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ORIGINAL PAPER

Silicatein-Mediated Polycondensation of Orthosilicic Acid: Modeling of a Catalytic Mechanism Involving Ring Formation

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Abstract The sponge protein silicatein is the first enzyme that has been described to form an inorganic polymer (silica) from a monomeric precursor (tetraethoxysilane or orthosilicic acid). The models proposed for silicatein-mediated silica formation are mainly based on the use of synthetic substrates (hydrolytic cleavage of tetraethoxysilane to silanol compounds) or only consider the formation of less reactive silicic acid dimers (disilicic acid). Here we propose a new model for the catalytic mechanism of silicatein that leads to the formation of reactive, cyclic silicic acid species (trisiloxane rings and higher-membered siloxane rings) which easily promote the silica polycondensation reaction.

Keywords Silicatein · Silica formation · Polycondensation · Nucleophilic attack · Cyclic trisiloxane

1 Introduction

The formation of silica that constitutes the hierarchically ordered skeletons of the siliceous sponges (demosponges and hexactinellid sponges) is an enzymatic process which is mediated by silicatein [1–3; for a review, see 4, 5]. This enzyme enables the synthesis of silica particles (nanospheres) at ambient-temperature, near-neutral-pH and low precursor concentrations. The silica is subsequently assembled / fused to microscopic and macroscopic structures of varying morphologies, often characterized by a lamellar organization [6–9].

The kinetic constants of silicatein which exhibits both silica-polymerase and silica-esterase activity have been determined using synthetic substrate molecules [10]. Although these studies have provided clear-cut evidence for the enzymatic nature of silicatein-mediated silica formation, the mode of action of the enzyme still remained enigmatic, in particular because of uncertainties about the physiological substrate of the enzyme. It is reasonable to assume that the mechanism of enzymatic silica formation will follow principles similar to those described for the chemical polycondensation reaction.

The starting molecule for the chemical polymerization / polycondensation reaction is the monomer orthosilicic acid in which the silicon atom is tetrahedrally co-ordinated to four hydroxyl groups [$Si(OH)_4$]. At neutral pH and concentrations above of 1–2 mM, this molecule undergoes a series of polycondensation reactions, resulting in the formation of siloxane (Si-O-Si) bonds [11, 12]. Various mechanisms have been proposed for the non-enzymatic condensation reaction which may be promoted by both acid and base catalysts. The reaction may take place either between two unionized silicic acid molecules or between an ionized and unionized silicic acid molecule. At physiological conditions (near neutral pH) the proportion of ionized silicic acid molecules is likely to be very small (approx. 0.2% of the silanol groups are present as ionized species for silicic acid at pH 7; [13]). The condensation reaction is based on a nucleophilic substitution (S_N2) reaction, involving the

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formation of a pentacoordinate intermediate, a proton transfer and the release of water [14].

The initial products of the polycondensation reaction are dimers which preferentially react with monomers to form trimers and higher oligomers. The proximity of the chain ends of small oligomers formed during the early stage of the polycondensation reaction facilitates the formation of rings consisting of three to six silicon atoms linked by siloxane bonds. The cyclic oligomers have a higher proportion of ionized silanol groups and a negative charge, as the pK_a of the silanol groups decreases with increasing size of the oligomers [14]; the monomer orthosilicic acid has a pK_a of 9.8 and is thus only weakly acidic [12]. These cyclic species thus become preferential sites for the addition of further silicic acid monomers, dimers and other small oligomers [15].

The mode of action of silicatein has hitherto mainly been studied using the synthetic substrate tetraethoxysilane (TEOS) which is commonly used in *in vitro* assays to determine the activity of the enzyme [16]. The mechanism of the presumable "physiological" substrate for silicatein, silicic acid, has not yet been considered.

Here we describe for the first time a mechanism for the silicatein reaction, based on modeling studies and using the presumable "physiological" substrate, orthosilicic acid, which results in the generation of a cyclic silicic acid species (three-membered or higher membered siloxane rings) which are reactive intermediates known to occur in the early phase of the silica polycondensation reaction.

