INTERACTION OF PCDD/F AND C_{60} FULLERENES

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Introduction

The realization that the field of nanotechnology has the ability to provide many socioeconomic benefits has instigated the rapid growth of the types and quantities of available nanomaterials that are anticipated to be exploited in numerous diverse applications. Carbon nanomaterials (CNMs), namely buckminsterfullerene (C_{60}) and its derivatives have shown great promise in many areas of applications ranging from solar cells and drug delivery to water purification. In view of its large production and wide applications, fullerene will inevitably be released into the environment and thus raise environmental and health concerns due to its probable toxicity. Indeed, even without intended production, it is found in particulates emitted from coal-burning power plants. After discharge, C_{60} may interact with other xenobiotic compounds as demonstrated by sorption studies with phenanthrene carried out by Yang et al. [1]. Long et al. reported that carbon nanotubes as superior sorbent for dioxin removal [2]. On the other hand, the potential use of C_{60} and other nanoparticles as drug carriers indicate that compounds associated with nanoparticles are available as also shown in a recent study by Zhang et al., who found that bioaccumulation of cadmium in fish was enhanced in the presence of TiO_2–nanoparticles [3]. Nevertheless, studies carried out with antibodies against fullerenes suggested that water-soluble fullerenes can readily pass cell membranes and are preferentially located at or near mitochondria [4].

TCDD is one of the most potent AhR agonists known. Therefore, it is used as a model compound for AhR binding kinetics as well as to study downstream effects, such as CYP1A1 induction. TCDD induces CYP1A1 and CYP1A2 in rats up to 50–100 folds. The best known AhR signaling pathway is initiated by a ligand binding to the receptor. In this process, proteins associated with the AhR are released. Mediated through translation, the AhR signaling cascade elicits a biological response, such as CYP1A1 induction.CYP1A1 activity is most frequently measured by its ethoxyresorufin-O-deethylase (EROD) activity.

In conclusion, co-existence of C_{60} and its marketed and environmental derivatives with contaminants such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) pose the following question; *I*) Do the nanoparticles and TCDD interact synergistically and/or *II*) Do they trap TCDD and inhibit its action and/or *III*) Does a nanoparticle itself act as an AhR agonist?

Materials and methods

Chemicals

Pure (99.9%) C_{60} was purchased from MER Corporation (Tucson, Arizona). TCDD (purity 99%) was procured from Cambridge Isotope Laboratories, Inc., Woburn, MA. Other chemicals and solvents (if not stated otherwise) were purchased from Sigma Aldrich Chemie GmbH Laboratory, Germany, at the highest purity available.

Preparation of Aqu-nC_{60} and its Blank

Aqu-nC_{60} stock nanoparticle formulation was prepared by solvent exchange method as shown in Fig.1 and the concentration was determined by UV-visible spectrophotometric method as descibed by Andrievsky et al. [5]. Specifically, C_{60} fullerene powders (20 mg) were allowed to dissolve completely into 20 ml of toluene using a magnetic stirrer. This solution was referred to as the C_{60}-toluene stock solution. The purple C_{60}-toluene stock solution was transferred to a beaker containing 50 ml ultrapure water and 1.5 ml ethanol. The mixture then was

sonicated using a dip probe sonicator (Bandline SONOPULS GM 70) operated until the toluene phase disappeared. The resulting yellowish/ brown suspension was filtered through a 0.7-µm glass fiber. Blank Aqu-nC_{60} (Treated water) was prepared as mentioned above except addition of fullerene (C_{60}). Now 200 µl of 50 ng/ml TCDD stock solution (TCDD stock solution was prepared by dissolving in dimethylsulfoxide/ isopropanol 4/1 v/v) was added separately to 10 ml of each nC_{60} and its blank. After mixing, different dilutions of these were prepared by using their corresponding solution and, kept at cool temperature and protected from light.

