

The Molecular Basis of Diatom Silica Biomineralization: Insights from Silicanin-1

Damian Pawolski*

B CUBE Center for Molecular Bioengineering, CMCB, TU Dresden, Arnoldstrasse 18, 01307, Dresden, Germany

Abstract

Background: A lipid bilayer membrane commonly encloses specialised compartments where biological mineral formation (biomineralization) takes place. The function of membranes in the biomineralization process is currently poorly understood.

Results: We discovered Silicanin-1 (Sin1) as a conserved diatom membrane protein found in silica deposition vesicles (SDVs) of *Thalassiosira pseudonana* while researching the biomineralization of SiO₂ (silica) in diatoms. For the first time, silica synthesis *in vivo* may be followed by fluorescent microscopy of GFP-tagged Sin1, which is a biomineralization protein. The investigation showed that the interaction of the N-terminal domain of Sin1 with the organic matrix within the SDVs resulted in integration of Sin1 into the biosilica. Via a synergistic interaction with long-chain polyamines, *in vitro* tests revealed that the recombinant Sin1 N-terminal domain undergoes pH-triggered assembly into sizable clusters and encourages silica formation.

Conclusions: Sin1 is the first SDV transmembrane protein to be discovered, and because it is highly conserved throughout the diatom kingdom, it may play a crucial part in the biomineralization of diatom silica. Sin1 might act as a molecular link via which the SDV membrane regulates the formation of organic matrices that create biosilica in the SDV lumen through interactions with long-chain polyamines.

Introduction

The cell walls of diatoms, which are unicellular photosynthetic eukaryotes, are composed of amorphous, hydrated silicon dioxide (silica) and related macromolecules. The protoplast is entirely enclosed by the diatom cell wall, which is extracellular to the plasma membrane. The biomineral building blocks of diatoms are created inside the cell in specialised vesicles and are then exocytosed and incorporated into the cell wall, just like in many other species (such as radiolaria, coccolithophores, sponges, and foraminifers). Girdle bands (ring-shaped silica) and valves are two different types of nanopatterned porous biosilica building components found in diatom cell walls (complex shaped silica often with plate-, bowl-, or dome-like structure). Specifically opposite the region of the cell wall where a new biomineral building block would be incorporated, silica deposition vesicles (SDVs) are positioned at the cytosolic location of the plasma membrane during the biosilica formation process. A notable illustration of polarised intracellular membrane trafficking is the site-specific [1-6] assembly of SDVs and exocytosis of the biosilica building pieces. Although a number of the factors involved in silica production have been previously identified (see below), the molecular mechanisms behind SDV biogenesis and exocytosis are still unknown. The actin filaments and microtubules that are closely linked to the SDVs probably contribute to their location and shape.

Materials and Methods

Long-chain polyamines (LCPA) and distinctive proteins (silaffins, cingulins, and silacidins) have been identified as organic components of diatom biosilica through previous biochemical investigation. Several of the biosilica-associated proteins have been demonstrated to significantly speed up silica synthesis from monosilicic acid solutions *in vitro*. The majority of these proteins are highly charged, hydrophilic, and predicted to be inherently disordered. The diatom *Thalassiosira pseudonana* has a number of unique biosilica-associated proteins that have unidentified activities, according to a recent proteomics investigation. One of these, SiMat7, has a predicted secondary structure that differs significantly

from silaffins, cingulins, and silacidins in terms of amino acid content. In the current study, we examined the role of SiMat7 by identifying its intracellular sites at various cell cycle stages, examining its associations with cellular membranes and biosilica, and [7-11] evaluating its silica production activity and self-assembly characteristics. Here, we show that SiMat7 is the original member of the silicanins, a new family of silica biomineralization proteins. SiMat7 was changed to silicanin-1 as a result (abbreviated Sin1).

