Physica E 70 (2015) 198–202

Contents lists available at ScienceDirect

Physica E

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

Physical consequences of the mitochondrial targeting of single-walled carbon nanotubes probed computationally

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HIGHLIGHTS

- Interaction of single-walled carbon nanotube and H atom was simulated with DFT.
- H atom was found to make a bond with the nanotube from outside of the carbon cage.
- Possible implications and mechanism of intra-mitochondria potential alteration are discussed.

ARTICLE INFO

Article history:
Received 8 November 2014
Received in revised form
25 February 2015
Accepted 6 March 2015
Available online 7 March 2015

Keywords:
Single-walled carbon nanotubes
Mitochondria
Antioxidant activity
Protons
Sculachev ions
Density functional theory

ABSTRACT

Experiments by F. Zhou and coworkers (2010) [16] showed that mitochondria are the main target of the cellular accumulation of single-walled carbon nanotubes (SWCNTs). Our *in silico* experiments, based on geometrical optimization of the system consisting of SWCNT+proton within Density Functional Theory, revealed that protons can bind to the outer side of SWCNT so generating a positive charge. Calculation results allow one to propose the following mechanism of SWCNTs mitochondrial targeting. SWCNTs enter the space between inner and outer membranes of mitochondria, where the excess of protons has been formed by diffusion. In this compartment SWCNTs are loaded with protons and acquire positive charges distributed over their surface. Protonation of hydrophobic SWCNTs can also be carried out within the mitochondrial membrane through interaction with the protonated ubiquinone. Such "charge loaded" particles can be transferred as "Sculachev ions" through the inner membrane of the mitochondria due to the potential difference generated by the inner membrane. Physiological consequences of the described mechanism are discussed.

1. Introduction

The discovery of the mitochondrion role in producing adenosine triphosphate (ATP) through oxidative phosphorylation was one of the main achievements of biology in the 20th century [1]. Later these organelles were found to participate in the production of other substances (besides ATP) of no less importance for the maintenance of normal cellular function and such biochemical pathways as hemesynthesis and iron-sulfur (Fe/S) proteins assembling in the majority of cells [2], gluconeogenesis and urea cycle in hepatocytes [3,4], glyceroneogenesis in adipocytes [5], and others. Providing life of the cell, mitochondria nevertheless get involved in the processes of endogenous toxin generation and cell destruction. It is commonly accepted that mitochondria and their electron transport chains act as the major source of oxygen radicals in cells. Initial estimates [6] suggested that, during the normal transfer of electrons, 2–5% of total molecular oxygen consumed by mitochondria is converted into superoxide due to its incomplete reduction and electron escaping during the process of mitochondrial respiration. Furthermore, mitochondria play a pivotal role in the development of programmed cell death phenomena, since mitochondria may release pro-apoptotic signals in the particular intra- or intercellular environment [7].

Because of the above facts, mitochondrial targeting by a wide spectrum of drugs has become very attractive for fundamental pharmacology [8]. Currently, the most successful project aimed at developing such drugs exploits the lipophilic cation – plastoquinone-conjugated triphenylphosphonium (also known as SkQ1). This substance manifested great multiplicity of adaptogenic effects on both living creatures and their functions [9]. Clinical approbation of SkQ resulted in a release of the first officially approved

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http://dx.doi.org/10.1016/j.physe.2015.03.005
1386-9477/

mitochondrial-targeted medication (eye-drops) visomitin [10]. However, triphenylphosphonium is not optimum for drug targeting because of its high toxicity. Nowadays, the SkQ-project team is striving to replace it with berberine, which is a natural substance [11].

Other promising compounds for mitochondrial targeting are carbon nanostructures. Foley et al. [12] showed that a fullerene derivative can cross the outer cellular membrane and localize preferentially in the mitochondria. Recently we suggested [13] a possible mechanism of the fullerene C_{60} accumulation in mitochondria. We proposed that C_{60} is able to acquire positive charge by absorbing several protons, and this complex could penetrate mitochondria. Such a process facilitates mild uncoupling of respiration and phosphorylation. This, in turn, leads to the decrease of ROS (reactive oxygen species) production. We also suggested that this mechanism underlies the ability of C_{60} to prolong significantly the lifespan of rats, as described in [14]. Both C_{60} fullerenes and their ten atoms clusters were found to penetrate lipid bilayers mimicking eukaryotic cell membrane based on molecular dynamics simulation in the very recent work of Bozdaganyan et al. [15], which also supports ROS-scavenging speculations.

Experiments by Feifan Zhou and coworkers [16] showed that mitochondria are the main target of the cellular accumulation of single-walled carbon nanotubes (SWCNTs). Nanotubes conjugated with fluorescent marker fluorescein isothiocyanate (FITC) dispersed in phospholipid-polyethylene glycol (PL-PEG) were added to tumor and normal cells. In the majority of tested cell lines SWCNTs–FITC, fluorescence was predominantly detected in mitochondria. The exceptions were macrophages due to phagocytosis, where SWCNTs accumulation was found mainly in lysosomes. Interruption of mitochondrial transmembrane potential ($\Delta\Psi m$) by a chemotherapeutic agent staurosporine (STS) led to inhibition of SWCNTs–FITC depending fluorescence of mitochondria. When STS penetrated cells where SWCNTs–FITC had been already accumulated, the authors of [16] observed a gradual distribution of the dye throughout the cell. Although STS is not protonophore-uncoupler of oxidative phosphorylation, its ability to decrease mitochondrial transmembrane potential, because of activation of mitochondrial "intrinsic" apoptotic signaling cascade in a wide spectrum of eukaryotic cells, has been demonstrated in a number of works [17–19].

