Molecular Dynamics Simulations on the Coplanarity of Quercetin Backbone for the Antioxidant Activity of Quercetin-3-monoglycoside

Hyunmyung Kim,[†] Karpjoo Jeong,[‡] and Seunho Jung[†§,*]

[†]*Department of Advanced Technology Fusion, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea*
[‡]*College of Information and Communication, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea*
[§]*Department of Microbial Engineering, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea*
[*]*E-mail: shjung@konkuk.ac.kr*
Received December 15, 2005

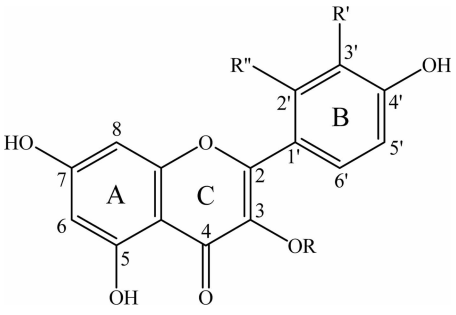
Key Words : Quercetin 3-monoglycoside, Flavonoid, Antioxidant activity, Coplanarity, Molecular dynamic simulations

Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. The family includes flavanols, flavanones, anthocyanidines, flavones, and flavonols. Most of the beneficial effects of flavonoids are attributed to their antioxidant abilities.[1] The flavonoids contain a number of phenolic hydroxyl groups attached to ring structures, conferring the antioxidant activity. Among these flavonoid families, flavonols are found in almost every plant.[2] The most commonly occurring flavonols are those with dihydroxylation in the 3' and 4' positions of B ring and the preferred glycosylation site on the flavonoids is the 3 position. It has been reported that the hydroxyl group at position 3 is required for the maximal radical scavenging activity of flavonols.[3] Quercetin is a representative flavonol with high antioxidant activity.[4] Therefore, we focused on the conformation of quercetin and quercetin-3-monoglycoside to investigate the effect of glycosylation of quercetin on their antioxidant activity.

Structure-antioxidant activity relationships of flavonoids have been extensively reported.[5] Generally, antioxidant activity depends on the number and positions of hydroxyl groups and other substituents, and glycosylation of flavonoid molecules. Glucose is the most usual sugar residue but others include galactose and rhamnose.[6] Aside from other factors which can affect the activity, it is generally known that glycosylation of flavonoids diminishes their antioxidant activity when compared to the corresponding aglycones, perhaps since this reduces the number of free hydroxyl groups and also the linkage of sugar may hinder access of the free radical scavengers to the radical center.[4]

As mentioned above, there are several and complicated factors which affect the antioxidant activity of flavonol. Conjugation between the A- and B-rings permits a resonance effect of the aromatic nucleus that lends stability to the flavonoid radical.[7] van Acker *et al.* suggested that the removal of the 3 hydroxyl group or other substituents interfere with the coplanarity of the B-ring with the rest of the flavonoid and the ability to delocalize electrons.[8] They calculated the geometry using quantum chemical calculation which cannot include the solvent effects and shows only optimized conformation. The flavonols are conformationally flexible and their conformations are influenced by the intermolecular environment.[9] Thus, a range of torsion angle of backbone can be observed for the same flavonol in different environments. Therefore, we pursue the trajectories during the molecular dynamics simulations in aqueous environment and investigate the coplanarity of quercetin's backbone and conformations in order to find out the relationship between the conformation and antioxidant activity.

It is difficult to compare the antioxidant activities of flavonols between the results of one author and others because of different experimental condition, methods, and skill. Therefore, we selected one reference which use two different methods and obtained similar results.[4] Six flavonols are investigated by molecular dynamic simulations and their chemical structures and abbreviations are shown in Figure 1. Quercetin aglycone has the most potent antioxidant activity in flavonol aglycones. The structure of quercetin was elucidated by crystal and molecular modeling.[10] The exocyclic B ring of quercetin exists in an almost planar conformation with respect to the rest of the molecule. Quercetin not only has 3-hydroxyl group in the C-ring and 3',4'-dihydroxy groups in the B-ring, but also possess the 2,3-double bond in conjugation with 4-oxo function in the C-ring (Figure 1), which are the essential structural elements for potent radical



R = H	R' = OH	R" = H	**QUE**
R = β-D-glucoside	R' = OH	R" = H	**Q3G**
R = β-D-galactoside	R' = OH	R" = H	**Q3L**
R = α-L-rhamnoside	R' = OH	R" = H	**Q3R**
R = CH_3	R' = OH	R" = H	**Q3M**
R = H	R' = H	R" = OH	**MOR**

Figure 1. The basic chemical structure of flavonols investigated.