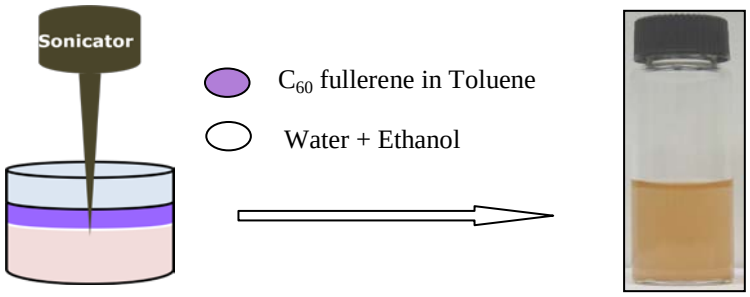

Fig.1 Colloidal Aqu-nC_{60} after complete removal of organic phase by sonication and filtration through 0.7 µm glass filter

In vitro induction of EROD activity

An in vitro EROD assay with the *H4IIE* rat hepatoma cell line was conducted according to method described by Hofmaier et al. [6] and Schwirzer et al. [7] with slight modification. In brief, cells were plated at a density of ~10,000 cells/well in 96well plates and cultured for 24 hr prior to exposure. Culture media were then replaced with 100 µl media containing TCDD or test compounds. TCDD standards were dissolved in dimethylsulfoxide/ isopropanol 4/1 (v/v). The concentration of organic solvents in media was 0.5% in the final assay mixture. Various concentrations of compounds were added and the plates were incubated for 24, 48, and 72 hr. Protein content was measured employing the Pierce assay. Cytotoxicity was determined by the resazurine assay, based on the potential of living cells to reduce this agent to resorufin. Immediately after quantification of EROD activity, the cells in each well were fixed with glutaraldehyde and exposed to resazurine in medium. Fluorescence was measured after incubation at 37 °C for 90 min and compared to controls.

Results and discussion

Fig. 2 depicts induction of EROD activity by TCDD in standard, in presence of fullerene C_{60}, and in presence of matrix without C_{60}. The enzyme induction after 24 hr of TCDD application was higher than of 72 hr. As reported in the literature that nanoparticles can pass the cell membrane and there is adsorption of dioxin on fullerenes which indicates that coexistence of C_{60} nanoparticles and TCDD might change the fate and behaviour of the later, but contrary to the hypothesis, we found that, C_{60} nanoparticles did not alter the induction of EROD activity by TCDD significantly, by their presence. This might be because of essentially planar and rigid stereochemistry of TCDD. Nevertheless, it can not ruled out that cell culture medium might interact with the surface of nanoparticles and have biased the findings.The affinity of C_{60} nanoparticles to the AhR was compared to the potency of these ligands to induce EROD activity. And it was found that, there was no induction of EROD activity when only nC_{60} was present. The concentration of C_{60} was 16 ppm as measured by UV-visible spectrophotometry. Fig. 3 shows the possible scheme of adsorption of TCDD for fullerene. Adsorbate molecules might be entrapped in closed interstitial spaces between small aggregates.While changes in toxicity and bioaccumulation due to the presence of C_{60}-aggregates may depend on several factors such as toxic mechanism and interaction of the compound with C_{60}-aggregates, our results show that Similar studies should be executed with a toxicologically similar compound to TCDD with more degrees of freedom in terms of stereochemistry.