2 Experimental Details

Earlier results showed that silicatein-α is more closely related to cathepsin S than to cathepsin L [17]. The identity/similarity score for the mature silicatein-α sequence from *Suberites domuncula* (accession number CAC03737.1) to the human procathepsin S sequence (GI:119389039) is 48.9%/62.6%. The gapped BLAST search was performed using http://swissmodel.expasy.org/workspace/index.php?func=tools_targetidentification [18]. Details on the BLAST search performed are given (see Figs. S1 and S2 in the Supplementary information). The crystal structure of the Cys25→Ser mutant of human cathepsin S (2.2 Å resolution) has been published [19]. Hence, the structure prediction of the mature silicatein-α molecule from the demosponge *Suberites domuncula* has been performed according to the published structure of cathepsin S (PDB database: www.PDB.org; as well as http://swissmodel.expasy.org). Alignment of the deduced amino acid sequence of silicatein-α (*S. domuncula*) against the Cys25→Ser mutant of human cathepsin S revealed an identity of 51.6% (see Fig. S2 in the Supplementary information). Silicatein-α was modeled on the structural template of cathepsin S (PDB ID: 1GLO) using the Modeller Program package ([20]; www.Salilab.org). The best fitting predicted structure with the lowest Discrete Optimized Protein Energy (DOPE) score (*S. domuncula* silicatein-α: −19578.26; [21]) was used for the silica precursor ligand docking analysis. Ramachandran plot analysis [22] revealed that in the modeled *S. domuncula* silicatein-α structure only glycine residues are located outside of the allowed regions, confirming that the predicted conformation of the protein is plausible (see Fig. S3 and Table S1 in the Supplementary information).

Energy minimization was performed using the Modeller software 9v4 (see Tables S2 and S3 in the Supplementary information).

The diameter of the silicatein substrates was measured using the Accelrys Discovery Studio Visualizer 2.5 [23].

Ligand docking analysis was performed using LibDock (Accelrys Discovery Studio package, version 2.5.0.9164) [24]. The number of conformers, polar and apolar Hot-Spots, and the LibDock score for the substrate molecules, $Si(OH)_4$ and TEOS, are given in the Supplementary information (Table S4).

3 Results and Discussion

Our considerations are based on the predicted three-dimensional structure of the mature silicatein-α molecule from the demosponge *S. domuncula* that has been obtained using the X-ray structure of the human procathepsin S as template for the modeling process [17, 18, 25, 26]; Fig. 1A. Silicatein differs from the cathepsins by the replacement of a cysteine residue by a serine residue in the catalytic center of the enzyme, which is thought to be essential for the catalytic mechanism [1, 2].

The modeling studies revealed that the synthetic substrate, TEOS, fits very well into the substrate pocket of the *S. domuncula* silicatein-α molecule (Fig. 1A), which comprises the amino acids of the catalytic center, Ser, His and Asn. The calculated distance between the C-α atom of Ser to the C-α of His is 7.8 Å; between Asn und Ser, and between Asn and His this distance is 9.6 Å and 9.77 Å, respectively. In order to find the optimal position of TEOS within the catalytic center of the enzyme, the diameter of the TEOS sphere (5.76 Å) has been calculated. Assuming an effective diameter of 8 Å for that molecule including its aqueous environment, the predicted three-dimensional structure of silicatein-α was found to allow this substrate to enter into the catalytic pocket and to interact with the oxygen atom of the Ser residue of the catalytic center of the molecule. The calculated distance between the Ser hydroxyl group involved in the nucleophilic attack at the silicon atom of the TEOS molecule was found to be 2.93 Å (Fig. 1B),

