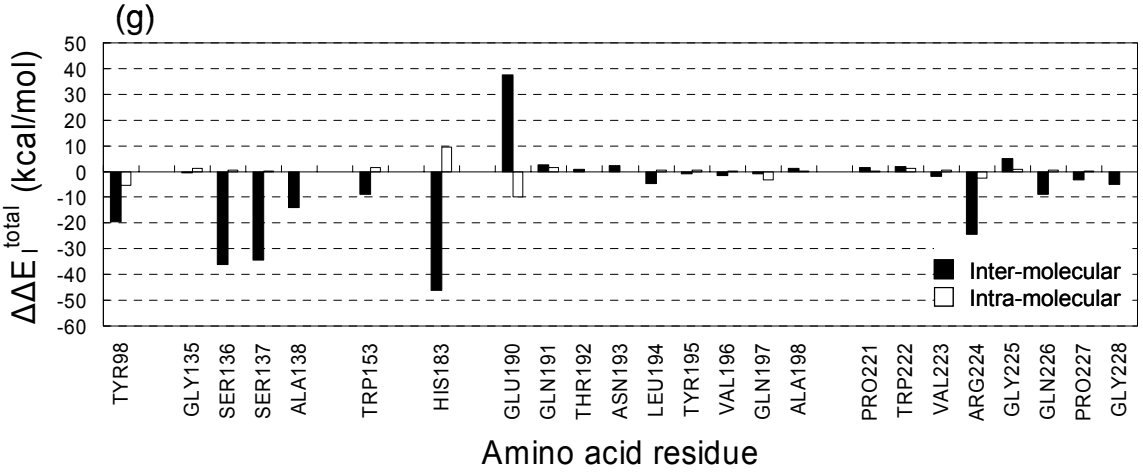
PRO227
GLY228
Amino acid residue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Intra-molecular
TYR98
GLY135
SER136
SER137
ALA138
TRP153
HIS183
GLU190
GLN191
THR192
ASN193
LEU194
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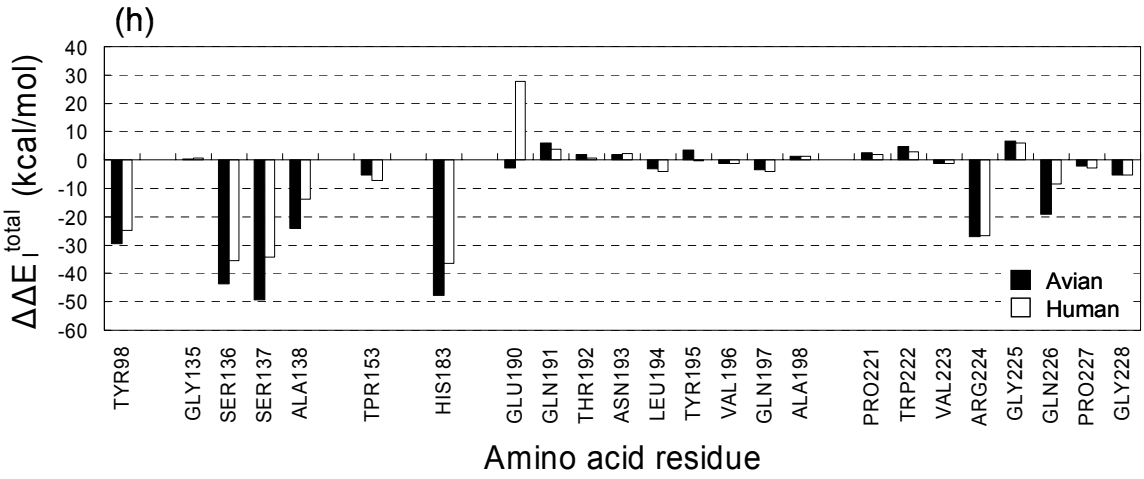
GLY228
Amino acid residue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TYR195
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VAL223
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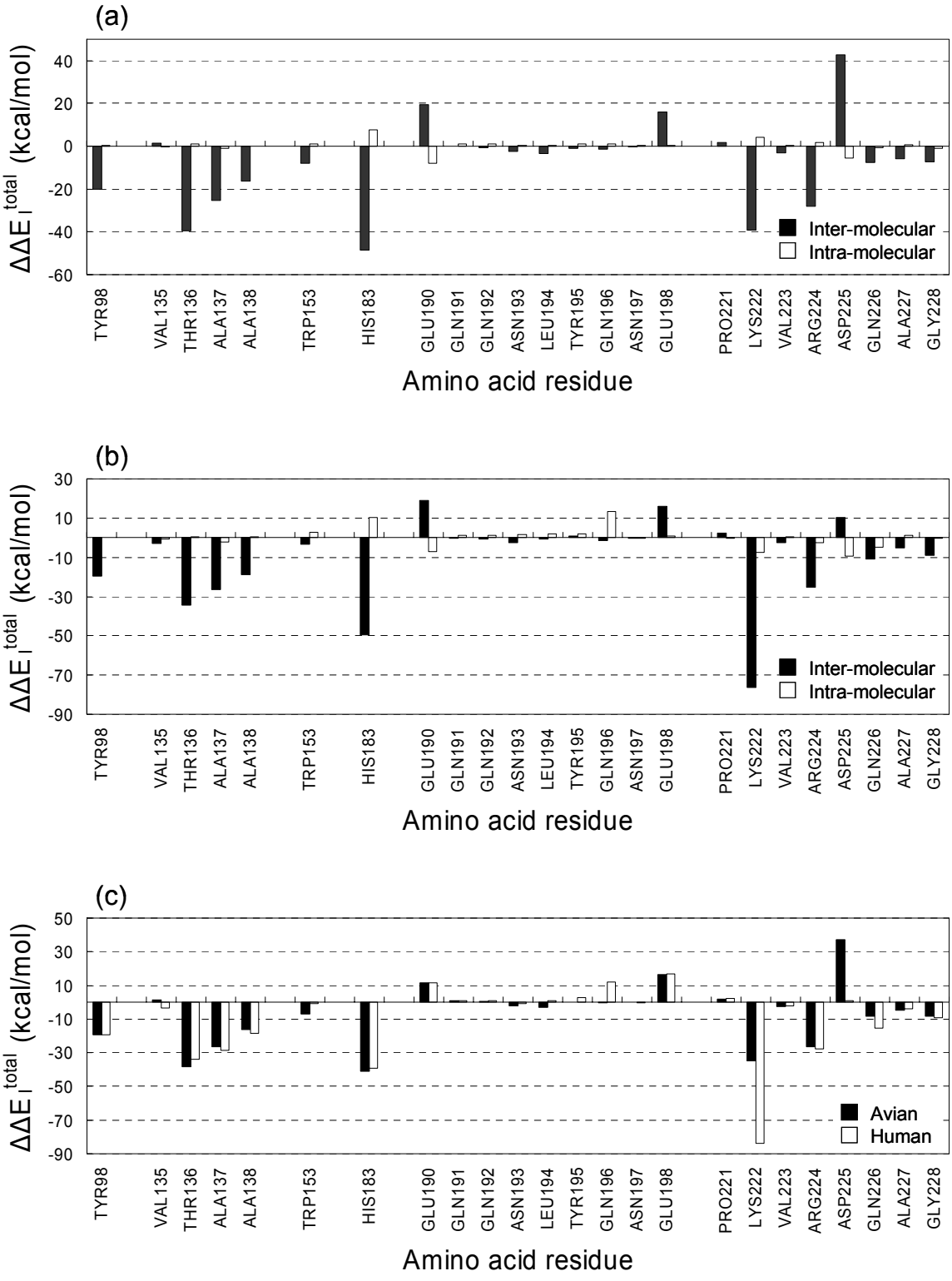
