External micro-PIXE analysis of Nd3+ accumulation in Euglena gracilis Postprint

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Abstract

External micro-PIXE measurements were conducted to investigate Nd3+ accumulation in the green alga Euglena gracilis. Analysis of Nd3+ distribution patterns within the cells revealed biosorption of Nd3+ to cellular compartments. Comparison of elemental maps of cells treated with varying concentrations of a 1 mg/mL Nd3+ solution indicated that Nd3+ uptake by Euglena gracilis cells was not dose-dependent. Examination of Ca and Mg distributions demonstrated that Ca was partially complementary to Nd3+, while Nd3+ and Mg distributions exhibited marked similarity, suggesting that Nd3+ may be primarily associated with chlorophyll molecules. The associated biochemical mechanisms are discussed.

Full Text

Preamble

External Micro-PIXE Analysis of Nd^{3+} Accumulation in *Euglena gracilis*

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Abstract: External micro-PIXE measurements were performed to investigate the accumulation of Nd^{3+} in the green alga *Euglena gracilis*. The Nd distribution patterns within *E. gracilis* cells reveal the biosorption of Nd^{3+} to various cellular compartments. Comparison of elemental maps from cells treated with different doses of 1 mg/mL Nd^{3+} solution shows that Nd uptake by *E. gracilis* is not dose-dependent. Analysis of Ca and Mg distributions indicates that Ca is partially complementary to Nd, while Nd and Mg exhibit similar distribution patterns, suggesting that Nd may be associated primarily with chlorophyll molecules. The relevant biochemical implications are discussed.

Keywords: *Euglena gracilis* cells, external micro-PIXE, Nd^{3+} accumulation
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Introduction

Biosorption by microorganisms such as bacteria, fungi, and algae represents an effective method for accumulating heavy metal ions from aqueous environments [1, 2]. *Euglena gracilis*, a unicellular green alga, demonstrates a remarkable capacity to accumulate rare earth element (REE) cations within cellular compartments. Previous studies have shown that these cells can actively transport REE cations such as Nd^{3+} against concentration gradients exceeding five orders of magnitude [3]. Beyond its intrinsic biological interest, this trait may have practical applications in bioremediation for removing toxic metal ions and organic contaminants from aquatic environments.

The cellular effects of lanthanides are diverse, influencing processes such as cell fusion, muscle relaxation, and blood coagulation [4]. Over the past decade, the biomedical aspects and toxicity of REE ions have been comprehensively reviewed [5–7]; however, the mechanisms underlying REE ion uptake, intracellular transport, and storage remain poorly understood.

Micro-PIXE has been successfully applied to numerous biological, medical, and environmental problems due to its high sensitivity and excellent spatial resolution. The use of an external beam offers additional advantages, including elimination of special sample preparation for vacuum environments, easier sample handling and observation, and reduced damage from heating and charging [8]. In this study, we investigate the bioaccumulation of REE cations (Nd^{3+}) in *Euglena gracilis* by mapping their distribution patterns using external micro-PIXE, aiming to better understand the accumulation process and its effects on other ions.

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II. Experimental

A. Cell Culture and Sample Preparation

Euglena gracilis strain 848 was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan). Detailed culture procedures are described in Ref. [9]. Cells were harvested by centrifugation and washed through three cycles of resuspension in deionized water to remove the culture medium. The *Euglena* cell suspension (3 mL) was then combined with either 1 mL or 0.1 mL of 1 mg/mL $NdCl_3$ solution. After loading with metal ions, the cells were washed again through three cycles of resuspension and fixed with 0.25% glutaraldehyde ($CHO(CH_2)_3CHO$). The fixed cell suspension in deionized water was deposited onto Mylar film at low density to enable single-cell external micro-PIXE measurements.

