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Biological effect of aqueous C_{60} aggregates on *Scenedesmus obliquus* revealed by transcriptomics and non-targeted metabolomics

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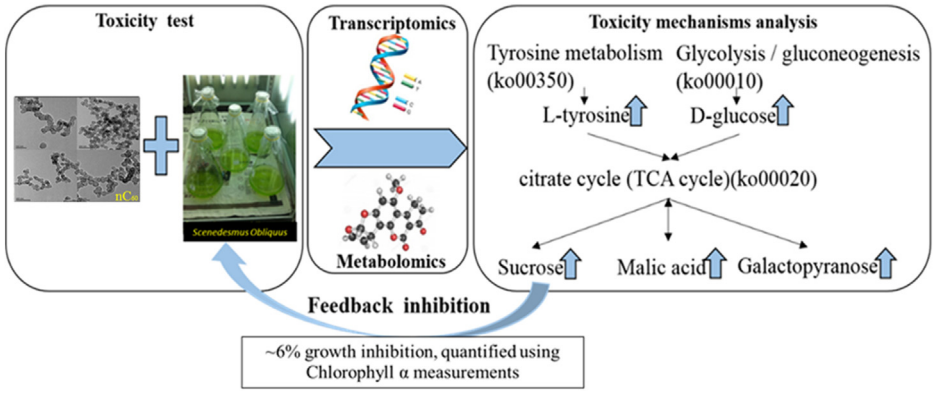
HIGHLIGHTS

- Low dose of nC_{60} had minor growth inhibition on *Scenedesmus Obliquus*.
- Omic technology was used to examine the molecular mechanism.
- nC_{60} increased TCA cycle activity and caused feedback inhibition of photosynthesis.

GRAPHICAL ABSTRACT



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ABSTRACT

This work evaluated biological effect of nC_{60} on *Scenedesmus obliquus*. The cells were exposed to various concentrations of nC_{60} for 7 days. Low-dose of nC_{60} was found to have a minor growth inhibitory effect. The transcriptomics and metabolomics were integrated to examine intricate molecular and cellular effects of nC_{60} on *Scenedesmus obliquus*. We found that *Scenedesmus obliquus* cells exposed to nC_{60} had several significant alterations in cellular transcription and biochemical processes. During the 7-day exposure to nC_{60}, 2234 and 2,448 unigenes were differentially expressed by 0.1 mg/L and 1 mg/L nC_{60}-treated groups compared with the control, including 2085 or 2247 up-regulated genes and 149 or 201 down-regulated genes, respectively. We successfully identified 22 metabolites, including 6 significantly changed metabolites, such as sucrose, D-glucose, and malic acid. The citrate cycle (TCA cycle) (ko00020) was the main target of both differentially expressed genes and metabolic change. However, accumulation of sucrose (end-product) could have induced feedback inhibition of photosynthesis in *Scenedesmus obliquus*, explaining the slight growth inhibition observed. The

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results provided a mechanistic understanding of the growth inhibition of nC_{60} toxicity. These genes and metabolites are useful biomarkers for future studies and offer new insights into the early detectable changes in *Scenedesmus obliquus* with nC_{60} exposure.

1. Introduction

Since the discovery of fullerenes in 1985, the third allotrope of carbon has been a promising compound for myriad applications, such as catalysts, cosmetics, and sensors. Stable C_{60} aggregates (nC_{60}) can be formed in water when fullerenes are released into aquatic environment, increasing the transport and potential ecological risk of fullerenes. nC_{60} has been reported to have acute toxic effects on bacteria [1], zebrafish[2], and human cell lines [3]. nC_{60} was also found to accumulate in aquatic organisms through aqueous exposure [4–6]. The concentration of nC_{60} in the environment was computed to be as low as 1 mg/L (ppm) [7]. Due to the stability of nC_{60}, it can persist in the environment for a long time, albeit current levels of nC_{60} release are low. Therefore, it is of great significance to evaluate the chronic biological effect of low concentration nC_{60}, a topic that is relatively unexplored in literature.

