

Can C₆₀ fullerene demonstrate properties of mitochondria-targeted antioxidant from the computational point of view?

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Abstract— Theoretical examination of one hypothesis explaining fullerene C₆₀ anti-oxidant potential has been done with Density Functional Theory (DFT). We found that a mechanism involving fullerene-mediated proton transport through inner mitochondrial membrane is feasible from the computational point of view. Such a hypothesis may explain recent experimental results on C₆₀ high antioxidant activity promoted by additional mechanism based on decreasing of the transmembrane proton concentration gradient.

Keywords— Fullerene C₆₀; Mitochondria membrane potential; Antioxidant activity; Density Functional Theory (DFT).

I. INTRODUCTION

Fullerene C₆₀ was recently found by Baati et al. [1] to be a powerful antioxidant demonstrating anti-aging activity towards Wistar rats' life span. Besides, rats treated with fullerene C₆₀ demonstrated high resistance to carbon tetrachloride toxin known for triggering generation of harmful reactive oxygen species in large amounts. Consequently, fullerene C₆₀ was proposed to have high antioxidant activity in vivo. Geroprotective activity of C₆₀ fullerene found experimentally in the work [1] turned out to excel that of the most powerful reactive oxygen species scavengers known to date.

Reactive oxygen species are harmful for organisms as they may cause oxidative damage to DNA, lipids and proteins leading to malfunctioning of cell components and, eventually, its death. Described effect is considered to be the main reason of living beings aging; currently free-radical theory is one of the most recognized among many concepts of aging [2].

However, why and how exactly fullerene acts as an antioxidant is still unclear. Fullerene C₆₀ is known to inactivate hydroxyl radicals by attaching to their double bonds [3]. Nevertheless, such mechanism does not explain that significant

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– twofold – increase in the rats' life duration.

In the current study we propose an additional mechanism of the fullerene anti-aging activity based on mild uncoupling of respiration and phosphorylation processes inside a cell associated with proton gradient alteration on the inner mitochondrial membrane. The majority of reactive oxygen species is generated in the mitochondrial respiratory chain; the latter is the source of superoxide anion that triggers a reaction chain resulting in formation of other harmful radicals. That is why the most effective antioxidants are mitochondrial-targeted ones.

Among such compounds are lipophilic cations (so-called Skulachev ions) which can be used as antioxidant species carriers [4]. Because of electron transport chain activity, fall of transmembrane electric potential is due to difference in concentrations of protons in- and outside of the inner mitochondrial membrane. The outer and inner surfaces of the latter are positively and negatively charged, respectively. Such difference in electric potential makes accumulation of Skulachev ions inside mitochondria possible. Hence, lipophilic cations concentrate in mitochondria via electric field forces [4].

Fullerene C₆₀ is also a lipophilic compound [5]. Molecular dynamics theoretical simulations indicated that C₆₀ may penetrate and accumulate inside a membrane [6]. Although such simulations did not take into account a charge of the mitochondrial membrane, fullerene seems to be likely able to cross the inner mitochondrial membrane.

Let us suppose that protons can penetrate fullerene's surface and give it a positive charge, which allows C₆₀ to cross the cell membrane. Thus, such fullerene confining protons within inside transfers them through the inner mitochondrial membrane leading to decrease of transmembrane electric potential. Korshunov et al. demonstrated [7] that even small (about 10%) decrease of transmembrane potential causes tremendous tenfold decline in harmful superoxide anion-radical generation. Therefore, such systems called "mild uncouplers of oxidative phosphorylation" can facilitate proton transport inside the mitochondria and thereby possess an excellent oxygen radicals-protective effect, although they are not pure antioxidants in terms of chemistry [5].

One may question described concept of proton transport via mitochondria membrane facilitated by proton-loaded

fullerenes with the following consideration. How verisimilar proton penetration of the C_{60} surface is? What are forces pushing a proton inside the fullerene and making it confined therein? Can C_{60} be a good proton transporting system indeed?

Fullerenes ionic conductivity has been already corroborated both experimentally and theoretically [8]. In the work [8] Ken Tasaki reported on unexpectedly low DFT-computed activation barriers for intra- and intermolecular proton-hopping between different carbon bonds along C_{60} surface and towards closely located another fullerene, respectively. Based on these findings, it was proposed that fullerenes themselves have high proton conductivity even without chemical functionalization with different groups [8]. Moreover, fullerenes membranes functionalized with hydroxyl-groups have been already patented by Sony Corporation as industry-available proton conductors [9].