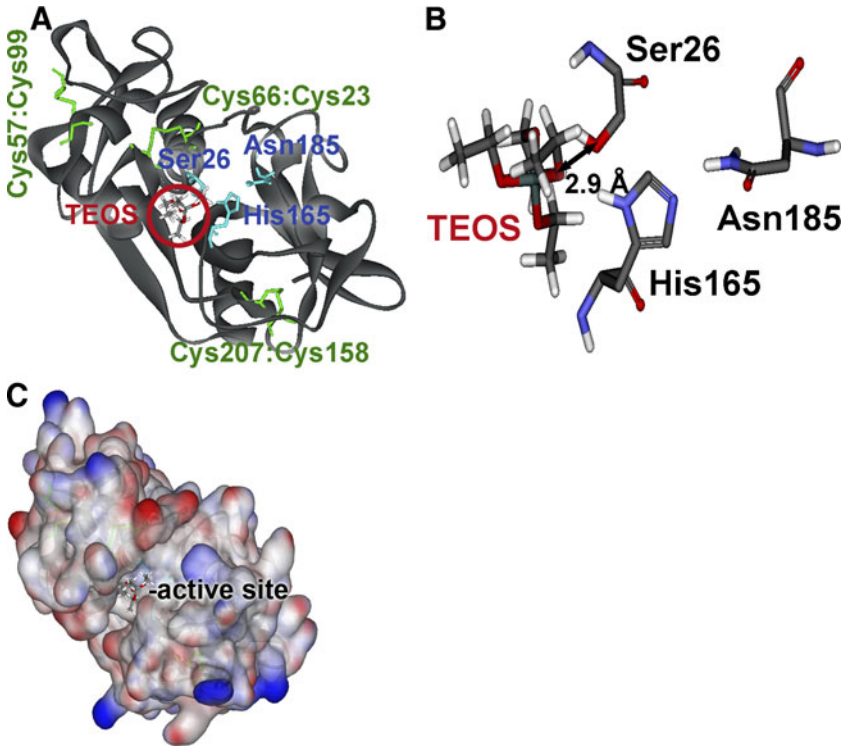

Fig. 1 Model of silicatein-α from *S. domuncula* and using TEOS as its substrate in the catalytic pocket. **A** Deduced structure of the enzyme from *S. domuncula* showing the most likely position for the insertion of TEOS into the catalytic center (*red circle*). The structure of the enzyme has been modeled using the X-ray structure of cathepsin S as the template for the prediction. Besides the amino acid residues (Ser26, His165 and Asn185; in blue) present in the catalytic center of silicatein-α, the cysteine (Cys) residues involved in the three disulfide bridges are indicated (in *green*). **B** Steric proximity of the three amino acids of the catalytic center to the TEOS molecule. The distance between the oxygen atom of the Ser hydroxyl group and the substrate (TEOS) is indicated. **C** Three-dimensional model of the electrostatic charge distribution of silicatein-α with the inserted TEOS molecule (positive charges: red; negative charges: blue; hydrophobic areas: *white*)

below that for hydrogen-bond interaction (3.6Å), thus permitting the enzymatic cleavage (hydrolysis) of the substrate molecule.

Even more space is available for the natural substrate, orthosilicic acid, which is much smaller than the synthetic silica precursor (TEOS). Our analysis revealed that oligomers of up to eight silicic acid units fit into the substrate pocket of the *S. domuncula* silicatein-α molecule; results from docking experiments with the cyclic silicic acid trimer and the cyclic silicic acid hexamer, as well as a silicic acid octamer containing a 6-membered siloxane ring, into the modeled silicatein-α are shown in Fig. 2.

Cha et al. [16] have proposed a two-step mechanism for silicatein-mediated silica formation from silicon alkoxide substrates. Step 1 is the (rate-limiting) hydrolysis of the alkoxide substrate resulting in the formation of silanol compounds; step 2 comprises the subsequent (poly)condensation reaction of the silanol compounds formed in step 1 to amorphous silica. In this mechanism, it is assumed that silicatein exhibits, like cathepsin, an esterase activity resulting in the hydrolytic cleavage of Si-O bonds in silicon alkoxides [10, 27]. The specific mechanism of the polymerisation/ polycondensation reaction catalyzed by silicatein (step 2) is not described or assumed to be based on purely chemical reactions.

