Putrescine homologues control silica morphogenesis by electrostatic interactions and the hydrophobic effect†

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A systematic model study on the role(s) of putrescine homologues on silicification is presented and it is proposed that electrostatic forces between additive and silicic acid, and the hydrophobic behaviour of the additives are both important in silicification.

In nature, several classes of biosilicifying organisms process soluble silicon to generate hierarchically organised ornate biogenic silica structures under mild conditions of pH and temperature and exert precise control in shaping biosilica.[1] In contrast, current synthetic procedures typically employ relatively harsh conditions for the preparation of silicas and exhibit relatively poor morphological control.[2] In order to gain insights into biosilicification, several studies have been carried out on biosilicifying organisms wherein organic biomolecules have been isolated and identified (reviewed in ref. 3). These bioextracts control *in vitro* silicification *via* catalysis, aggregation and/or scaffolding.[4]

In this model study on bioinspired silica, we have chosen to investigate the roles of simple amines—putrescine homologues—on silicification [1,2-diaminoethane (DA2), 1,4-diaminobutane or putrescine (DA4), 1,6-diaminohexane (DA6), 1,8-diaminooctane (DA8) and 1,10-diaminodecane (DA10)]. Although several biomimetic analogues possessing amine functionalities, including aminoalkanes, have been investigated for their roles in silica synthesis *in vitro*,[4,5] the current study is the first extensive report on the use of low molecular weight alkyldiamines in bioinspired silicification. We also provide an insight into the processes occurring during the transformation of monomers to oligomers to useful material. The silica precursor used was a water soluble silicon complex—dipotassium silicon triscatecholate†—which rapidly liberates orthosilicic acid at neutral pH unlike alkoxysilane precursors that produce a mixture of pre-condensed polysilicic acid and we are therefore able to monitor the condensation of "true" monosilicic acid.[6] Throughout this study, the silicon to amine molar ratio was kept constant at one.

During silicic acid polymerisation, orthosilicic acid initially polymerises to yield dimers and trimers that will eventually lead to the formation of stable nuclei. A colorimetric molybdic acid assay was used to monitor the kinetics of silica polymerisation. The rates of trimerisation (a third order reaction with respect to monomer concentration; rate constant k_3) and further oligomerisation (a first order reversible reaction; rate constants k_+, k_-) were monitored by this silicomolybdate blue assay.[6,7] The rate constants for the formation of trimers from monomer and dimer were found to increase by *ca.* two fold for the DA2 system and *ca.* 2.5 times for the longer chain diamines when compared with the blank (with no trend observed over these samples); Fig. 1a. Increases in the third order rate constants were found to be statistically significant, exceeding two standard deviations calculated using four 'blank'data-sets. The next stage of the reactions involves the reversible addition of orthosilicic acid to already formed oligomers and showed less variation for the silicas prepared in the presence of diamines (Fig. 1a). Previous work on silicic acid condensation in the presence of Group 1 cations has shown a rate dependency related to the size of the hydration sphere of the cations.[7] Here the cationic species and concentration were constant and only the hydrophobic portion of the additive varied as the chain length of the diamine increased. The increase in trimerisation rate can be explained by the formation of a clathrate cage-like water structure around the nonpolar surfaces of the alkyldiamines. The cage-like structure may tie up some of the free water molecules (*i.e.* those not associated with ion hydration shells)[8] resulting in higher reactant (silicic acid) concentrations in the bulk aqueous environment and also a possible reduction in the hydration shells around the anionic silica species. Under these conditions, reactions involving anions, such as the condensation of a silicate anion with a neutral silicate species, would be expected to show an increase in rate as observed for condensation reactions performed

