2015 IEEE 15th International Conference on Bioinformatics and Bioengineering (BIBE 2015)

Belgrade, Serbia
2 – 4 November 2015
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DFT investigation of the reaction of cyanidin with hydroxyl radical

Dejan A. Milenković, Jasmina M. Dimitrić Marković, and Zoran S. Marković

Abstract—Cyanidin, as an important plant pigment, was theoretically investigated for its ability to scavenge potentially, highly damaging hydroxyl radical. The applied method successfully reproduces the bond dissociation enthalpy (BDE), the ionization potential (IP) and proton affinity (PA). The HAT mechanism is most favorable reaction pathway for antioxidative action of cyanidin in the gas phase. On the other hand, the SPLET mechanism is most favorable reaction pathway for antioxidative action of cyanidin in the aqueous phase. Mechanistic investigations of antioxidative action of cyanidin in reaction with the hydroxyl radical confirmed that HAT is the dominant reaction pathway in the gas phase, and that the 4´ position is most reactive.

I. INTRODUCTION

Anthocyanidins and anthocyanins, as hydroxylated and glycosidized flavanol compounds, are plant pigments responsible for the red, blue, and purple hues of flowers and fruits in nature. These compounds are flavonoids that belong to the family of polyphenols. Important property of the anthocyanidins is that they are natural antioxidants. There are well known therapeutic effects of the antioxidants in the treatment for diabetes, atherosclerosis and cardiovascular diseases. Their protective role correlates with their antioxidant activity, which is manifested through different actions, like direct radical scavenging, transition metal chelation, inhibition of certain enzymes, or removing oxidatively changed and damaged molecules.

The scavenging of free radicals seems to play a notable role in the antioxidant activity of flavonoid compounds. The antiradical properties of flavonoids are related to their ability to transfer their phenolic H-atom to a free radical. The reactive radical species (R') in the radical scavenging mechanisms are inactivated by accepting a hydrogen atom from a hydroxyl group of the flavonoid. It has been recognized that this reaction proceeds via at least three different mechanisms: hydrogen atom transfer (HAT, eqn(1)), single electron transfer followed by proton transfer (SET-PT, eqn(2)) and sequential proton loss electron transfer (SPLET, eqn(3)). These mechanisms may co-exist, and they depend on solvent properties and radical characteristics.

In HAT mechanism, radical species (R') remove a hydrogen atom from the antioxidant molecule (ArOH) which converts to flavonoid radical (ArO'):

\[ \text{ArOH} + R' \rightarrow \text{ArO'} + \text{RH} \quad (1) \]

The second mechanism consists of two steps. The first step in the SET-PT mechanism is transfer of an electron to the radical while the primary antioxidant, the flavonoid molecule, is transformed into the radical cation (eqn (2a)). The next step is the heterolytic O–H bond dissociation of ArOH⁺ (eqn (2b)).

\[ \text{ArOH} + R' \rightarrow \text{ArOH}^+ + R^- \quad (2a) \]
\[ \text{ArOH}^+ + R' \rightarrow \text{ArO'} + \text{RH} \quad (2b) \]

In SPLET mechanism, antioxidant losses one proton and converts to anion, ArO⁻ (eqn (3a)). Further, electron transfer from the flavonoid anion to a radical leads to the formation of the flavonoid radical, ArO', and the corresponding anion R' (eqn (3b)), which is further protonated (eqn (3c)).

\[ \text{ArOH} \rightarrow \text{ArOH}^- + \text{H}^+ \quad (3a) \]
\[ \text{ArOH}^- + R' \rightarrow \text{ArO'} + \text{R}^- \quad (3b) \]
\[ \text{R}^- + \text{H}^+ \rightarrow \text{RH} \quad (3c) \]

Important thermodynamic parameters that determine the mechanism by which the flavonoids "scavenge" free radicals are:

1) Bond Dissociation Enthalpy (BDE) of ArOH molecule for HAT mechanism. The importance of BDE is due to the antioxidant activity estimation. The lower BDE value, the easier O–H bond rupture.