Table 1. The structures and antioxidant activities of flavonols

Flavonols	Hydroxyl substituents	Deviation from coplanarity (°)[a]	Antioxidant activity (mM)[b]
Quercetin (QUE)	3,5,7,3',4'-OH	1.2	4.42
Quercetin-3-glucoside (Q3G)	5,7,3',4'-OH	22.1	2.39
Quercetin-3-galactoside (Q3L)	5,7,3',4'-OH	25.6	–
Quercetin-3-rhamnoside (Q3R)	5,7,3',4'-OH	27.5	2.18
Quercetin-3-methyl ether (Q3M)	5,7,3',4'-OH	69.5	–
Morin (MOR)	3,5,7,2',4'-OH	34.1	2.68

[a]Deviated average torsion angle from coplanarity (0°, 180°, or −180°) of C3-C2-C1'-C2'. [b]TEAC (Trolox equivalent antioxidant capacity) from Ref. 4.

scavenging activities of the flavonols. The planar conformation means that quercetin is completely conjugated. The planarity can be described as the deviation from 0°, 180°, or -180° torsion angle of conjugated bond (C3-C2-C1'-C2') between B-ring and AC-ring. Morin is included in these simulations because of a very similar flavonol to quercetin, which has no other substituents and the same number of hydroxyl group (with different position in B ring).

Table 1 shows the average coplanarity in MD simulations of flavonol and their antioxidant activities. Quercetin has coplanarity and its deviation value from planarity is 1.2°. However, quercetin-3-monoglycosides are not the planar structure and their deviation from coplanarity is above 20°. The antioxidant activities of these glycosyl substituted quercetin decrease about the half of quercetin aglycone's one. Morin shows further deviation form planarity (34.1°) in aqueous MD simulations. In crystal structure of morin grown from an aqueous methanol solution, there are two molecular conformations and the deviation from planarity is 43.4° and 51.0°.[9] Although morin doesn't have the glycosyl substituent and contains an intact 3-hydroxyl group in the C-ring, its antioxidant activity is only similar to quercetin-3-monoglycoside. These results show that the rupture of coplanarity and overall conjugation is a decisive factor in the antioxidant activity of flavonol. Morin keeps a stable hydrogen bond between 3- and 2'-hydroxyl groups during the entire simulation time, which cause the steric hindrance between the hydroxyl groups. These result in the twisted ring conformation, which might be a major factor for reducing antioxidant activity in spite of the similar structure with quercetin aglycone.

In the case of quercetin-3-monoglycosides, the bulky monoglycoside moieties cause the loss of coplanarity of ring B with AC ring. To observe the effect of bulky moiety, quercetin-3-methyl ether (Q3M) which contains a small and no hydroxyl group subustiuent is also investigated. Q3M shows the angle between B ring and AC ring planes is 69.5° and the standard deviation the angle is higher than that of quercetin-3-monoglycoside, 20° and about 10°, respectively. These mean that the bulky monoglycoside moieties not only cause the loss of coplanarity of ring B with AC ring but also hold these structures to avoid further distortion. Although the methoxy moiety also causes the distortion by steric hindrance, this small moiety doesn't tightly interact with B ring. Figure 2 show the change of torsion angle change (C3-C2-C1'-C2') of Q3R and Q3M during the MD simulations. Q3R shows the restricted backbone structure with some twisted angle but the structure of Q3M's backbone cover overall conformation except coplanar structure. Wang et al. investigated the antioxidant activities of phenolics including Q3M and QUE by using DPPH (1,1-diphenyl-2-picryl-dydarazyl) radical scavenging assay.[11] Although Q3M was required 2.3 times of QUE for 50% reduction of the same concentration of DPPH radical,[11] Q3R only needs half of Trolox for equivalent antioxidant potential of the same concentration of QUE (Table 1). In the rough comparison between different assay results, Q3M shows lower antioxidant activity than Q3R, which is another support about the relationship between deviation from coplanarity and anti-

