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MOLECULAR DYNAMICS STUDY OF QUERCETIN AND SOME OF ITS SUCCINYL DERIVATIVES IN AQUEOUS SOLUTION

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Flavonoid quercetin (fig. 1) is a pleiotropic molecule that has multiple intracellular targets and effects

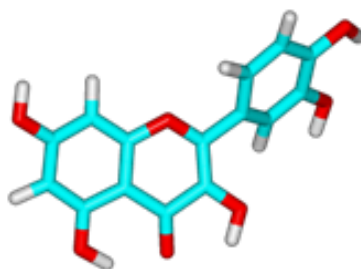


Fig. 1 – Quercetin. Oxygen, hydrogen and carbon atoms are colored in red, white and cyan, respectively.

In the solid state, quercetin is known to exist in two crystal forms, quercetin monohydrate [8] and quercetin dihydrate [9] (fig. 2.1A, B and 2C, D, correspondingly). But there are only scarce data on its structure in the aqueous solution [11]. To investigate the peculiarities of structure and intermolecular interactions of quercetin as well as some of its succinyl derivatives in liquid phase we carried out molecular dynamics simulation of their behavior in the aqueous solution.

Methods

Models building, refining, molecular dynamics simulations, and analysis as well as the result presentation by using molecular graphics were done by molecular modeling program YASARA Structure [15-18].

Molecular dynamics. The coordinates of quercetin molecule were retrieved from the crystal structure of human beta-hydroxyisobutyryl-CoA hydrolase in complex with quercetin (PDB code 3BPT). Succinyl derivatives of quercetin

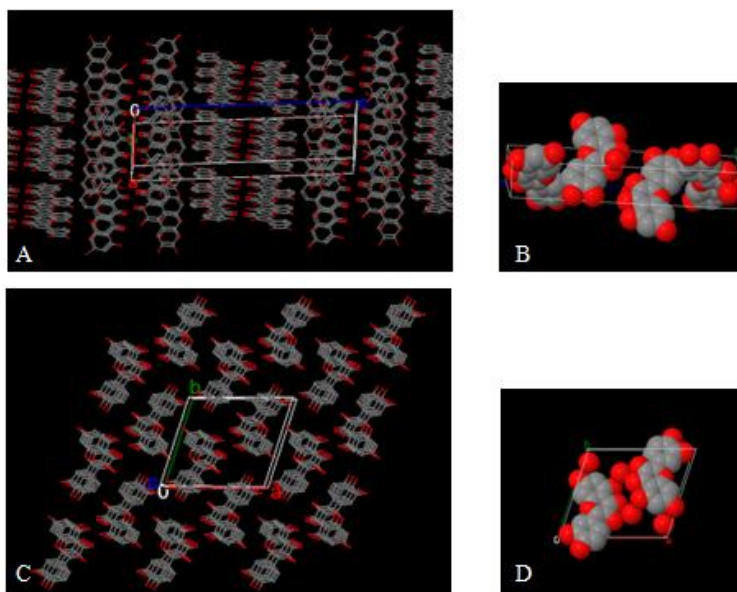


Fig. 2 – Crystal forms of quercetin: quercetin monohydrate (A, B) and quercetin dihydrate (C, D). A and C – crystal packing; B and D – unit cell. Oxygen and carbon atoms are colored in red and gray, respectively. Data were retrieved from Cambridge Structural Database (CSD).

(fig.3) were built by YASARA's small molecule builder. Molecules of quercetin or its succinyl derivatives were randomly placed within a dodecahedral simulation cell by manual translations to random distances in three mutually perpendicular directions and rotations by random angles. The distances between any atoms of the neighbor molecules were kept not less than 8 Å. Simulation cell was filled with TIP3P water molecules to reach 10 % concentration of quercetin or its succinyl derivatives (fig. 4). Na⁺ and Cl⁻ counterions were added to neutralize the system and to reach ion mass fraction 0.9% NaCl [19]. The

molecular system was energy-minimized using AMBER14 force field [20] with 8 Å force cutoff for dispersion interactions. To treat longrange electrostatic interactions the Particle Mesh Ewald algorithm [21] was used. After a short steepest descent minimization, the procedure continued by simulated annealing minimization. The molecular dynamics simulations were run in NPT ensemble at 300 K and pH 7.4 using a multiple timestep of 2.5 fs for intra-molecular and 5 fs for inter-molecular forces [22]. Trajectories were computed for 100 ns.