Other models for silicatein reaction including the mechanism proposed by Fairhead et al. [28], which is based on the crystal structure of a "chimeric" mutant of human cathepsin L ("cathsilicatein"), showing silica polycondensation activity, are only able to explain the formation of disilicic acid dimers. This mechanism does not involve the intermediary formation of a covalently (serine) linked enzyme-substrate (silicic acid) intermediate [28].

The mechanism of silicatein reaction proposed by us is based on the assumption that the presence of the serine and histidine residues in the catalytic center of the silicatein molecule, as well as their interaction through formation of a hydrogen bond between the Ser-OH and the imidazole nitrogen of the His residue are essential for the catalytic mechanism (Fig. 3A). This assumption is supported by site-directed mutagenesis experiments with either of the two specific amino acids [29].

According to previous models [13, 26, 29] we assume that the initial step of the catalytic mechanism mediated by silicatein is the nucleophilic attack (S_N2 type) of the (electronegative) oxygen atom of the hydroxyl group of

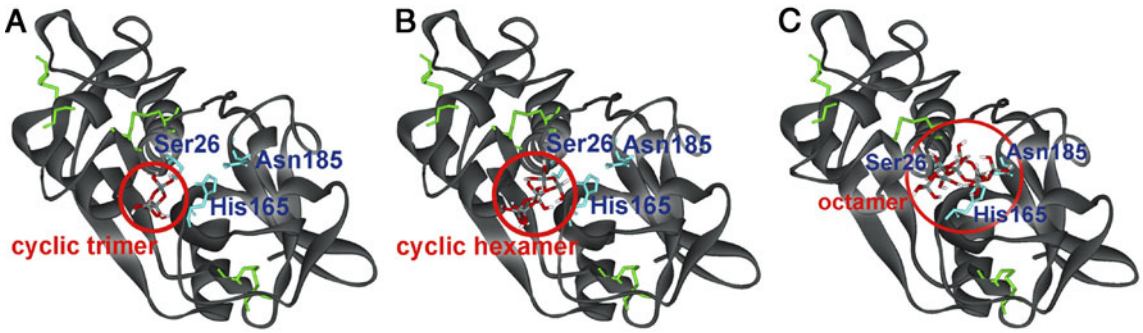

Fig. 2 Docking of cyclic silicic acid oligomers into the modeled silicatein-α from *S. domuncula*. The cyclic silicic acid trimer (**A**) and hexamer (**B**) and a silicic acid octamer containing a 6-membered siloxane ring (**C**) were docked into the catalytic pocket of the enzyme. The catalytic center (*red circle*) and the catalytic triad of amino acids Ser26, His165 and Asn185 (in *blue*) are indicated

the serine residue at the (electropositive) silicon atom of a silicic acid molecule in the catalytic pocket of the silicatein molecule; Figs. 3A and 4 (step 1). This mechanism is presumably facilitated by the hydrogen bond between Ser and His, which increases the nucleophilicity of the serine OH group.

The proton transfer from the histidine nitrogen (Ser-His hydrogen bond) to one of the silicon OH ligands of the pentavalent intermediate formed (transition stage; Fig. 4, step 1) then leads to the release of a water molecule (Fig. 4, step 2), resulting in a silicic acid species, covalently bound to the serine residue in the catalytic center of silicatein (Fig. 3B).

This serine-bound silicic acid molecule then performs a nucleophilic attack at the silicon atom of a second orthosilicic acid molecule entering the substrate pocket of the enzyme, whereby the nucleophilicity of the attacking oxygen atom of the OH ligand of the first silicic acid molecule is thought to be increased by the formation of a hydrogen bond to the nitrogen imidazole of the His residue of the catalytic center of the enzyme (Figs. 3C and 4, step 3). This assumption is also supported by previous molecular simulation experiments indicating that two orthosilicic acid units can be brought together in the catalytic pocket of the silicatein molecule by the rotation of the serine and histidine residues of the catalytic center of the silicatein molecule, through the formation of hydrogen bonds, favouring the condensation reaction [30].

