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ScienceDirect
Journal of Structural Biology 161 (2008) 55–63
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

Silicon uptake and metabolism of the marine diatom *Thalassiosira pseudonana*: Solid-state ^{29}Si NMR and fluorescence microscopic studies

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Received 4 May 2007; received in revised form 12 September 2007; accepted 13 September 2007
Available online 20 September 2007

Abstract

Uptake and metabolism of silicon by diatoms are studied by the combined use of solid-state ^{29}Si NMR spectroscopy and confocal laser fluorescence microscopy especially with respect to the presence and nature of an intracellular silicon-storage pool. Cells of the marine diatom *Thalassiosira pseudonana* were synchronized by silicon starvation and frozen without any freeze-drying or chemical treatment in order to analyze integer and unmodified diatoms. The frozen samples were investigated by solid-state ^{29}Si NMR spectroscopy to identify potential silica precursors. The developmental state of the cell culture and the formation of new siliceous girdle bands and valves were monitored by laser fluorescence microscopic studies. A comparison of fluorescence microscopic and NMR data allows the assignment of NMR spectra to the various developmental stages of the dividing diatom cells. A detailed analysis of solid-state ^{29}Si NMR spectra suggests that the silicon-storage pool—if present—consists of four-coordinated, condensed silicon; possibly a silica sol.

Keywords: Diatoms; Biomineralization; Silicification; Silicon-storage pool; Solid-state ^{29}Si NMR spectroscopy; Confocal laser fluorescence microscopy

1. Introduction

Diatoms are unicellular, eukaryotic algae which can be found in almost every sea and fresh water habitat on earth. They are responsible for approximately 40% of the marine primary production (Falkowski et al., 1998) and play a key role in the ocean's silicon cycle (Treguer et al., 1995). In particular, diatoms are well-known for the intricate micro- and nano-structured patterns of their silica-based cell walls. This makes them an outstandingly interesting system for the study of silica biomineralization processes (Sumper and Brunner, 2006). The species-specific patterns are reproduced during each cell division cycle (Zurzolo and Bowler, 2001) which is closely correlated with the silicon metabolism of the cell. Monosilicic acid is taken up from the surrounding seawater which on average exhibits silicic acid concentrations of ca. 70 μM (Hildebrand, 2000). Special silicic acid-binding proteins were discovered which are involved in the silicon transport process into the cell (SIT-proteins, Hildebrand et al., 1997). The intracellular silicon transport and the transfer into the silica deposition vesicle are rather poorly understood (Hildebrand, 2000). The synthesis of new valves takes place in a highly specialized intracellular vesicle, the so-called silica deposition vesicle (SDV, see Fig. 1; Drum and Pankratz, 1964). Numerous papers report the existence of intracellular silicon-storage pools in diatoms that may represent up to 50% of the total cellular biosilica depending on the species (Werner, 1966; Chisholm et al., 1978; Martin-Jezequel et al., 2000; Martin-Jezequel and Lopez, 2003). The silicon-storage pool in diatom cells—if present—is supposed to accumulate silicon for the production of a new valve (Hildebrand, 2000). The accumulated silicon is then transported to the SDV where the new cell wall is synthesized.

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doi:10.1016/j.jsb.2007.09.010

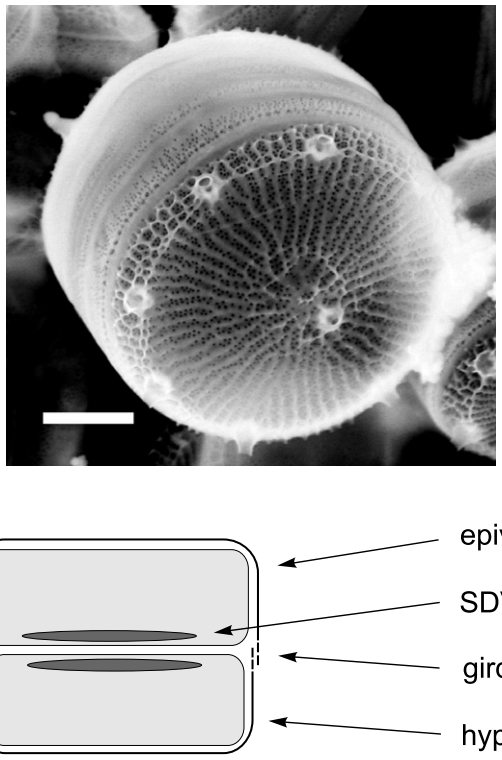

Fig. 1. Top: scanning electron microscopic image of extracted silica shell of *Thalassiosira pseudonana*. Scale bar: 1 μm. Bottom: scheme showing a diatom cell during cell division at the stage where new valves are formed in the SDV.

