Solid-State $^{29}$Si VACP/MAS NMR Studies of Silicon-Accumulating Plants: Structural Characterization of Biosilica Deposits

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A series of silicon-accumulating plants [different *Equisetum* (horse tail) species, *Echium vulgare*, and *Symphytum officinale*] were studied by solid-state $^{29}$Si NMR experiments. For this purpose, selected parts of these plants were freeze-dried and then investigated by solid-state $^{29}$Si VACP/MAS NMR spectroscopy. The $^{29}$Si NMR spectra of these plants are quite similar and exhibit the typical pattern characteristic of polysilicic acid (amorphous silica).

Introduction

It has been generally accepted that silicon is an essential element for many biological systems, being required for the production of structural materials and/or metabolic processes (see for example ref. [1], and literature cited therein). Biomineralized silica (biosilica) of the general formula type [$\text{SiO}_{2/3}(\text{OH})_{4-n}$]$_{m}$ ($n = 0$ to $4$) has been recognized to have a structural function in certain marine organisms (such as diatoms, silicoflagellates, radiolarians, and sponges) and silicon-accumulating plants [such as *Equisetum* (horse tail) species]. As shown by various physical methods (including high-resolution transmission electron microscopy [2], solid-state $^{29}$Si NMR spectroscopy [2], and SiK XANES spectroscopy [3]), the structural nature of biosilica is best described as an amorphous material. However, some kind of mid-range ordering at a length-scale of 4 - 10 Å has been claimed for silicas isolated from rice husks (*Oryza sativa*) [3]. Here we report on the application of solid-state $^{29}$Si VACP/MAS NMR spectroscopy (VACP = Variable Amplitude Cross Polarization) to the structural investigation of biogenic silica deposits of plants [4]. This particular method allows a direct structural characterization of the biosilica without its isolation and enrichment [5]. The plants examined in this study were a series of *Equisetum* species, *Echium vulgare*, and *Symphytum officinale*.

Experimental Section

Different parts of the plants investigated (see Table 1) were separated mechanically and subsequently freeze-dried. The synthetic silica was purchased from Merck (silica gel 60; particle size, 0.015 - 0.040 mm). All samples were studied by solid-state $^{29}$Si VACP/MAS NMR spectroscopy. For this purpose, the samples were measured at 23 °C on a Bruker DSX-400 NMR spectrometer with bottom layer rotors (ZrO$_2$; diameter, 7 mm) containing ca. 200 mg of sample ($^{29}$Si, 79.5 MHz; spinning rate, 5 - 7 kHz; contact time, 5 ms; 90° $^1$H transmitter pulse length, 3.6 µs; repetition time, 4 s; number of scans, 22000 - 150000; external standard, TMS ($\delta = 0$)).

Results and Discussion

The biosilica deposits of the silicon-accumulating plants *Equisetum arvense*, *Equisetum giganteum*, *Equisetum hyemale*, *Equisetum palustre*, *Equisetum telmateia*, *Echium vulgare*, and *Symphytum officinale* were structurally characterized by solid-state $^{29}$Si NMR experiments. For this purpose, certain parts of these plants (see Table 1) were separated and freeze-dried and then investigated by $^{29}$Si VACP/MAS NMR spectroscopy. For reasons of comparison, synthetic silica was also studied by this method.

The $^{29}$Si NMR spectra obtained in these studies look quite similar for all plants. Fig. 1 (leaves...
Table 1. Isotropic $^{29}$Si chemical shifts ($\delta$ values in ppm) of biosilica deposits of *Equisetum arvense*, *Equisetum giganteum*, *Equisetum hyemale*, *Equisetum palustre*, *Equisetum telmateia*, *Echium vulgare*, *Symphytum officinale*, and synthetic silica obtained by solid-state $^{29}$Si VACP/MAS NMR studies (estimated error $\pm 1$ ppm).

<table>
<thead>
<tr>
<th>Species</th>
<th>(part of the plant)</th>
<th>$\delta$Si(O$\equiv$)$_4$[Q$^4$]</th>
<th>$\delta$Si(O$\equiv$)$_3$OH [Q$^3$]</th>
<th>$\delta$Si(O$\equiv$)$_2$(OH)$_2$ [Q$^2$]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Equisetum arvense</em></td>
<td>(stems and blossoms)</td>
<td>-111</td>
<td>-101</td>
<td>-92</td>
</tr>
<tr>
<td><em>Equisetum giganteum</em></td>
<td>(leaves)</td>
<td>-111</td>
<td>-101</td>
<td>-92</td>
</tr>
<tr>
<td><em>Equisetum giganteum</em></td>
<td>(stems)</td>
<td>-111</td>
<td>-101</td>
<td>-92</td>
</tr>
<tr>
<td><em>Equisetum hyemale</em></td>
<td>(blossoms)</td>
<td>-111</td>
<td>-101</td>
<td>-92</td>
</tr>
<tr>
<td><em>Equisetum hyemale</em></td>
<td>(stems)</td>
<td>-111</td>
<td>-101</td>
<td>-91</td>
</tr>
<tr>
<td><em>Equisetum palustre</em></td>
<td>(stems and leaves)</td>
<td>-110</td>
<td>-101</td>
<td>-92</td>
</tr>
<tr>
<td><em>Equisetum telmateia</em></td>
<td>(stems and blossoms)</td>
<td>-111</td>
<td>-101</td>
<td>-91</td>
</tr>
<tr>
<td><em>Echium vulgare</em></td>
<td>(stem leaves)</td>
<td>-110</td>
<td>-101</td>
<td>-91</td>
</tr>
<tr>
<td><em>Symphytum officinale</em></td>
<td>(stem leaves)</td>
<td>-110</td>
<td>-101</td>
<td>-91</td>
</tr>
<tr>
<td>Synthetic silica</td>
<td></td>
<td>-111</td>
<td>-102</td>
<td>-92</td>
</tr>
</tbody>
</table>

Fig. 1. Solid-state $^{29}$Si VACP/MAS NMR spectrum of the leaves of *Equisetum giganteum* (spinning rate, 5 kHz; number of scans, 150000; for further details, see Experimental Section).

Fig. 2. Solid-state $^{29}$Si VACP/MAS NMR spectrum of the stem leaves of *Echium vulgare* (spinning rate, 5 kHz; number of scans, 80000; for further details, see Experimental Section).

Fig. 3. Solid-state $^{29}$Si VACP/MAS NMR spectrum of synthetic silica (spinning rate, 5 kHz; number of scans, 2072; for further details, see Experimental Section).

It should be mentioned that the ratio of the Q$^2$, Q$^3$, and Q$^4$ moieties in the biosilica samples studied does not correspond to the intensity ratio of the respective resonance signals in the spectra, because the VACP/MAS technique does not lead to quantitative spectra [7, 8]. Nevertheless, the results obtained in this study clearly demonstrate that solid-state $^{29}$Si VACP/MAS NMR spectroscopy is a valuable non-destructive method for the structural characterization of biosilica in intact biological systems. In contrast to other experimental methods reported, this particular $^{29}$Si NMR technique allows the direct investigation of intact (freeze-dried) silicon-
accumulating plants and does not need the isolation and enrichment of the biosilica by destructive methods. We are planning to extend our solid-state $^{29}$Si NMR studies to biosilica deposits of marine organisms, such as diatoms, radiolarians, and sponges.

Acknowledgements

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[5] The solid-state $^{29}$Si NMR studies described in ref. [2] were not performed with the VACP/MAS technique and the biosilica samples investigated were isolated from the plants.
[8] Because of the poor signal to noise ratio, contact time depending or quantitative NMR experiments were not performed.