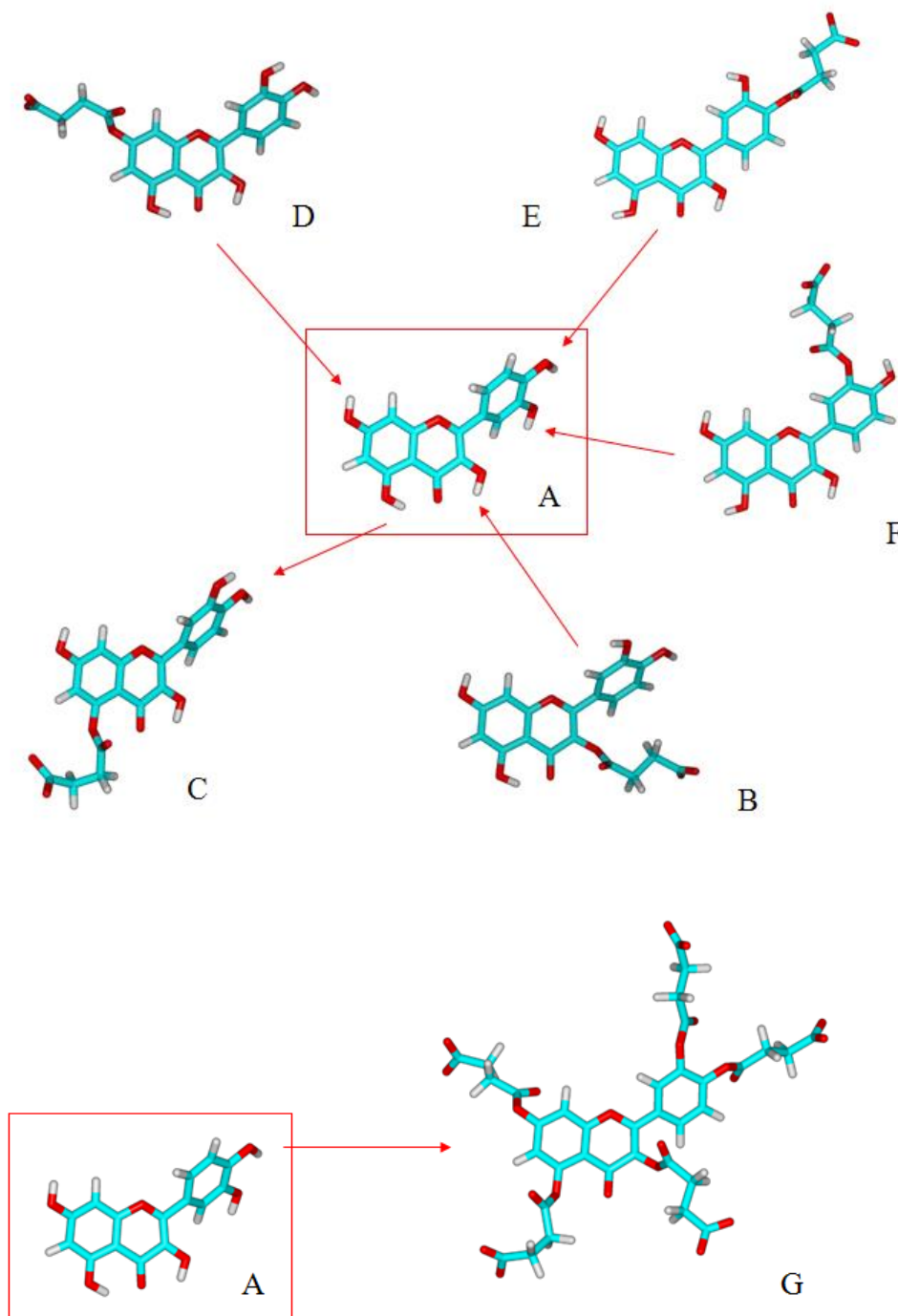


Fig. 3 – Succinyl- quercetin derivatives. A – quercetin; B – 3-succinyl-quercetin; C - 5- succinyl-quercetin; D - 7- succinyl-quercetin; E – 4'- succinyl-quercetin; F – 5'- succinyl- quercetin and G – 3,5,7,4'5'- succinyl-quercetin.

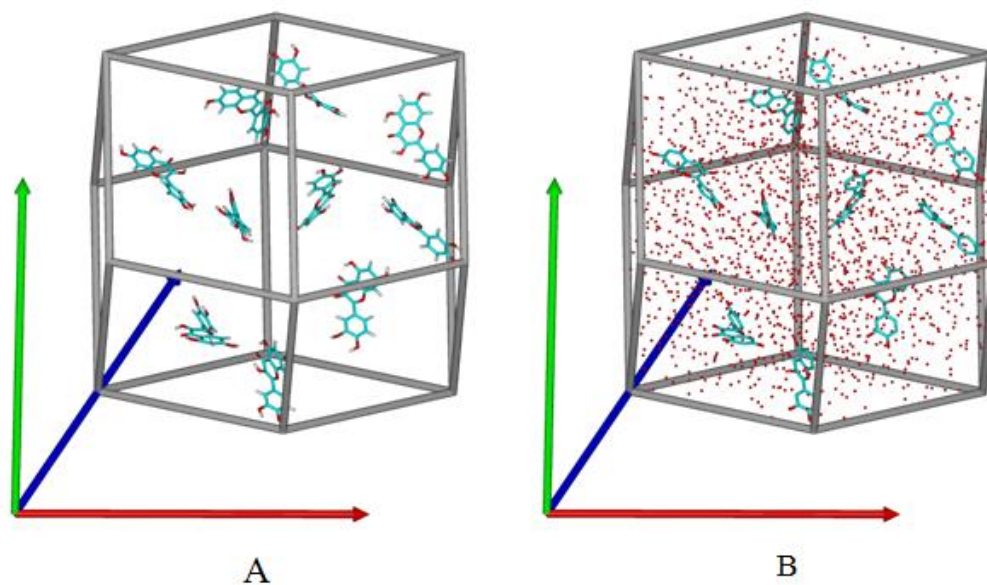


Fig. 4 – Initial configuration of quercetin molecules (A) in aqueous solution (B). Red dots are oxygen atoms of water molecules. Hydrogen atoms were not shown for clarity.

Results and discussion

Quercetin. From the first nanoseconds of simulations, quercetin molecules approached one another and approximately at 3 ns began to self-organize into a compact aggregate that remained stable during further simulation time (fig. 5). As a result, the value of total

molecular surface of quercetin sharply decreased correspondingly and then oscillated insignificantly near its average value during the further simulation (fig. 6 and table 1).

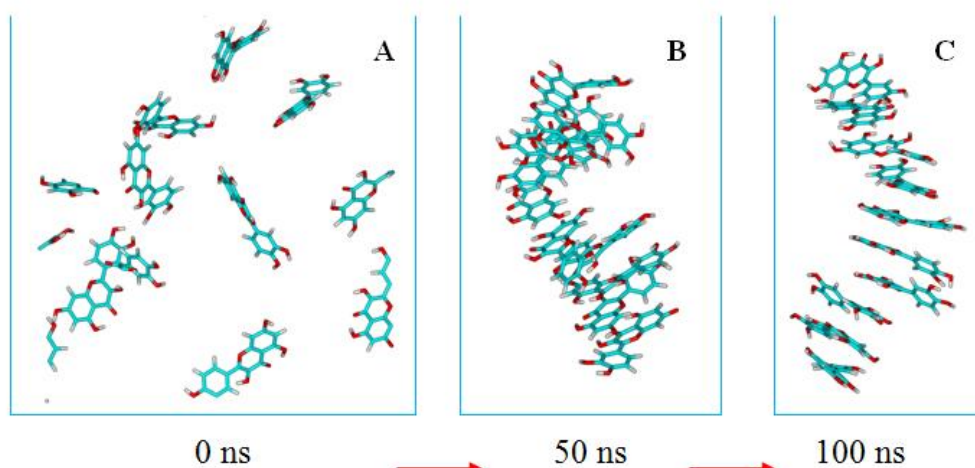


Fig. 5 – Snapshots of quercetin configuration at 0, 50 and 100 ns.