Discussion

The first SDV transmembrane protein has been identified as Sin1 in the current study. According to bioinformatics studies, Sin1 is highly conserved across the whole diatom kingdom, and two non-diatom organisms also have homologous proteins. One of them, the amoeboid alga *Rhizochromulina marina*, belongs to the taxon Dictyochophyceae, which also includes silicoflagellates that create siliceous skeletons but is not known to produce biosilica. The colepid ciliate *Tiarina fusa*, a protozoan that creates a calcium carbonate shell, contains the other non-diatom homologue of Sin1. This shows an evolutionary link between the mechanisms for the biomineralization of silica and calcium carbonate, which has recently been also demonstrated for three coccolithophore species. These coccolithophore species produce proteins that resemble silicic acid transporters, and germanic acid

*Corresponding author: Damian Pawolski, B CUBE Center for Molecular Bioengineering, CMCB, TU Dresden, Arnoldstrasse 18, 01307, Dresden, Germany, Email: oawolski@gmail.com

Received: 01-Apr-2023, Manuscript No: jabt-23-90534, Editor assigned: 03-Apr-2023, Pre QC No: jabt-23-90534(PQ), Reviewed: 17-Apr-2023, QC No: jabt-23-90534, Revised: 21-Apr-2023, Manuscript No: jabt-23-90534(R), Published: 28-Apr-2023, DOI: 10.4172/2155-9872.1000508

Citation: Pawolski D (2023) The Molecular Basis of Diatom Silica Biomineralization: Insights from Silicanin-1. J Anal Bioanal Tech 14: 508.

Copyright: © 2023 Pawolski D. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

interfered with the biomineralization of their calcium carbonate scales (i.e., an inhibitor of silica metabolism). Sin1 is not always necessary for the synthesis of biological silica, as evidenced by the absence of Sin1 genes in other non-diatom species that create biosilica, such as the synurophyte *Mallomonas* sp., chrysophyte *Paraphysomonas* *imperfurata*, and silica sponge *Amphimedon queenslandica*.

Results

Molecular architecture and sequence conservation of Sin1

Sin1 has 426 amino acids and is a projected type 1 transmembrane protein. It has a cytosolic domain of 20 amino acids at the C-terminus and a single transmembrane helix that is also 20 amino acids long. There are no cytoskeleton binding sites or any recognised motifs in the cytosolic domain. Due to the presence of an N-terminal signal peptide for co-translational import into the endoplasmic reticulum, it is projected that the remaining portion of Sin1 will be exposed to the extracellular environment or the lumen of a secretory compartment. The RXL domain, which is typical of many diatom biosilica-associated proteins and [7-11] is designated by the tripeptide RRL, is found 30 amino acids after the signal peptide. A 341 amino acid polypeptide portion of Sin1 that makes up the majority of the protein is abundant in asparagine and glutamine, which are frequently seen in clusters (NQ-rich domain). In addition to 18 cysteine residues, the NQ-rich domain of Sin1 also contains 28% helix, 14% sheet, and 58% disordered areas, according to secondary structure research. In contrast to silaffins, cingulins, and silacidins, which have only one or no cysteine residues and are projected to adopt completely random coil configurations, the 3D structure of Sin1 is likely significantly different. No other silica-associated proteins that have been previously characterised share a significant amount of sequence similarity with Sin1. We named Sin2 the protein that the *T. pseudonana* genome encodes and shares 55% sequence similarity with Sin1. Only diatom-specific homologous proteins were found after searching the NCBI database (note that only proteins having an E-value lower than 10⁻⁵⁰ were regarded as homologs). By using the Basic Local Alignment Search Tool (BLAST) to scan the Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) database, which has a huge number of eukaryotic microbe gene sequences not included in the NCBI database, we furthered our search for Sin1 homologs. Research found Sin1 homologs in two non-diatom creatures and in 70 different species of diatoms. The 18 cysteine residues in the NQ domains of all the found Sin1 homologues are conserved in their locations, and all of them are expected type 1 transmembrane proteins (Additional file 1: Figure S1). As a result, we consider these proteins to be silicanin protein family members and assume that they have biological properties and 3D structures that are extremely similar to Sin1's.