The loss of a water molecule which is facilitated by proton transfer from the nitrogen imidazole of the His

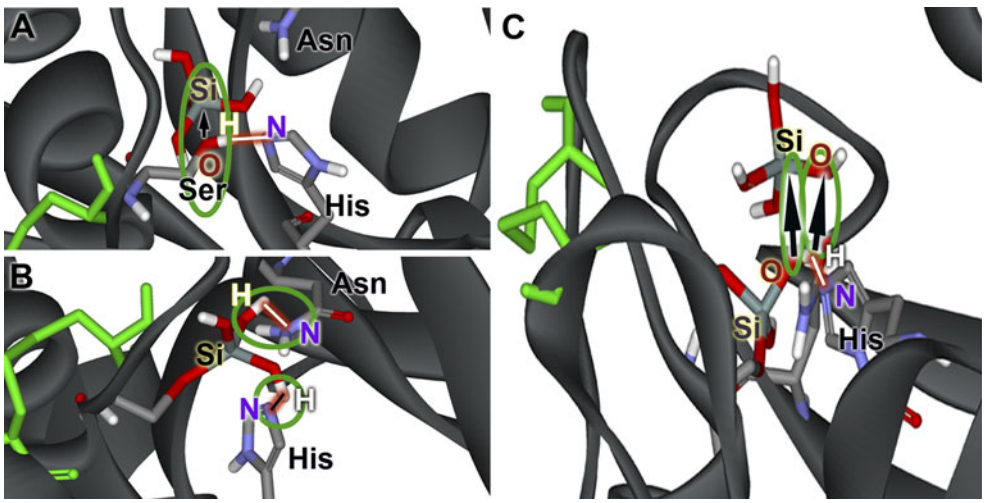

Fig. 3 Detail of the silicatein-α structure (*S. domuncula*) showing the interaction of the three amino acids of the catalytic center (Ser, His and Asn) with orthosilicic acid during different steps of the proposed catalytic mechanism of the enzyme. **A** Nucleophilic attack (*arrow*) of the oxygen atom of the Ser hydroxyl group at the silicon atom of the orthosilicic acid substrate modeled in the catalytic pocket of the enzyme. The nucleophilicity of the Ser oxygen is increased by the formation of a hydrogen bridge to the nitrogen of the His imidazole group (*red-framed white bar*). **B** Formation of hydrogen bridges (*red-framed white and black bars*) of the silicic acid molecule covalently bound to the serine residue to the two other amino acids of the catalytic center of the enzyme (Asn and His). **C** The formation of the latter hydrogen bond increases the nucleophilicity of the oxygen of the corresponding OH ligand of the silicic acid molecule, thus allowing the nucleophilic attack to a second orthosilicic acid species, and a proton transfer from the His residue to an OH ligand of that molecule (*arrows*)