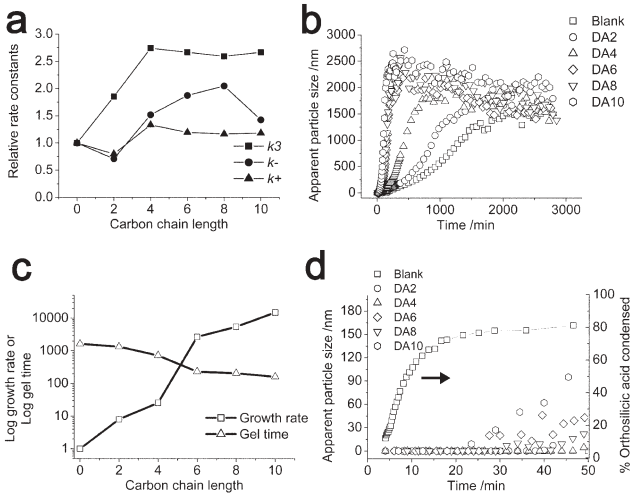

Fig. 1 (a) Effect of diamine chain length on trimerisation and oligomerisation reactions. (b) Particle growth with time, and (c) relative growth rate and gel time as a function of diamine chain length. (d) Comparison of particle aggregation with the free silicic acid as functions of time.

† Electronic supplementary information (ESI) available: Experimental methods and additional data from SEM, TEM, gas adsorption, TGA and FTIR analyses. See http://www.rsc.org/suppdata/cc/b5/b504310g/

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in the presence of the diamines.[9] Although the silicic acid condensation rates were higher in the presence of the diamines, the rates showed little dependence on carbon chain length which can be explained as follows. As the hydrophobic nature of the longer chain diamines increases, there is a greater reduction in entropy as a result of the re-assembly of water molecules around the nonpolar regions, and as a consequence micellisation is favoured.[8,10] The reduction in free water around silicic acid is limited by micellisation and as a consequence no significant change was observed in the trimerisation reaction rates in the presence of longer chain diamines (DA6–DA10).

Once the stable nuclei are formed, particle growth and aggregation can occur. This process has been monitored using photon correlation spectroscopy (PCS) (Fig. 1b). Duplicate analyses were conducted for each sample to confirm the reproducibility of the data. The relative growth rates determined in the presence of diamines were enhanced up to *ca.* 15 000 times as the carbon chain length increased. In addition, there was a distinct step change in the relative growth rate between the additives DA4 and DA6 (Fig. 1c). Gelation times decreased with diamine chain length and also showed the step change in behaviour between DA4 and DA6. Comparison of orthosilicic acid concentration with PCS data (Fig. 1d), shows that it is particle aggregation and not particle growth that is being monitored, as in excess of 80% of monomer is already condensed before significant growth is observed by this technique (*ca.* 23 min for DA10). The observed behaviour can be explained as follows. For the shorter chain diamines (DA2 and DA4) the initial increase in the aggregation rates are probably due to surface charge neutralisation of the negatively charged primary silica particles by the cationic diamines—the electrostatic effect. As the diamine chain length increases, the diamines may additionally be able to bridge the particle double layer, resulting in accelerated growth (observed as the step change in the growth rate). The coacervation of diamine coated silica particles (the hydrophobic effect), which increases with increasing chain length, may be manifesting itself as the continued rate and size increase observed.

The process of maturation and evolution of structure, in the presence of diamines, has been studied using nitrogen gas adsorption and electron microscopy.† Surface areas decreased with time of reaction and with increasing chain length of the additives (Fig. 2a and Fig. S1c of the ESI†). Surface area reduction with time is the expected consequence of Ostwald ripening. Reduction of surface area with increasing diamine chain length in silicas isolated at shorter maturation times may be a consequence of the degree of condensation of the silicas produced since pore radii in the isolated material were unaffected (Fig. 2b). Thermogravimetric analysis did not show significant uptake of organic material (Fig. 2d and Fig. S2†) which could otherwise be the cause of surface area reduction through complete infilling of some pores. Measurements of pore radii show the beginning of the permanent structure after 1 day (before 1 day pore radii were around 20 Å for all silicas); see Fig. S1d.† By 7 days this resulted in pore radii of up to 50 Å for DA6 and DA8 (Fig. 2b) possibly representing a templating effect of micelles, and 37 Å for DA10. The reduced pore radii of the material produced with DA10 is thought to be a function of greater flexibility of the diamine compared with the other diamines used, thus allowing some degree of coiling of the hydrocarbon chains to produce smaller micelles