Conventional approaches utilize endpoints such as mortality, reproductive dysfunction, impaired growth and aberrant behavior to assess the toxicity of chemicals. However, these conventional endpoints may not show significant stress response in view of the low concentration and long-term occurrence of nC_{60}. Under these circumstances, "omics" is the best choice. Even at low concentrations of exposure, transcriptomic can display the differential expression levels of many genes in response to profound physiological changes, and metabolomics analyses can reveal the changes in metabolites, which are the end products of genomic, transcriptomic, and proteomic regulatory processes [8]. Omics has been found to be feasible in evaluating the toxic effect of nanoparticles on biological organisms [9–11]. Choudhury et al. investigated antimicrobial physiology of nanoallotropes of sulfur at the interface of transcriptome and metabolome [12]. Taylor et al. studied the molecular toxicity of cerium oxide nanoparticles to the freshwater alga *Chlamydomonas reinhardtii* [13]. In these studies, the metabolite profile showed a positive correlation with the transcript profile, further indicated the sensitivity of omics technology.

Algae remains one of the most important classes of living organisms in the aquatic ecosystems, as it is often the primary producer and plays an important role in the global carbon cycle [14]. Algal toxicity tests have been used to evaluate the effect of toxicants in water. *Scenedesmus obliquus* is recommended by Organization of Economic Cooperation and Development (OECD) as a standard test alga in growth inhibition tests for toxic compounds [15]. Tao et al. found that nC_{60} particles blocked Mg^{2+} channels and resulted in a decrease of the photosynthetic pigment and photosynthetic products in *Scenedesmus obliquus* cells at sublethal concentrations [16]. However, Wei et al. and Van Hoecke et al. found the algal cells did not change morphologically under the optical microscope even in the highest concentration groups of more than 20 mg/L [17,18]. Therefore, omic technology has become particularly important and necessary to provide insights into the biological effect of nanoparticles, especially at low concentrations.

Our work aims to elucidate the chronic biological effect and mechanism of low concentration nC_{60} on *Scenedesmus obliquus*. The effects of different concentrations of nC_{60} on the growth of algae cells were compared and assessed. The gene expression levels and metabolites were measured upon the exposure of algae to nC_{60} to analyze the inner physiological effects. We expect that this study could provide insights into the effects and mechanisms of nanotoxicity in the aquatic environment.

2. Materials and methods

2.1. Materials

C_{60} was purchased from MER Corporation (purity 99.90%, America). The chemicals of BG11 were procured from sinopharm chemical reagent Co., Ltd, China (Analystical grade, China). *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide(MSTFA) and methanol was obtained from Sigma-Aldrich (Chromatographic grade, America). Ultrapure water (18.2 MU/cm) was produced by a water filtration equipment (Hitach, Japan).

2.2. Preparation and characterization of nC_{60}

The nC_{60} used in all experiments was freshly prepared according to the previously reported protocol [19] with minor modifications. 40mgC_{60} powder in 100 ml Milli-Q water was stirred at 1000 rpm with low heat (40 °C) for 2 weeks. The brown suspension was filtered through a 700 nm glass fiber membrane to remove suspended C_{60} powder. This suspension was stored at 4 °C till use. The nC_{60} were characterized by TEM (model JEM-2100), and Asymmetrical Flow Field-flow Fractionation (Wyatt Technology Corporation, Santa Barbara, CA, USA) coupled with multi-angle light scattering detectors (MALS) (Dawn Eos, Agilent Technology, USA). The zeta potential of nC_{60} in ultrapure water was measured using Delsa nano C (Beckman Coulter, USA).

2.3. Scenedesmus obliquus *culture*

Scenedesmus obliquus (FACHB-417) was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Batch cultures of *Scenedesmus obliquus* were grown in BG11 medium [20]. All glassware and BG11 medium used in the experiment were sterilized in a Sanyo autoclave (MLS-3750, Sanyo, Japan) at 121 °C for 30 minThe *Scenedesmus obliquus* was axenic cultured under standard temperature and lighting conditions in an incubator (25 ± 1 °C; illumination 2500 lx; 12:12 light: dark cycle) with agitation three times a day.

2.4. *Exposure of* Scenedesmus obliquus *to* nC_{60}

For the growth test, 100 ml BG11 medium containing exponentially growing algae cells was distributed into sterile 250 ml flasks. The initial *Scenedesmus obliquus* density was 10^4 to 10^5cell/mL. The algal cells were exposed to a series of concentrations (0, 0.1, 1, 2, 5, 7 mg/L) of nC_{60} for 7 days. The control experiments were performed in the absence of nC_{60}. Each treatment was done in 7 parallels. Each flask was shaken three times and repositioned daily to prevent the clumping of algal cells.

The cell count of *Scenedesmus obliquus* and chlorophyll α were monitored every day at $A_{\lambda=680nm}$ using an Agilent UV-7500 spectrometer and a PHYTO-PAM Phytoplankton Analyzer (Water-PAM, Walz, Germany), respectively. Based on the OECD guidelines, the

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