Consequently, there might be one more mechanism of the proton conveyance inside inner mitochondria membrane with the fullerene: inter- C_{60} -attached groups' proton transport in the case of functionalized fullerenes. Trying to distinguish between two mentioned possible mechanisms, one should note functionalized fullerenes can be toxic. For example, hydroxylated fullerenes (fullerenols or fullerols [$C_{60}(OH)_n$]) demonstrated high cytotoxicity owing to too excessive loss of mitochondrial membrane potential caused mitochondrial dysfunction and, eventually, cell death [10]. In other words, C_{60} might be the golden mean between moderate transmembrane potential decrease and significant inhibition of ATP synthesis and other detrimental effects.

In order to prove proposed mechanism of proton transport through-mitochondria membrane theoretically, we performed here theoretical analysis of the fullerene C_{60} ability to acquire positive charge and absorb protons using Density Functional Theory (DFT). Similar calculations are powerful as they may give an insight on how fullerenes interact and are functionalized with different compounds. For instance, in the paper [11] an optimal configuration of the system C_{60} fullerene + ascorbic acid is found using a combination of classical molecular mechanics and DFT; authors proposed feasibility of non-covalent functionalization of C_{60} with ascorbic acid and determined ascorbic acid to be an electron acceptor based on electric charge population analysis. Below we describe our theoretical findings proving the proposed mechanism of C_{60} fullerene anti-oxidant activity.

II. METHODS

Density Functional Theory (DFT) is a powerful and widely used method for quantum-chemistry calculations [12]. It is based on the numerical solution of the quantum-mechanical problem for a given system of atoms. Similarly to Hartree-Fock method, DFT allows one to compute an electronic structure of relatively small systems with a decent precision. The difference between DFT and Hartree-Fock approach is that one does not solve the Schrödinger equation by looking for a solution in the form of many-electron wavefunction

represented by a set of Slater determinants, but reformulates the problem for the electronic density functional $n(\mathbf{r})$. Such a trick is based on the Hohenberg-Kohn theorem, and has important consequence: instead of dealing with the wavefunction $\Psi(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N)$ that depends on coordinates of all N electrons comprising a system under study, one has to find the electronic density $n(\mathbf{r})$ depending on Cartesian coordinates only. Then system's total energy E and all other properties become dependent upon $n(\mathbf{r})$. For more details on mathematical formalism please refer to [13]. DFT enables one to accurately compute electronic and structural properties of a variety of molecular systems within reasonable computer time. In the present work, DFT implemented in ADF 2012 program suite [14] was used to simulate the interaction between the single fullerene and surrounding proton(s) by searching for the most probable atomic configuration of the whole system. Such configuration is found by minimizing the total energy of the system during the so-called process of the geometry optimization, which means evaluation of the system stable configuration corresponding to the minimum on the total energy hypersurface among multitude of close structures.

One of the most important matters when it comes to using DFT is how one treats exchange and correlation interaction between electrons. The former is a special kind of interaction due to Pauli principle or, more generally, to the particles' identity principle in quantum mechanics. The latter is an inter-electronic coupling because of the pairwise electronic Coulomb repulsion term of the exact system's Hamiltonian. These are interactions which cannot be found strictly mathematically, and thus, have to be approximated in some way in DFT. There are multitude of ways available for approximating exchange-correlation functional in DFT. Some of them are suitable for certain problems only, others are more general but less precise. In the present work, we applied so-called General Gradient Approximation (GGA) for the exchange-correlation part of potential in two recently developed forms proved to be efficient for many molecular systems: GGA-BLYP [15] and GGA-BLYP-D3 [16]. Both of them include dependence of the exchange-correlation part of energy not only on electronic density $n(\mathbf{r})$ like in the less precise "local density approximation", but also on its derivatives (gradients, to be more precise, that is why corresponding branch of potentials is called "general gradient approximation").

