DALTON
FULL PAPER

Silicon-29 NMR evidence of a transient hexavalent silicon complex in the diatom *Navicula pelliculosa*

Stephen D. Kinrade,*[a] Ashley-M. E. Gillson[a] and Christopher T. G. Knight[b]

[a] *Department of Chemistry, Lakehead University, Thunder Bay, ON P7B5E1, Canada. E-mail: Stephen.Kinrade@lakeheadu.ca*
[b] *School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana IL 61801, USA*

Received 19th June 2001, Accepted 26th November 2001
First published as an Advance Article on the web 3rd January 2002

Cultures of the freshwater diatom *Navicula pelliculosa* were synchronized by silicon starvation, fed a ^{29}Si-enriched silicate solution, and then studied by ^{29}Si NMR spectroscopy. Two NMR resonances could be detected reproducibly, the ever-present orthosilicic acid peak at −71 ppm and a weak signal at −131.5 ppm that was only seen after the diatoms had been allowed six hours to accumulate the ^{29}Si. When the initial culture medium was enriched in ^{15}N, the −131.5 ppm resonance narrowed significantly which, taken with the peak's unique chemical shift, implies the existence of an organosilicon complex containing hexavalent silicon coordinated to at least one nitrogen. The signal is the first direct evidence of an organosilicon complex formed during the life cycle of an organism.

Introduction

Certain primitive life forms such as diatoms, radiolaria, some sponges and the Equisetaceae (horsetails) require silicon to complete their reproductive cycle.[1] Silicon is also utilized by many higher plant species to protect against abiotic stresses (wilting, lodging, metal toxicity, salinity) and biotic stresses (fungal diseases, insect damage).[2] In mammals, Si-deficiency has been linked to bone[3] and heart[4] diseases, cancers[5] and neurodegenerative disorders.[6] However, the biochemistry underlying silicon essentiality is almost totally unknown and, to date, no organosilicon compound has been detected in a living system. Indeed, some workers now regard *silicon biochemistry* as a contradiction in terms, and attribute the element's biological importance to its role in producing solid oxide support structures along with an ability to alter toxic metal activity.[7]

Recently we demonstrated that, contrary to expectation, organosilicon compounds *easily* form between silicate anions and aliphatic carbohydrate molecules in aqueous alkaline solution, provided that the carbohydrate molecules contain at least four adjacent hydroxy groups, with two in *threo* configuration.[8,9] Extracellular matter, cell membranes, bio-fluids and soil solutions are generally rich in such potential ligands. Surprisingly, the resulting organosilicon complexes contain pentaoxo- or hexaoxo-silicon sites rather than the four-coordinated Si centres typical of aqueous silicate anions.

Here we report results from a ^{29}Si nuclear magnetic resonance study of the fresh water diatom *Navicula pelliculosa*, providing the first direct evidence of an organosilicon compound formed *in-vivo*.

Experimental

Navicula pelliculosa (Bréb.) Hilse (UTEX 674, Univ. of Texas Culture Collection, Univ. of Texas at Austin) was cultured in Si-starvation synchrony using the procedure of Darley and Volcani.[10] The cultures were grown at 298 K in magnetically stirred 1 L polycarbonate flasks containing 500 mL of freshwater Tryptone liquid medium, at a pH between 8.3 and 8.5, and under a constant light source of 40 W m^{-2}. In the case of ^{15}N enriched cultures, the calcium nitrate in the medium was substituted with Ca(^{15}NO$_3$)$_2$ (Aldrich, 99.6 atom% ^{15}N). Silicon levels in the media were monitored by ICP (Jarrell-Ash ICAP 9000).

Silicon-starvation was terminated by the addition of aqueous potassium silicate, enriched 75 or 99.8 atom% in ^{29}Si (recovered and purified in-house), bringing the initial Si concentration in the medium to a nominal concentration of 16 mg L^{-1}. The cultures were maintained under normal growing conditions for a specified period, centrifuged (15 min at 3000 rpm and 5 °C) to a solid, and then re-suspended in just enough deuterium oxide (Aldrich, 99.8 atom% ^{2}H) to produce a thick slurry that was poured into 10 mm Kel-F NMR tubes (9 mm ID).

