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Acclimation of *Tetrahymena thermophila* to bulk and nano-TiO_2 particles by changes in membrane fatty acids saturation

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ABSTRACT

We provide experimental evidence that changes in the membrane fatty acid profile of *Tetrahymena thermophila* incubated with nano- or bulk TiO_2 particle are not accompanied by ROS generation or lipid peroxidation. Consequently these changes are interpreted as acclimation to unfavorable conditions and not as toxic effects. *T. thermophila* cells were exposed to TiO_2 particles at different concentrations for 24 h at 32 °C. Treatment of cultures with nano- and bulk TiO_2 particles resulted in changes of membrane fatty acid profile, indicating increased membrane rigidity, but no lipid peroxidation or ROS generation was detected. There were no differences in membrane composition when *T. thermophila* was exposed to nanosized or bulk-TiO_2 particles. We also observed reversible filling of food vacuoles, but this was different in case of nano- or bulk TiO_2 exposure. Our results suggest that interactions of particles and cell membranes are independent of oxidative stress.

1. Introduction

There have been many studies on the effects of nanosized TiO_2 (nano-TiO_2) on aquatic and terrestrial organisms [1,2]. The most frequently studied consequences of exposure to nano-TiO_2 are cytotoxicity and genotoxicity *via* oxidative stress [3,4].

Irrespective of the exposure route, the first contact between the cells and nanoparticles must involve the cell membranes [5]. Accordingly, we investigated whether a suspension of TiO_2 particles could affect the cell membrane composition of a eukaryotic microorganism *Tetrahymena thermophila* in the absence of light (when ROS generation due to catalytic activity of TiO_2 was expected to be minimized) and whether this is evidence of a toxic response or of acclimation to unfavorable environmental conditions. A significant decrease in membrane fluidity after exposure of *Tetrahymena* sp. to TiO_2 has already been documented [6,7].

There are numerous reports on functional alterations of cell membranes occurring under unfavorable environmental conditions involving for example, temperature or chemicals. Bearden et al. [8] and Schultz et al. [9], described membrane fatty acid profile alterations in *Tetrahymena* sp. resulting from exposure to chemicals, such as pentachlorophenol and 1-octanol, acting non-covalently. Shug et al. [10] detected a marked effect of iron ions on the desaturation of fatty acids in the membrane of *T. thermophila*. When exposed to methyl mercury, small but distinct changes in the profile of membrane fatty acids were detected (personnel communication) and it was suggested that profiling of fatty acid methyl esters (FAME-s) could be used for identification of different groups of chemicals in *T. thermophila*. The study of effects of an aqueous suspension of fullerene (C60) on bacteria also showed changes in lipid composition which were dependent on the C60 concentration [11]. The same authors also successfully employed profiling of FAME-s. Alterations in membrane lipid profiles have been interpreted as a physiological adaptation or an acclimation to extreme conditions [10,12–14]. Mortimer et al. [7] demonstrated changes in the fatty acid profile of protozoon *T. thermophila* exposed to nano-CuO.

Functional alterations of cell membranes could also be interpreted as acclimation to unfavorable conditions. However, acclimation is not linked only to membranes. This phenomenon acclimation has been defined as a short-term phenotypic change, which allows survival in suboptimal environmental conditions, including pollution [8,15,16]. When suboptimal conditions result from exposure to chemicals, the primary stress response of an organism compensates for the potential adverse effects on cells, but with elevated concentrations of chemicals and prolonged exposure

Abbreviations: RM, nutrient rich medium; PM, nutrient poor medium.

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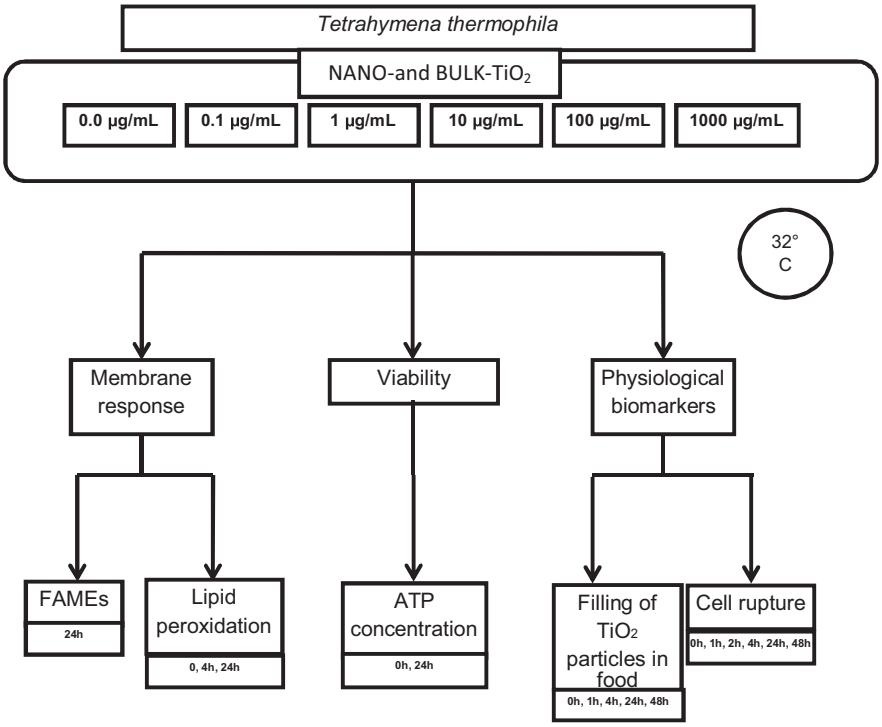

