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

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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 [illegible] 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: [illegible] 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.

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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 imperforata, 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. ere 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. e 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 a er 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 sila ns, cingulins, and silacidins, which have only one or no cysteine residues and are projected to adopt completely random coil con gurations, the 3D structure of Sin1 is likely signi cantly di erent. No other silica-associated proteins that have been previously characterised share a signi cant 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-speci c homologous proteins were found a er searching the NCBI database (note that only proteins having an E-value lower than 1 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 di erent species of diatoms. e 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 le 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 di erence 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. us, 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 modi cations, its apparent molecular mass is slightly higher than rSin1lum, which is consistent with Sin1 having the transmembrane helix and cytosolic domain. e apparent molecular mass of Sin1 did not change a er O-linked glycans and O-phosphoryl moieties were taken out of T. pseudonana membranes using anhydrous HF. is shows that native Sin1 does not contain signi cant levels of glycan and phosphate moieties. e minor di erence 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. e rst SDV membrane protein to be found, Sin1, interacts with LCPA, suggesting a potential method through which the SDV membrane may a ect silica morphogenesis in the SDV lumen. It is believed that in addition to silicanins, diatom SDVs also contain other membrane proteins. e 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) V@^Áå^,}ãcã[}Á[~Áæ}^{ãæKÁ¸@æcÁã•Ác@^Á|[¸^¦Á|ã{ãcÁ[~Á 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.ÁŒ{ÁRÁÔ|ã}ÁÚæc@[|ÁIHKÁJËFÍÈ
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 cyanhaemiglobin method. II. Standardisation of the method using pure chlorohaemin. Clin Chim Acta 136: 95-104.
5. Ù@æ@ÁXÓÊÁÙ@æ@ÁÓÙÊÁÚ˘¦æ}ã\ÁÕXÁÇG€FFDÁEvaluation of non cyanide methods for hemoglobin estimation.ÁQ}åãæ}ÁRÁÚæc@[|ÁTã&¦ÁÍIKÁÏÎIËÏÎÌÈ
6. Sæ¦æ\[&@˘\ÁÔÖÊÁP^••ÁÙŸÊÁT[[¦c@ˆÁÖÊÁÞæ{æ•c^ÁÙÊÁÚæ¦\^¦ÁTÒÊÁ^cÁæ|ÈÁÇG€FJDÁ Measurement and interpretation of hemoglobin concentration in clinical and ¸^|åÁ•^ccã}*•KÁæÁ}æ¦¦æcãç^Á¦^çã^¸È 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) Ò^&c•Á[~Á]¦^æ}æ|ˆcã&æ|Á~æ&c[¦•Á[}Á@^{[*|[àã}Á{^æ•˘¦^{^}cKÁŒÁ 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.ÁRÁÔ|ã}ÁÚæc@[|ÁÍHKÁJHHËJHÏÈ
10. U•à[¦}ÁZVÊÁXã||æ|àæÁÞÊÁÖ^¦ã&\•[}ÁÚÜÊÁÙ^¸æc•\ˆÁVÚÊÁYæ*}^¦ÁŒÚÊÁ^cÁæ|ÈÁÇG€FJDÁ Œ&&˘¦æ&ˆÁ[~ÁÚ[ã}cË[~ËÔæ¦^ÁV^•cã}*Á~[¦ÁŒ}^{ãæÁã}Ác@^ÁÒ{^¦*^}&ˆÁÖ^]æ¦c{^}cÈ Resp Care 64: 1343-1350.
11. Dybas J, Alcicek 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.