Computational Molecular Docking Study of Interactions between Anion of Kaempferol and Procalcitonin

Milenković, Dejan; Stanojević Pirković, Marijana; Jeremić, Svetlana;
Dimitrić Marković, Jasmina; Dimić, Dušan; Amić, Dragan; and Marković, Zoran

Abstract: ***Molecular docking analysis was carried out in order to identify the inhibition potency of the kaempferol-anion against human Procalcitonin. The ligand was prepared for docking by minimizing its energy using B3LYP-D3/6-311+G(d,p) level of theory. The inhibition activity was obtained for ten conformations of ligand inside the protein. For the most effective conformation, the active positions for nucleophilic, electrophilic and radical attack are determined by Fukui indices. This study proved that the molecular docking analysis is very important tool in analyzing interactions of biologically important compounds, kaempferol-anion and human procalcitonin in this case.***

Index Terms: ***Kaempferol-anion, Procalcitonin, Molecular docking, Ligand, Fukui function***

1. INTRODUCTION

Flavonoids are natural polyphenolic compounds reported to exert a wide range of positive health effects mainly arising from their antioxidant ability. The protective role of flavonoids is manifested in their capability to "sacrifice" first in the oxidative processes transforming free radicals into stable, deprotonated forms. The antioxidant activity of flavonoids generally has broader significance. The protection role of flavonoids against diet-related oxidative stress could have nutritional significance in preservation of dietary lipids essential to cell functioning and protection against toxicity of potentially harmful lipid oxidation products. As antioxidants, flavonoids can i) bind metal ions, ii) inhibit enzymes involved in free radical production, and iii) directly scavenge free radicals. According to their bioavailability and chemical properties they may scavenge most of oxygen species that are produced during oxidation stress i.e., singlet oxygen, carbon and mainly oxygen centered free radicals such as hydroxyl, superoxide anion, peroxy, alkoxy, as well as nitric oxide radicals [1-3].

Kaempferol (3,5,7-trihydroxy-2-(4`-hydroxyphenyl)-4*H*-chromen-4-one) (Figure 1) is a natural flavonoid that can be found mainly in vegetables (broccoli, cabbage, leek, beans, tomato), fruits (strawberries, grapes), tea, gingko, and many medical herbs used in traditional medicine [4, 5]. It is known as a strong antioxidant that helps to prevent arteriosclerosis by inhibiting the oxidation of low density lipoprotein and the formation of platelets in the blood [4]. It also reduces the risk of developing some types of cancer, induces apoptosis in glioma cells, and shows anti-viral activity against cytomegalovirus, influenza virus, herpes simplex virus and human immunodeficiency virus (HIV) [4].

At the pH values higher than 7.00, it can be expected that polyphenols undergo to deprotonation, forming monoanions. The reaction of deprotonation can be presented by the following equation:

$$AOH \rightarrow AO^{-} + H^{+} \quad (1)$$

Manuscript received May 31, 2017. This work is supported by the Ministry of Education, Science and Technological Development in Serbia, with projects OI172015, OI172040, and OI174028.

Dejan Milenković is with the Bioengineering Research and Development Center (BioIRC), Kragujevac, Serbia (e-mail: deki82@kg.ac.rs).

Marijana Stanojević Pirković is with the Faculty of Medical Sciences University of Kragujevac, Serbia (e-mail: marijanas14@gmail.com)

Svetlana Jeremić is with the Department of Biochemical and Medical Sciences, State University of Novi Pazar, Republic of Serbia (e-mail: sjeremic@np.ac.rs)

Jasmina Dimitrić Marković is with the Faculty of Physical Chemistry, University of Belgrade, Republic of Serbia (e-mail: markovich@ffh.bg.ac.rs)

Dušan Dimić is with the Faculty of Physical Chemistry, University of Belgrade, Republic of Serbia (e-mail: ddimic@ffh.bg.ac.rs)

