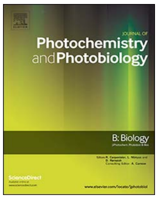
Journal of Photochemistry & Photobiology, B: Biology 180 (2018) 268–275

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol



Investigating the scavenging of reactive oxygen species by antioxidants via theoretical and experimental methods

Hifza Jabeen[a,1], Samia Saleemi[a,1], Humaira Razzaq[b,1], Azra Yaqub[c], Saira Shakoor[a], Rumana Qureshi[a,b,*]

[a] *Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan*
[b] *Nanoscience and Catalysis Division, National Centre for Physics, Islamabad 44000, Pakistan*
[c] *Chemistry Division, Directorate of Science, Pakistan Institute of Nuclear Science and Technology (PINSTECH), P.O. Nilore, Islamabad 45650, Pakistan*

ARTICLE INFO

Keywords:
Antioxidant
Reactive oxygen species (ROS)
DFT
Vitamins
UV–visible spectrophotometry
Cyclic voltammetry

ABSTRACT

Reactive oxygen (hydroxyl ·OH, hydroperoxyl ·OOH) species are highly unstable to be studied experimentally under normal conditions. The present study reports the antioxidant potential of the vitamins namely ascorbic acid, riboflavin and nicotinic acid against these reactive oxygen species (ROS) using the predictive power of Density Functional Theory (DFT) (B3LYP with 6311G basis set) calculations. The order of reactivity of aforementioned vitamins was assessed by determining the bond dissociation enthalpy (BDE) of the OH bond, which is the controlling factor, if hydrogen atom transfer (HAT) mechanism is considered. Transition state calculations were also carried out to determine the reaction barrier for the radical scavenging reaction of vitamins by calculating the forward and the backward activation energies using the same level of theory as mentioned above.

The theoretical methodology was first validated by taking a model stable free radical, 2, 2-diphenyl-1, picrylhydrazyl radical (DPPH·) and applying the proposed approach followed by the experimental studies using UV–visible spectroscopy and cyclic voltammetry. The close agreement between the theoretical prediction and experimental observations proved the authenticity of theoretical approach.

1. Introduction

The species with an unpaired electron are termed as free radicals which pose much harm to the living system when present at higher concentration. Many sources contribute to the generation of free radicals. Respiratory chain and the activated leukocytes are the natural metabolic processes occurring in the body which are responsible for the production of endogenous free radicals, while smoking, factory emission, car fumes, alcohol, pesticides, solvents and even some illness or infection generate the exogenous free radicals [1–3]. Oxygen-centered species known as reactive oxygen species (ROS) are most commonly found in the living organism. They include superoxide anion ($O_2^{-}\cdot$), hydroxyl radical (·OH), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), alkoxy radicals (RO·) and peroxide radicals (ROO·) [4,5].

The species that scavenge the free radicals and protect the body from their adverse effects are termed as antioxidants. The antioxidants interact with free radicals and transform them to stable products. In literature, various mechanisms have been proposed for quenching of free radicals by antioxidants [6–10]. The three major mechanisms are discussed here. The first one is single-electron transfer followed by the proton transfer (SET-PT) which is a stepwise process. In this mechanism, first step involves the transfer of electron from antioxidant to the active radical resulting in the generation of cation of antioxidant and mono anion of the radical followed by transfer of proton from the cation radical to the anion in the subsequent step. The mechanism can be generalized as

$$R^{\cdot} + AOH \rightarrow R- + AOH^{\cdot+} \rightarrow RH + AO^{\cdot} \quad (1)$$

This mechanism is governed mainly by various factors including the ionization potential of the antioxidant, electronegativity of the free radical/scavenger, and the reactivity of cation radical.

The second mechanism involves the direct hydrogen atom transfer (HAT) from the antioxidant to the radical. This mechanism can be presented as Eq. (2).

$$R^{\cdot} + AOH \rightarrow RH + AO^{\cdot} \quad (2)$$

Bond dissociation energy (BDE) for OH bond of the antioxidant AOH is responsible for controlling this type of mechanism.

The third mechanism has been as associated with sequential proton

---

[*] Corresponding author at: Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan.
E-mail address: r_quresh@qau.edu.pk (R. Qureshi).
[1] These authors have contributed equally.

https://doi.org/10.1016/j.jphotobiol.2018.02.006
Received 28 September 2017; Received in revised form 23 December 2017; Accepted 5 February 2018

loss – electron transfer (SPLET) which is pH dependent process and occurs stepwise. In the first step, the antioxidant releases its proton and hence converts to its anion. In the following step, the electron is transferred from the anion to the free radical resulting in the formation of radical's anion which is stabilized by the proton lost in the first step.

$$\begin{aligned} &AOH \rightarrow AO^{-} + H^{+} \\ &AO^{-} + R^{\cdot} \rightarrow R^{-} + AO^{\cdot} \\ &R^{-} + H^{+} \rightarrow RH \end{aligned} \tag{3}$$

In SPLET mechanism, the controlling factors are BDE of the OH bond of the antioxidant and the ionization potential of AO^{-}. It is possible that all the mechanisms may occur in parallel, but at different rates and under different conditions.

Many experimental methods exist to investigate the radical scavenging activity of antioxidants [11–14]. With the recent advances in theoretical chemistry, it is now possible to investigate the interaction studies of very short lived species, including ROS which are difficult to handle experimentally [15–21]. However, the validation of the theoretical results in case of study of extremely short lived radicals is intricate. This problem can be addressed by taking a model free radical that can be investigated both experimentally and computationally. In this way, the same theoretical approach is first applied to the interactional study with the model radical and the results are compared with the experimental findings. If the agreement is found then the theoretical approach can be satisfactorily applied to examine the interactional studies with highly unstable radicals. The stable nature of 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) makes it a nice model for aforementioned purpose.