GLY228
Amino acid residue

Figure 5.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-60
Inter-molecular
Intra-molecular
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VAL135
THR136
ALA137
ALA138
TRP153
HIS183
GLU190
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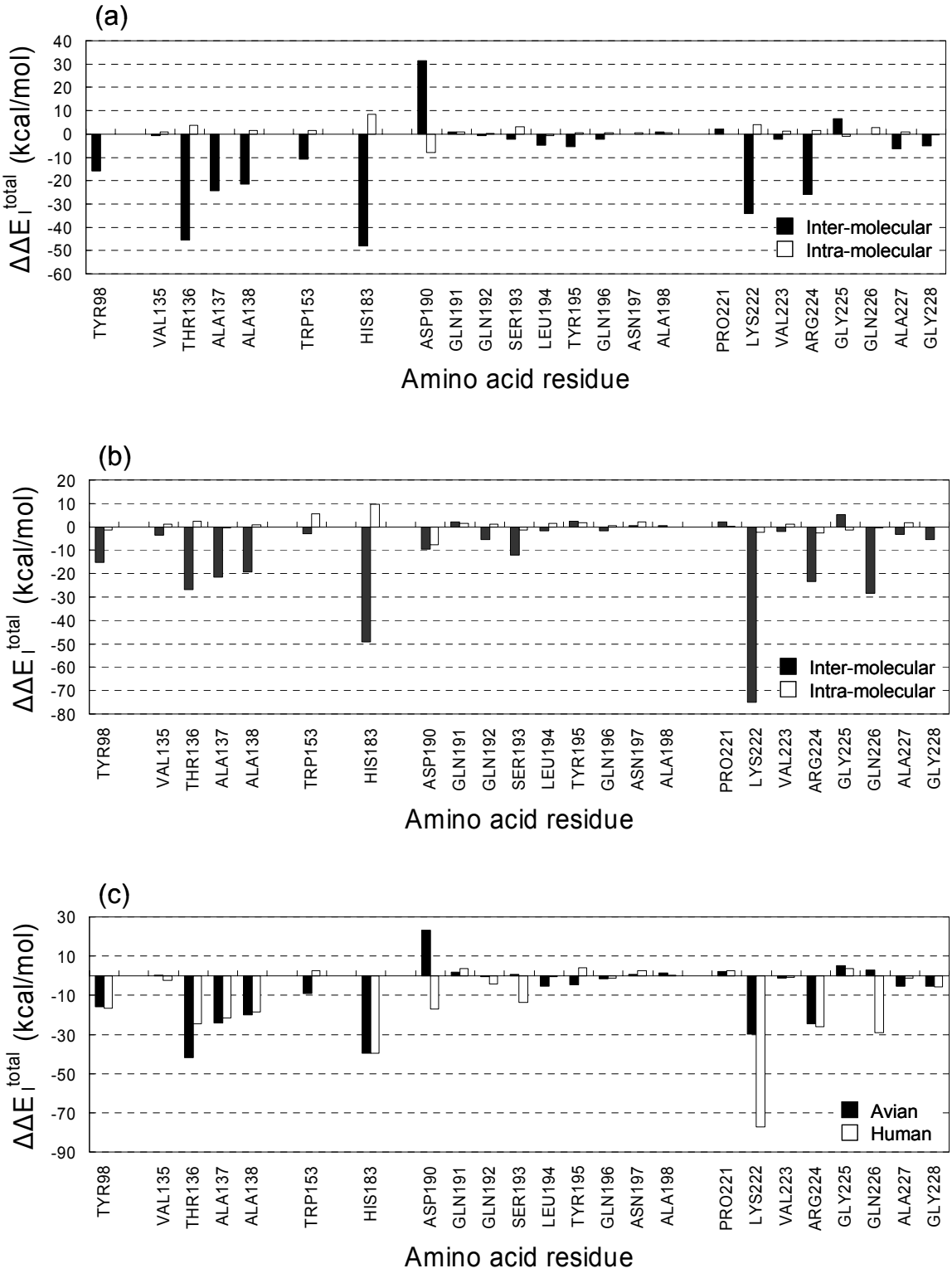
GLN192
ASN193
LEU194
TYR195
GLN196
ASN197
GLU198
PRO221
LYS222
VAL223
ARG224
ASP225
GLN226
ALA227
GLY228
Amino acid residue
(b)
ΔΔE_I^total (kcal/mol)
30
10
-10
-30
-50
-70
-90
Inter-molecular
Intra-molecular
TYR98
VAL135
THR136
ALA137
ALA138
TRP153
HIS183
GLU190
GLN191
GLN192
ASN193
LEU194
TYR195
GLN196
ASN197
GLU198
PRO221
LYS222
VAL223
ARG224
ASP225
GLN226
ALA227
GLY228
Amino acid residue
(c)
ΔΔE_I^total (kcal/mol)
50
30
10
-10
-30
-50
-70
-90
Avian
Human
TYR98
VAL135
THR136
ALA137
ALA138
TRP153
HIS183
GLU190
GLN191
GLN192
ASN193
LEU194
TYR195
GLN196
ASN197
GLU198
PRO221
LYS222
VAL223
ARG224
ASP225
GLN226
ALA227
GLY228
Amino acid residue

Figure 6.


