The Characterisation of the Nature of Silica in Biological Systems

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The silica in some biominerals has been investigated by electron microscopy and i.r. and n.m.r. spectroscopy and has been shown to be amorphous and to contain many Si–OH units.

The characterisation of silicon atoms in silicates and zeolites has been recently developed by Lippmaa[1,2] and subsequently by Fyfe.[3,4] These authors have used solid-state n.m.r. methods to ascertain the *chemical* environment of the Si atom bound by four oxygens which link either to further Si atoms in Si–O–Si or to aluminium Si–O–Al where characteristic chemical shift ranges are found[2,4] for the five possible Si environments in these systems: Si[4Al], Si[3Al, 1Si], Si[2Al, 2Si], Si[1Al, 3Si], and Si[4Si]. When these data are correlated with electron microscope studies of the aluminosilicates substantial insight is obtained into the detailed structures of these materials.[5] In recent work, Maciel and co-workers[6] have studied silica gel and have detected peaks due to silicon atoms functionalized by one and two hydroxy-groups $Si(OSi\equiv)_3(OH)$ and $Si(OSi\equiv)_2(OH)_2$ as well as $Si(OSi\equiv)_4$ atoms in the 'bulk' of the sample.

We have been especially interested in the forms of silica which occur in biological systems for example in the choanoflagellate, *Stephanoeca diplocostata* Ellis, and in various grasses. As we shall show, the silicon environments are of the same type as found in synthetic silica gel, and can also contain various amounts of tightly held water. Using silica, biological systems can make almost any shape of object from opaline spheres in plants, to rods in choanoflagellates and plates of a variety of dimensions in diatoms. Peculiarities of silica have been stressed elsewhere but here we refer to studies of its nature by electron microscopy, and solid-state n.m.r. and i.r. spectroscopy.

We have reported elsewhere that the very high resolution electron microscope study of the rods of *Stephanoeca diplocostata* shows that the silica present was amorphous down to the highest level of resolution.[9] The same experimental procedure using a 200CX Jeol electron microscope, has now been employed to examine the nature of the silica in the grass *Phalaris canariensis*. The image produced is shown in Figure 1. The opaline bodies have no order down to the level of 10 Å. Using the *X*-ray fluorescence attachment on the electron

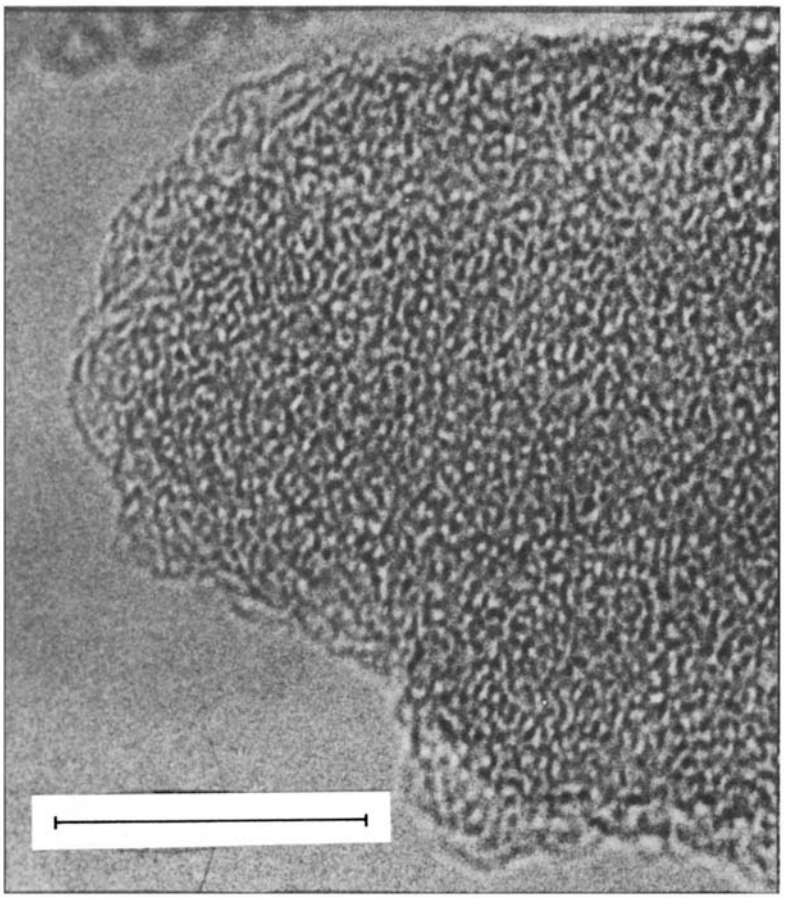

Figure 1. High resolution electron micrograph of a thin edge from a fractured silica hair of *Phalaris canariensis* (×6 100 000). The bar on the figure is 10 nm.