In the present research work, antioxidant activity of three different vitamins namely; ascorbic acid (AA), nicotinic acid (NA) and riboflavin (RF) towards the short-lived oxygen centered radicals; ·OH and ·OOH has been predicted by DFT. The calculations are based on determining the −OH bond dissociation enthalpy and ionization potentials of vitamins. The structures of the investigated vitamins and the stable free radical DPPH· has been given in Fig. 1.

The theoretical approach is verified by taking the stable DPPH radical and performing the theoretical calculations and then validating the results experimentally by UV–Vis and Cyclic Voltammetry. The agreement between the theoretical and experimental results gives support to the theoretical approach used for investigating the interactional study with the ROS.

2. Experimental

2.1. Materials and Methods

Ascorbic acid, (Merck, 99%), Riboflavin (BDH. 98%), Nicotinic acid (MP Biomedical, 97%), 2, 2-diphenyl-1, picrylhydrazyl radical (DPPH ·-Sigma, 98%), and methanol (Sigma, 95%) were used without further purification. Supporting electrolyte TBAP (Tetra *n*-butyl ammonium perchlorate) (Sigma, 98%) was used in electrochemical studies. The estimation of the antioxidant activity of the vitamins was carried out using UV–Vis spectroscopy and cyclic voltammetry.

Shimadzu 1601 UV–visible spectrophotometer was used for investigating the interactional studies. Methanol as solvent was used to prepare 80 μM DPPH· solution. This solution was taken in the cuvette and its spectrum was recorded. Upon subsequent addition of respective vitamins, it was noticed that absorbance of DPPH· decreased after the equilibrium time. The time at which absorbance becomes stable with no further change is the equilibrium time. Different concentrations of the vitamins varying from (20 μM to 10^{-2} M) were used according to their antioxidant capacity. All the measurements were carried out at 25 °C.

Cyclic voltammetry (CV) was performed on an EcoChemie Autolab PGSTAT-10 potentiostat/galvanostat. The electrochemical experiments were carried out at room temperature (25 °C) using a conventional three electrode cell. The working electrode was a glassy carbon (GC) electrode of 0.071 cm^2. Prior to measurements, the electrode was cleaned and polished with 0.5 μm alumina powder slurries (Buehler GmbH, Germany), rinsed and sonicated for 15 min in chromasolv water to remove the loose alumina particles. A coiled platinum wire served as the counter electrode and saturated calomel electrode (SCE) as reference in an aqueous media. All the solutions were degassed with oxygen-free nitrogen (BOC Gases) and a flow of N_2 was maintained over the solution during the measurements.

To carry out electrochemical experiments, 1 mM solution of DPPH· was prepared in the presence of 0.05 M TBAP as supporting electrolyte in methanol. The potential range of + 0.3 to − 0.5 V was set for DPPH· studies. Initially a blank run measured in the working range of CV cycle was performed to mark inactivity of the supporting electrolyte. The CV cycle was performed again after addition of the DPPH. Decrease in the current was observed after subsequent addition of various concentrations of the vitamins to the DPPH solution. All the experiments were carried out at the scan rate of 100 mVs^{-1} at 25 °C.

2.2. Computational Details

All quantum chemical calculations were performed using the GAUSSIAN computational package. Geometry optimization and frequency calculation of all stationary points (reactants, complexes, transition states and products) were carried out using the DFT method. The geometry of each compound/radical was optimized in the gas phase by using B3LYP functional (Becke's three parameter hybrid functional with the LYP correlation functional) with the 6-31G basis set, while 6-311G basis set was used to perform the frequency calculations. These calculations were used to validate the suggested transition state structure and also to establish the exact zero-point energy and change in Gibbs free energy.

It was assumed that the reaction of the vitamins with free radicals

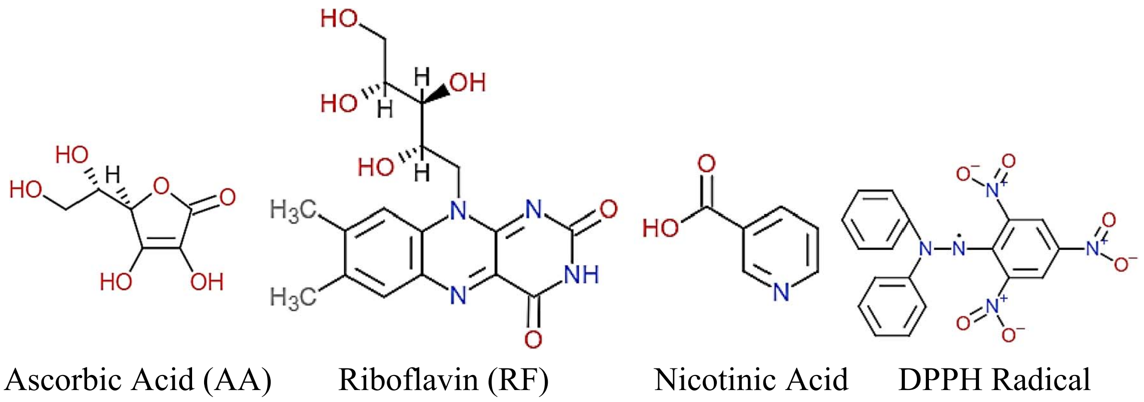


Fig. 1. Structure of investigated vitamins with labeled hydroxyl groups and of 2, 2-diphenyl-1, picrylhydrazyl radical (DPPH·).