(a)
ΔΔE_I^total (kcal/mol)
40
30
20
10
0
-10
-20
-30
-40
-50
-60
Inter-molecular
Intra-molecular
TYR98
VAL135
THR136
ALA137
ALA138
TRP153
HIS183
ASP190
GLN191
GLN192
SER193
LEU194
TYR195
GLN196
ASN197
ALA198
PRO221
LYS222
VAL223
ARG224
GLY225
GLN226
ALA227
GLY228
Amino acid residue
(b)
ΔΔE_I^total (kcal/mol)
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
Inter-molecular
Intra-molecular
TYR98
VAL135
THR136
ALA137
ALA138
TRP153
HIS183
ASP190
GLN191
GLN192
SER193
LEU194
TYR195
GLN196
ASN197
ALA198
PRO221
LYS222
VAL223
ARG224
GLY225
GLN226
ALA227
GLY228
Amino acid residue
(c)
ΔΔE_I^total (kcal/mol)
30
10
-10
-30
-50
-70
-90
Avian
Human
TYR98
VAL135
THR136
ALA137
ALA138
TRP153
HIS183
ASP190
GLN191
GLN192
SER193
LEU194
TYR195
GLN196
ASN197
ALA198
PRO221
LYS222
VAL223
ARG224
GLY225
GLN226
ALA227
GLY228
Amino acid residue

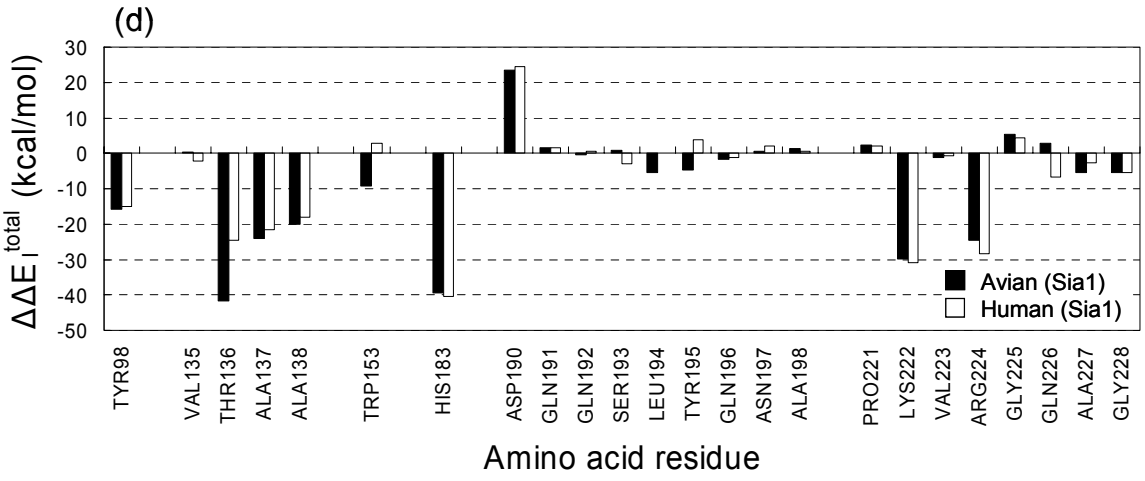
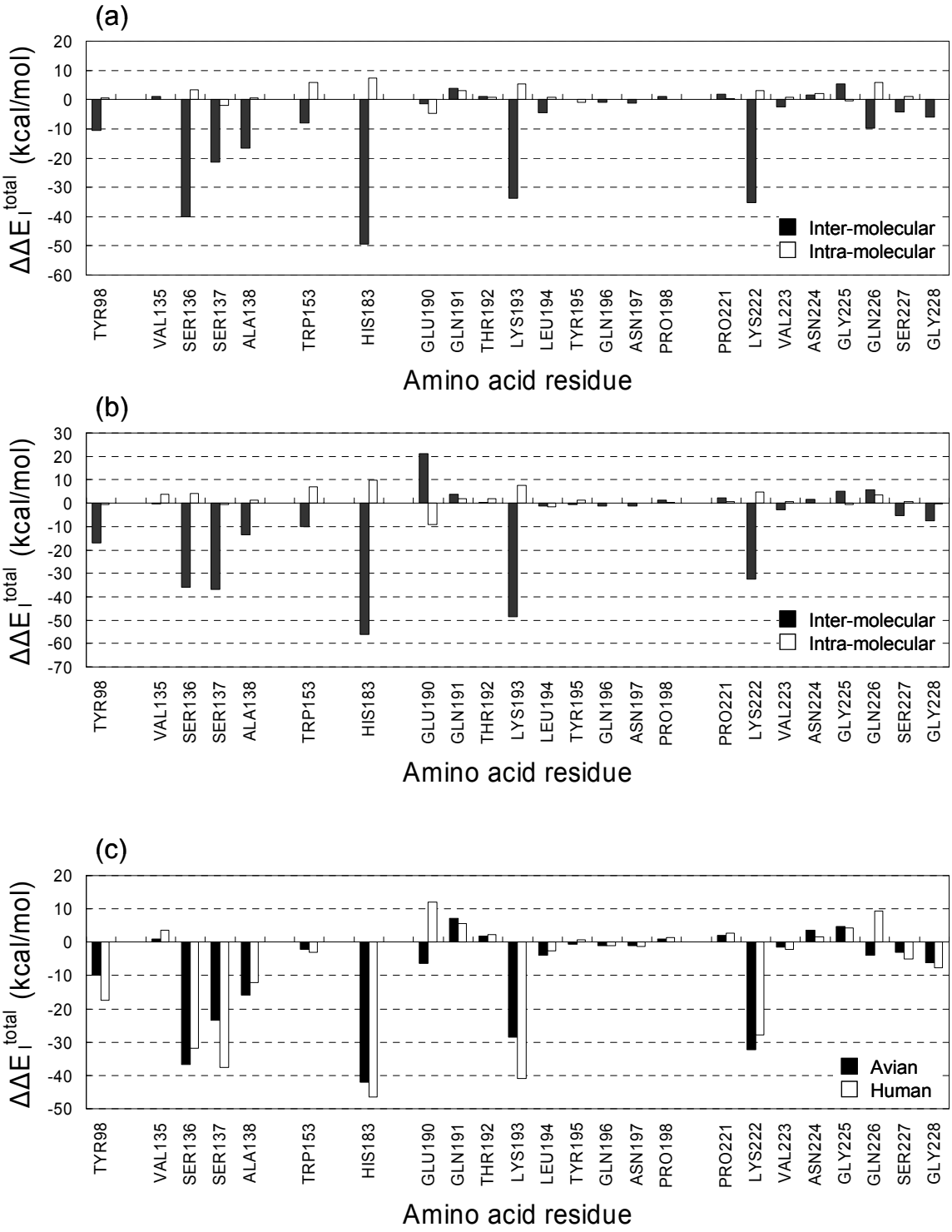
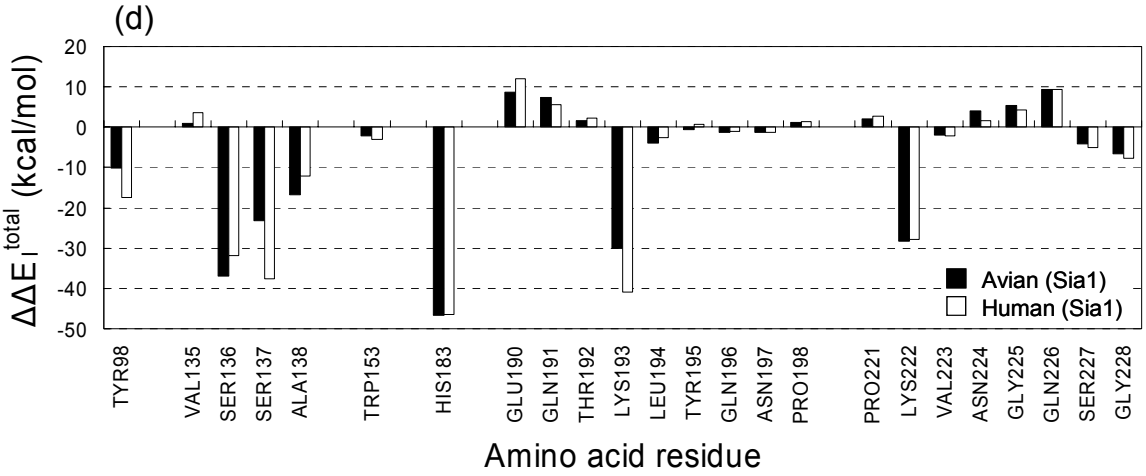

(d)
30
20
10
0
-10
-20
-30
-40
-50
ΔΔE_I^total (kcal/mol)
Avian (Sia1)
Human (Sia1)
TYR98
VAL135
THR136
ALA137
ALA138
TRP153
HIS183
ASP190
GLN191
GLN192
SER193
LEU194
TYR195
GLN196
ASN197
ALA198
PRO221
LYS222
VAL223
ARG224
GLY225
GLN226
ALA227
GLY228
Amino acid residue

Figure 7.


(a)
ΔΔE_I^total (kcal/mol)
20
10
0
-10
-20
-30
-40
-50
-60
Inter-molecular
Intra-molecular
TYR98
VAL135
SER136
SER137
ALA138
TRP153
HIS183
GLU190
GLN191
THR192
LYS193
LEU194
TYR195
GLN196
ASN197
PRO198
PRO221
LYS222
VAL223
ASN224
GLY225
GLN226
SER227
GLY228
Amino acid residue
(b)
ΔΔE_I^total (kcal/mol)
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
Inter-molecular
Intra-molecular
TYR98
VAL135
SER136
SER137
ALA138
TRP153
HIS183
GLU190
GLN191
THR192
LYS193
LEU194
TYR195
GLN196
ASN197
PRO198
PRO221
LYS222
VAL223
ASN224
GLY225
GLN226
SER227
GLY228
Amino acid residue
(c)
ΔΔE_I^total (kcal/mol)
20
10
0
-10
-20
-30
-40
-50
Avian
Human
TYR98
VAL135
SER136
SER137
ALA138
TRP153
HIS183
GLU190
GLN191
THR192
LYS193
LEU194
TYR195
GLN196
ASN197
PRO198
PRO221
LYS222
VAL223
ASN224
GLY225
GLN226
SER227
GLY228
Amino acid residue


(d)
20
10
0
-10
-20
-30
-40
-50
ΔΔE_I^total (kcal/mol)
Avian (Sia1)
Human (Sia1)
TYR98
VAL135
SER136
SER137
ALA138
TRP153
HIS183
GLU190
GLN191
THR192
LYS193
LEU194
TYR195
GLN196
ASN197
PRO198
PRO221
LYS222
VAL223
ASN224
GLY225
GLN226
SER227
GLY228
Amino acid residue