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Bioinspired control of colloidal silica *in vitro* by dual polymeric assemblies of zwitterionic phosphomethylated chitosan and polycations or polyanions

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ABSTRACT

This paper focuses on the effects of biological and synthetic polymers on the formation of amorphous silica. A concise review of relevant literature related to biosilicification is presented. The importance of synergies between polyelectrolytes on the inhibition of silicic acid condensation is discussed. A specific example of a zwitterionic polymer phosphonomethylated chitosan (PCH) is further analyzed for its inhibitory activity. Specifically, the ability of PCH to retard silicic acid condensation at circumneutral pH in aqueous supersaturated solutions is explored. It was discovered that in short-term studies (0–8 h) the inhibitory activity is PCH dosage-independent, but for longer condensation times (>24 h) there is a clear increase in inhibition upon PCH dosage increase. Soluble silicic acid levels reach 300 ppm after 24 h in the presence of 160 ppm PCH. Furthermore, the effects of either purely cationic (polyethyleneimine, PEI) or purely anionic (carboxymethylinulin, CMI) polyelectrolytes on the inhibitory activity of PCH is systematically studied. It was found that the action of inhibitor blends is not cumulative. PCH/PEI blends stabilize the same level of silicic acid as PCH alone in both short-term (8 h) and long-term (72 h) experiments. PCH/CMI combinations on the other hand can only achieve short-term inhibition of silicic acid polymerization, but fail to extend this over the first 8 h. PCH and its combinations with PEI or CMI affect silica particle morphology, studied by SEM. Spherical particles and their aggregates, irregularly shaped particles and porous structures are obtained depending on additive or additive blend. It was demonstrated by FT-IR that PCH is trapped in the colloidal silica matrix.

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1. Introduction

Nature directs the formation of amorphous hydrated silica (biosilica) in living organisms, such as marine/freshwater diatoms and terrestial plants via the important process of biosilicification [1–3]. One can put this in perspective by considering that the gross production of biogenic silica in surface waters was estimated to be ~240 ± 40 terramoles of silicon per year. This means, marine biological systems process the breathtaking amount of about 6.7 gigatonnes of silicon annually [4]. Biosilicification is a unique kind of biomineralization. Its uniqueness and differentiation from a plethora of other biogenic, metal-containing minerals (*e.g.* calcite, aragonite, vaterite, octacalcium phosphate, hydroxyapatite, iron sulfides, *etc.*) lies not only with the simple, albeit unique, structure of the final product, amorphous silicon dioxide (silica), but also with the intricate (and enigmatic) mechanism of its formation. Whereas metal carbonate and phosphate solids are crystalline ionic materials whose formation is governed by cation–anion association and solubility equilibria, silica is an oxide of amorphous nature formed by a complicated inorganic polymerization process that is controlled by organic macromolecules, resulting in "exotic" morphologies at the micron scale [5].

Silicon, in the form of silicic acid, is a fundamental nutrient not only for diatoms, but also for silicoflagellates, radiolaria, and many sponges, all of which polymerize it to build skeletons of biogenic silica. According to Maldonado et al. [6], our current understanding of the silicon cycle in the ocean assumes that diatoms dominate not only the uptake of silicic acid, but also the production and recycling of biogenic silica, and that other organisms with siliceous skeletons, including sponges, radiolarians, and silicoflagellates, play a negligible role. These authors showed that the retention of "Si" by siliceous sponges in some sublittoral and bathyal environments is substantial and that sponge populations function as "Si" sinks. Therefore, sponges may affect "Si" cycling dynamics and "Si" availability for diatoms, particularly in Si-depleted environments. It was strongly suggested by Maldonado that the role of sponges in the benthopelagic coupling of the "Si" cycle is significant. For example, Antarctic giant hexactinellids, such as *Rossella nuda* and *Scolymastra joubini*, which may be up to 2 m tall, 1.4 m in diameter, and up to 600 kg wet weight, containing up to 50 kg biogenic silica each.

Silica deposition is also a fundamental process in sponges. According to the modern point of view, two different mechanisms of silicification in sponges are proposed: enzymatic (silicatein-based) and non-enzymatic, or self-assembling (chitin- and collagen-based). There are two possible mechanisms for enzyme catalysis [7]: (i) stabilization of one molecule of deprotonated silicic acid (the nucleophile) at the active site, which will then react with another molecule of silicic acid; or (ii) stabilization of a protonated silicic acid (the electrophile) which will then react with another molecule of silicic acid.