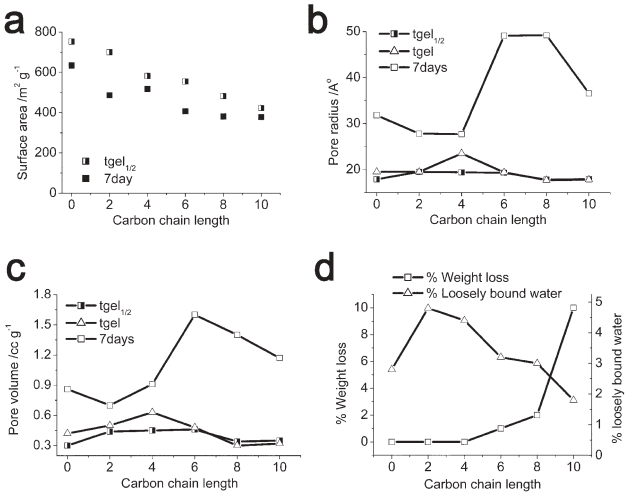

Fig. 2 (a) Surface area, (b) pore size, and (c) pore volume measurements of silica samples with varying diamine chain lengths at two/three time intervals. (d) Weight loss from samples during thermogravimetric analysis of silica.

(unpublished data). Pore volumes followed the same trends; material with larger pores had larger pore volumes, *i.e.* some conservancy of overall pore populations (Fig. 1c).

Thermogravimetric analysis on samples isolated after different periods of reaction showed a virtual absence of significant levels of entrained organic material up to one day. However after 7 days maturation the level of organic entrainment increased with the additive chain length due to the increasingly condensed silica matrix (Fig. 2d and Fig. S2†). The percentage of loosely bound water determined as the weight loss below 373 K decreased with increasing diamine carbon chain length—increasing hydrophobicity. The presence of hydrophobic groups associated with the aggregating silica particles would be expected to squeeze out water between the forming pores.

Scanning electron microscopic data showed an ordering of silica structure with time for all samples. In particular, the silica prepared in the presence of DA10 (Fig. 3 and Figure S5), which initially had a very open structure due to very rapid aggregation, but became more ordered and close packed during the Ostwald ripening phase. An increasingly open structure with diamine chain length was also observed. Primary particles measured after 7 days maturation by

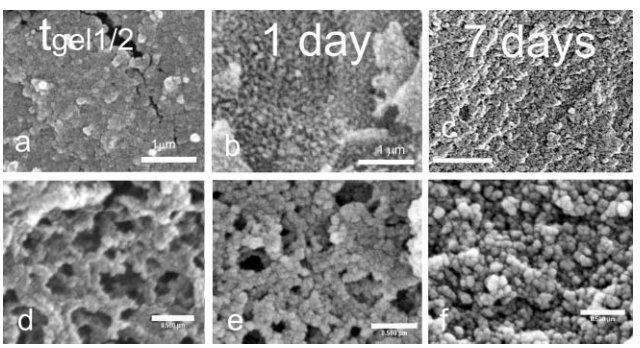

Fig. 3 Representative SEM micrographs from selected silica samples. (a)–(c) blank (bar = 1 μm) and (d)–(f) 1,10 diaminodecane (DA10) (bar = 500 nm).†

