Supporting Information

for

Angew. Chem. Int. Ed. Z52212

© Wiley-VCH 2003
69451 Weinheim, Germany
Biomimetic control of size in the polyamine-directed formation of silica nanospheres

Manfred Sumper* , Sonja Lorenz, Eike Brunner

* Prof. Dr. M. Sumper, Lehrstuhl Biochemie I, Universität Regensburg, D-93053 Regensburg, Germany, Phone: +49 941 943-2833, Fax : +49 941 943-2936
  e-mail: manfred.sumper@vkl.uni-regensburg.de
Prof. Dr. E. Brunner, S. Lorenz, Institut für Biophysik und physikalische Biochemie
Universität Regensburg, D-93053 Regensburg, Germany
Replacement of phosphate by polyvalent anions.

Since the size of silica nanospheres is controlled by the concentration of multivalent anions, replacement of phosphate by polyvalent anions or negatively charged surfaces can be expected to influence silica morphology as well. This possibility was tested by replacing phosphate by double-stranded DNA and anionic surfactants, respectively. DNA directed the precipitation of strings of small silica nanoparticles (a). EDXA measurements (data not shown) proved the incorporation of carbon, nitrogen, oxygen, silicon, and phosphorus into this structure. Thus, the anionic backbone of the DNA molecule appeared to guide its own encapsulation with silica. Even more complex silica morphologies were observed by using sodium dodecyl sulfate as the anionic component. Depending on the concentration of the surfactant, collapsed vesicular or spongy silica precipitates (b and c) could be produced. Some of these structures are reminiscent of silica obtained in a chemically different approach using cetyltrimethylammonium chloride as surfactant template and tetraethoxysilane as silica precursor (H. Yang, N. Coombs, G. A. Ozin, *Nature* 1997, 386, 692-695).

**Figure O1:** SEM images of silica precipitates obtained in the presence of polyanionic substances. In the silica precipitation assay (see Fig. 1 in main text), phosphate was replaced by polyanionic substances and the buffer system was 30 mM sodium acetate, pH 5.5. a, 10 µg/ml dsDNA from salmon sperms. b, Sodium dodecyl sulfate (0.6 mM). c, Sodium dodecyl sulfate (1.2 mM). Scale bars, 200 nm.
$^{31}$P NMR spectroscopy of phosphate-containing samples.

$^{31}$P NMR spectroscopy could be applied to the phosphate-containing samples. As the phosphate ions are not covalently bound to the polyamine molecules, rapid exchange between bound and free phosphate was observed. Therefore, only one single signal is observed. This signal changes its chemical shift and linewidth (full width at half maximum) as a function of orthophosphate concentration. The chemical shift decreases from ca. 2.3 ppm for very low orthophosphate concentrations to ca. 1.4 ppm for high phosphate concentrations. The former value approximately corresponds to the chemical shift in the polyamine-bound state while the latter value is characteristic for free phosphate. A similar behaviour is observed for the linewidth (see figure below). An estimation shows that the $^{31}$P NMR linewidth of orthophosphate ions bound to a single polyamine molecule of 1.55 kDa should not exceed 1 Hz. In contrast, a linewidth of ca. 7 Hz was measured for phosphate ions bound to the polyamines which indicates phosphate binding to larger polyamine aggregates.

Figure O2: Dependence of the linewidth (full width at half maximum) of the $^{31}$P NMR signal of orthophosphate added to an aqueous solution of polyamine from S. turris.
Analysis of partial reaction steps in silica formation.

The kinetics of monosilicic acid polymerization in the physiologically relevant pH range was determined in the absence or presence of *S. turris* polyamines (Figure O3, a).

Silica formation was also followed by the inspection of intermediate silica-containing structures by SEM and EDXA (Figure O3, b and c). The first structures to be observed (*t* = 8 min) were large aggregates of interconnected nanospheres with diameters down to 30 nm. These structures contained silica as well as carbon, nitrogen, and phosphate as determined by EDXA analysis. Under the acidic conditions of this experiment (pH 5.0), it took 20 min until most of the intermediate aggregates were consumed and converted into a nearly monodisperse population of 500 nm-spheres.

