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Enantioselective oxidative stress caused by chiral ionic liquids forms of 1-alkyl-3-methyl imidazolium tartrate on *Scenedesmus obliquus*

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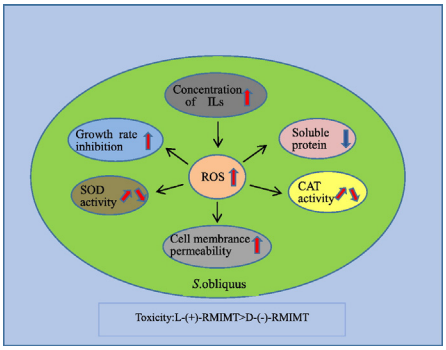
HIGHLIGHTS

- ROS, SOD, and CAT were stimulated by RMIM T in *S. obliquus*.
- ROS and growth inhibition/membrane permeability/soluble protein content correlated.
- RMIM T destroyed the cell wall, membrane, nucleus, and chloroplast structure.
- The toxicity of L-(+)-RMIM T treatment was greater than D-(+)-RMIM T with enantioselectivity.

GRAPHICAL ABSTRACT



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ABSTRACT

Ionic liquids (ILs) are widely used, but their potential threat to the environment has recently gained more attention. The enantioselective oxidative stress caused by chiral ionic liquids (CILs), such as 1-alkyl-3-methyl imidazolium tartrate (RMIM T), on *Scenedesmus obliquus* was demonstrated in this study. Stronger green fluorescence was observed in response to L-(+)-RMIM T treatment than to D-(+)-RMIM T treatment, which suggested that more reactive oxygen species (ROS) were stimulated by L-(+)-RMIM T. Significantly higher ROS levels were recorded during the RMIM T treatments than in the control. There were 1.13-, 1.25-, 1.43-, 1.68-, and 1.96-fold increases over levels in the control in the 3, 5, 10, 15, and 25 mg/L D-(−)-HMIM T treatments, respectively, and 1.26-, 1.37-, 1.58-, 1.86- and 2.08-fold increases over levels in the control in the 3, 5, 10, 15, and 25 mg/L L-(+)-HMIM T treatments, respectively. The total soluble protein content decreased as the RMIM T concentration increased. The SOD and CAT activities were stimulated at lower concentrations, but were inhibited at higher concentrations. Regression analysis implied that ROS is the major factor responsible for the oxidative damage caused by RMIM T. The ultrastructural morphology analysis showed that plasmolysis and damage to the chloroplasts, starch granule decreases, and lipid granule increased, and pyrenoid and nucleoid damage had occurred. These results showed that enantioselective oxidative stress and oxidative damage were caused by D-(+)-RMIM T and L-(+)-RMIM T, and that L-(+)-RMIM T caused more damage than D-(+)-RMIM T.

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1. Introduction

Ionic liquids (ILs) have gained considerable attention as novel alternatives to traditional organic solvents because of their good solubility, catalytic properties, non-flammability, low vapor pressure, wide electrochemical window, and high stability (Kárászová et al., 2014; Santos et al., 2014; Zhuo et al., 2015). A potentially large number of ILs can be prepared by varying the combination of cations and anions (Amde et al., 2015). For example, there are over 30,000 imidazolium salts in the CAS database (Holbrey et al., 2003). The large number of available ILs and their widespread application means that their potential effect on environment should be investigated. Ionic liquids are unlikely to act as air contaminants or inhalation toxins, but their stability and water solubility means that they can easily accumulate in water, which leads to contamination and potential risks to the aquatic environment (Cvjetko et al., 2014). IL toxicity towards aquatic organisms at different trophic levels have been studied recently (Ventura et al., 2010; Amde et al., 2015), including toxicity to aquatic animals (Tsarpali et al., 2015; Samori et al., 2007), aquatic plants (Kumar et al., 2011; Deng et al., 2015), and aquatic microorganisms (Ventura et al., 2012; Viboud et al., 2012). These studies have shown that ILs are toxic or even highly toxic towards aquatic organisms, and their environmental impact merits more attention (Amde et al., 2015; Egorova and Ananikov, 2014; Pham et al., 2010).

Chiral ionic liquids (CILs) are used as chiral solvents, catalysts, or chiral inducers during asymmetric synthesis, stereoselective polymerization, and enantioseparation (Biedroń and Kubisa, 2005; Li et al., 2008; Rahim et al., 2016). The environmental load and ecological risks caused by CILs have been increasing as their application has become more widespread. It has been reported that the enantiomers of the chiral materials may have very different toxicological characteristics in the environment (Liu et al., 2005; Singh et al., 2016). CILs have the same properties as chiral materials. Chen et al. (2014) found that chiral ionic liquid 1-alkyl-3-methylimidazolium lactate has an enantioselective toxicity towards *Scenedesmus obliquus* and *Euglena gracilis*. A previous study on the effect of the chiral ionic liquid forms of 1-alkyl-3-methyl imidazolium tartrate (RMIM T) on *S. obliquus* showed that L-(+)-RMIM T was more toxic towards *S. obliquus* than D-(−)-RMIM T (Liu et al., 2015b). The assessment of chiral pollutants needs to take full account of the enantiomer effects. However, the enantioselective toxicity of CILs towards the ecosystem is poorly understood, and understanding of the mechanism responsible for the enantioselective toxicity of CILs is still limited.