As is usually done in Kohn-Sham DFT, molecular orbitals were expanded into localized basis set that means using atomic-like wavefunctions centered on atoms during calculations. Slater-type basis sets called double-zeta and triple-zeta with polarization function were used. Such basis functions are tailored and exponentially decay with increase of the distance from atom, which guarantees fast convergence and allows one to use restricted number of functions (several to tens) for every atom. Since all chemistry properties of interest are determined by valence electrons only, so-called frozen core approximation, implying ignoring calculation of changes

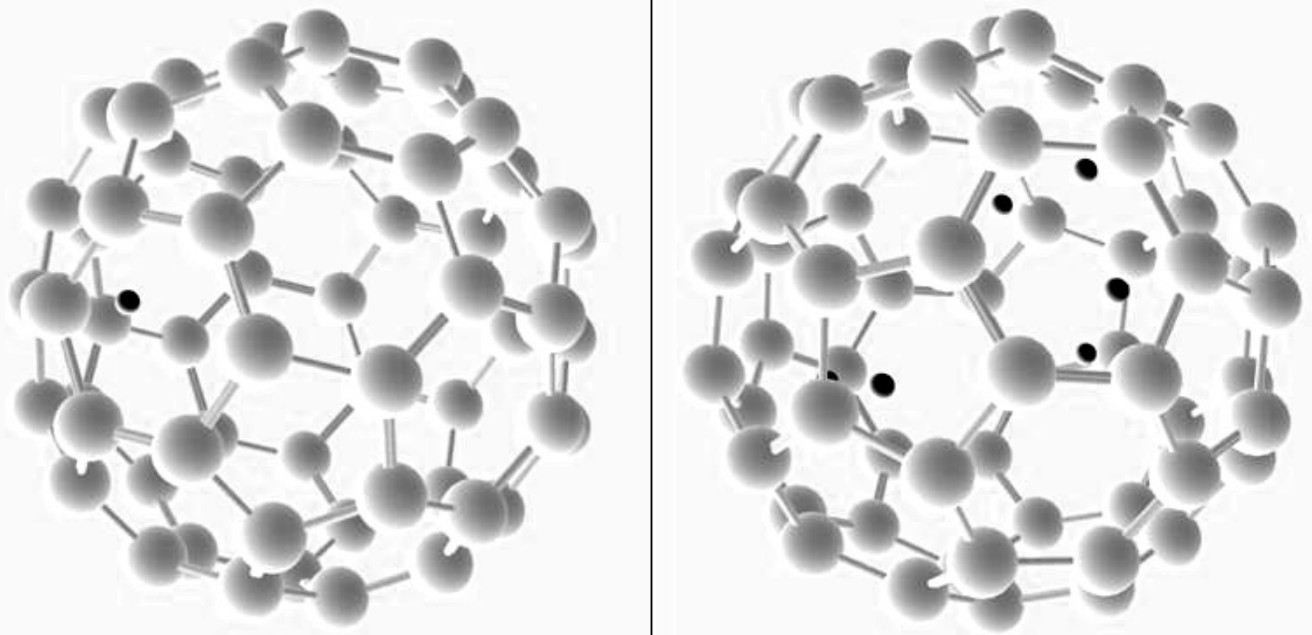


Fig. 1 The results of DFT geometry optimization for one (left) and six (right) protons and fullerene

in core electrons' state, was employed to shorten computation time.

III. RESULTS AND DISCUSSION

Initially, interaction between single proton and fullerene was simulated. The proton was placed outside of the C_{60} above one of the carbon pentagons at the distance about 1Å from the pentagon plane. Calculation resulted in the proton passing through the fullerene and finally appearing inside C_{60} (Fig. 1, left panel). Next, more and more protons were consecutively added by allocation them above both carbon pentagons and hexagons in the initial configuration before the geometrical optimization. In all cases when there were from one to six protons present, they penetrated into the fullerene. However, the seventh proton added to the system failed to enter space inside the fullerene. One may conclude then that maximum amount of protons fullerene can accommodate is equal to six (Fig. 1, right panel).

To be able to penetrate into the mitochondrial membrane according to the mechanism described in the Introduction the fullerene must have positive charge distributed on its surface. Thus, it is crucial to confirm the positive charge distribution over C_{60} surface for each tested configuration of protons. Fig. 2 illustrates charge distribution obtained with DFT within Mulliken scheme for two, four, and six protons caught inside the fullerene. One can note that when amount of protons stuck inside fullerene increases, its surface accumulates more positive charge.

As the next step, the presence of water molecules in real cell was taken into account by surrounding fullerene with water molecules. The simulation was carried out for a fullerene with single proton placed above a pentagon and 47 water molecules randomly distributed around the fullerene. We found water molecules do not influence the fullerene's capability of absorbing protons.