Dragan Amić is with the Faculty of Agriculture, Josip Juraj Strossmayer University of Osijek, Croatia (e-mail: damic@pfos.hr)

Zoran Marković is with the Department of Biochemical and Medical Sciences, State University of Novi Pazar, Republic of Serbia (e-mail: zmarkovic@np.ac.rs)

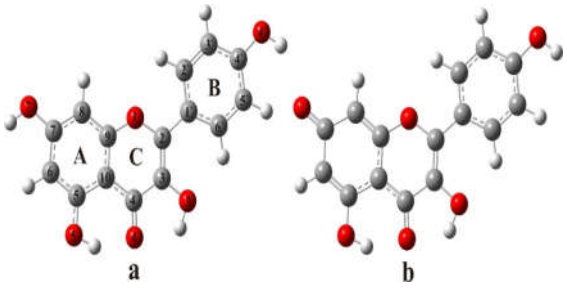

Fig. 1 Optimized structures of kaempferol (left) and its corresponding anion (right).

The equilibrium constant of deprotonation process can be calculated as is shown in Equation 2. The important value for estimation the acidity of observed compound is negative logarithm of deprotonation constant, labeled as *pKa* (Equation 3):

$$Ka = \frac{[H^+][AO^-]}{[AOH]} \quad (2)$$

$$pKa = -\log Ka \quad (3)$$

Compounds with more than one hydroxyl group undergo to deprotonation process until it is thermodynamically plausible under the certain conditions. Usually, the first dissociation step flows the most easily, and each subsequent is more difficult. Since *pKa* values can be treated as the measure of acidity, or as the measure of the possibility of compound to realize proton, the lower *pKa* values indicate higher affinity of compound to form anionic specie.

For kaempferol, the experimentally obtained *pKa* values are: pKa_1 = 7.05, pKa_2 = 9.04 and *pKa*3 = 11.04 [6].

Determination of *pKa* values is important for evaluation of quantity of every ionic moiety of molecule. Due to it, it is necessary to calculate β_1, β_2 and β_3 values using the equations 4, 5 and 6:

$$\beta_1 = 10^{pKa_3} \quad (4)$$

$$\beta_2 = 10^{pKa_3 + pKa_2} \quad (5)$$

$$\beta_3 = 10^{pKa_1 + pKa_2 + pKa_3} \quad (6)$$

Taking on mind that at physiological conditions (*pH* = 7.4), the concentration of H^+ ion is $3.98 \cdot 10^{-8}$ [7], the population of every ionic form can be quantified calculating its molar fraction (*f*) (Equations 7, 8, 9 and 10):

$$f(A^{3-}) = \frac{1}{1 + \beta_1[H^+] + \beta_2[H^+]^2 + \beta_3[H^+]^3} \quad (7)$$

$$f(HA^{2-}) = \beta_1[H^+] f(A^{3-}) \quad (8)$$

$$f(H_2A^-) = \beta_2[H^+]^2 f(A^{3-}) \quad (9)$$

$$f(H_3A) = \beta_3[H^+]^3 f(A^{3-}) \quad (10)$$

Based on previously presented calculations, the amount of mono-ionic moiety of kaempferol at physiological environment is 68.05%, and it is expected that at physiological environment, monoanionic species primarily acts as a ligand during the docking with here investigated protein.

In the earlier investigations, it was estimated that O-H group in the position 7 of kaempferol molecule presents the most plausible position for deprotonation [8]. For monoanion obtained at this way, labeled here as KMP-7A, are calculated Fukui functions. Fukui function or frontier function presents the function that describes the electron density in the frontier orbitals, as a result of a small change in the total number of electrons [9]. Fukui parameters can be used for the prediction of the active sites for biologically active molecules, in our case of KMP-7A. It is mathematically defined by the following equation:

$$f(r) = \left[\frac{\delta\mu}{\delta\upsilon(r)}\right]_N = \left[\frac{\partial\rho(r)}{\partial N}\right]_{\upsilon(r)} \quad (11)$$