Silicon was found associated with glycosaminoglycans bound as an ether or ester-like silicate with C – O – Si or C – O – Si – O – Si – O – C bonds, in amounts of one Si atom/130–280 repeating units of the organic [8]. Recently, Ehrlich et al. isolated and identified chitin from skeletal formations of some marine glass sponges for the first time. The presence of chitin within the framework skeleton of *Farrea occa* [9] and *Euplectella aspergillum* [10] as well as separate spicules *Rossella fibulata* [11] could also be revealed by gentle NaOH-based desilicification technique established by Ehrlich et al. [12,13]. It was suggested that silicate ions and silica oligomers preferentially interact with glycopyranose rings exposed at the chitin surface, presumably by polar and H-bonding interactions [14].

Furthermore, Ehrlich et al. reported recently and for the first time an example of *Hyalonema sieboldi* glass sponge whose spicules are a biocomposite containing a silicificated collagen matrix. The high collagen content is the origin of the unique mechanical flexibility of the spicules [12,13].

2. Macromolecules that affect silicification

The *in vivo* process of silicic acid condensation to yield silica nanopatterns is profoundly influenced by complex organic biomolecules. These, (mainly studied in sponges and diatoms) play an integral and complicated role in biosilica morphosynthesis [15,16]. Insight into the nature of this intricate organic matrix has been realized through characterization of diatom biosilica-associated peptides (silaffins, natSil-1 and natSil-2) and long-chain polyamines (LCPA) [17]. Silaffins contain repeated peptide sequences that are rich in basic amino acids (lysine and arginine) and unusual alkylamine post-translational modifications, see Fig. 1.

Silaffins assist in the formation of the silica wall that encompasses the diatom [18]. Furthermore, it has been suggested that formation of organic matrices composed of polyanionic natSil-2-like phosphoproteins and polycationic silica-forming components may represent a widespread mechanism in diatom biosilica morphogenesis [19].

Morse and coworkers have extensively studied the silica-producing proteins, silicateins, derived from sponge spicules. Through point mutation studies, histidine and serine were identified as the catalytic component in the active site of the protein [20]. Silaffins, isolated from *Cylindrotheca fusiformis*, have been shown to form silica at pH ~5.5. Structural analysis of one such silaffin, NatSil1A, revealed that serine residues were phosphorylated and the lysine were post-translationally modified forming N-dimethyl-lysine, N-trimethyl-hydroxy-lysine, and long-chain polyamines, derivatives of polypropylenimine [21].

HF-based extraction experiments allowed the isolation of three silaffins [22]. Two of them, the silaffin-1A1 and silaffin-1A2, were sequenced by mass spectrometry and were shown to be encoded by the same sil1 gene from *C. fusiformis* [23]. These silaffin-1A peptides are enriched in lysine and serine groups. Moreover, amino groups of the lysine residues are modified by introduction of long-chain polyamines, N,N-dimethyl-lysine or N,N,N-trimethyl-hydroxy-lysine. Another fraction of methylated polyamines were also recovered and identified from a HF extraction procedure [24]. When put in contact with HF extracts, aqueous solutions of prehydrolyzed silicon alkoxides at pH 7 form and precipitate silica nanoparticles.

Of particular interest is the fact that, at pH ~5, unsubstituted polyamines did not induce silica formation, whereas alkylated ones did. This is to be linked with previous reports on a possible acid pH within the Silica Deposition Vescicle (SDV) [25–28]. More recently, HF treatment in milder conditions revealed that the hydroxy groups of the silaffin serine residues were phosphorylated, illustrating the possible degradation of pristine molecules during the initial extraction process [20–22]. These zwitterionic proteins exhibit self-assembly properties that may be involved in the frustule morphogenesis [29].

In addition to silaffins, a biomimetic analogue derived from a repeat unit of the Natsil gene, the R5 peptide (SSKKSGSYSGSKGSKRRIL), induces precipitation of silica from monosilicic acid [30]. Mutation studies of the R5 peptide have shown that the RRIL motif is critical for silica formation, as it causes the peptide to self assemble, providing a locally high amine concentration, and promoting the subsequent condensation of monosilicic acid. Moreover, diatoms also contain long-chain polyamines such as N-methylated poly(propylene imines) attached to putrescine cores that are capable of precipitating silica [31].

The discovery that positively charged biomacromolecules (zwitterionic to be precise) can direct biosilica synthesis *in vivo* and *in vitro* was followed by a battery of research efforts to discover and test synthetic