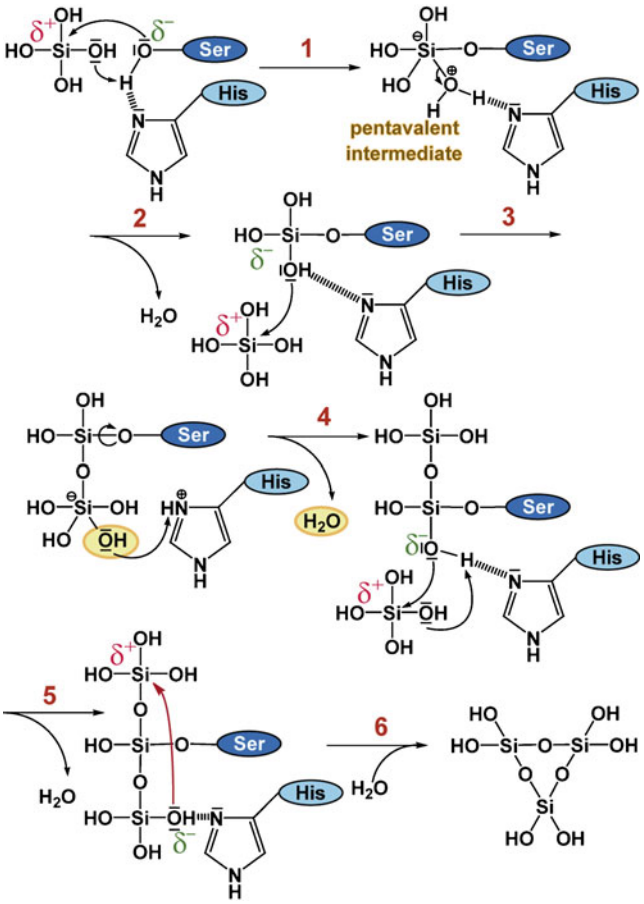

Fig. 4 Proposed mechanism of silicatein-catalyzed polymerization of orthosilicic acid. *Step 1*: Nucleophilic attack of the (*negatively charged*) Ser oxygen atom at the (*positively charged*) silicon atom of the orthosilicic acid substrate and transfer of a proton (originating from the Ser-His hydrogen bridge) from the imidazole nitrogen of the His residue to an OH ligand of the silicic acid molecule. *Step 2*: Release of a water molecule from the formed pentavalent intermediate. *Step 3*: Nucleophilic attack of the oxygen atom of one of the OH ligands of the covalently bound silicic acid molecule at the silicon atom of a second orthosilicic acid molecule, facilitated by hydrogen bridge formation of the bound silicic acid to the imidazole nitrogen. *Step 4*: Loss of water after proton transfer from the imidazole group. *Step 5*: Nucleophilic attack of the oxygen atom of a further OH ligand of the first silicic acid molecule, after rotation of the Si-O-C bond between this molecule and the enzyme Ser residue, at the silicon atom of a third orthosilicic acid species, again facilitated by hydrogen bridge formation to the imidazole nitrogen. *Step 6*: Cyclization of the resulting (enzyme-bound) trisilicic acid by nucleophilic attack of the (negatively charged) oxygen of an OH ligand of the lastly incorporated silicic acid species at the (positively charged) silicon of the second silicic acid species and release of the formed reactive trisiloxane ring after hydrolysis of the Si-O-C bond

residue, generates a disilicic acid molecule (Fig. 4, step 4) that is bound to silicatein through an ester-like linkage. Rotation of the ester bond may then allow interaction of a second OH ligand of the enzyme-bound silicic acid unit with the nitrogen imidazole of the catalytic center His residue, facilitating further growth of the disilicic acid by nucleophilic attack to a third orthosilicic acid molecule (Fig. 4, step 5). Repetition of this reaction cycle (nucleophilic attack, proton transfer, loss of water, rotation) may result in the generation of higher membered silicic acid oligomers (tetrasilicic acid etc.; not shown).

The release of silicatein-bound trisicilic acid (or tetrasilicic acid and higher oligomers) is proposed to be initiated by cyclization of the oligomers (Fig. 4, step 6). Cyclization reactions of siloxane polymers and their mechanisms have long been studied [31, 32]. The strain in the enzyme-bound three-membered (and higher membered) cyclic siloxane oligomers [33, 34] may facilitate this reaction. The covalent linkage between the enzyme and the silicic acid oligomer (ring) is hydrolyzed by water (Fig. 4, step 6).

In the proposed model, the initial step is a nucleophilic substitution reaction at the silicon of the silicic acid substrate. The stereochemistry of nucleophilic substitution reactions at tetracoordinated silicon compounds has been intensively discussed by Holmes [35]. It is assumed that nucleophilic attack results in the transient formation of a trigonal bipyramid, whereby the entering and leaving groups occupy the apical positions.