In the previous equation μ represents the chemical potential, while *N* is the number of electrons in the system, and $\upsilon(r)$ is the external potential. The approximately calculations of Fukui function is often calculated as is presented in following equations (12, 13 and 14):

$$f^-(r) = \rho_N(r) - \rho_{N\text{-}1}(r) \approx \rho^{HOMO}(r) \quad (12)$$

$$f^+(r) = \rho_{N+1}(r) - \rho_N(r) \approx \rho^{LUMO}(r) \quad (13)$$

$$f^0(r) = \left[f^-(r) + f^+(r)\right]/2 \approx \approx \left[\rho^{HOMO}(r) + \rho^{LUMO}(r)\right]/2 \quad (14)$$

Labels $\rho_N(r)$, $\rho_{N-1}(r)$ and $\rho_{N+1}(r)$, appeared in previous equations, represent separately, and in the case of anion, that is the object of our investigations, the electron densities of the system with *N* electrons (investigated anion), *N–1* (radical) and *N+1* (radical dianion) electrons. The values of $\rho^{HOMO}(r)$ and $\rho^{LUMO}(r)$ indicate the electron densities of the HOMO and LUMO orbitals of investigated anion, respectively.

A more convenient way to predict the reaction site in a molecule is function named condensed Fukui function (CFF) [9]. CFF can be obtained by integrating the equations 12, 13 and 14 for individual atoms in a molecule. The definition of CFF for an atom, noted as A, can be written as:

$$f_{\mathrm{A}}^{-} = p_{N}^{\mathrm{A}} - p_{N-1}^{\mathrm{A}} \quad (15)$$

$$f_{\mathrm{A}}^{+} = p_{N+1}^{\mathrm{A}} - p_{N}^{\mathrm{A}} \quad (16)$$

In the previous equation, p_N^A presents the electron population number of atom A. If atomic charge is defined as $q^A=Z^A-p^A$ (Z is the charge of atomic nucleus), then f^- and f^+ can be expressed as the difference of atomic charges in two states. Analogously, CFFs for an atom A can be easily formulated as is shown in the following equations:

$$f_{\mathrm{A}}^{-} = q_{N-1}^{\mathrm{A}} - q_{N}^{\mathrm{A}} \quad (17)$$

$$f_{\mathrm{A}}^{+} = q_{N}^{\mathrm{A}} - q_{N+1}^{\mathrm{A}} \quad (18)$$

$$f_{\mathrm{A}}^{0} = \left[f_{\mathrm{A}}^{+} + f_{\mathrm{A}}^{-} \right] / 2 = \left[q_{N-1}^{\mathrm{A}} - q_{N+1}^{\mathrm{A}} \right] / 2 \quad (19)$$

In the previous equations, q_N^A, q_{N-1}^A and q_{N+1}^A present the charges at atom A of the neutral, anionic and cationic species, respectively, if they are calculated for neutral molecule. In our case, they present charges at atom A of the inspected anion, radical and radical-dianionic species, respectively. Calculated values f_A^-, f_A^+ and f_A^0 are used to predict the position of electrophilic, nucleophilic and free-radical attack to the investigated moiety. The larger CFF value indicates the greater reactivity of that corresponding site towards reactive specie. There are many methods for the calculation of the atomic charges, and here for that intent are used Natural charges obtained from NBO analysis [10].

Procalcitonin (PCT) (~13 kDa) is a peptid consisting of 116 amino acids. PCT is enzymatically degraded into lower molecular weight peptides. Calcitonin is a final product that consists of 32 amino acids. All precursors including PCT and mature hormone peptide can be detected in serum of healthy humans. In septic patients, only a fragment of 3-116 amino acids is detectable, not a complete PCT molecule [11]. Biological effect of this protein was proven in the study [12], which showed that elevated concentrations of PCT can lead to sepsis that can be treated by the anti-PCT antibodies.