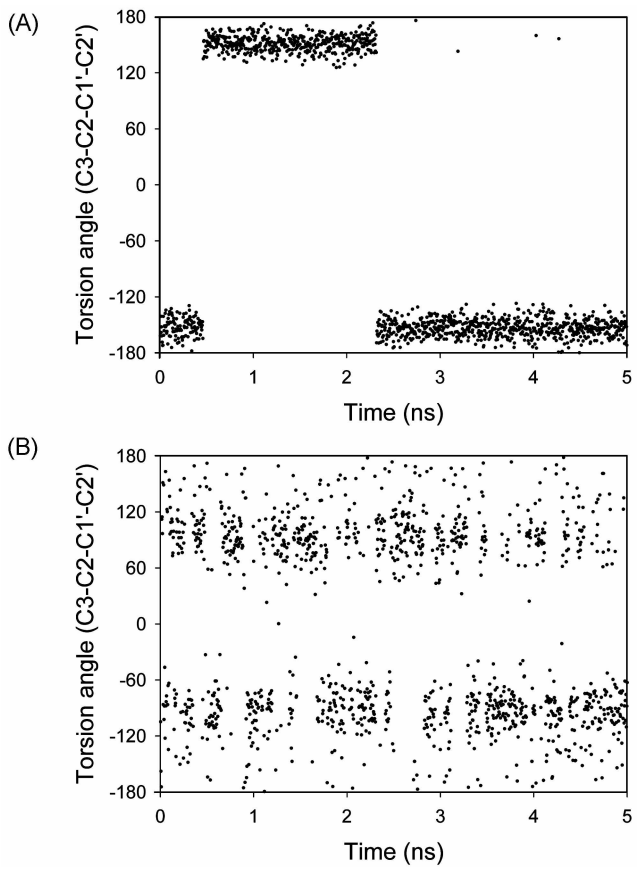


Figure 2. The change of torsion angle (C3-C2-C1'-C2') of Q3R (A) and Q3M (B) during the MD simulations.

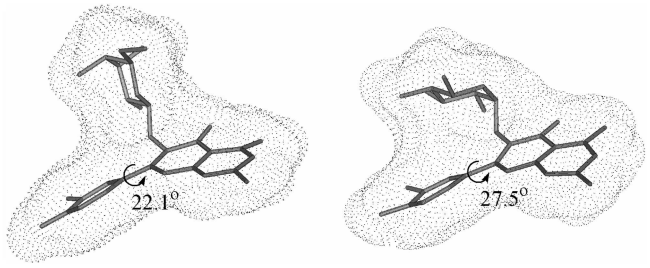


Figure 3. Representative Conformations of Q3G (left) and Q3R (right) in molecular dynamic simulations. Solvent accessible surface area of these quercetin-3-monoglycosides was represented as a dotted picture. All hydrogen atoms have been omitted for clarity.

oxidant activity.

The twisted conformations might be caused by the interaction of glycosyl moiety with backbone ring. Different glycosyl residues show the different antioxidant activities and conformations. The distance and angle of the center of geometry or the best fit plane between 6 ring atoms in B ring and in glycosyl moiety were calculated. Q3R shows smaller distance and angle than that of other quercetin-3-monoglycoside. The distance and angle are 4.9 Å and 29° in Q3R and 5.6 Å and about 76° in other quercetin-3-monoglycoside (Q3G and Q3R). Figure 3 show the representative conformations of Q3G and Q3R in MD simulations. Rhamnose of Q3R is a α-L-sugar which interacts with B ring like as stacking interaction and the deviation of Q3R's B ring is higher than that of β-D-sugar substitutent (Q3G and Q3R). The higher deviation angle is the lower antioxidant activity in quercetin-3-monoglycoside (Table 1).

We investigate the conformations of quercetin and quercetin substituents by molecular dynamic simulations in aqueous environment. Other previous quantum chemical studies calculated the optimized conformation without solvent despite the flexibility of flavonoids.[8,9] In the molecular dynamics simulations, the conformations of flavonols are fluctuated and changed. Therefore, the average values of different conformations in the ensemble from MD simulations were used for the conformational analysis. Quercetin's planar structure results from the increased conjugation.[8,10] However, the glycosylations of quercetin aglycone at 3-hydroxyl position cause the distorted conformation of flavonol backbone and their antioxidant activity is decreased as a function of the degree of distortion. Although the glycosyl moieties cause the distorted conformation, these bulky groups hold B ring and prevent further free rotation (distortion). In conclusion, the coplanarity of flavonol backbone can be an important factor to access the conformation-activity relationship. The series of the conformational analysis of other flavonoids are needed to obtain the detailed relationship with their antioxidant activities. These results will give us useful information for antioxidant drug design.