2) Ionization Potential (IP) of ArOH and Proton Dissociation Enthalpy (PDE) of radical cation ArOH⁺ for SET-PT mechanism. The lower the Ionization Potential of ArOH, the easier the electron transfers from antioxidant to radical species.

3) Proton Affinity (PA) of molecule ArOH and Electron Energy Transfer (ETE) corresponding to anion ArO⁻ for SPLET mechanism.

Cyanidin (Cy) 2-(3,4-dihydroxyphenyl) chromenyl-3,5,7-triol is one of the major water-soluble anthocyanins. One theory is that dietary intake of Cy may inhibit development of obesity and diabetes as well as provide anti-inflammatory effects. Cy and its glycosides are very strong antioxidants and they are active at pharmacological...
concentrations. The antioxidant activity is stronger than that of vitamin E, vitamin C and resveratrol and similar to other commercial antioxidants. Cy quickly neutralizes reactive oxygen species such as hydrogen peroxide, reactive oxygen and hydroxyl radical [10].

This paper addresses the DFT investigation of the reaction of Cy and hydroxyl radical. The reaction is quantified in terms of BDE, IP and PA values.

II. METHODOLOGY SECTION

The majority of theoretical investigation of Cy is focused on all rings where OH groups are located. Geometry optimizations for all species under investigation and frequency calculations have been carried out using a DFT method M052X [11] combined with the 6-311+G(d,p) basis set. The influence of water as polar solvent was approximated by SMD solvation model [12]. Geometries were fully optimized without imposing any restriction. Local minima were confirmed by the absence of imaginary frequencies. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies.

In the case of the transition states it was verified that the imaginary frequency (the number of imaginary frequencies is 1 for transition state) corresponds to the expected motion along the reaction coordinate, by Intrinsic Coordinate Calculations (IRC). The optimization and energy calculation of all geometries were performed without any constraints. All the electronic calculations were performed with the Gaussian 09 program package [13]. Natural bond orbital (NBO) analysis was performed for all species [14, 15].

Transition state theory (TST) affords one of the simplest theoretical approach for estimating the rate constants ($k$), which requires only structural, energetic, and vibrational frequency information for reactants and transition states [16]. The main advantage of using conventional TST is that it requires very limited potential energy information (only on reactants and the transition state), which makes it practical for a wide range of chemical reactions. Despite of its relative simplicity, this theory has been proven to be good enough to reproduce experimental rate constants of free radical scavenging reactions [17].

The rate constant for the hydroxyl radical-cyanidin reaction was calculated using TST, implemented in TheRate program [18] and 1 M standard state is calculated as follows:

$$k = \sigma \frac{k_B T}{h} \exp \left( -\frac{\Delta G^*}{RT} \right)$$

where $k_B$ and $h$ stand for the Boltzmann and Planck constants, $\Delta G^*$ is the free activation energy of activation, which is calculated as the difference in energies between transition states and reactants, $\sigma$ represents degeneracy accounting for the number of equivalent reaction paths, and $\kappa$ accounts for tunneling corrections. The tunneling corrections, defined as the Boltzmann average of the ratio between the quantum and classical probabilities, were calculated using the zero-curvature tunneling (ZCT) approach [19, 20].

III. RESULTS AND DISCUSSIONS

Reaction enthalpies, using DFT methods, for all three antioxidant mechanisms of Cy were calculated using the following equations:

$$\text{BDE} = H(\text{Cy} - \text{O}^*) + H(\text{H}^*) - H(\text{Cy} - \text{OH})$$

$$\text{IP} = H(\text{Cy} - \text{OH}^*) + H(e^-) - H(\text{Cy} - \text{OH})$$

$$\text{PA} = H(\text{Cy} - \text{O}^*) + H(\text{H}^*) - H(\text{Cy} - \text{OH})$$

$$\text{ETE} = H(\text{Cy} - \text{O}^*) + H(e^-) - H(\text{Cy} - \text{O}^*)$$

The species necessary to perform these calculations were generated from the most stable conformation of Cy (Fig.1, Cy-OH). Calculations were performed in the gaseous and aqueous phases. Which of the mechanisms is preferred can be estimated from the lowest value of BDE, IP, and PA. The calculated thermodinamical properties are presented in TABLE I:

<table>
<thead>
<tr>
<th>Site</th>
<th>Gas-phase</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAT</td>
<td>SET-PT</td>
</tr>
<tr>
<td></td>
<td>BDE</td>
<td>IP</td>
</tr>
<tr>
<td></td>
<td>HAT</td>
<td>SET-PT</td>
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<tr>
<td></td>
<td>BDE</td>
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<td>365</td>
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<td>3'</td>
<td>364</td>
<td>627</td>
</tr>
<tr>
<td>4'</td>
<td>394</td>
<td>656</td>
</tr>
<tr>
<td>5</td>
<td>402</td>
<td>665</td>
</tr>
</tbody>
</table>

As can be seen from TABLE I, the preferred mechanism of action in the gas-phase is HAT, because the BDE values of OH groups of Cy are significantly lower than the corresponding IP and PA values. On the basis of obtained values for BDE, it is clear that 4′–OH group should be more reactive OH group of Cy. The 4′–OH group has the lowest BDE value in both phases, so it represents the first site that can donate its H-atom, followed by 3′–OH and 3′–OH in gaseous phase [21–23] and 3′–OH and 3–OH in water.

PAs values of all present OH groups of Cy indicating proton transfer from C4′ group is easier comparing to other OH groups. In water PAs are significantly lower than corresponding BDE values. This indicates that SPLLET mechanism thermodynamically represents the more probable reaction pathway in polar solvent. The BDE and PA values show that the 4′–OH group is the most reactive OH group of Cy [22].

The IP value of Cy is significantly lower in the aqueous than in the gas phase, which is a consequence of the stabilization of charged species in polar solvents. It can be
supposed that reaction (2a) will be faster in aqueous solution, compared to the gaseous phase [21, 24].

On the basis of the obtained BDE values (TABLE I), indicating the reactivity of Cy OH groups, the potential antioxidant activity of Cy in gaseous phase is simulated in the reaction with hydroxyl radical. Since, the obtained BDE values are much higher in positions C5 and C7, the reactions were studied only in positions C4’, C3’ and C3. Three possible reaction pathways of the hydroxyl radical-Cy reaction are shown in Figure 1:

![Figure 1. Three possible reaction pathways for the reaction of Cy with hydroxy radical](image)

TABLE II shows values of the partial negative charge and the spin density, obtained by NBO analysis, on corresponding oxygen atoms for the reactant complexes (RCs), the transition states (TSs), and product complexes (PCs). During the reaction the partial negative charge on O11 increases and has the highest value in PCs. As opposed to O11, the partial negative charge on the phenolic oxygens decrease. The partial positive charges on phenolic hydrogens amount to around 0.5, with small variations during the reactions.

On the other hand, the spin density value on O11 is very close to unity in all RCs, while the spin density values on all other atoms are practically equal to zero. In TSs the spin density is shared between O11 and the phenolic oxygen (TABLE II). Since all revealed TSs are early transition states the spin density values on O11 are much larger. Finally, the spin density is distributed over the Cy moiety, and its value on O11 is equal to zero in PCs. This finding shows that each PC consists of water molecules and the corresponding Cy–O•. During the all reaction pathways the spin density on the phenolic hydrogens remains equal to zero. Distribution of spin densities in PC4’ shows that the unpaired electron is delocalized over O4’, C3’, C5’, and C1’ (Fig.2).

All these facts indicate that reactions of cyanidin OH groups with *OH radical proceed by HAT mechanism.

![Figure 2. Distribution of spin densities in the product complex PC4’](image)

The rate constants $k^{\text{TST}}$ and $k^{\text{ZCT}}$ were calculated for all hydroxyl radical-Cy reactions using the equation (4). The activation energies and rate constants at 298K, as well as the reaction free energies for all hydrogen atom transfer reactions are presented in TABLE III. Obtained results underline tunneling effects as those responsible for making the reaction between Cy and hydroxyl radical faster. With increasing the temperature this effect rapidly decreases. This behavior is to be expected since the abstraction reaction involves the motion of a light particle (hydrogen atom) that can easily tunnel through the reaction barrier. As also expected, the reactions are distinctly exothermic, while the activation energies are low and corresponding rate constants high. The O4’–H4’ homolytic bond cleavage requires the lowest activation energy (and shows the highest rate constant values), which can be attributed, due to the involvement of O4’ in the relatively strong hydrogen bond with H3’, to the weakness of the O4’–H4’ bond. In addition, this reaction path yields the most stable PC. The optimized geometries of RC, TS, and PC, for the most favorable hydrogen atom transfer reaction, are depicted in Fig. 3.