Future studies must include possible structural changes of the silicatein molecule following interaction of the silica precursor with the catalytic triad. Such conformational changes are indicated by the results of FTIR analysis of protein present in sponge spicules, which revealed a large percentage of β-sheet conformations [30].

In the modeling studies, water has been omitted from the catalytic site, though it cannot be excluded that water acts as a hydrogen bond acceptor/donor in the polycondensation reaction [36]. In the proposed mechanism (Fig. 4) water is only involved in the release step of the final product formed during the reaction cycle, like in the mechanism of TEOS hydrolysis proposed by Cha et al. [16], which involves the hydrolytic release of an enzyme-bond triethoxysilanol species. The latter reaction model does not explain the catalytic (enzyme-mediated) mechanisms of the subsequent condensation step.

Further polymerization of silica formed by the silicatein-mediated reaction may be driven by chemical processes which may be guided *in vivo* by further proteins (silintaphin; [37]). The (chemical) processes can be divided into three distinct phases [13]: First, formation of critical-sized seeds by monomer silicic acid polymerization; second, growth of the nuclei; and third, aggregation of the formed spherical particles. In sponges, the first step is initiated / catalyzed by the enzyme silicatein (which also contributes to the second step), while the latter step is likely directed by silintaphin molecules [5].

The growth of the particles by the so-called Ostwald ripening process finally leads to the formation / deposition of larger, less soluble particles on the expense of silicic acid released from smaller, more soluble particles [14, 38]. In

sponges, this process is accompanied by fusion / sintering of silica particles, most likely mediated by silicatein (and other proteins) incorporated in the formed hybrid structure [5, 39].

4 Conclusions

The silicatein-mediated formation of cyclic (ionized) silicic acid species which are much more reactive than the silicic acid dimer, as proposed in our model, may help to understand the catalytic mechanism of enzymatic (silicatein-mediated) silica polycondensation occurring in sponges at ambient temperature, near neutral pH and comparatively low substrate (orthosilicic acid) concentration.