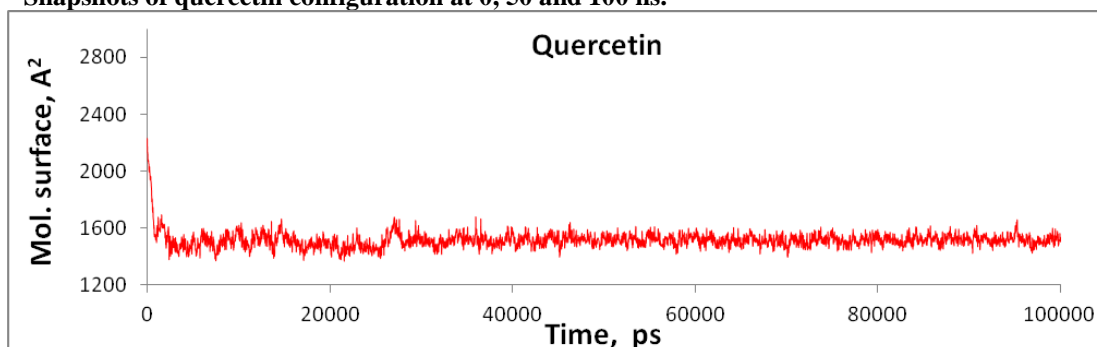


Fig. 6 – Molecular surface of quercetin.

Table 1 – Average values of H-bonds number, total molecular surface and radius of gyration for quercetin, combinatorial mixture of succinyl quercetins, and completely succinylated quercetin.

№	Molecular system	Number of H-bonds	Molecular surface, Å ²	Radius of gyration, Å
1	Quercetin	0.1±0.3 (3)	1513.6±57.6	11.4±2.9
2	Combinatorial mixture of succinyl quercetins	2.8±1.7 (10)	2085.3±118.7	11.4±1.6
3	3,4',5,5',7'-succinyl quercetin	–	2304.8±52.1	16.4±1.9

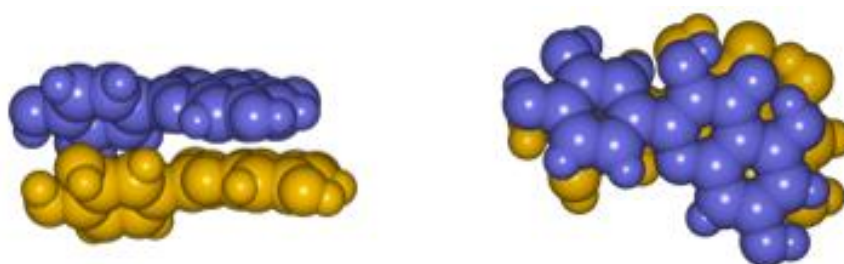


Fig. 7 – Stacking interactions between quercetin molecules in face-to-face ring arrangement.

Hydrogen bonds between quercetin molecules were practically absent in this molecular system (fig. 8 and table 1).

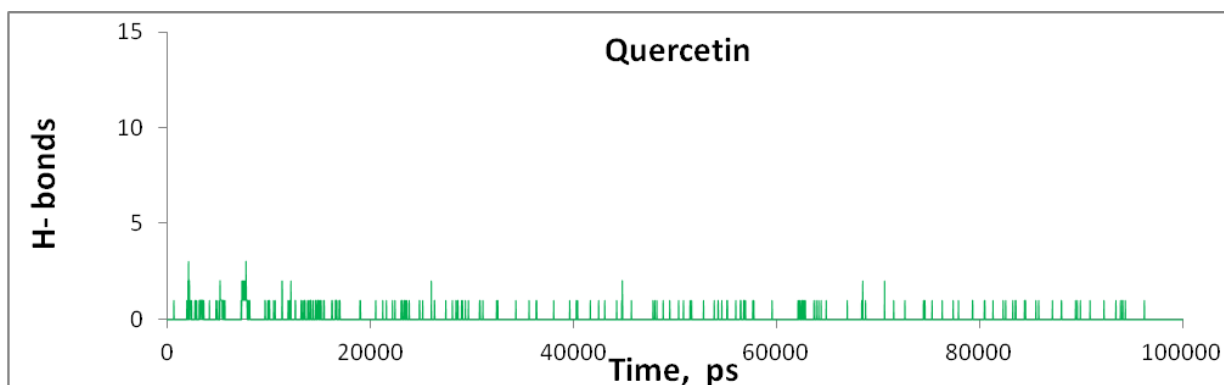


Fig. 8 – Hydrogen bonds between quercetin molecules.

Driving forces of quercetin aggregation are hydrophobic stacking interactions between the flat aromatic systems of neighbor molecules (fig. 7).

Combinatorial mixture of succinyl-quercetin derivatives. Similar to quercetin, molecules of succinyl-quercetin derivatives approached one another from the first nanoseconds of simulations and began to self-organize into a compact stable aggregate (fig. 9). However, the flat aromatic systems of neighbor succinyl-quercetin

molecules, owing to bulky succinyl substituents, could not be placed one above another optimally (fig. 10) to ensure efficient stacking interactions (as it was in case of quercetin (fig. 7)). As a result, these interactions were weaker compared to quercetin, the molecules in the aggregate were more mobile, and the system equilibrated slower.

Correspondingly, values of total molecular surface being higher than ones for quercetin oscillated around average value with higher amplitude (fig.11).