In this paper, the most stable kaempferol-monoanion, obtained by deprotonation of O-H group in position 7 (KMP-7A) (Figs. 1a and 1b), is investigated for the reactivity toward Procalcitonin protein by means of the Molecular Docking analysis.

2. METHODOLOGY

Molecular docking simulation was carried out using AutoDock 4.0 software [13]. The structure of human PCT was adapted from literature data [11]. Discovery Studio 4.0 [14] was used for the preparation of protein for docking by removing the co-crystallized ligand, water molecules and co-factors. To calculate Kollman charges and to add polar hydrogen, AutoDockTools (ADT) graphical user interface was used. Title compound, KMP-7A, was prepared for docking by minimizing its energy using B3LYP-D3 [15,16] local density functional method and 6-311+G(d,p) basis set as implemented in the Gaussian 09 package [17]. Flexibility of the ligand was considered, while the protein remained as a rigid structure in the ADT. All bonds of KMP-7A were set to be rotatable. The Geistenger method for calculation of partial charges was employed. All calculations for protein - ligand flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method [18]. A grid box with dimensions 92.007x0.202 x0.111 of human PCT was used in order to cover protein binding sites and accommodate ligand to move freely. Inhibition potency of title anion was investigated and discussed.

3. RESULTS AND DISCUSSION

Protein-ligand binding energy and the identification of potential ligand binding sites were determined from this study as well. The ligand conformation which showed the lowest binding energy (best position) was determined based on ligand docking results. The position and orientation of ligand inside protein receptor and the interactions with amino acids bound to the ligand were analyzed and visualized with Discovery Studio 4.0 and AutoDockTools.

Table 1 presents the values of the estimated free energy of binding, and the inhibition constants (Ki) for the investigated ligand in ten different conformations are given. Lower value of Ki indicates better inhibition.

Table 1: Estimated free energy of binding (ΔG_{bind}) in kcal/mol, estimated inhibition constant (K_i) (μM) of different poses of KMP-7A against PCT.

Position	*ΔG_{bind} (kcal/mol)*	*K_i (μM)*	*Hydrogen Bond*	*Hydrophobic Contact*
1	-2.80	9.1×10^{3}	A:ASP53	A:ASP53 A:LEU52

2	-2.47	$1.5x10^4$	A:ASP53	A:ASP53 A:LEU52 A:ARG56
3	-2.46	$1.6x10^4$	A:ASP53 A:ARG56	A:ASP53 A:LEU52 A:ARG56
4	-2.46	$1.6x10^4$	A:ASP53 A:ARG56	A:ASP53 A:LEU52 A:ARG56
5	-2.45	$1.6x10^4$	/	A:ASP53 A:ARG56
6	-2.45	$1.6x10^4$	A:ASP53	A:LEU52 A:ARG56
7	-2.40	$2.2x10^4$	A:ASP53	A:LEU52 A:ARG56
8	-2.36	$1.6x10^4$	A:ASP53	A:ARG56
9	-2.30	$2.1x10^4$	A:ASP53 A:ARG56	A:ARG56
10	-2.29	$2.1x10^4$	A:ASP53 A:ARG56	A:ARG56

The lowest values of ΔG_{bind} and K_i are found for conformation **1** (Table 1). Analyzing the position of active amino acids, it can be concluded that ligand binds at the catalytic site of substrate by weak non-covalent interactions. The most prominent are H-bonds, alkyl-*π,* *π*-anion and *π*-*π* interactions. ASP in position 53 in the primary structure of Procalcitonin chain, has predominant role as active inhibition site of human PCT, regardless of the conformation of investigated ligand (Fig. 2). ASP53 forms one H-bond by length of 1.91 Å with O-H group of the KMP-7A and one weak *π*-anion interaction (Fig. 2). LEU52 forms weak alkyl-*π* interaction with chroman ring (Fig. 2). Autodock results predict that anion of kaempferol in position 7 (KMP-7A) forms very stable complex with PCT based on the K_i values (Table 1).