Specific biomolecules could meanwhile be extracted from diatom cell walls which are capable of inducing silica precipitation *in vitro* (silaffins, polyamines, Kröger et al., 1999, 2000, 2002; Sumper and Brunner, 2006). These molecules are, therefore, assumed to be involved in silica biogenesis.

However, the reported concentrations of the intracellular silicon within the storage-pool (Martin-Jezequel et al., 2000; Martin-Jezequel and Lopez, 2003) strongly exceed the stability of monosilicic acid (Iler, 1979). A number of concepts are discussed in the corresponding literature: Azam et al. (1974) suggest that silicic acid is associated with some kind of organic material or special proteins to form a soluble silicic acid pool inside the cells. Schmid and Schulz (1979) proposed the existence of special silicon-storing vesicles since they observed certain cytoplasmic vesicles fusing with the developing SDV. Such silica containing vesicles were recently found during the formation of the siliceous spicules of sponges (Schröder et al., 2007). Until now, however, there is no evidence for the presence of silicon inside these vesicles observed in diatoms. Another possible silicon-storage mechanism would be some kind of pre-polymerization of silicic acid inside the cells, for example so-called polyamine-stabilized silica sols as suggested by Sumper (2004). A major reason for the uncertainty concerning the silicon-storage mechanisms is that the techniques commonly applied for the determination of internal silicon-storage pools are not able to distinguish between the different condensation states of silicon. This is particularly true for silicon bound to organic matter. The molybdate method (Martin-Jezequel et al., 2000, and references therein) exclusively detects mono- and disilicic acid and requires the destruction of the diatom cells and chemical treatment of the remaining compounds. On the other hand, the radioisotopic analogue ^{68}Ge (Martin-Jezequel et al., 2000, and references therein) is used as a tracer for silicon. This method can be applied to investigate intact cells but suffers from the fact that there is no possibility to distinguish between silicic acid, polymerized silica, and silicon bound to organic molecules.

^{29}Si NMR spectroscopy is a powerful and non-destructive tool to investigate and distinguish the different silicon species because the chemical shift is very sensitive to changes in the chemical environment (Williams and Cargioli, 1979; Marsmann, 1981), e.g., if silicon is bound to organic material. Several solid-state ^{29}Si NMR studies were performed on extracted diatom cell walls or freeze-dried cells (Bertermann et al., 2003; Gendron-Badou et al., 2003; Lutz et al., 2005; Christiansen et al., 2006). But, to the best of our knowledge, only one ^{29}Si NMR spectroscopic study on intact diatom cells has been carried out so far. In this study, liquid-state ^{29}Si NMR was applied (Kinrade et al., 2002). A dominant signal at −71 ppm corresponding to free monosilicic acid and a weak and transient signal at −131 ppm could be observed. The latter signal has been assigned to a hypercoordinated organo-silicon complex which was suggested to be a silica precursor compound. However, liquid-state ^{29}Si NMR spectroscopy is not able to identify slowly tumbling or immobilized species such as silicic acid attached to organic components or polysilicic acid species.

In this paper we present solid-state ^{29}Si MAS NMR experiments on untreated, integer[1] diatoms (*Thalassiosira pseudonana*, Fig. 1). Solid-state ^{29}Si NMR spectroscopy in combination with magic angle spinning offers the great advantage to detect all silicon species present in the samples without damaging the cells. Preliminary tests on polysilicic acid solutions (unpublished results) showed that the polymerization state of the silica is changed during freeze-drying the samples. Lowly condensed silicon species are transformed into species of higher condensation state. Therefore, in contrast to former ^{29}Si NMR studies of diatoms (see above), a much gentler method for sample preparation preventing the application of any freeze-drying or chemical procedure has been used within the present paper. As it was shown by Frigeri et al. (2006), a centrifuged culture of *T. pseudonana* inoculated into fresh artificial sea water grows up. Microscopic investigations have shown that the cells were not damaged under the centrifugal forces (*g*-forces) applied here (see Section 2.2). Therefore, diatom cells were directly centrifuged into the MAS rotors. The

[1] Integer means that the cells are neither chemically treated nor destroyed by mechanical treatments such as ultrasonic treatment, for details, see Section 2.