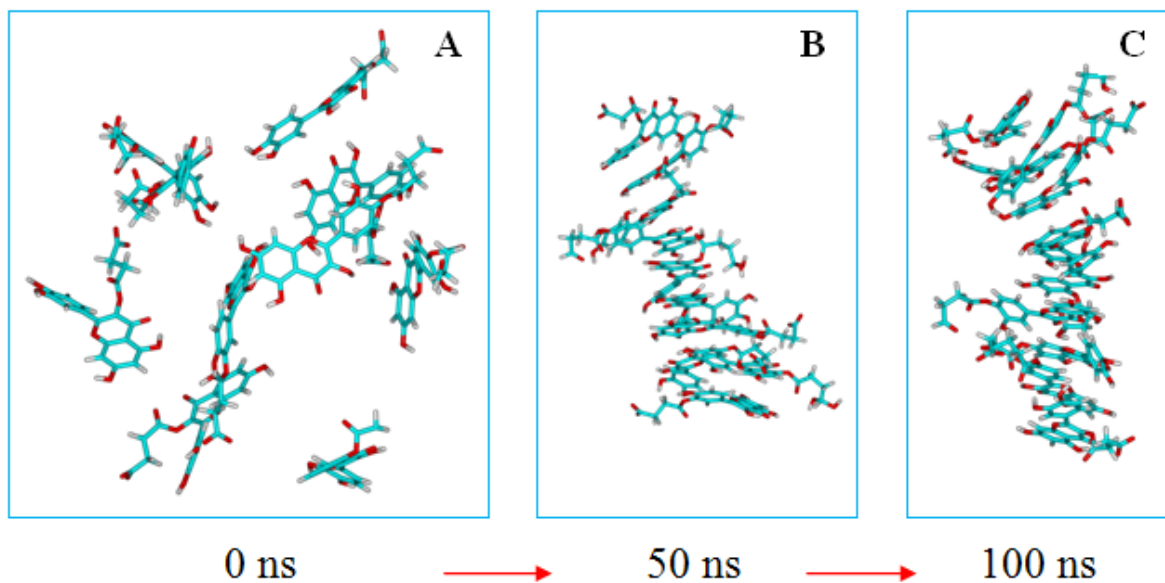


Fig. 9 –Mixture of succinyl-quercetins: snapshots at 0, 50 and 100 ns.

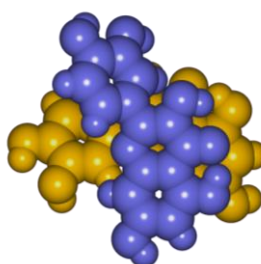


Fig. 10 – Stacking interactions between succinyl-quercetin molecules in face-to-face ring arrangement.

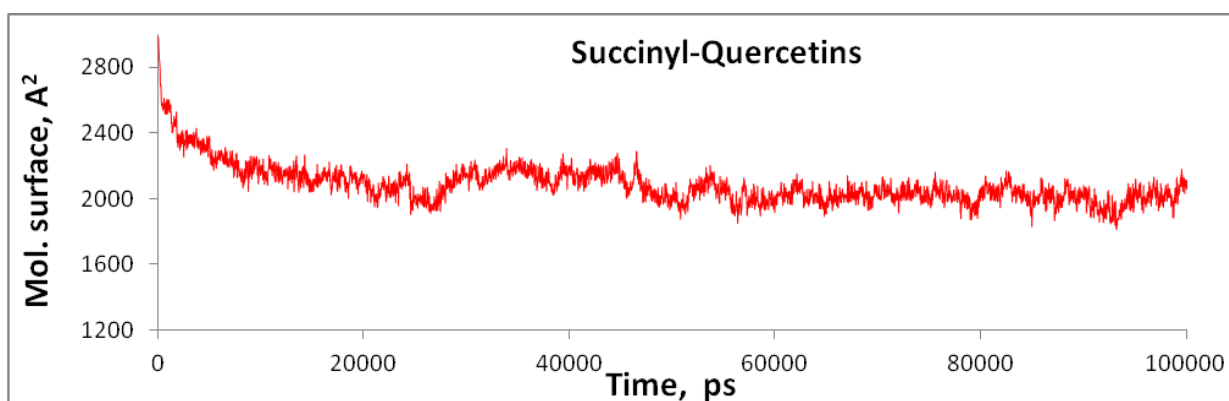


Fig. 11 – Molecular surface of succinyl-quercetins.

In contrast to quercetin, molecules of succinyl-quercetin derivatives formed hydrogen bonds easier due to its higher mobility (fig. 12 and table 1). Radius of gyration in this

case remained practically unchanged compared to quercetin suggesting the size similarity for these two molecular systems.