Silicon-29 NMR spectra were recorded of the diatoms *in-vivo* at either 149 MHz (Varian Unity Inova 750, Keck Facility, Univ. of Illinois at Urbana–Champaign) or 99.4 MHz (Bruker AMX500 with Si-free probe, Univ. of Manitoba, Winnipeg). All spectra were sequentially acquired in blocks of 1000 pulses using ^{1}H-decoupling (gated to prevent nuclear Overhauser distortion). The temperature was calibrated (±0.5 K) using the ^{1}H spectrum of ethylene glycol.[11] Chemical shifts were determined relative to tetramethylsilane, employing the orthosilicate monomer peak, here assigned at −71.0 ppm, as a secondary reference.

Results

Fig. 1 shows the evolution of the *Navicula pelliculosa* cell population and the silicon concentration of the culture medium during a Si-starvation synchrony experiment. As the diatom population increases the concentration of silicon falls, ultimately reaching a minimum of 0.2 ppm after 36 h. Previous workers[10] have demonstrated that, when starved of Si, the bulk of the cell population is synchronized at the biprotoplastic stage of the life cycle, with each diatom frustule containing two daughter protoplasts surrounded by new plasmalemma. Without a fresh supply of aqueous silicon the cells are incapable of dividing. The objective of this study was to detect and identify molecular compounds that may be involved in the uptake and deposition of freshly added Si in a synchronized, living diatom culture. This was accomplished by adding ^{29}Si-enriched silicon to starvation-synchronized diatoms at 298 K, waiting for

DOI: 10.1039/b105379p

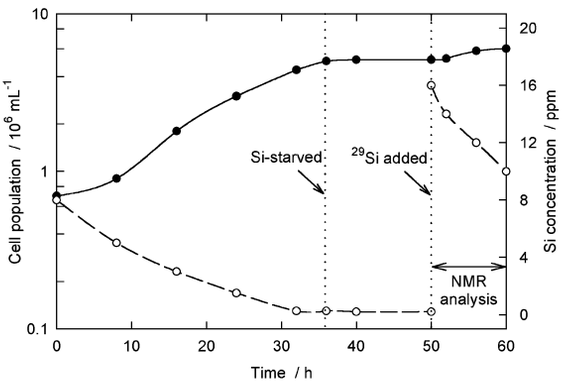

Fig. 1 Time dependence of *Navicula pelliculosa* cell population (solid line) and of Si concentration in the culture medium (dashed line) during a silicon-starvation synchrony experiment at 298 K. The culture was analyzed by ^{29}Si NMR spectroscopy at various intervals following the addition of ^{29}Si-enriched silicon to the Si-starved colony.

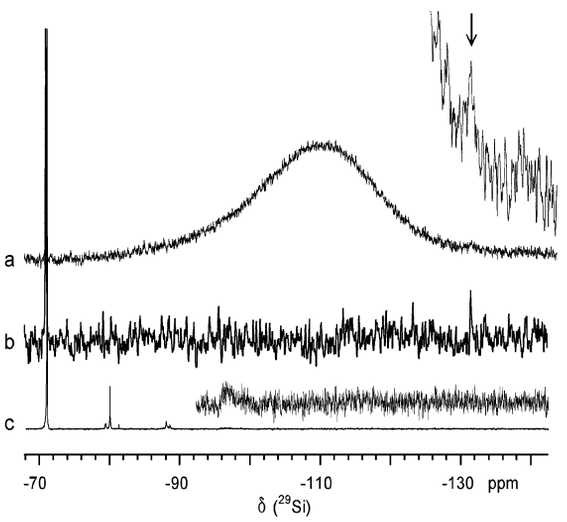

Fig. 2 (a) Silicon-29 ^{1}H-decoupled NMR spectrum (149 MHz) of a synchronized culture of *Navicula pelliculosa* following 6 h feeding on ^{29}Si-enriched (75 atom%) silicon at 298 K. The spectrum was acquired over 9.3 h at 278.2 K using π/2 (16 μs) pulses with a cycling period of 11 s. The dominant feature centred at −110 ppm arises from Si-containing components in the NMR probe. The sample itself yielded a sharp orthosilicic acid peak at −71.0 ppm and a weak signal at −131.5 ppm with a half-height peak width of 25 Hz (see vertical expansion). (b) The last of four successive 3.3 h silicon-29 ^{1}H-decoupled NMR spectra (99.4 MHz) acquired of another synchronized *Navicula pelliculosa* culture, following 6 h exposure to ^{29}Si (99.8 atom%) at 298 K in a medium also enriched (99.6 atom%) in ^{15}N. It was acquired at 273.5 K using π/2 (14 μs) pulses with a cycling period of 12 s. The strong orthosilicate peak at −71.0 ppm appeared in every spectrum. However, the peak at −131.5 ppm occurred only in the fourth spectrum and was considerably narrower than in Fig. 2a, the half-height line-width being only 6 Hz. The signal accounted for *ca.* 5–10% of the detected Si. (c) Silicon-29 ^{1}H-decoupled NMR spectrum (99.4 MHz) at 273.5 K of the nutrient medium in Fig. 2b immediately following the addition of 16 ppm ^{29}Si. No peak is apparent in the vicinity of −131.5 ppm (see vertical expansion).