IV. CONCLUSION

We have applied DFT quantum mechanical calculations of the system "fullerene C_{60} plus proton(s)" to theoretically analyze the possible way of C_{60} ability to transport protons through inner membrane of the cell mitochondria. To sum up, DFT simulations allowed us to propose the following mechanism. C_{60} fullerene molecules enter the space between inner and outer membranes of mitochondria, where there is excess of protons due to diffusion. In this compartment, fullerenes are loaded with protons and acquire positive charge distributed over their surface. Such "charge-loaded" particles can be pushed through the inner membrane of the mitochondria due to the potential difference generated by the inner membrane, using electrochemical mechanism described in detail by Skulachev et al. [4], [17]. In this case, the transmembrane potential reduces, that, in turn, significantly lowers the intensity of superoxide anion radical production.

While mitochondrial uncoupler and protonophore 2,4-dinitrophenol is known to be toxic in high doses, it demonstrates significant adaptogenic activity in low doses. Mice treated with 1 mg/L of 2,4-dinitrophenol revealed enhanced longevity. Moreover, this substance promotes higher tissue respiratory rates, citrate synthase activity, cytochrome c oxidase and mitofusin-2 expression, improved serological glucose, triglyceride and insulin levels, decrease of reactive oxygen species (ROS) levels in tissues, DNA and protein oxidation, as well as losing of body weight [18], [19]. These findings are in line with the "uncoupling to survive" hypothesis proposed by Brand, suggesting that uncoupling could be an approach to promote lifespan extension due to its ability to prevent formation of the reactive oxygen species [20].

The key role of mitochondria in the cellular regulation makes such "charge-loaded" fullerenes of great scientific interest not to say about prospective route for development of

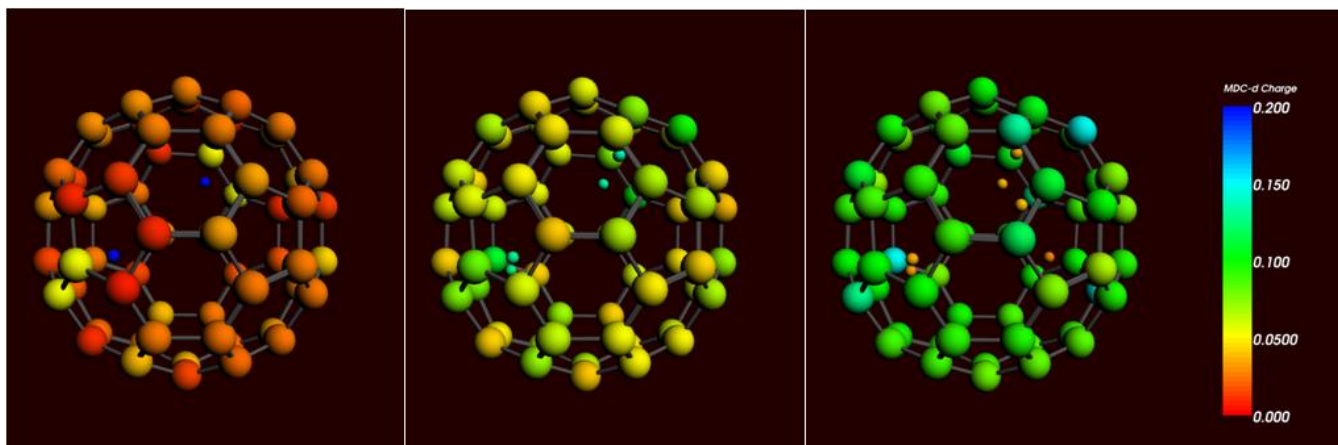


Fig. 2 The distribution of charge for two, four and six protons inside the fullerene, from the left to the right, respectively

novel anti-aging drugs. There are number of issues to be addresses further including fullerenes' toxicity and consideration of the more realistic theoretical models. More sophisticated theoretical simulations of the fullerenes' anti-oxidant activity are under way and to be published elsewhere.

Another attractive particular line of research would be research of such carbon low-dimensional structures as nanotubes concerning their possibility to promote through-mitochondria membrane proton transport. Single-walled carbon nanotubes have already been experimentally studied by Zhou et al. who pointed out on the nanotubes localization inside mitochondria membranes because of mitochondrial transmembrane potential [21]. The next step on the way to more realistic simulations would be including of the water and subcellular structures in modeling yet it may quickly make computations formidable. A recent nice example of similar simulations for the system integrating nanoparticles (including fullerenes), water molecules, and a biomembrane is the paper by Lai et al. [22], where authors applied molecular dynamics to analyze the pore formation process of the lipid bilayer membrane in the presence of nanoparticles. Although all theoretical models should also be backed up by biological experimental proofs, we hope that presented here initial theoretical insight on the intriguing anti-oxidant mechanism of C₆₀ fullerene will stimulate more profound research.

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