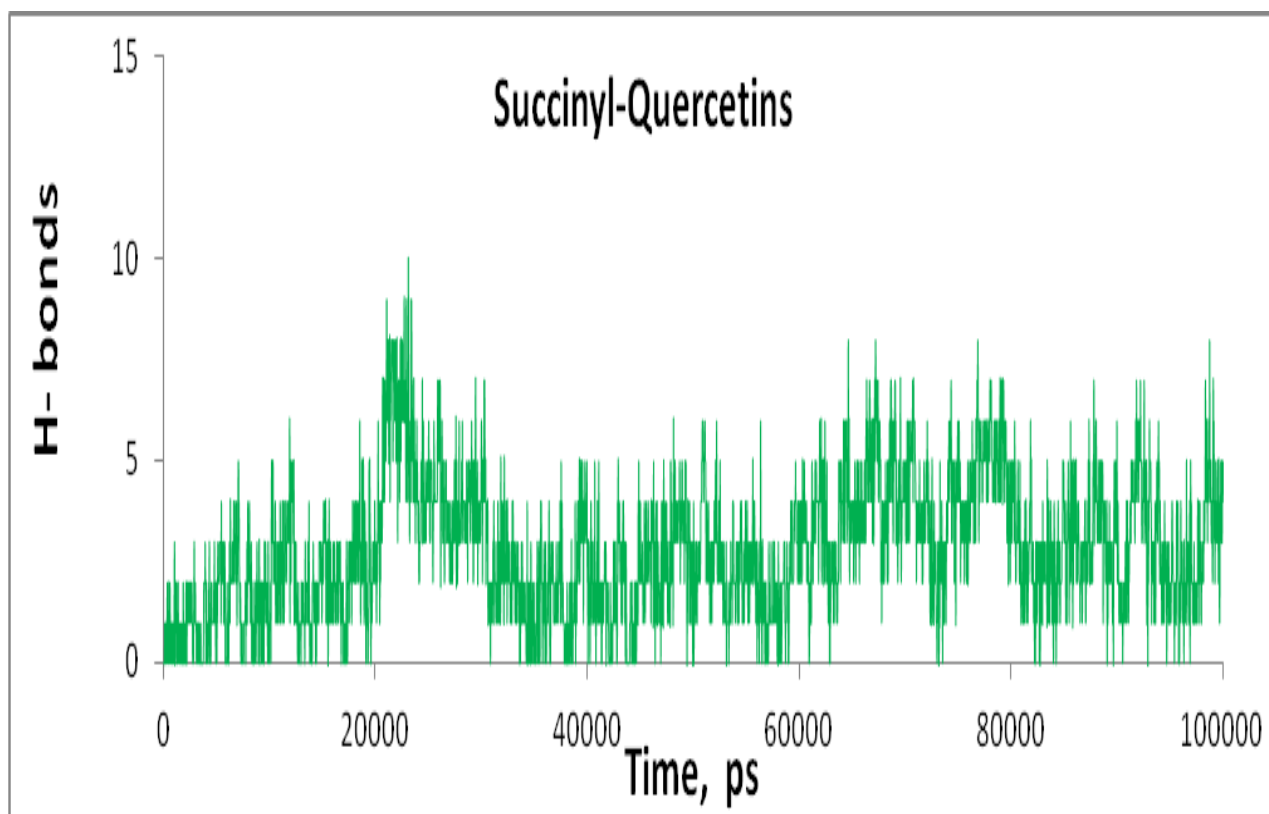


Fig. 12 – Hydrogen bonds between succinyl-quercetins.

Completely succinylated quercetin (3,4',5,5',7-succinyl quercetin). Behavior of completely succinylated quercetin, 3,4',5,5',7-succinyl quercetin (fig. 3G), in aqueous solution was essentially different from one of unmodified quercetin (fig. 3A) or the mixture of succinyl quercetins (fig. 3B-3F). In this case, molecules remained free the majority of time and sometimes there were formed short-lived complexes between two or three molecules (fig. 13). The driving forces of the complex formation are hydrophobic and ionic intermolecular interactions (fig. 14). Hydrophobic interactions occurred between aliphatic residues (fig. 14 A), between aliphatic residues and aromatic systems (fig. 14 C, D), and also between aromatic systems (fig. 14 C, D). The later took place in T-shape ring arrangement (fig. 14 E), in contrast to face-to-face (stacking) interactions in quercetin or succinyl-quercetins systems (fig. 7 and 10, respectively). Ionic interactions between molecules of completely succinylated quercetin are mediated by their interactions with one or two Na^+ ions (fig. 14 B). In contrast to quercetin or succinyl-quercetins, molecules of completely succinylated quercetin do not form hydrogen bonds between each other (table 1) because of lack the H-bond donors. The value of total molecular surface of completely succinylated quercetin, which is higher than one of succinyl quercetin (table 1), oscillated near its average value with lower amplitude (fig. 15). Radius of gyration for molecular system of 3,4',5,5',7-succinyl quercetin is higher compared to two previous systems considered (table 1).

Discussion. The search of new drugs is usually focused on organic compounds that specifically, as a single molecule, interact with a protein. But at the same time there exist ligands of another type, such as promiscuous inhibitors, which are supramolecular aggregates comprising of many individual molecules [23-32]. These aggregates are formed by self-assembly of individual molecules and interact with protein targets non-specifically at the sites which differ from typical binding sites [25-27]. An example of a promiscuous inhibitor is dye Congo red which is known to form ribbon-like supramolecular structures [30, 31].

According to high-throughput screening, quercetin belongs to this type of ligands [23, 24] and, therefore, in corresponding concentrations it supposed to form the aggregates. What is a structure of these aggregates? How individual molecules are arranged inside these aggregates? In case of quercetin, contrary to Congo red, these questions remained unanswered.

Our molecular dynamics simulations of the behavior of quercetin and some of its succinyl derivatives in aqueous solution are in line with the hypothesis of the self-organized aggregate formation by molecules of promiscuous inhibitor quercetin and reveal the peculiarities of the structure and intermolecular interactions within the aggregates of both quercetin and the combinatorial mixture of its succinyl derivatives. These data may be useful for the search of new drugs based on quercetin as a lead compound.

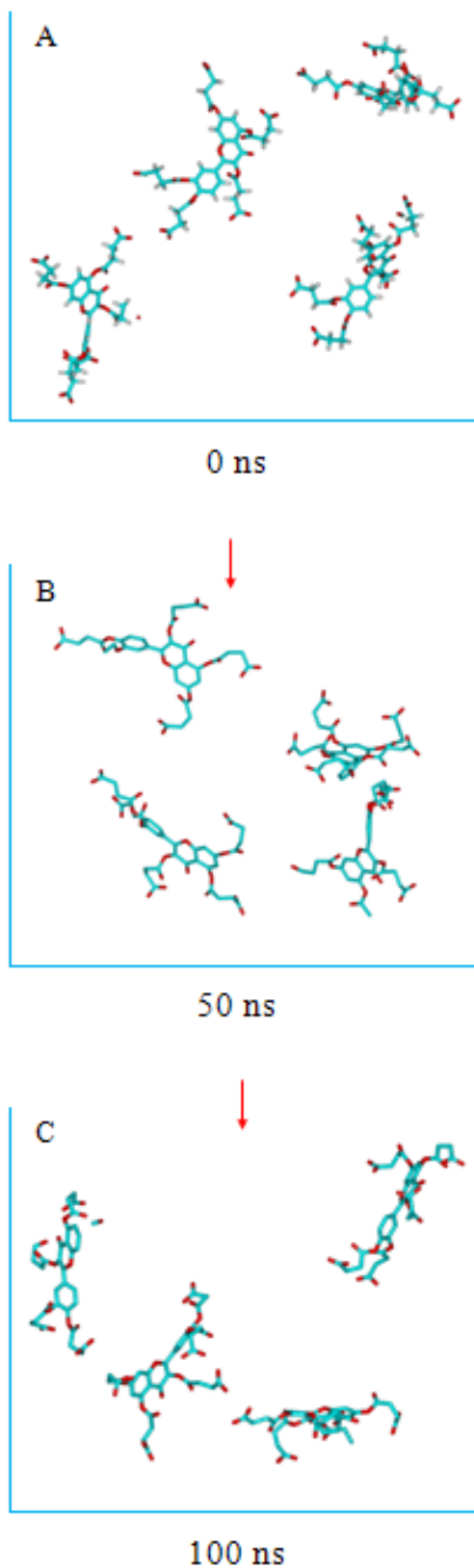


Fig. 13 – Completely succinylated quercetin (3,4',5,5',7-succinyl quercetin) in aqueous solution: snapshots at 0, 50 and 100 ns.