Sin1 membrane association

We recovered complete membranes from *T. pseudonana* in accordance with a predetermined methodology in order to determine whether Sin1 is membrane-associated as predicted (see above). An antibody against the luminal region of Sin1 was used to conduct a Western blot analysis on the membranes to check for the presence of Sin1 (i.e., the combined RXL and NQ domains; Fig. 1b). Around 10 kDa more than was predicted for a Sin1 molecule devoid of the signal peptide, a single strong band of apparent molecular mass of 55 kDa was seen. We generated two recombinant Sin1 proteins in *E. coli* to test whether the difference in apparent molecular mass was due to an atypical migratory behaviour of Sin1 on sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE). Protein rSin1lum (molecular mass: 40.7 kDa) only contained the luminal region,

whereas protein rSin1-SP (molecular mass: 45.1 kDa) comprised all Sin1 domains aside from the signal peptide. Both proteins displayed apparent molecular weights of about 55 kDa on SDS-PAGE, proving that Sin1 does, in fact, cause an abnormal migratory behaviour there. Thus, we deduced that Sin1 is represented by the 55 kDa band in *T. pseudonana*'s membrane fraction. If this protein doesn't have a lot of post-translational modifications, its apparent molecular mass is slightly higher than rSin1lum, which is consistent with Sin1 having the transmembrane helix and cytosolic domain. The apparent molecular mass of Sin1 did not change after O-linked glycans and O-phosphoryl moieties were taken out of *T. pseudonana* membranes using anhydrous HF. This shows that native Sin1 does not contain significant levels of glycan and phosphate moieties. The minor difference in apparent molecular mass between Sin1 and rSin1-SP raises the possibility that Sin1 does not include the RXL domain.

Conclusions

Insights into the intracellular sites of the biomineralization protein Sin1 during silica synthesis have never before been possible according to the current research. The first SDV membrane protein to be found, Sin1, interacts with LCPA, suggesting a potential method through which the SDV membrane may affect silica morphogenesis in the SDV lumen. It is believed that in addition to silicanins, diatom SDVs also contain other membrane proteins. The team of Mark Hildebrand presented a family of putative SDV membrane proteins from *T. pseudonana* in 2015 at the meeting "Molecular Lives of Diatoms" (in Seattle, WA, USA) (Scripps Institution of Oceanography, UCSD, USA). Although these proteins have predicted transmembrane domains, they do not have any sequence similarities to Sin1 (Mark Hildebrand, personal communication).

References

1. Beutler E, Waalen J (2006) The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? *Blood* 107: 1747-1750.
2. US Department of Health and Human Services. (n.d.). Blood tests - blood tests. National Heart Lung and Blood Institute.
3. Sundermann FW (1956) Status of clinical hemoglobinometry in the United States. *Am J Clin Pathol* 43: 9-15.
4. Wolf HU, Lang W, Zander R (1984) Alkaline haematin D-575, a new tool for the determination of haemoglobin as an alternative to the cyanhaemoglobin method. II. Standardisation of the method using pure chlorohaemin. *Clin Chim Acta* 136: 95-104.
5. Shah VB, Shah BS, Puranik GV (2011) Evaluation of non cyanide methods for hemoglobin estimation. *Indian J Pathol Micro* 54: 764-768.
6. Karakochuk CD, Hess SY, Moorthy D, Namaste S, Parker ME, et al. (2019) Measurement and interpretation of hemoglobin concentration in clinical and field settings: a narrative review. *Ann NY Acad Sci* 1450: 126-146.
7. Kang SH, Kim HK, Ham CK, Lee DS, Cho HI (2008) Comparison of four hematology analyzers, CELL-DYN Sapphire, ADVIA 120, Coulter LH 750, and Sysmex XE-2100, in terms of clinical usefulness. *Int J Lab Hem* 30: 480-486.
8. Whitehead Jr RD, Zhang M, Sternberg MR, Schleicher RL, Drammeh B, et al. (2017) Effects of preanalytical factors on hemoglobin measurement: A comparison of two HemoCue point-of-care analyzers. *Clin Biochem* 50: 513-520.
9. Ingram CF, Lewis SM (2000) Clinical use of WHO haemoglobin colour scale: validation and critique. *J Clin Pathol* 53: 933-937.
10. Osborn ZT, Villalba N, Derickson PR, Sewatsky TP, Wagner AP, et al. (2019) Accuracy of Point-of-Care Testing for Anemia in the Emergency Department. *Resp Care* 64: 1343-1350.
11. Dybas J, Alciček FC, Wajda A, Kaczmarska M, Zimna A, et al. (2022) Trends in biomedical analysis of red blood cells - Raman spectroscopy against other spectroscopic, microscopic and classical techniques. *Trend Anal Chem* 146: 116481-116508.