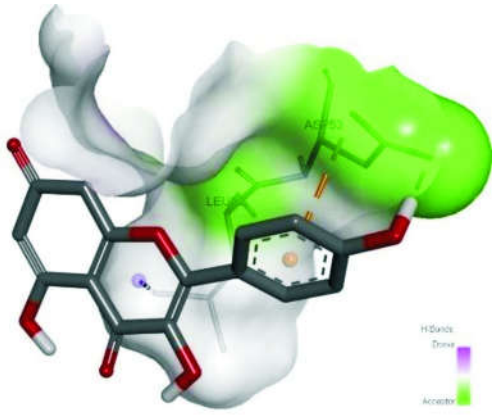

Fig. 2 Picture is showing interaction between KMP-7A (conformations 1, the lowest K_i) and amino acids in PCT.

The most probable sites for electrophilic (***f*$_{nbo}$-**), nucleophilic (***f*$_{nbo}$+**), and radical attack (***f*$_{nbo}$0**) are given in Table 2. The NBO charges predict that C6, C8, C10, O4, and O7 are the most reactive atoms both for electrophilic and free-radical attack. For the nucleophilic attack, three positions are favored: C4', C5', and H4' (bold in Table 2). These results are in perfect agreement with docking positions predicted by Docking analysis.

Table 2: The condensed Fukui functions for electrophilic (***f*$_{nbo}$-**), nucleophilic (***f*$_{nbo}$+**), and radical attack (***f*$_{nbo}$0**) for ligand KMP-7A, predicted by NBO analysis.

Position	f_{nbo}^+	f_{nbo}^-	f_{nbo}^0
O1	0.008	0.029	0.018
C2	0.013	0.064	0.039
C3	0.034	0.012	0.023
O3	0.011	0.036	0.024
C4	0.028	0.009	0.019
O4	0.043	**0.076**	**0.059**
C5	0.005	-0.004	0.000
O5	0.020	0.039	0.030
C6	0.033	**0.077**	**0.055**
C7	0.008	-0.043	-0.018
O7	0.052	**0.253**	**0.152**
C8	0.017	**0.166**	**0.091**
C9	-0.007	-0.023	-0.015
C10	0.015	**0.111**	**0.063**
C1'	0.042	-0.031	0.006
C2'	0.031	0.015	0.023
C3'	0.016	0.011	0.013
C4'	**0.114**	0.035	0.075
O4'	0.059	0.024	0.042
H4'	**0.149**	0.009	0.079
C5'	**0.175**	0.012	0.094
C6'	0.026	0.020	0.023

4. CONCLUSION

To evaluate the inhibitory nature of kaempferol-anion towards Procalcitonin, the molecular docking study was performed. According to the results of the molecular docking study, the investigated ligand forms stable complex with Procalcitonin as evident from the binding energy (ΔGbind -2.80 kcal/mol). Atoms C6, C8, C10, O4, and O7 are favorable sites for electrophilic and free-radical attack, while those labeled as C4', C5', and H4' are probable positions for nucleophilic attack. The most important

interactions are H-bonds, π-π and π-alkyl. Atoms that form bonds are predicted as the most reactive sites by the Fukui indices. These preliminary results suggest that kaempferol at physiological conditions (pH = 7.4) might exhibit significant inhibitory activity against Procalcitonin acting as monoanion.

REFERENCES

[1] Cos, P., Ying, L., Calomme, M., Hu, J. P., Cimanga, K., Van Poel, B., Pieters, L., Vlietinck, A. J., Vanden Berghe, D., "Structure−Activity Relationship and Classification of Flavonoids as Inhibitors of Xanthine Oxidase and Superoxide Scavengers," Journal of Natural Products, 1998, pp. 71–76.

[2] Pietta, P. G., "Flavonoids as antioxidants," Journal of Natural Products, 2000, pp. 1035–1042.