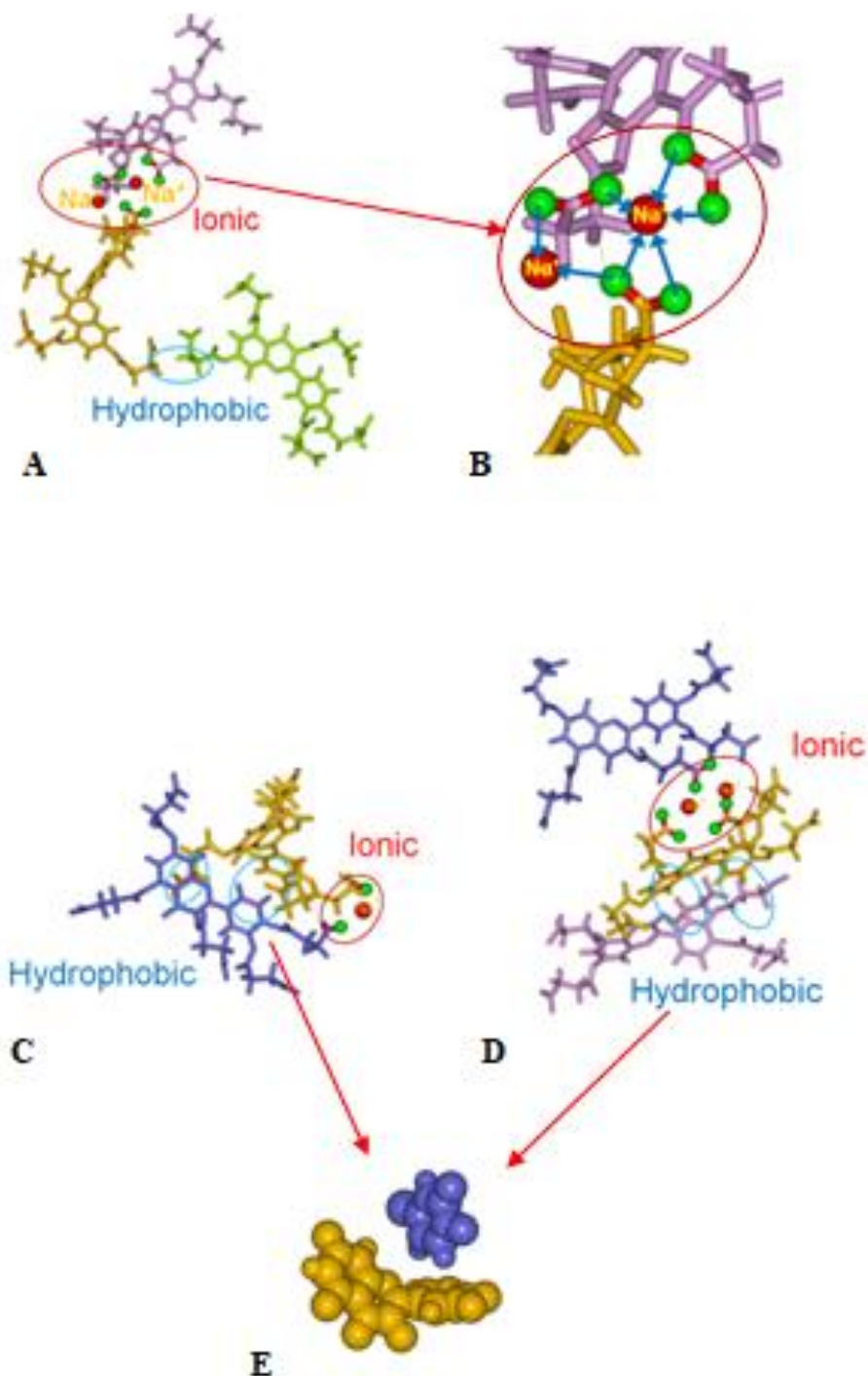


Fig. 14 – Intermolecular interactions within the shot lived complexes of completely succinylated quercetin in aqueous solution. For clarity, molecules in complexes are differently colored. Oxygen atoms of carbamates and Na⁺ ions which form ionic bonds are shown as a ball-and-stick model and colored in green and red, respectively. Quercetin's aromatic systems of neighbor molecules which interact in T-shape ring arrangement are represented as a ball model and colored in orange and light-blue.

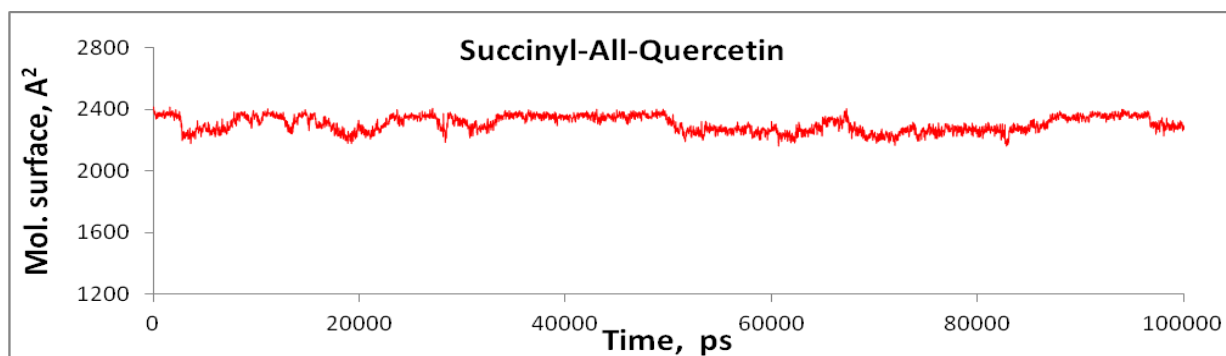


Fig. 15 – Molecular surface of 3,4',5,5',7-succinyl-quercetin.