various time intervals to allow Si uptake, and then obtaining the ^{29}Si NMR spectrum of the living colony at temperatures close to the system's freezing point. Our results are shown in Fig. 2.

Fig. 2a is the ^{29}Si NMR spectrum of a slurry of *Navicula pelliculosa* recorded after the addition of 75 atom% ^{29}Si-enriched silicon solution. The diatoms were allowed to accumulate silicon for 6 hours prior to being centrifuged and transferred to the NMR spectrometer. A sharp peak is apparent at −71.0 ppm, resulting from orthosilicic acid $Si(OH)_4$, along with a weak, but clearly integratable, signal at −131.5 ppm. Orthosilicic acid was detected in all spectra acquired following ^{29}Si addition, although the signal at −131.5 ppm was only seen in spectra acquired after 6 hours of ^{29}Si uptake. After 10 hours, for example, there was no trace of the signal. No other ^{29}Si resonances were detected with any degree of certainty.

In an attempt to confirm the existence of the −131.5 ppm signal, we repeated the experiments using a higher level of ^{29}Si enrichment (99.8 atom%), a Si-free NMR probe, and a lower acquisition temperature (to optimize the Boltzmann sensitivity factor and mitigate chemical exchange broadening). Additionally, the initial culture medium was enriched to 99.6 atom% in ^{15}N so as to obviate any possible effects of coupling to the quadrupolar ^{14}N nucleus. Fig. 2b shows the resulting NMR spectrum, again acquired after the colony was allowed 6 hours to accumulate the isotopically enriched silicon. As before, the signal of orthosilicic acid is apparent, at −71.0 ppm, together with the weak resonance at −131.5 ppm. The linewidth of the latter is considerably less than that in Fig. 2a, falling from 25 Hz to 6 Hz, although that of orthosilicic acid is unchanged. No other signals are visible,† and again the peak at −131.5 ppm only appears when the diatoms are allowed to accumulate silicon for 6 hours. Spectra of the colony obtained after longer accumulation periods fail to show any peaks except for that of orthosilicic acid. Moreover, the signal at −131.5 ppm seems to be that of a transient species, since it is only observed in the fourth block of pulses (600 to 800 min after transferring the diatoms to the NMR tube) and at no other time.

Fig. 2c is the ^{29}Si NMR spectrum of the nutrient medium in the second set of experiments, immediately following the addition of 16 ppm ^{29}Si. The main signal is that of orthosilicic acid, although some 28% of the dissolved silicon exists as silicate oligomers including the dimer (−80.0 ppm), linear trimer (−79.3 and −88.0 ppm) and the cyclic trimer (−81.2 ppm). However, there is no trace of any signals corresponding to hypervalent silicon, as shown in the vertical expansion.

Discussion

Silicon-29 NMR analysis of living, isotopically enriched diatoms reveal that: a) orthosilicic acid is, by far, the dominant *freely mobile* Si-containing species occurring within *Navicula pelliculosa*, as the organism takes up and deposits silicon from its aqueous surroundings; b) a small amount (*ca.* 5–10%) of the silicon exists temporarily within a hypervalent organosilicon complex; and c) no other species are apparent to within the detection limits of the spectrometer.

The signal at −131.5 ppm is seen only in the presence of the diatoms, and, indeed, only when the diatoms have been allowed to accumulate silicon for 6 hours. After 10 hours, for example, it is no longer detected. The signal must therefore arise from a transient species, although the structure and function of the molecule cannot be determined at this time. Nevertheless, because the signal's chemical shift lies well outside the frequency range of four-coordinated silicon, it clearly does not correspond to any of the silicate anions typically found in aqueous solution. Moreover, aqueous species known to contain five-coordinated silicon yield peaks some 30 ppm to higher frequency, and so the signal is also unlikely to be the result of a pentaoxosilicon compound.