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References

1. Shimizu K, Cha J, Stucky GD, Morse DE (1998) Proc Natl Acad Sci USA 95:6234–6238
2. Krasko A, Lorenz B, Batel R, Schröder HC, Müller IM, Müller WEG (2000) Eur J Biochem 267:4878–4887
3. Müller WEG, Wang X, Kropf K, Boreiko A, Schloßmacher U, Brandt D, Schröder HC, Wiens M (2008) Cell Tissue Res 333:339–351
4. Schröder HC, Boreiko A, Korzhev M, Tahir MN, Tremel W, Eckert C, Ushijima H, Müller IM, Müller WEG (2006) J Biol Chem 281:12001–12009
5. Müller WEG, Wang X, Cui FZ, Jochum KP, Tremel W, Bill J, Schröder HC, Natalio F, Schloßmacher U, Wiens M (2009) Appl Microbiol Biotechnol 83:397–413
6. Müller WEG, Rothenberger M, Boreiko A, Tremel W, Reiber A, Schröder HC (2005) Cell Tissue Res 321:285–297
7. Müller WEG, Wang X, Sinha B, Wiens M, Schröder HC, Klaus Jochum P (2010) ChemBioChem 11:1077–1082
8. Woesz A, Weaver JC, Kazanci M, Dauphin Y, Aizenberg J, Morse DE, Fratzl P (2006) J Mater Res 21:2068–2078
9. Schröder HC, Natalio F, Shukoor I, Tremel W, Schloßmacher U, Wang XH, Müller WEG (2007) J Struct Biol 159:325–334
10. Müller WEG, Schloßmacher U, Wang XH, Boreiko A, Brandt D, Wolf SE, Tremel W, Schröder HC (2008) FEBS J 275:362–370
11. Mann S (2001) Biomineralization: principles and concepts in bioinorganic materials chemistry. Oxford University Press, Oxford
12. Iler RK (1979) Solubility, polymerisation, colloid and surface properties, and biochemistry. Wiley, New York
13. Perry CC, Belton D, Shafran K (2003) In: Müller WEG (ed) Silicon biomineralization: Biology – Biochemistry – Molecular biology – Biotechnology. Prog Mol Subcell Biol 33:269–299
14. Perry CC (2003) Rev Mineral Geochem 54:291–327
15. Tarutani T (1989) Anal Sci 5:245–252
16. Cha JN, Shimizu K, Zhou Y, Christianssen SC, Chmelka BF, Stucky GD, Morse DE (1999) Proc Natl Acad Sci USA 96:361–365
17. Müller WEG, Wang XM, Belikov SI, Tremel W, Schloßmacher U, Natoli A, Brandt D, Boreiko A, Tahir MN, Müller IM, Schröder HC (2007) In: Bäuerlein E (ed) Handbook of biomineralization, vol 1: Biological aspects and structure formation. Wiley-VCH, Weinheim, pp 59–82
18. Arnold K, Bordoli L, Kopp J, Schwede T (2006) Bioinformatics 22:195–201
19. Turkenburg JP, Lamers MB, Brzozowski AM, Wright LM, Hubbard RE, Sturt SL, Williams DH (2002) Acta Crystallogr D Biol Crystallogr 58:451–455
20. Sali A, Blundell TL (1993) J Mol Biol 234:779–815
21. Shen MY, Sali A (2006) Protein Sci 15:2507–2524
22. Ramachandran GN, Ramakrishnan C, Sasisekharan V (1963) J Mol Biol 7:95–99
23. Hodis E, Sussman JL (2009) Trends Biochem Sci 34:100–101
24. Diller DJ, Merz KM (2001) Proteins 43:113–124
25. Schröder HC, Brandt D, Schloßmacher U, Wang X, Tahir MN, Tremel W, Belikov SI, Müller WEG (2007) Naturwissenschaften 94:339–359
26. Schröder HC, Wang XH, Tremel W, Ushijima H, Müller WEG (2008) Nat Prod Rep 25:455–474
27. Wolf SE, Schloßmacher U, Pietuch A, Mathiasch B, Schröder HC, Müller WEG, Tremel W (2010) Dalton Trans 39:9245–9249
28. Fairhead M, Johnson KA, Kowatz T, Mcmahon SA, Carter LG, Oke M, Liu H, Naismith JH, Van Der Walle CF (2008) Chem Commun 1765–1767
29. Zhou Y, Shimizu K, Cha JN, Stucky GD, Morse DE (1999) Angew Chemie [Int Ed] 38:780–782
30. Croce G, Frache A, Milanesio M, Marchese L, Causà M, Viterbo D, Barbaglia A, Bolis V, Bavestrello G, Cerrano C, Benatti U, Pozzolini M, Giovine M, Amenitsch H (2004) Biophys J 86:526–534
31. Jacobson H, Stockmayer WH (1950) J Chem Phys 18:1600–1606
32. Flory PJ, Semlyen JA (1966) J Am Chem Soc 88:3209–3212
33. Wallace S, West JK, Hench LL (1993) J Non-Cryst Solids 152:101–108
34. Hench LL, West JK (1995) Ann Rev Mat Sci 25:37–68
35. Holmes RR (1995) Phosphorus, Sulfur, and Silicon 99:207–238
36. Garofalini SH, Martin G (1994) J Phys Chem 98:1311–1316
37. Wiens M, Bausen M, Natalio F, Link T, Schloßmacher U, Müller WEG (2009) Biomaterials 30:1648–1656
38. Perry CC, Keeling-Tucker T (2000) J Biol Inorg Chem 5:537–550
39. Müller WEG, Wang X, Burghard Z, Bill J, Krasko A, Boreiko A, Schloßmacher U, Schröder HC, Wiens M (2009) J Struct Biol 168:548–561