References

1. Bischoff, S. C. Quercetin: potentials in the prevention and therapy of disease [Text] / S. C. Bischoff // Current Opinion in Clinical Nutrition & Metabolic Care. -2008 – Vol. 11, N 6. – P. 733–740.
2. Russo, G. L. Quercetin: kinase inhibitor against cancer [Text] / G. L. Russo, M. Russo, C. Spagnuolo, I. Tedesco et al. // Cancer Treatment and Research. – 2014. – Vol. 159. – P. 185-205.
3. Carlini, C. R. Ureases as multifunctional toxic proteins: A review [Text] / C. R. Carlini, R. Ligabue-Braun // Toxicon. – 2016. – Vol. 110. – P. 90-109.
4. Carvalho, D. Structural evidence of quercetin multi-target bioactivity: A reverse virtual screening strategy [Text] / D. Carvalho, M. Paulino, F. Polticellic, F. Arredondob et al. // European Journal of Pharmaceutical Sciences. – 2017. – Vol. 106. – P. 393–403
5. Xiao, Z.-P. Molecular docking, kinetic study and structure-activity relationship analysis of quercetin and its analogues as *Helicobacter pylori* urease inhibitors [Text] / Z.-P. Xiao, X.-J. Wang, Z.-Y. Peng, S. Huang et al. // J. Agric. Food Chem. – 2012. – Vol. 60. – P. 10572-10577.
6. Xiao, Z.-P. Synthesis, structure-activity relationship analysis and kinetics study of reductive derivatives of flavonoids as *Helicobacter pylori* urease inhibitors [Text] / Z.-P. Xiao, Z.-Y. Peng, J.-J. Dong, J. He et al. // Eur. J. Med. Chem. – 2013. – Vol. 63. – P. 685-695.
7. Cornard, J. P. Structural study of quercetin by vibrational and electronic spectroscopies combined with semiempirical calculations [Text] / J. P. Cornard, J. C. Merlin, A. C. Boudet, L. Vrielynck // Biospectroscopy. – 1997. – ol. 3, N 3. – P. 183-193.
8. Domagala, S. Structural analysis and multipole modelling of quercetin monohydrate – a quantitative and comparative study [Text] / S. Domagala, P. Munshi, M. Ahmed, B. Guillot et al. // Acta Cryst. B. – 2011. - Vol. 67. – P. 63-78.
9. Jin, G.-Z. Structure of quercetin dihydrate [Text] / C.-Z. Jin, Y. Yamata, K. Tomita // Acta Cryst. C. – 1990. - Vol. 46. – P. 31-313.
10. Cao, H. X-ray crystal structure of a xanthine oxidase complex with the flavonoid inhibitor quercetin [Text] / H. Cao, J. M. Pauff, R. Hille // J. Nat. Prod. – 2014. – Vol. 77. – P. 1693-1699.
11. Álvarez-Diduka, R. Electrochemical characterization of quercetin in aqueous solution [Text] / R. Álvarez-Diduka, M. T. Ramirez-Silvab, G. Alarcón-Angelesc, A. Galanod et al. // ECS Trans. – 2009. - Vol 20, N 1. – P. 115-122.
12. Walker, E. H. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine [Text] / E. H. Walker, M. E. Pacold, O. Perisic, L. Stephens et al. // Molecular Cell. – 2000. – Vol. 6. – P. 909-919.
13. Macomber, L. Reduction of urease activity by interaction with the flap covering the active site [Text] / L. Macomber, M. S. Minkara, R. P. Hausinger, K. M. Merz, Jr. // J. Chem. Inf. Model. – 2015. – Vol. 55. – P. 354-361.
14. Precupas, A. Quercetin influence on thermal denaturation of bovine serum albumin [Text] / A. Precupas, R. Sandu, V. T. Popa // J. Phys. Chem. B. – 2016. – Vol. 120, N 35. – P. 9362–9375.
15. Krieger, E. Assignment of protonation states in proteins and ligands: combining pKa prediction with hydrogen bonding network optimization [Text] / E. Krieger, R. L. Dunbrack, R. W. Hooft, B. Krieger // Methods Mol. Biol. – 2012. – Vol. 819. – P. 405-421.
16. Krieger, E. Improving physical realism, stereochemistry, and side-chain accuracy in homology modelling: four approaches that performed well in CASP8 [Text] / E. Krieger, K. Joo, J. Lee, et al. // Proteins. – 2009. – Vol. 77, Suppl. 9. – P. 114-122.
17. Krieger, E. Making optimal use of empirical energy functions: force field parameterization in crystal space [Text] / E. Krieger, T. Darden, S. Nabuurs, et al. // Proteins. – 2004. – Vol. 57. – P. 678-683.
18. Krieger, E. Increasing the precision of comparative models with YASARA NOVA - a self-parameterizing force field [Text] / E. Krieger, G. Koraimann, G. Vriend // Proteins. – 2002. – Vol. 47. – P. 393-402.
19. Krieger, E. Fast empirical pKa prediction by Ewald summation [Text] / E. Krieger, J. E. Nielsen, C. A. Spronk, G. Vriend // J. Mol. Graph. Model. – 2006. – Vol. 25, N 4. – P. 481-486.
20. Hornak, V. Comparison of multiple AMBER force field and development of improved protein backbone parameters [Text] / V. Hornak, R. Abel, A. Okur, B. Strockbine, et al. // Proteins. – 2006. – Vol. 65. – P. 712-725.
21. Essman, U. A smooth particle mesh Ewald method [Text] / U. Essman, L. Perera, M. L. Berkowitz, et al. // J. Chem. Phys. B. – 1995. – Vol. 103. – P. 8577-8593.
22. Krieger, E. New ways to boost molecular dynamics simulations [Text] / E. Krieger, G. Vriend // J. Comput. Chem. – 2015. – Vol. 36. – P. 996-1007.
23. McGovern, S. L. A common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening [Text] / S. L. McGovern, E. Caseli, N.