Table 1. ^{29}Si Chemical shifts (p.p.m. from Me_4Si) in silicas.

Compound	$Si(OSi\equiv)_4$	$Si(OSi\equiv)_3(OH)$	$Si(OSi\equiv)_2(OH)_2$	Ref.
Synthetic silica gel	−109.3	−99.8	−90.6	6
Biological silica from *Phalaris canariensis*[a]	−110.8	−101.8	−91.8	This work
Dealuminated zeolite	−110	−101	−91	7
	−110	−100	−90	7

[a] The synthetic silica gel and the biological silica give rise to broadline spectra which is to be expected if the co-ordination around the Si deviates significantly and irregularly from the fixed tetrahedra of crystalline zeolites and minerals such as quartz and cristobalite.

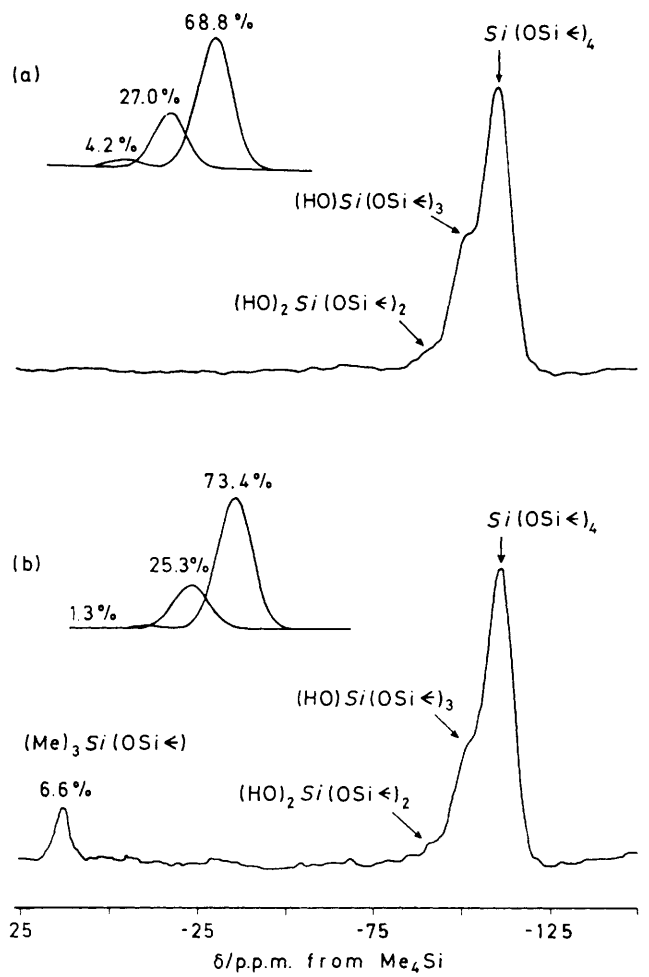

Figure 2. (a) ^{29}Si M.a.s. n.m.r. spectrum obtained at 79.5 MHz of fibres from *Phalaris canariensis* with the peak assignments as indicated and (inset) the calculated deconvolution of the spectrum in terms of Gaussian peaks of the indicated relative intensities. (b) ^{29}Si M.a.s. n.m.r. spectrum of the same sample as in (a) after reaction with trimethylchlorosilane Me_3SiCl and extraction and drying of the product with (inset) the calculated deconvolution of the high-field region as before with the relative peak intensities (total 100%). The relative intensity of the peak at +13.5 p.p.m. due to the attached silylating agent is indicated above the absorption. This is approximately equal to the change in relative intensities of the three peaks in the high field region as a result of Me_3SiCl reacting with the hydroxy-groups present to convert some of the $Si(OSi\equiv)_2(OH)_2$ species into the $Si(OSi\equiv)_3(OH)$ species and some of the $Si(OSi\equiv)_3(OH)$ species into the $Si(OSi\equiv)_4$ species.

microscope, elemental analysis showed no atoms other than silicon to be present in these materials. Note that lighter atoms than sodium cannot be observed.