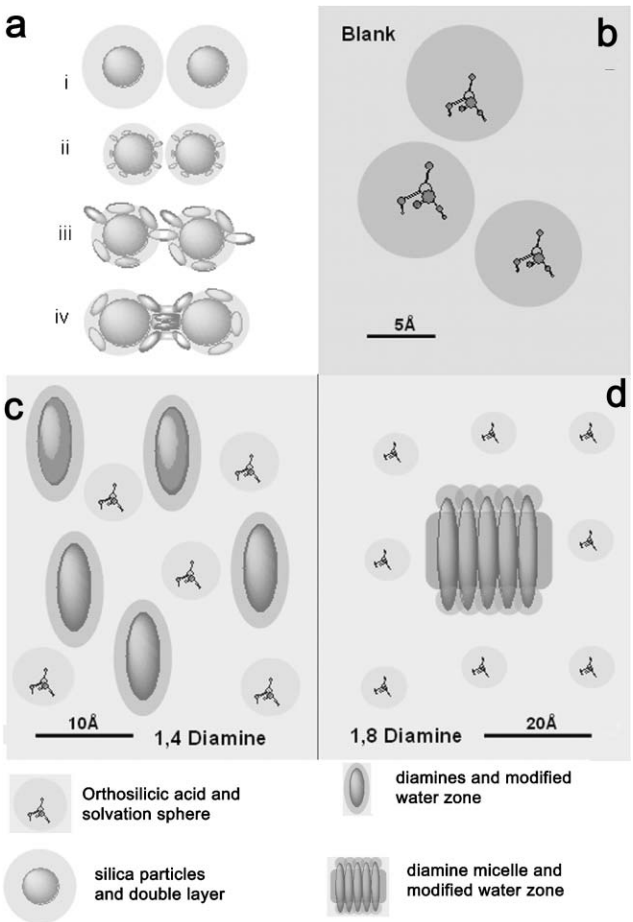

Fig. 4 Schematic representation of the effect of n-alkyldiamines on the condensation of silicic acid and subsequent silica particle aggregation modes. (a) Aggregation modes: i. in the absence of diamines, ii. charge neutralisation and reduction in the thickness of the double layer in the presence of shorter diamines, iii. particle bridging by longer chain diamines and iv. coacervation due to increased micellisation with increasing carbon chain length and hence hydrophobic character. (b)–(d) Kinetic control of diamines: (b) Blank, showing solvation barrier to condensation, (c) Addition of diamines with hydrophobic domains reduces the solvation barrier and hence increases the condensation rate. (d) Subsequently, the formation of micelles by longer chain diamines limits this effect by minimising the hydrophobic water surface. Colour shades are used to indicate the different 'ordering' of water (the darkest shade represents the most organised water).

TEM were slightly bigger (5–7 nm) when compared with the blank sample (4 nm) but with no specific trend (Figure S3). Confirmation that the structures observed were siliceous was made by EDS and FTIR analyses (Figure S3 and Figure S4). Selected area electron diffraction carried out on selected samples showed no evidence of ordering on the atomic or molecular scale, confirming the amorphous nature of the silica produced (data not shown).

The current *in vitro* bioinspired silicification study confirms the importance of amines in silica synthesis as has been proposed for biosilicas produced by diatoms in nature.[11] The alkyldiamines chosen for study were found to catalyse silica formation and promote silica aggregation, the latter contributing greatly to the structure and physical properties of the silicas generated. The electrostatic forces between the amine end-groups of the diamines and the silica oligomers are important in regulating silicification. This study has, however, shown that the hydrophobic behaviour exhibited by diamines on silica formation is in direct relation to the carbon chain length. The hydrophobic effect is a fundamental factor regulating *in vivo* processes, such as protein folding and protein–substrate interactions.[10,12] The stability and hence the function of proteins, for example, is altered by the presence of solute due to the rearrangement of water molecules. This effect is particularly enhanced when the solutes added are hydrophobic in nature. In the study described here, the addition of diamines to the reaction medium alters the water structure around silicic acid by the hydrophobic effect, thus changing the stability of silicic acid, modifying condensation and aging, and generating siliceous materials that differ greatly in their physical properties. Fig. 4 shows schematically the proposed effects of the n-alkyldiamines on the condensation of silicic acid and subsequent particle aggregation modes together with the effects of the diamines on the 'ordering' of water in the vicinity of the reactive species. It is thus thought that the results presented herein on controlled silica formation using alkyldiamines may help understand the role(s) such organic molecules (functionalities) may play in (bio)silicification. Current research is directed towards understanding the effects of amines found widely *in vivo* on silica formation *in vitro* and will be reported in due course.

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