- Grigorieff, B. K. Shoichet // J. Med. Chem. – 2002. – Vol. 45. – P. 1712-1722.
24. Seidler, J. Identification and prediction of promiscuous aggregating inhibitors among known drugs / J. Seidler, S. L. McGovern, T. N. Doman, B. K. Shoichet // J. Med. Chem. – 2003. – Vol. 46. – P. 4477-4486.
25. Stopa, B. Supramolecular ligands: monomer structure and protein ligation capacity [Text] / B. Stopa, M. Gorny, L. Konieczny, B. Piekarska et al. // Biochemie. – 1998. – Vol. 80. – P. 963-968.
26. Piekarska, B. Congo red – stabilized intermediates in the λ light chain transition from native to molten state [Text] / B. Piekarska, M. Skowronek, L. Konieczny, J. Rybarska et al. // Biochemie. – 1996. – Vol. 78. – P. 183-189.
27. Roterman, I. Congo red bound to α -1-proteinase inhibitor as a model of supra-molecular ligand and protein complex [Text] / I. Roterman, J. Rybarska, L. Konieczny, M. Skowronek et al. // Comput. Chem. – 1998. – Vol. 22. – P. 61-70.
28. Krol, M. Local and long-range structural effects caused by the removal of the N-terminal polypeptide fragment from immunoglobulin L chain [Text] / M. Krol, I. Roterman, B. Piekarska, L. Konieczny, M. Skowronek et al. // Biopolymers. – 2003. – Vol. 69. – P. 189-200.
29. Piekarska, B. The heat induced formation of a specific binding sites for self-assembled Congo red in V domain immunoglobulin chain λ [Text] / B. Piekarska, Konieczny, B. Stopa et al. // Biopolymers. – 2001. – Vol. 59. – P. 446-456.
30. Skowronek, M. Self-assembly of Congo red – a theoretical and experimental approach to identify its supramolecular organization in water and salt solutions [Text] / M. Skowronek, B. Stopa, L. Konieczny, J. Rybarska et al. // Biopolymers. – 1998. – Vol. 46. – P. 267-281.
31. Stopa, B. The use of supramolecular structures as protein ligands [Text] / B. Stopa, A. Jagusiak, L. Konieczny, B. Piekarska et al. // J. Mol. Mod. – 2013. – Vol. 19. – P. 4731-4740.
32. Auweter, H. Supramolecular structure of precipitated nanosize β -carotene particles [Text] / H. Auweter, H. Haberkorn, W. Heckman, D. Horn et al. // Angew. Chem., Int. Ed. – 1999. – Vol. 38. – P. 2188-2191.

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Introduction. Numerous and diverse biological activity of flavonoid quercetin motivates interest to the peculiarities of its structure as a prerequisite for understanding its structure-function properties which are not fully understood today and remain an object of physico-chemical, biological and structural studies. In the solid state, quercetin is known to exist in two crystal forms, quercetin monohydrate and quercetin dihydrate. But there are only scarce data on its structure in the aqueous solution. High-throughput screening studies indicated that quercetin belongs to promiscuous inhibitors and is hypothesized to form aggregates comprising of many individual molecules. To investigate the

peculiarities of structure and intermolecular interactions of quercetin as well as some of its succinyl derivatives in liquid phase we carried out molecular dynamics simulation of their behavior in the aqueous solution.

Methods . Molecular dynamics simulations and the data analysis were performed by molecular modeling program YASARA Structure. Molecules of quercetin or its succinyl derivatives were randomly placed within a dodecahedral simulation cell. Simulation cell was filled with TIP3P water molecules to reach 10 % concentration of quercetin or its succinyl derivatives. Na^+ and Cl^- counterions were added to neutralize the system and to reach ion mass fraction 0.9% NaCl. The molecular system was energy-minimized using AMBER14 force field with 8 Å force cutoff for dispersion interactions. To treat long-range electrostatic interactions the Particle Mesh Ewald algorithm was used. After a short steepest descent minimization, the procedure continued by simulated annealing minimization. The molecular dynamics simulations were run in NPT ensemble at 300 K and pH 7.4 using a multiple timestep of 2.5 fs for intra-molecular and 5 fs for inter-molecular forces. For each molecular system, trajectories were computed for 100 ns.

Results and discussion. From the first nanoseconds of simulations, molecules of quercetin as well as succinyl-quercetins approached one another and began to self-organize into a compact aggregate that remained stable during further simulation time. Driving forces of the aggregate formation were the hydrophobic stacking interactions between aromatic systems of neighbor molecules. Due to a bulky succinyl substituent the aromatic systems of neighbor succinyl-quercetin molecules could not overlap optimally to ensure efficient stacking interactions that resulted in the higher mobility of the molecules in the aggregate and the possibility to form easily intermolecular H-bonds. Behavior of completely succinylated quercetin, 3,4',5,5',7-succinyl quercetin, in aqueous solution was essentially different. In this case, molecules remained free the majority of time and sometimes formed short-lived complexes between two or three molecules. The driving forces of the complex formation are hydrophobic and ionic intermolecular interactions. Hydrophobic interactions occurred between aliphatic residues, between aliphatic residues and aromatic systems, and also between aromatic systems. The later took place in T-shape ring arrangement. Ionic interactions between molecules of completely succinylated quercetin are mediated by their interactions with one or two Na^+ ions.

Conclusions. Results of our molecular dynamics simulations of the behavior of quercetin and some of its succinyl derivatives in aqueous solution are in line with the hypothesis of the self-organized aggregate formation by molecules of promiscuous inhibitor quercetin and reveal the peculiarities of the structure and intermolecular interactions within the aggregates in case of quercetin and the combinatorial mixture of its succinyl derivatives. These data may be useful for the search of new drugs based on quercetin as a lead compound.

Key words: quercetin, succinylated quercetin, supramolecular aggregates, molecular dynamics.