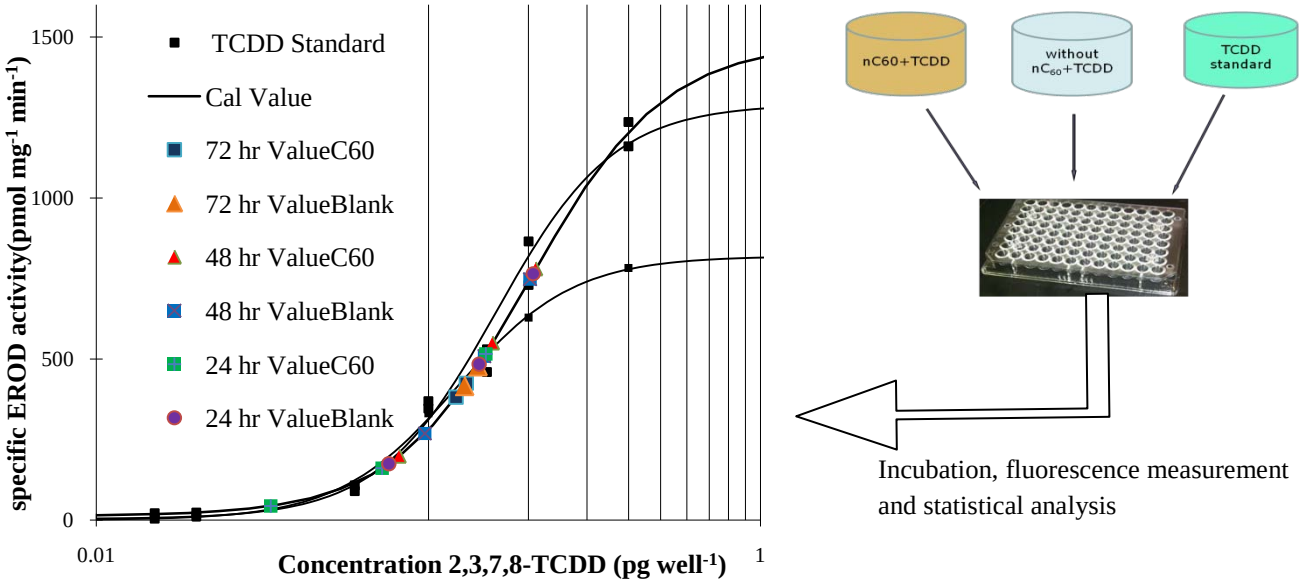

Fig. 2 Dose-response curves of EROD. Induction of EROD activity *in vitro* by TCDD with nanoparticle formulation of C_{60} and TCDD with blank matrix of nanoparticle formulation after 24, 48, and 72 hr of incubation. Blanks were prepared as shown in Fig. 1 but without C_{60} and amount of TCDD added were similar to that of experimental sample. Regression curves are shown.

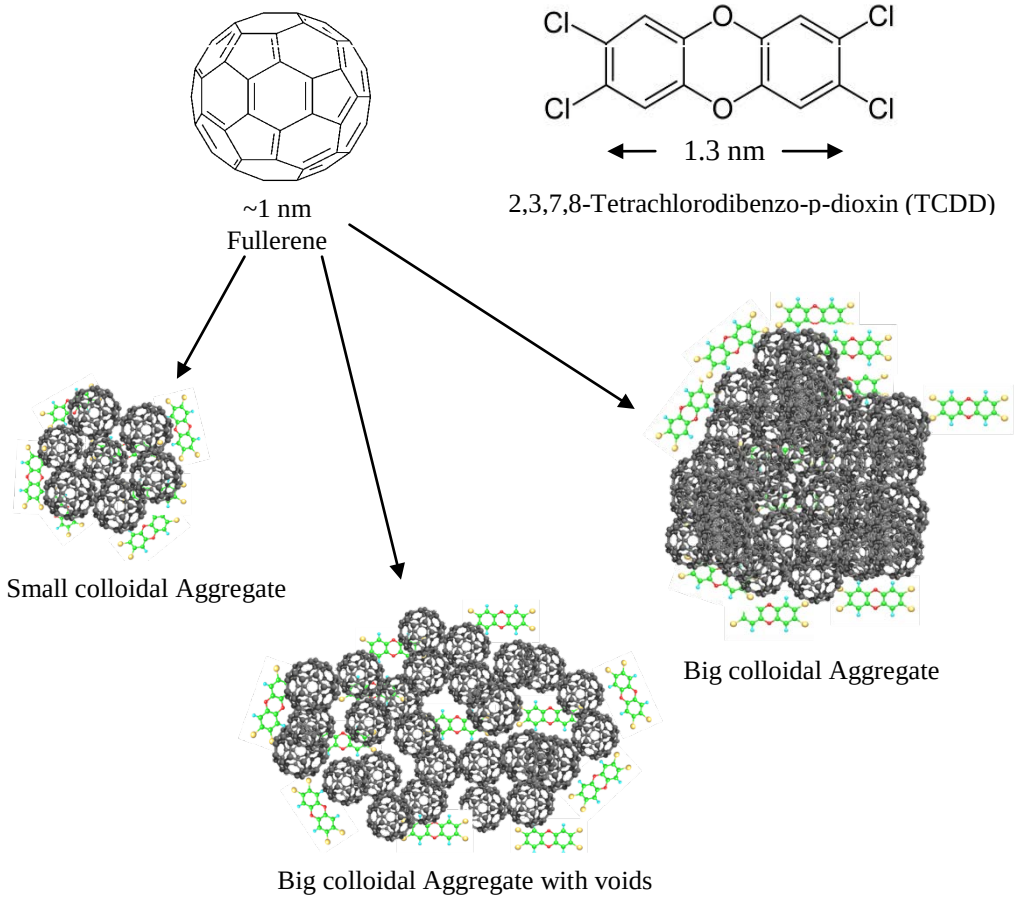

Fig. 3 Adsorption and entrapment of TCDD with fullerene nanoparticles

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