**Figure O3:** a. Plot of the concentration of Si(OH)₄ (60 mM at *t* = 0 min) against time in 30 mM sodium phosphate, pH 5.5 (red line), and in the presence of 0.3 mM polyamine (blue line). The temperature was 25 °C. Si(OH)₄ was determined as its trimethylsilyl derivative by capillary gas chromatography (D. Hoebbel, G. Garzo, G. Engelhardt, R. Ebert, E. Lippmaa, M. Alla, *Z. Anorg. Allg. Chem.* 1980, 465, 15-33). b. Time-resolved analysis of silica morphogenesis in vitro (30 mM sodium phosphate, pH 5.0, 0.2 mM polyamine, and 40 mM silicic acid). SEM images of silica structures obtained after 8 min (left) and 20 min (right). Scale bars, 300 nm. c. Elemental composition of silica structures obtained after 8 min as determined by EDXA. The Al and Cu signals are caused by the sample holder.
EXPERIMENTAL SECTION

PURIFICATION OF POLYAMINES
The initial purification steps of polyamines from *S. turris* were as described (N. Kröger, R. Deutzmann, C. Bergsdorf, M. Sumper, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14133-14138). The polyamines eluted by 2M NaCl/20mM Na₂CO₃ from the HighS cation exchange column (Bio-Rad) were extracted twice with chloroform/methanol (3/2 by volume). The organic phase was evaporated and the residue dissolved in water. This material was size fractionated on a Superdex-Peptide HR 10/30 column (Amersham Pharmacia) equilibrated with 250 mM ammonium acetate. The flow rate was 0.25 ml/min and fractions were analysed for polyamines by Tris-Tricine-SDS-PAGE (H. Schägger, G. von Jagow, *Anal. Biochem.* **1987**, *166*, 368-379) with subsequent Coomassie blue staining. Polyamine containing fractions were dialyzed against water (Spectra/Por CE dialysis tubing; molecular mass cut off 500 Da) and concentrated by lyophylization.

MASS SPECTROMETRY OF POLYAMINES
Electrospray ionization/MS and fragmentation analysis were performed by using an Ion Trap ESQUIRE LC instrument (Bruker). The chain length distribution of *S. turris* polyamines ranged from 15 to 21 N-methyl-propyleneimine repeated units exhibiting a maximum at 18 repeated units.

QUANTIFICATION OF POLYAMINES
Polyamines were semi-quantitatively determined by a previously described staining method that detects primary, secondary, and tertiary amines on TLC plates (E. Stahl, *Thin-layer chromatography*, Springer, Berlin 1969). 1 µl of a polyamine solution was dotted onto a silica gel 60 TLC plate. After drying, the plate was sprayed with an aqueous cobalt thiocyanate solution (2 M ammonium thiocyanate, 200 mM cobalt chloride hexahydrate). The synthetic polyamine DAB-Am-16 (Aldrich) served as a reference. This compound is a dendrimer of 1686.8 Da molecular mass that is made up of propyleneimine units attached to a putrescine residue.

PREPARATION OF SILICIC ACID
A freshly prepared solution of 1 M tetramethoxysilane in 1 mM HCl was incubated at 20 °C for exactly 15 min and immediately used as a source of monosilicic/ disilicic acid (R.K. Iler, *The chemistry of silica*, John Wiley & Sons, New York 1979).
IN VITRO PRECIPITATION OF SILICA AND SEM ANALYSIS
A typical precipitation assay contained in 50 µl: 30 mM sodium acetate, pH 5.5, 0.2 mM polyamine, and a multivalent anionic compound like phosphate (0.1 to 100 mM). Silica formation was initiated by the addition of 2 µl silicic acid, prepared as described above. After 12 min at 25 °C, the reaction was terminated by adjusting the pH to 3.0 with HCl. Silica was collected by centrifugation (4 min, 12,000 rpm). The precipitate was washed twice with water, then suspended in water, applied to an aluminium sample holder, and air dried. Silica precipitates were analysed without sputter-coating with a LEO1530 field-emission scanning electron microscope equipped with energy dispersive X-ray analysis (EDXA, Oxford instruments).

NMR ANALYSIS
NMR spectra were acquired on a DMX-500 spectrometer (Bruker) operating at 500 MHz $^1$H resonance frequency. Diffusion measurements were carried out by pulsed field gradient experiments (E.O. Stejskal, J.E. Tanner, J. Chem. Phys. 1965, 42, 288-292; J. Kärger, D. Ruthven, Diffusion in zeolites and other microporous solids, John Wiley & Sons, New York 1992).

DYNAMIC LIGHT SCATTERING (DLS) EXPERIMENTS
Dynamic light scattering experiments were performed on a MALVERN HPPS5001 nanosizer.