NANOHOLES REGULATE THE BIOENERGETIC IMPACT OF TWO-DIMENSIONAL LAYERED METAL TO SOIL INVERTEBRATE

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ABSTRACT

Two-dimensional molybdenum disulfide (2D MoS_2) nanomaterials are increasingly utilized in various fields, inevitably leading to their release into soils. We combined traditional toxicity endpoints with targeted energy metabolomics to compare the mechanistic differences in the toxicity of nanohole-free (DF-MoS_2) and nanohole-rich MoS_2 nanosheets (DR-MoS_2) to *Eisenia fetida* using a coelomocyte-based *in vivo* assessment model. Our findings indicate that MoS_2 nanosheets exert particle-specific effects on *Eisenia fetida*, and highlight the necessity of fully considering the role of surface defects, whether from synthesis or environmental accumulation, in evaluating the toxicity of these 2D materials.

KEY WORDS

Metal nanosheets, nanoholes, invertebrate coelomocyte, homeostasis, Mechanisms.

1. INTRODUCTION

Transition metal dichalcogenide (TMD) nanomaterials have emerged as promising nanoplatforms[1]. As a typical TMD, two-dimensional molybdenum disulfide (2D MoS_2) has been widely applied in various fields. However, accumulating reports have shown that MoS_2 nanosheets can negatively affect organisms. Therefore, the development and use of MoS_2 nanosheets must be assessed in terms of potential environmental impacts.

Exogenous contamination of soil ecosystems is characterized by universality, complexity, and irreversibility. Like many contaminants, soil will be a significant sink for MoS_2 nanosheets. Once released into the soil, MoS_2 nanosheets are not easily transported or diffused and may exert persistent toxicity to soil-dwelling organisms. Hence, there is an urgent need for mechanistic studies on the exposure and effects of MoS_2 nanosheets on sensitive soil biota. Earthworms, acting as "soil engineers", constitute over 60% of the soil biomass[2]. They spend their entire life cycle in the soil and respond to contamination through activities like ingestion and excavation, making them representative indicator organisms for soil ecotoxicity assessment[3]. In biota, energy supply and homeostasis are fundamental for maintaining cellular activity and health[4]. The effects of nanomaterials on energy homeostasis are closely linked to observed toxicity, making energy-based assays a valuable tool for impact assessment.

In this study, traditional toxicity endpoints and targeted energy metabolomics were combined to reveal the toxicity of MoS_2 nanosheets to earthworm coelomocytes. Our findings contribute to understanding the toxicity of MoS_2 nanosheets to soil organisms, including the potential role of surface defects in their toxicity and risk profile.

2. MATERIALS AND METHODS

2.1 Synthesis of DR-MoS_2 nanosheets

DF-MoS_2 nanosheets were produced through a chemical exfoliation approach. UV irradiation was chosen to generate DR-MoS_2 nanosheets in this study as it is an important approach in "defect engineering", as well as a common environmental aging factor.

2.2 Material properties

The morphologies of DF-MoS_2 and DR-MoS_2 nanosheets were observed using an atomic force microscope (AFM, MFP-3D, Oxford Instruments plc., U.K.) and a high-resolution field emission transmission electron microscope (HR-TEM, Talos F200X G2, Thermo fisher, U.S.A.). Brunauer-Emmett-Teller (BET) analysis (Autosorb-IQ, Quantachrome Instruments, U.S.A.) was conducted to obtain the specific surface area, hole size distribution, and total hole volume of DF-MoS_2 and DR-MoS_2.

2.3 Experimental design

One percent agar was selected as the test medium. The selected test concentrations (10 and 100 mg Mo/L) were obtained based on our comprehensive survey of current MoS_2 nanosheets *in vivo* toxicity studies and further screening of our pre-experimental results. Earthworm exposure was continued at 25 ± 1°C for 96 h; light was excluded to not affect earthworm activity. The low mortality rate of the earthworms (0–6.2%) proved their stable survival at all exposure levels.

2.4 Cell activity and ROS detections

WST-8 (#CA1210, Solarbio, China) and DCFH-DA (#S0033S, Beyotime, China) were used for the detection of cell activity and ROS levels, respectively.

2.5 Energy metabolism analysis

Coelomocytes isolated after earthworm treatment were resuspended in aqueous methanol acetonitrile (2:2:1, v / v) and vortexed for 60 s. After

centrifugation at 14,000 rpm for 20 min at 4℃, the supernatant was isolated and freeze-dried at -80℃ for 24 h. The samples were separated using an Agilent 1290 Infinity LC ultra-performance liquid chromatography system. Mass spectrometry was performed with a 5500 QTRAP mass spectrometer (AB SCIEX) in negative ion mode.

3. RESULTS AND DISCUSSION

3.1 Characterization of two types of MoS_2 nanosheets

Atomic force microscopy and transmission electron microscopy images showed that the DF-MoS_2 possessed an intact and uniform basal plane and produced almost no surface defects in chemical exfoliation. UV irradiation induced abundant surface defects on the basal plane of the MoS_2 nanosheets, mainly in the form of irregularly shaped nanoholes.

3.2 Cellular activity and reactive oxygen species (ROS) levels

At 100 mg Mo/L DF-MoS_2, no significant effects on coelomocyte activity were observed, but 10 mg Mo/L DF-MoS_2 significantly increased cellular activity. Conversely, the inhibition rates of coelomocyte activity with 10 and 100 mg Mo/L DR-MoS_2 exposure were 22.7% and 29.8%. In addition, both DF-MoS_2 and DR-MoS_2 induced a significant and concentration-dependent increase of intracellular ROS levels in earthworm coelomocytes, while the ionic controls showed no such changes.

3.3 Targeted energy metabolism detection

Targeted energy-focused metabolomics was used to further interrogate cellular response to Mo exposure. Biased PLS-DA clearly separated DF-MoS_2 and DR-MoS_2 both at low and high concentrations, revealing the contribution of surface defects to cellular metabolic patterns. Quantification results showed that DF-MoS_2 and DR-MoS_2 disturbed similar metabolic pathways at low concentrations, with pyruvate metabolism and glycolysis being the most significantly impacted shared pathway. Only purine metabolic pathways were inhibited by the 100 mg Mo/L DF-MoS_2 exposure, which may be related to the mitochondrial compensation mechanism under high stress. Unlike DF-MoS_2, 100 mg Mo/L DR-MoS_2 inhibited the riboflavin metabolic pathway. Interestingly, the surface defects mitigated the inhibition of the TCA cycle by MoS_2 nanosheets. In the Na_2MoO_4 group, quantification of metabolites and pathway enrichment analysis demonstrated the inhibitory effect of Na_2MoO_4 on glycolysis.

4. CONCLUSIONS

In this study, a rapid technique was employed to collect earthworm coelomocytes, enabling a rapid and mechanistic assessment of MoS_2 nanosheets' toxicity. Through this approach, we successfully uncovered the mechanism by which MoS_2 nanosheets impact the energy supply of coelomocytes *in vivo*, along with the specific toxicity linked to surface defects. Our results indicate that MoS_2 nanosheets exhibit particle-specific toxicity to earthworms compared to conventional Mo ions, and that artificially or environmentally induced surface defects in MoS_2 nanosheets can enhance their toxicity potential.

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