

Substrate Recognition by Enzymes: a Theoretical Study

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Abstract

We previously reported that a series of gangliosides inhibited the activity of an enzyme NAD glycohydrolase (CD38), and that those with tandem sialic acid residues in the sugar chain had great inhibitory effect. We describe the results of computer simulations on three-dimensional and electronic structures of gangliosides to clarify the causative factors of difference in the inhibitory effect and the recognition mechanisms of the enzyme.

We found that dipole moments and HOMO were correlated with inhibitory effect by conformational analyses and molecular orbital (MO) calculations. CD38 is likely to recognize the two carboxyl groups in tandem sialic acid residues of gangliosides, as well as the phosphate groups in NAD. A strong correlation was found between the orbital energies of HOMO by MO calculations and the extent of the inhibitory effect. Solvation effects were also considered to interpret the substrate recognition mechanisms in the biological system, which supported the above results.

1. Introduction

Substrate recognition by enzymes has been of great interest in biochemistry. Enzymatic regulation system involves highly structured pathways of signal transfer triggered by specific contact events. Oligosaccharides of glycosphingolipids on cell surface show diversity in three-dimensional structures, which is closely associated with specific interactions in the extracellular domain. Reports on higher order structures of glycosphingolipids have been limited because of the experimental difficulties to deal with oligosaccharides, documenting only the primary structures of them.

Gangliosides are sphingolipids containing sialic acids. Some enzymes may recognize oligosaccharides in gangliosides. One of the authors reported previously that gangliosides inhibit the activity of an enzyme NAD (Nicotinamide Adenine Dinucleotide) glycohydrolase, CD38, and that those with tandem sialic acid residues in

the sugar chain have great inhibitory effect[1]. However, the recognition mechanisms remain elusive.

In this paper, the results of computer simulations on three-dimensional structures and electronic structures of gangliosides are described in order to clarify the causative factors of difference such as topological factor of oligosaccharide moieties in the inhibitory effect and to elucidate the recognition mechanisms of the enzyme.

2. Similarities between dimeric sialic acid and NAD

Similarities between dimeric sialic acid and NAD in the calculated structures are assessed by conformational analyses and MO calculations, on the assumption that CD38, an NAD reacting enzyme, cross-reacts with the tandem sialic acid residues in gangliosides.

Conformational analyses were performed with molecular mechanics (MM3) calculation combined with a systematic structure generation algorithm using CONFLEX program [2]. A partial change in the first step was made on an initial conformation with flip-flap operations, followed by an energetically feasible conformation selection with minimization using MM3 force field. The lowest energy conformer for each molecule was optimized with ab initio and PM3 MO calculations.

The three-dimensional structure of dimeric sialic acid is analogous to that of NAD, though one of the O-O distances in dimeric sialic acid is longer than that in NAD. The HOMO of dimeric sialic acid is localized at either of two carboxyl groups. Similarly, the HOMO of NAD is localized at two phosphate groups that are considered to be anionic sites. Furthermore, dimeric sialic acid and NAD have similar dipole moments in the same direction in a molecule, though the magnitude of dipole moment of dimeric sialic acid is larger than that of NAD. As a result, we concluded that CD38 is likely to recognize the two carboxyl groups in tandem sialic acid residues of gangliosides as well as the two phosphate groups in NAD.

3. Mechanism of substrate recognition by NAD glycohydrolase

A series of gangliosides reported in our previous experimental work[1] have been studied theoretically to clarify the effects of topology of oligosaccharide moiety on the inhibitory effects. Their initial geometries were constructed using the modeling tool CAChe3.2 (Fujitsu Ltd, Tokyo, Japan, 1999) considering the interaction model proposed by one of the authors[3]. Dipole moments were calculated at the RHF level of theory with the STO-3G basis set.

We found that magnitude of the dipole moments was well correlated with the inhibitory effects. A good correlation was also found between the orbital energy of HOMO and the degree of the inhibitory effect. These results suggest the following mechanisms of substrate recognition: (1) when a substrate is far from the enzyme, the enzyme recognizes the substrate with a large dipole moment, (2) an electrostatic interaction would bring them closer, and (3) a HOMO-LUMO interaction between the enzyme and the substrate would be important at a close range.

4. Solvation Effect

Solvation effects were explored to interpret the substrate recognition mechanisms in biological systems. ONIOM (Our own N-layered Integrated molecular Orbital and molecular Mechanics) method [4], which is one of the QM/MM methods, was applied to the system containing a ganglioside and the surrounding H₂O molecules. The computational procedure is depicted in Figure 1. Solvation effects were also interpreted by considering the system including water as a super-molecule and by using one of the dielectric model, Conductor-like Screening Model (COSMO)[5].

We compared the calculated orbital energies of frontier orbitals of monosaccharides as components of gangliosides. The energy profiles of molecular orbitals were similar qualitatively among the methods, ONIOM(PM3:UFF), ONIOM(RHF/3-21G:PM3), and super-molecule method. This consistency suggests that ONIOM is appropriate to consider the solvation effects. In contrast, COSMO method, which is one of the dielectric models, gave the different energy profile. Therefore, it is not appropriate to apply it.

We analyzed the orbital energies of frontier orbitals of hydrated gangliosides with ONIOM(PM3:UFF) and super-molecule method and found the qualitative characteristics of the order of orbital energy of HOMO were the same as those obtained in isolated systems.

The structural change by the solvation was considered and that of tandem sialic acid residues, which might be important in the inhibitory effect, did not change so much by the solvation..

5. Conclusion

We have described computational results concerning the inhibitory effects of gangliosides. Relationship between the electronic structure of gangliosides and the inhibitory effects can be clarified by quantum chemical calculations.

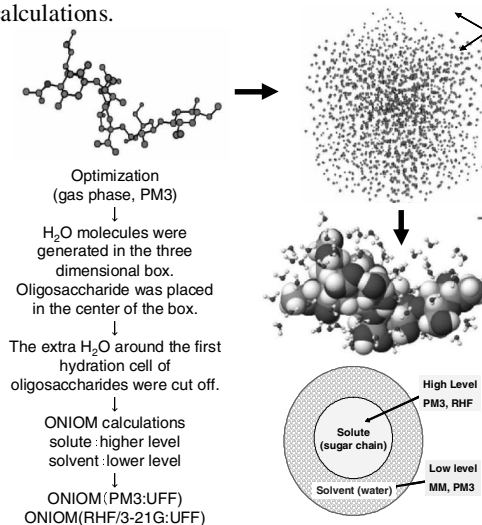


Figure 1. The procedure of ONIOM calculations.

6. References

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