We turn next to the solid-state n.m.r. study of these silicas (Table 1). Unfortunately an adequate size sample of silica from *Stephanoeca diplocostata* was not available. However we have been able to obtain good ^{29}Si n.m.r. spectra from the biological silica from *Phalaris canariensis*. ^{29}Si Magic-angle spinning (m.a.s.) n.m.r. spectra were obtained on a conventional Bruker WH-400 high-resolution spectrometer using a home-built probe which we have shown gives excellent spectra for a wide range of inorganic nuclei.[10] The solid-state ^{29}Si n.m.r. spectrum of this biological material is shown in Figure 2(a) together with peak assignments. The spectra were obtained under conditions quantitatively reliable to within 10% and the relative peak areas from a deconvolution of the spectrum into overlapping Gaussian peaks is also indicated. A substantial number of the silicon atoms have OH groups attached.

Since there is some ambiguity in the spectrum {there could be a contribution to the peak at −101.8 p.p.m. assigned to $Si(OSi\equiv)_3(OH)$ from Si[1Al, 3Si] if there was any residual aluminium in the system} we attempted to obtain a ^{27}Al n.m.r. spectrum of this material. Absolutely no aluminium was detected in contrast to other silica samples we have examined. The ^{27}Al nucleus is very sensitive and we have shown that Si/Al ratios in excess of 2000:1 can be easily measured so the biological sample is unique in this regard.

Confirmation of the presence of hydroxy-groups may be obtained by silylation of the sample with trimethylchlorosilane Me_3SiCl which reacts with Si–OH groups to yield Me_3Si–O–Si groupings. Figure 2(b) shows the appearance of a peak at +13.5 p.p.m. due to the Me_3Si–O–Si moiety in agreement with previous findings[8] and a systematic change in the intensities of the other resonances consistent with reaction of some of the hydroxy-groups. The percentage of hydroxy-groups which react (24%) indicates clearly that these are not all present as 'surface' hydroxy-groups, at least in terms of their accessibility to the reagent Me_3SiCl.

The ^{29}Si n.m.r. absorptions of quartz, stishovite, dealuminated zeolites, and other polymorphs of silica where only single sites are present are very narrow reflecting the crystallinity of the sample. In crystalline silica polymorphs which have multiple sites, narrow signals for different sites may be observed.[11]

The width of the absorptions (6—8.8 p.p.m.) observed in the present work correspond closely with those observed in synthetic silica gels and we consider this to reflect the non-crystalline nature of this biological material.

In support of the results from the solid-state n.m.r. studies the i.r. spectra of the silica hairs from *Phalaris canariensis* have been examined. Two references were used to relate the physical characteristics of the system under investigation to known silicas. The spectrum of quartz showed absorption at 1172(s), 1082, 790(s), and 460(m) cm^{-1} but virtually no absorption in the OH region around 3300 and 1600 cm^{-1}. The second reference chosen was that of silicic acid which has broader absorption peaks at 1210, 1090(s), 800(w), and 470(m) cm^{-1} as well as peaks at 954 and 966(m) cm^{-1} and a broad region of absorption from 3220 to 3640 cm^{-1} with minor peaks. The new peaks arise from Si–O–H and OH stretching and bending modes. The hairs from the Phalaris seeds give spectra very similar to those of silicic acid and similar to other forms of opaline amorphous SiO_2. The Si–OH peaks are quite distinct from those of bound water at 3435 and 1600 cm^{-1}. Similar results have been obtained for silica from diatoms.[12]

After prolonged acid treatment of the biological fibres to remove any organic matter the resulting SiO_2 i.r. spectrum closely resembles that of industrial amorphous silicas which

still contain $Si(OSi\equiv)_3(OH)$ units. The absorption peaks due to Si–OH bending and stretching modes are considerably weaker than in the untreated sample, owing to the dehydration of the sample during this treatment.

We now return to the nature of silica in biological materials which we can write as $[Si(OSi\equiv)_n(OH)_{4-n}]$ allowing for some $Si(OSi\equiv)_3(OH)$ units and many $Si(OSi\equiv)_4$ species. This formula readily accounts for the following properties of these silicas: (a) the amorphous nature, (b) the mobility and the very ready dissolution of silica in certain biological products, (c) the bonding to organic matrices which can be through donor as well as acceptor hydrogen-bonding, and (d) the ready formation of compounds with divalent metal ions owing to the acidic nature of the Si–OH unit.

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