This would imply that the transient species contains a hexavalent silicon centre. Indeed, hexaoxosilicon-carbohydrate complexes are known to yield peaks as near as 5 ppm to low frequency of the −131.5 ppm signal.[8,9,12] Factors such as ring strain may account for the unique chemical shift. Another strong possibility, however, is that nitrogen occupies one of the silicon coordination sites. Zhou *et al.* recently proposed a nitrogen-containing (albeit five-coordinated) silicon complex

† No spectral features other than the peaks at −71.0 and −131.5 ppm are enhanced through artificial line broadening.

to account for "silicatein"-mediated condensation of tetraalkoxysilanes.[13] The magnitude and direction of the chemical shift from those of known hexaoxosilicon complexes is entirely consistent with published accounts of nitrogen's shielding effect on ^{29}Si.[14–16] Moreover, direct coupling to the quadrupolar ^{14}N nucleus (I = 1, 99.6% natural abundance) would significantly broaden the ^{29}Si resonance, and removal of ^{14}N by enriching the system in ^{15}N (I = 1/2) appears to have caused the peak to narrow significantly. It is unlikely that the small decrease in NMR acquisition temperature between Fig. 2a and 2b could account for such a narrowing, and it is therefore tempting to speculate that the ^{29}Si NMR signal at −131.5 ppm arises from an organic pentaoxo-azo-silicon complex.

Acknowledgements

We thank R. J. Kirkpatrick (Univ. of Illinois at Urbana-Champaign) for the generous loan of isotopically enriched silica, C. C. Perry (Nottingham Trent Univ.) for helpful discussions, and L. Malek (Lakehead Univ.), K. Marat (Univ. of Manitoba), P. Molitor, V. Mainz, S. Fay and W. M. Schnitzlein (Univ. of Illinois at Urbana-Champaign) for their experimental assistance. This work was supported in part by the National Institutes of Health (PHS 1 S10 RR 10444–01, GM-42208, RR01811) and the Natural Sciences and Engineering Research Council of Canada.

References

1 D. Evered and M. O'Connor, eds., *Silicon Biochemistry*, Ciba Foundation Symp. 121, Wiley, Chichester, 1986.
2 E. Epstein, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1999, **50**, 641.
3 E. M. Carlisle, in *Handbook of Nutritionally Essential Mineral Elements*, eds. B. L. O'Dell and R. A. Sunde, Marcel Dekker, New York, 1997, pp. 603–618.
4 M. R. Peluso and B. O. Schneeman, *J. Nutr.*, 1994, **124**, 853.
5 A. C. Burton, J. F. Cornhill and P. B. Canham, *J. Environ. Pathol. Toxicol.*, 1980, **4**, 31.
6 M. Belles, D. J. Sanchez, M. Gomez, J. Corbella and J. L. Domingo, *Alzheimer Dis. Assoc. Disord.*, 1998, **12**, 83.
7 C. Exley, *J. Inorg. Biochem.*, 1998, **69**, 139.
8 S. D. Kinrade, R. J. Hamilton, A. S. Schach and C. T. G. Knight, *J. Chem. Soc., Dalton Trans.*, 2001, 961.
9 S. D. Kinrade, J. W. Del Nin, A. S. Schach, T. A. Sloan, K. L. Wilson and C. T. G. Knight, *Science*, 1999, **285**, 1542.
10 W. M. Darley and B. E. Volcani, in *Methods in Enzymology*, ed. A. San Pietro, Academic, New York, 1971, pp. 85–96.
11 C. Ammann, P. Meier and A. E. Merbach, *J. Magn. Reson.*, 1982, **46**, 319.
12 S. D. Kinrade, E. Deguns, A.-M. E. Gillson, R. J. Hamilton and C. T. G. Knight, unpublished work.
13 Y. Zhou, K. Shimizu, J. N. Cha, G. D. Stucky and D. E. Morse, *Angew. Chem., Int. Ed.*, 1999, **38**, 780.
14 V. F. Sidorkin, V. A. Pestunovich and M. G. Voronkov, *Magn. Reson. Chem.*, 1985, **23**, 491.
15 J. H. Iwamiya and G. E. Maciel, *J. Am. Chem. Soc.*, 1993, **115**, 6835.
16 A. Chandrasekaran, R. O. Day and R. H. Holmes, *J. Am. Chem. Soc.*, 2000, **122**, 1066.