B. External Micro-PIXE

External micro-PIXE experiments were conducted using the 3 MV single-ended accelerator at TIARA (Takasaki Ion Accelerators for Advanced Radiation Application) to generate 3.0 MeV proton beams [8, 10]. A 2-μm-thick Mylar foil served as the exit window to withstand the pressure differential. Cells were attached directly to the Mylar foil, eliminating air gaps between the target and detector. The final proton beam resolution was 1.2 μm. A Si(Li) X-ray detector (30 mm^2 active area, 135 eV energy resolution) was positioned at 140° relative to the beam incidence within a vacuum chamber to avoid interference from argon K X-rays. The detector window consisted of 8 μm beryllium. An annular absorber (100-μm Mylar with a Φ3 mm hole) was employed to enable detection of Mg and P. Data acquisition software processed signals from the detector simultaneously. The beam current was maintained at 100–200 pA, and cells were scanned with an integrated charge of 130–200 nC to obtain distributions of matrix and trace elements.

III. Results and Discussion

PIXE spectra were extracted from raw scanning data based on cell morphology. The intensity of neodymium L X-rays was used to generate elemental maps of the cells [1]. Figure 1 shows elemental distributions in *E. gracilis* cells treated with different doses of $NdCl_3$ solution: (a) control, (b) treated with 1 mL, and

(c) treated with 0.1 mL of 1 mg/mL $NdCl_3$ solution. Cells in each group were randomly selected.

The phosphorus distribution closely matched the cell shape observed under microscopy, as phosphorus is a major component of cell membranes. For Nd distribution, no obvious local enrichment was observed in cells treated with 1 mL $NdCl_3$ (1 mg/mL) compared to the control, whereas Nd was distributed throughout cellular compartments in cells treated with 0.1 mL $NdCl_3$ (1 mg/mL), clearly indicating that Nd^{3+} had been transported into the cells. This result is consistent with our previous work at the Centre for Ion Beam Applications, National University of Singapore [11, 12], as well as with cryosection studies of I4TCF-Nd^{3+}-stained cells, EDAX analysis of fast-frozen ultrathin cryosections, and electron microscopy experiments on the alga [13]. Lansman [14] proposed that lanthanide ions can traverse ion channels to enter the cell interior, and each algal cell possesses a single transport apparatus [15, 16] that enables Nd^{3+} uptake, sharing some characteristics with calcium ion channels [13].

Comparison of Figs. 1(b) and 1(c) reveals that cellular uptake of Nd^{3+} is independent of Nd^{3+} concentration in the bulk solution, a finding previously confirmed by ICP-AES [9, 13]. Chloroplasts are known to be the primary compartments where Nd is localized [13].

In nature, magnesium serves as the coordination center in chlorophyll molecules. Lanthanide-substituted chlorophyll has been investigated previously [17, 18], and EXAFS (extended X-ray absorption fine structure) results suggest that exogenous lanthanides may substitute for magnesium in chlorophyll after transport into algal cells [19]. Energy-dispersive X-ray microanalysis (EDXA) of chloroplasts has shown a characteristic lanthanide peak [13]. Furthermore, Figs. 1(b) and 1(c) demonstrate similar distribution patterns for Nd and Mg, providing strong evidence that accumulated neodymium associates with chlorophyll. Additionally, compared to the control, Mg content decreased by approximately 70% in treated cells (both Figs. 1(b) and 1(c)), indicating that Nd substitutes for Mg in chlorophyll after cellular uptake.

Figure 2 shows Ca and Nd distributions in single cells from control and Nd^{3+}-treated groups. Although the overall Ca and Nd distributions differ, the red rectangles highlight that Ca is partially complementary to Nd in Nd-loaded cells, a phenomenon not clearly observed in control samples. This suggests that Ca content decreases following Nd cation uptake. The ionic radius of rare earth ions is very close to that of Ca^{2+}, allowing them to bind biomacromolecules, form coordination compounds, and replace Ca^{2+} at protein binding sites [4, 20].

IV. Conclusion

External micro-PIXE analysis reveals Nd^{3+} accumulation in *Euglena* 848 cells and elucidates relationships between Nd distribution patterns and those of Ca

and Mg. Neodymium ions traverse ion channels to enter cells, likely replacing calcium ions at protein binding sites and magnesium ions in chlorophyll, ultimately becoming enriched in chloroplasts. Further investigation is required to achieve a deeper understanding of the Nd transport mechanism.

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Note: Figure translations are in progress. See original paper for figures.