[3] Rice-Evans, C., "Flavonoid Antioxidants," Current Medicinal Chemistry, 2001, pp. 797–807.

[4] M. Calderon-Montano, J., Burgos-Moron, E., Perez-Guerrero, C., Lopez-Lazaro, M., "A Review on the Dietary Flavonoid Kaempferol," Mini-Reviews in Medicinal Chemistry, 2011, pp. 298–344.

[5] Andersen, M., Markham, KR., "Flavonoids : chemistry, biochemistry, and applications," CRC, Taylor & Francis, 2006, pp. 1256.

[6] Matei, I., Tablet, C., Ionescu S., Hillebrand M., "Close-lying pKa Values of Kaempferol Determinated by Second-derivative Synchronous Fluorescence," Revue Roumaine de Chimie, 2014, pp. 401–405.

[7] Galano, A., Alvarez-Idaboy, J. R., "A Computational Methodology for Accurate Predictions of Rate Constants in Solution: Application to the Assessment of Primary Antioxidant Activity," Journal of Computational Chemistry, 2013, pp. 2430–2445.

[8] Dimitrić Marković, J., Milenković, D., Amić, D., Popović-Bijelić, A., Mojović, M., Pašti, I., Marković, Z., "Energy requirements of the reactions of kaempferol and selected radical species in different media: towards the prediction of the possible radical scavenging mechanisms," Structural Chemistry, 2014, pp. 1795–1804.

[9] Tiznado, W., Chamorro, E., Contreras, R., Fuentealba, P., "Comparison among Four Different Ways to Condense the Fukui Function," Journal of Physical Chemistry A, 2005, pp. 3220–3224.

[10] Reed, A. E., Curtiss, L. A., Weinhold, F., "Intermolecular interactions from a naturalbond orbital donor-acceptor viewpoint," Chemical Review, 1988, pp. 899–926.

[11] Weglöhner, W., Struck, J., Fischer-Schulz, C., Morgenthaler, N. G., Otto, A., Bohuon, C., Bergmann, A., "Isolation and characterization of serum procalcitonin from patients with sepsis," Peptides, 2001, pp. 2099–2103.

[12] Nylén, E. S., Rohatgi, P., Becker, K. L., Snider, R. H., Thompson, K. A., Rohatgi, P., Becker, K. L., "Pneumonitis-Associated Hyperprocalci-toninemia," The American Journal of the Medical Sciences, 1996, pp. 12–18.

[13] Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., Olson, A. J., "AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility," Journal of Computational Chemistry, 2009, pp. 2785–2791.

[14] BIOVIA Discovery Studio 2016, Accelrys Studio Inc, San Diego, 2016.

[15] Becke, A. D., "Density-functional thermochemistry. III. The role of exact exchange," The Journal of Chemical Physics, 1993, pp. 5648–5652.

[16] Becke, A. D., Johnson, E. R., "A density-functional model of the dispersion interaction," The Journal of Chemical Physics, 2005, pp. 154101.

[17] Frisch, M. J., Trucks, GW., Schlegel H. B., et al. Gaussian 09, Revision D.1, Gaussian, Inc, Wallingford CT, 2013.

[18] Fuhrmann, J., Rurainski, A., Lenhof, H-P., Neumann, D., "A new Lamarckian genetic algorithm for flexible ligand-receptor docking," Journal of Computational Chemistry, 2010, pp. 1911-1918.

Dejan MILENKOVIĆ, PhD in Chemistry, achieved his PhD at the Faculty of Science (University of Kragujevac) at the Department of Chemistry in 2014. He graduated at the same faculty at the Department of Chemistry in 2007. He is working as Research Assistant at the Bioengineering Research and Development Center BioIRC in Kragujevac. He is interested in molecular modeling implemented in investigations of the mechanisms of antioxidative activity of some nature molecules, primarily from the group of polyhydroxy phenols, preferentially flavonoids and flavones, as the metal complexes of mentioned molecules.