![Table II](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Site</th>
<th>Atom</th>
<th>RC</th>
<th>TS</th>
<th>PC</th>
<th>RC</th>
<th>TS</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>O3</td>
<td>-0.69</td>
<td>-0.64</td>
<td>-0.55</td>
<td>0.01</td>
<td>0.18</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>O11</td>
<td>-0.43</td>
<td>-0.61</td>
<td>-0.97</td>
<td>1.02</td>
<td>0.70</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>O3’</td>
<td>-0.71</td>
<td>-0.66</td>
<td>-0.56</td>
<td>0.01</td>
<td>0.19</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>O11’</td>
<td>-0.43</td>
<td>-0.57</td>
<td>-0.98</td>
<td>1.01</td>
<td>0.75</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>O4’</td>
<td>-0.69</td>
<td>-0.64</td>
<td>-0.55</td>
<td>0.01</td>
<td>0.18</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE III**

<table>
<thead>
<tr>
<th>Site</th>
<th>$\Delta G^\circ$ (kJ/mol)</th>
<th>$k^{\text{TST}}$ (M⁻¹ s⁻¹)</th>
<th>$k^{\text{ZCT}}$ (M⁻¹ s⁻¹)</th>
<th>$\Delta G_f$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy–O•</td>
<td>30.1</td>
<td>4.5x10⁷</td>
<td>4.18x10⁹</td>
<td>-118.1</td>
</tr>
<tr>
<td>Cy–O•</td>
<td>39.8</td>
<td>9.1x10⁴</td>
<td>5.7x10⁶</td>
<td>-110.9</td>
</tr>
<tr>
<td>Cy–O•</td>
<td>28.7</td>
<td>7.9x10⁷</td>
<td>1.1x10¹⁰</td>
<td>-118.3</td>
</tr>
</tbody>
</table>

**The Calculated Values Of Thermochemical Parameters For The Reaction Of Cy And Hydroxyl Radicals, $\Delta G^\circ$, $k^{\text{TST}}$, $k^{\text{ZCT}}$ And $\Delta G_f$ Denote Activation Free Energy, Rate Constants, And Reaction Free Energy, Respectively**
On the basis TABLE III it is possible to conclude that the C4′–OH is most favorable position for the reaction with hydroxyl radical. The reaction path is shown in Fig. 3. The distances between CO, OH and H–O11 bonds are given in pm.

![Fig. 3. Reaction path for the H-atom transfer from the C4′-OH position of Cy to the hydroxyl radical](image)

These findings are in accordance with the results presented in TABLE I, as well as with results obtained in the investigation of the Marković et al. [22]

IV. CONCLUSIONS

The results of the present work were obtained using the DFT method M05-2X combined with the 6-311+G(d,p) basis set. The calculations were performed for the gaseous phase and aqueous solution. To estimate the effects of water as solvent a SMD solvation model was used. BDE, PA, and IP values served to determine thermodynamically most favorable reaction pathway. The HAT mechanism is most favorable reaction pathway for antioxidative action of Cy in gaseous phase. In the aqueous phase the SPLET mechanism is most favorable reaction pathway for antioxidative action of Cy. SET-PT is not favorable mechanism of antioxidative action of Cy in either of phases.

Mechanistic investigations of antioxidative action of cyanidin have been commenced. A simulation of the reaction of cyanidin with the hydroxyl radical confirmed that HAT is the dominant reaction pathway in the gaseous phase and that the 4′OH group is the most reactive.

ACKNOWLEDGMENT

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (projects No 172016, 174028).

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