Fig. 1. Experimental arrangement. Three major toxicity endpoints were studied: changes in cell membrane, viability and physiological responses of *T. thermophila* to different exposure concentrations of TiO_2 particles. The culture of *T. thermophila* was preincubated in the PM for 24 h before beginning the exposure to TiO_2. Changes in membrane composition were assessed by FAME-s method (at 0.1, 10 and 1000 μg TiO_2/ml) and by the extent of lipid peroxidation (measuring MDA content). Viability was assessed by ATP concentration. Two physiological biomarkers distinctive of *T. thermophila*, namely filling of TiO_2 particles in food vacuoles and cell rupture were also assessed as described by Dai et al. [24].

to them, toxic effects are unavoidable. An association of alterations in membrane structure with cytotoxicity has been reported by Clarke et al. [17] for human breast cells and also by Mortimer et al. [7] for *T. thermophila*. So far, *Tetrahymena pyriformis* has been shown when exposed to temperature changes, to respond through changes in membrane fatty acid profiles [15] and to organic chemicals considered to act *via* a nonpolar toxic action [16] [8]. Very recently, changes in membrane lipid composition in terms of lowering membrane fluidity were demonstrated by Mortimer et al. [7] and explained as an adaptation mechanism to exposure to CuO nanoparticles [7]. These authors studied the effects of toxic concentrations of nano-CuO on the membrane of *T. thermophila* [7].

The aim of this research was to assess the total membrane fatty acid profile of *T. thermophila* after exposure to TiO_2 particles in a range of concentrations. Organisms were exposed to particles *via* food as well as substratum. In parallel experiments, some additional biomarkers such as lipid peroxidation, ATP concentration, cell morphology and filling of particles in vacuoles were analyzed in an attempt to correlate changes in membrane fatty acid saturation with potential cytotoxic effects. The effects of nano- and bulk TiO_2 particles were compared. We hypothesize that if changed membrane fatty acid profile of *T. thermophila* exposed to TiO_2 particles is not accompanied by a cytotoxic response this is direct evidence of acclimation to the particles present in media and not an indication of particle toxicity.

2. Materials and methods

2.1. Chemicals

All reagents were purchased from Sigma Aldrich Co. (St. Louis, MO, USA), Merck (Darmstadt, Germany) or Biolife (Milan, Italy), unless specifically stated otherwise.

2.2. T. thermophila growth and exposure conditions

Axenic cultures of *T. thermophila* from the Protoxkit F™ (MicroBioTests Inc.) were grown for 24 h in the dark at 32 °C in a semidefined proteose-peptone based medium [18] – a nutrient rich medium; RM. The nutrient rich medium contains 5 g D-glucose, 5 g proteose-peptone, 1 g yeast extract, 1.2 g Trisma-base, chlorides (2.28 μM $CaCl_2{\cdot}2H_2O$, 0.29 μM $CuCl_2{\cdot}2H_2O$, 0.05 μM $FeCl_3{\cdot}6H_2O$, 0.03 μM $MnCl_2{\cdot}6H_2O$, 0.004 μM $ZnCl_2$) and sulphates (4.1 μM $MgSO_4{\cdot}7H_2O$, 0.64 μM $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$), up to 1000 ml doubly distilled H_2O, pH corrected to 7.35 with HCl. The cell density obtained in these culture conditions was approximately 10^5 cells/ml.

The cells were harvested by centrifugation (3 min, 60 rcf), washed and resuspended in a medium specifically modified for this experiment: semidefined proteose-peptone based medium by Schultz [18] lacking yeast extract and bacteriological peptone – a nutrient poor medium; PM. This nutrient poor medium contains 5 g D-glucose, 1.2 g Trisma-base, 1000 ml doubly distilled H_2O. The pH of the medium was adjusted to 7.4 with HCl and temperature was held constant at 32 °C for the entire experiment. All experiments were performed in batch cultures of 100 ml in Erlenmeyer flasks, and aerated by shaking (90 rpm) in darkness.

After 24 h in the PM, cells were treated with bulk or nano-TiO_2. The final concentrations of particles in the medium were: 0.1, 1, 10, 100, 1000 μg/ml. Following the addition of TiO_2, *T. thermophila* cultures were incubated at 32.0 °C for 48 h. Assays of ATP concentration, total protein concentration, filling of vacuoles with TiO_2 and morphological characterizations were performed at several time intervals (Fig. 1). For each concentration of nano- or bulk TiO_2, three independent assays were carried out. A supplementary set of three replicates, without TiO_2 particles, was set up for each assay as a control.