Experimental Section

The molecular models of flavonols were built with the InsightII/Biopolymer program (version 2000, Accelrys Inc. San Diego, USA). Molecular dynamics simulations were carried out with the AMBER8[12] software to explore the conformational preferences of flavonol. MD simulations were based on the GAFF,[13] whose parameters cover most of the organic chemistry space. The initial coordinates of flavonols were determined using simulated annealing molecular dynamics (SA-MD) and lowest energy conformations were used for molecular dynamics simulations.[14,15] In SA-MD simulations, the temperature was alternated between 200 and 900 K 100 cycles. The total time for the SA-MD simulation was 10 ns. Structures were saved and fully energy-minimized at the end of each cycle, and the lowest-energy conformation among the 100 structures was selected for the initial structure of MD simulations in water.

Flavonols are solvated in a rectangular box of pre-equilibrated TIP3P[16] water molecules which extended 9 Å × 9 Å × 9 Å further than the flavonols atoms. The resulting system was then minimized using steepest descent and conjugated gradient minimization for 3500 cycles. MD simulations were performed and the long-range electrostatic interactions were treated via the particle-mesh Ewald (PME) method.[17] The dielectric constant was set to 1 All MD simulations were performed using periodic boundary conditions in the NPT ensemble at constant pressure (1 atm) and at temperatures maintained at 300 K by coupling the system to a thermal bath with Berendsen's algorithm.[18] The integration time step was set to 2 fs, and the X-H stretching modes were frozen with the SHAKE algorithm.[19] After minimization, the system was heated under constant-volume conditions to 300 K over 100 ps. This first step was followed by a 200 ps equilibration at constant pressure. Starting from the equilibrated system, 5 ns production dynamics was carried out in the NPT ensemble. All the dynamic simulations and final MD trajectories were processed using a computational Grid system,[20] called MGrid (http://www.mgrid.or.kr), to process a large number of MM calculations simultaneously.

Acknowledgements. This study was supported by a grant of the Korea Research Foundation (KRF2004-F00019) and partially supported by MIC (Ministry of Information and Communication) through e-Science Project of KISTI (Korea Institute of Science and Technology Information). SDC.

References

1. Heim, K. E.; Tagliaferro, A. R.; Bobilya, D. J. *J. Nutr. Biochem.* **2002**, *13*, 572.
2. Herrmann, K. J. *Food Technol.* **1976**, *24*, 117.
3. Bors, W.; Michel, C.; Saran, M. *Methods Enzymol.* **1994**, *234*, 420.
4. Cai, Y. Z.; Sun, M.; Xing, J.; Luo, Q.; Corke, H. *Life Sci.* **2005**, in press.
5. Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radical Biol. & Med.* **1996**, *20*, 933.
6. Cook, N. C.; Samman, S. *J. Nutr. Biochem.* **1996**, *7*, 66.
7. Bors, W.; Heller, W.; Michel, C.; Saran, M. *Methods Enzymol.* **1990**, *186*, 343.

8. van Acker, S. A. B. E.; De Groot, M. J.; van den Berg, D. J.; Tromp, M. N. J. L.; den Kelder, G. D. O.; van der Vijgh, W. J. F.; Bast, A. *Chem. Res. Toxicol.* **1996**, *9*, 1305.
9. Cody, V.; Luft, J. R. *J. Mol. Struct.* **1994**, *317*, 89.
10. Rossi, M.; Rickles, L. F.; Halpin, W. A. *Bioorg. Chem.* **1986**, *14*, 55.
11. Wang, K. J.; Zhang, Y. J.; Ynag, C. R. J. *Ethnopharmacol.* **2005**, *99*, 259.
12. Case, D. A.; Darden, T. A.; Cheatham, III, T. E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Wang, B.; Pearlman, D. A.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, J. W.; Ross, W. S.; Kollman, P. A. *AMBER 8*; University of California: San Francisco, 2004.
13. Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **2004**, *25*, 1157.
14. Kim, H.; Jeong, K.; Lee, S.; Jung, S. *J. Comput. Aid. Mol. Des.* **2002**, *16*, 601.
15. Jung, E.; Jeong, K.; Lee, S.; Kim, J.; Jung, S. *Bull. Korean Chem. Soc.* **2003**, *24*, 1627.
16. Jorgensen, W. L. *J. Am. Chem. Soc.* **1981**, *103*, 335.
17. Darden, T.; York, D.; Pedersen, L. *J. Chem. Phys.* **1993**, *98*, 10089.
18. Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; Dinola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684.
19. Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327.
20. Choi, Y.; Kim, D. W.; Park, H.; Hwang, S.; Jeong, K.; Jung, S. *Bull. Korean Chem. Soc.* **2005**, *26*, 769.