When algaes are exposed to pollutants, they develop defense reactions, and under certain conditions, stress effects are observed (Liu et al., 2017). The immediate responses of algaes to adverse environmental conditions includes the excessive production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), the superoxide radical (O_2^-), and the hydroxyl radical (OH^-) (Lesser, 2006). Algaes have developed an elaborate system to maintain a healthy ROS equilibrium. This system can involve non-enzymic antioxidant molecules, such as glutathione, ascorbate, flavonoids, α-tocopherol, and carotenoids, and effective antioxidant enzymes, such as ascorbate peroxidase (APX), glutathione reductase (GR), glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) (Obermeier et al., 2015; Qian et al., 2016).

Our previous study published results on growth rate inhibition, chlorophyll contents and cell membrane permeability (Liu et al., 2015b), while the mechanism of toxicity caused by L-(+)-RMIM T and D-(−)-RMIM T remained unknown. In this study, the enantioselective oxidative stress caused by CIL forms of 1-alkyl-3-methylimidazolium tartrate (RMIM T) with chiral anions on *S. obliquus* was evaluated. The ROS level and the distribution of ROS in *S. obliquus* cells were measured to evaluate the oxidative damage caused by D-(−)-RMIM T and L-(+)-RMIM T. The total soluble protein levels and antioxidant enzyme activities (SOD and CAT) were determined so that the oxidative stress induced by D-(−)-RMIM T and L-(+)-RMIM T could be evaluated. The regression analysis between ROS level and other indexes were analyzed. Changes in the ultrastructural morphology of *S. obliquus* cells grown in the presence of D-(−)-RMIM T and L-(+)-RMIM T were also studied to confirm the presence of oxidative damage.

2. Materials and methods

2.1. Chemicals

D-(−)-tartrate 1-hexyl-3-methyl imidazolium (D-(−)-HMIM T), L-(+)-tartrate 1-hexyl-3-methyl imidazolium (L-(+)-HMIM T), D-(−)-tartrate 1-octyl-3-methyl imidazolium (D-(−)-OMIM T), L-(+)-tartrate 1-octyl-3-methyl imidazolium (L-(+)-OMIM T), D-(−)-tartrate 1-decyl-3-methyl imidazolium (D-(−)-DeMIM T), and L-(+)-tartrate 1-decyl-3-methyl imidazolium (L-(+)-DeMIM T) were purchased from the Chengjie Chemical Co. Ltd., Shanghai, China, and the purities of all the RMIM T products were 99%. The structures of the RMIM Ts are shown in Fig. 1. Fluorescein acetate (FDA) was purchased from Sigma Aldrich. All other reagents met the analysis purity grade.

2.2. Algal culture

The microalga *S. obliquus* was obtained from the Institute of Hydrobiology of Chinese Academy of Sciences (Wuhan, China). The algae were cultured and tested in an environmental chamber with standard lighting and temperature conditions (16:8 light: dark cycle; illumination 3000–4000 lx; 25 °C). The HB-4 medium for *S. obliquus* growth was prepared according to the Chinese National Environmental Protection Agency Guidelines 201 (CNEPA, 1990), and was sterilized in an autoclave (Hirayama HVE-50) before use. The composition of the HB-4 medium including distilled water and the following chemical ingredients: $(NH_4)_2SO_4$ 200 mg/L; $Ca(H_2PO_4)_2 \cdot H_2O$ + $(CaSO_4 \cdot H_2O)$ 30 mg/L; $MgSO_4.7H_2O$ 80 mg/L; $NaHCO_3$ 100 mg/L; KCl 23 mg/L; $FeCl_3$ 1.5 mg/L; and 0.5 mL of soil leaching solution (Liu et al., 2015b). The *S. obliquus* cells has been precultured to the log phase before exposed to a series of IL concentrations in a sterilized flasks to reach a final density of 8.0×10^5 cells/mL in the final 100 mL solution. All flasks were shaken many times a day and repositioned daily within the culture chamber to minimize any possible spatial differences. The algal cells were collected after 96 h for endpoint analysis. Three replicates were tested for each treatment.

2.3. Determination of the reactive oxygen species (ROS) levels

The ROS levels were determined using a method modified from Knauert and Knauer (2008). The H_2DCFDA stock solution (10 mM) was prepared in methanol and kept at −20 °C. The final concentration was diluted 1000-fold in HB-4 medium before use. After the algal cells had been treated for 96 h, algal cell samples (3 mL) were centrifuged at 6000 rpm for 10 min. The supernatant was discarded, and 0.3 mL H_2DCFDA (10 μM) was added to the cell pellet. Then the volume was made up to 3 mL with HB-4 medium. The samples were incubated in a water bath at 37 °C for 30 min in the dark and then centrifuged at 14,840 *g* for 10 min. The resulting pellet was washed twice with HB-4 medium and then resuspended in HB-4 medium. The cell permeability indicator H_2DCFDA is hydrolyzed by cellular esterases to form H_2DCF after penetrating the *S. obliquus* cell. H_2DCF is then immediately transformed into DCF, which is highly fluorescent in the presence of ROS (Wang et al., 2011). The ROS levels were quantitatively determined using a fluorescence spectrophotometer, with excitation at 485 nm and emission at 530 nm. Changes in ROS content compared to the control were evaluated using the following equation (Hong et al., 2009):

$$\text{Relative ROS sensor level (\%)} = \frac{1 + (\text{Mean DCF fluo.[RMIM T]-Mean DCF fluo.[control]})}{\text{Mean DCF fluo.[control]}} \times 100\%$$