In subsequent works Feifan Zhou and co-workers proposed application of SWCNTs as an anti-cancer modality: upon irradiation of the near-infrared laser, SWCNTs, localized mainly in mitochondria, induced their either photothermal [20] or photoacoustic [21] damage thus causing mitochondrial pathway of apoptosis. Interestingly, laser treatment or SWCNTs incubation alone did not result in such a significant response. Moreover, SWCNTs did not harmfully affect normal tissues or increase oxidative stress. The findings of Zhou and coworkers open the way for using SWCNTs for delicate manipulation of cellular processes. Therefore the word "insight" in the title of reference [16] seems quite relevant.

However, even a hypothetical mechanism leading to the accumulation of SWCNTs in the mitochondria is not present in the paper [16]. In the current study we perform a theoretical analysis of the SWCNTs ability to acquire positive charge and bind protons in order to prove the mechanism, proposed earlier for fullerene C_{60} [13]. We apply Density Functional Theory simulation to check whether a proton can be bound to the carbon nanotube, which could facilitate the charge transfer between the mitochondria membranes.

2. Materials and methods

Theoretical modeling of the "SWCNT+Hydrogen atom" system was done within Kohn–Sham Density Functional Theory (DFT) implemented in the ADF2013 package [22,23]. In order to simulate a CNT-proton interaction, a structure of the CNT and H atom was optimized by minimizing the total energy of such system in the Cartesian space. General Gradient Approximation with Becke exchange potential part [24] and the Lee–Yang–Parr 1988 correlation correction [25] was applied. So called "DZ" and "DZP" Slater-type orbitals basis sets with 1s carbon frozen core were used as basis sets; larger basis sets led to overwhelming computer resources demands.

SWCNT with (6,5) chirality comprising 362 carbon atoms of ~40 Å length was optimized; this type of CNT is one of the most prevalent types of CNT probed in experimental study by Zhou et al. [16,26]. Although during these experiments SWCNTs with phospholipid-polyethylene glycol (PL-PEG) were applied as is general for biological application because it allows one to make nanotubes less hydrophobic, reduce toxicity and tendency towards aggregation, and facilitate their penetration inside cells, we studied SWCNTs without PL-PEG groups. The reasoning behind that is a small probability of PL-PEG molecules to completely hinder protons transferring through the SWCNTs walls not to say about negligible size of H^+. In fact, PL-PEG might be stronger bound to the ends of nanotubes, while their interaction with the rest of SWCNT may be characterized as more solution- but not chemical-like. Results of the molecular dynamics simulations showed PEGylated phospholipids wrap SWCNTs only partially so that there is enough surface space left unaffected to let us consider bare nanotubes [27–29].

The "capping" H atoms were added to the ends of CNT to compensate for the cut bonds. A hydrogen atom was put near the middle part of CNT, as far as possible from the tube's ends, at different distances from the tube's wall varying from 1.5 Å to 3 Å. The +1 total system's charge allowed us to simulate a proton presence instead of the hydrogen atom, as in work [13]. Calculations were repeated for different hydrogen locations inside and outside of the SWCNT: above the single carbon atom, C–C bond, and the middle of the benzene ring. Both initial and preliminary optimized SWCNT structures were optimized with H atoms in the described way.

3. Results and discussion

The purpose of calculation was to check (i) if protons can penetrate through the SWCNT from the outside forming structures resembling endohedral fullerenes; (ii) if a stable structure of $CNT+H^+$ may be formed. Affirmative answer to either of these questions would allow intriguing biological consequences discussed here. In a nutshell, the answer to the first of the abovementioned questions seems to be negative, that to the second question is positive.

None of the calculations, starting from the configuration when the hydrogen was put outside of the CNT cage, resulted in the proton penetrating the SWCNT. Yet one cannot exclude proton passing through the ends of the SWCNT; this scenario is less probable because of the very large ratio (SWCNT side surface space / ends surface space) for the CNTs in real experiments. It is interesting to note that fullerenes are quite able of incorporating protons. Ganji et al. calculated formation of such fullerenes as (B, N or Si)–doped C_{60}, C_{120} and $(BN)_n$ ($n=24$, 36, 60) with H atom (s) incorporated inside the cage and found that from two to eight hydrogen molecules can be bound to the inner surface of the fullerene [30–32]. As we calculated earlier [13], up to a few protons may penetrate C_{60} fullerene from outside, which is not the case with SWCNT as studied in the present work. Furthermore, all calculations for the hydrogen atoms put into the nanotube in order