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Calculation of ^{29}Si NMR shifts of silicate complexes with carbohydrates, amino acids, and MuHicarboxylic acids: Potential role in biological silica utilization

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Abstract—The existence of ether or ester-like complexes of silicate with organic compounds has long been debated in the literature on biological utilization of silicon. Comparison of theoretically calculated ^{29}Si NMR chemical shifts for such complexes with experimentally measured values in biological systems could provide a diagnostic tool for identifying which, if any of these molecules exist under physiological conditions. Results are presented here for ab initio molecular orbital calculations of ^{29}Si NMR shifts and formation energies of silicate complexes with polyalcohols, sugar-acids, pyranose sugars, amino acids and multicarboxylic acids. The effects of functional group and molecular structure including ligand size, denticity, ring size, silicon polymerization and coordination number on calculated ^{29}Si shifts were considered. The potential role of such compounds in biological silica utilization pathways is discussed.

^{29}Si NMR shifts and energies were calculated at the HF/6-311+G(2d,p)//HF/6-31G* level. The main result is that only five-membered rings containing penta- and hexa-coordinated Si can explain experimentally observed resonances at ~ -101 and -141 ppm. Further, the heptet observed in ^{1}H-^{29}Si coupled spectra can only be explained by structures where Si bonds to oxygens atoms in H-C-O-Si linkages with six symmetrically equivalent H atoms.

While compounds containing quadra-coordinated silicon may exist in intracellular silicon storage pools within diatoms, calculated reaction energies suggest that the organism has no thermodynamic advantage in taking up extracellular organ-silicate compounds, instead of silicic acid, from the ambient aqueous environment. Hyper-coordinated complexes are deemed unlikely for transport and storage, though they may exist as transient reactive intermediates or activated complexes during enzymatically- catalyzed silica polymerization, as known previously from sol-gel silica synthesis studies.

1. INTRODUCTION

1.1. Silicon in Biology

Silicon is utilized in diverse ways by both unicellular and multicellular eukaryotic organisms. Microscopic dinoflagellates, radiolaria, diatoms, and sponges precipitate amorphous silica as an exoskeleton, and possibly also use the silica as a pH buffer for the enzymatic conversion of HCO_3^- to CO_2 (Milligan and Morel, 2002). It is estimated that marine organisms annually cycle ~6.7 gigatonnes of silicon, the most abundant element in the earth's crust after oxygen (Tacke et al., 1999). Diatoms contribute ~ 40% of the primary productivity of the oceans, thus significantly affecting the silicon and carbon global budgets (Nelson et al., 1995).

Silica (SiO_2) or dissolved silicon can provide nutrition and internal rigidity to the organism as in the case of higher plants such as bamboo, wheat, oat, barley, and horse-tails; provide a physical defense mechanism as in stinging nettles; chemical resistivity to fungal diseases in plants; and play a role in controlling aluminum and heavy metal toxicity in plants and animals (Iler, 1979; Frausto da Silva and Williams, 1991; Epstein, 1993; Marschner, 1995; Hodgson and Sangster, 1999; Exley et al., 2002, Richmond and Sussman, 2003). Silicate-organic complexes have been postulated to play a role in the evolution of early life (Bernal, 1951; Pierson et al., 1993; Phoenix et al., 2001).

Silica bioceramics may be directly involved in bone-growth in vertebrates by providing reactive surface nucleation sites for amorphous calcium phosphate (e.g., Sahai and Tossell, 2000), and silicon may indirectly affect bone-growth by influencing the activity of a Cu-enzyme (Birchall, 1995). Silicon was found associated with glycosaminoglycans and polyuronides bound as an ether or ester-like silicate with C-O-Si or C-O-Si-O-Si-O-C bonds, in amounts of 1 Si atom/130–280 repeating units of the organic (Schwartz, 1973).

The chemical form in which silicon is utilized biologically has been a long-standing question in the literature. Hyper-coordinated silicate-polyalcohol and silicate-sugar acid complexes have been proposed to play a role in biologic silicon transport and uptake, based on unexpected ^{29}Si NMR resonances observed at -101 and -141 ppm in aqueous solutions (Kinrade et al., 1999, 2001a,b, 2002). Others have questioned the existence of such compounds in biologic systems as a major means of silicon utilization, because ^{29}Si NMR of diatom tests showed no evidence for resonances characteristic of expected silicate-organic complexes (e.g., Perry and Mann, 1989; Birchall, 1989).

1.2. Biologic Silica Precipitation and Mesoporous Biomimetic Silica Synthesis

Focussing on controlled mesoporous SiO_2 precipitation by organisms such as diatoms, sponges, radiolaria and dinoflagellates, there are two approaches to understanding biologic silica precipitation. One approach is to assume that the diatom poly-

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merizes simple silicic acid. This idea is supported by the recent discovery that the enzyme, silaffin, and polyamine chains, isolated from diatoms are capable of polymerizing silicic acid at neutral to acidic pHs (Mitzutani et al., 1998; Kroger et al., 1999, 2000; Pohnert, 2002) characteristic of freshwater (pH ~ 7) and of the silica deposition vesicle of the diatom (pH ~ 5–6) (Vrieling et al., 1999a). The uptake of silicic acid across the cell membrane from extracellular seawater with a mildly basic pH of ~8.5 has also been shown in several diatom species (Amo and Brzezinski, 1999). A different perspective is that the starting compound is a silicate-organic compound. An enzyme named silicatein was identified in a sponge spicule. Silicatein catalyzes the hydrolysis and polymerization of tetraethylorthosilicate, a presumed starting silicate-organic compound (Shimizu et al., 1998, Zhou et al., 1999; Cha et al., 1999).

Early experiments found dissolved silicon in diatoms at levels two to three orders of magnitude greater than is allowed by silica solubility. Silica precipitation with strict control on pore size and pore geometry, as seen in diatoms requires a slow reaction. At the high silicon concentrations, however, slow precipitation should be difficult because inorganic silicic acid tends to polymerize quickly. It was suggested, therefore, that silicon is stored as a silicate-organic compound in intracellular silicon "pools" until precipitation begins in the silica deposition vesicle of the diatom (Azam et al., 1974; Sullivan, 1979; Bhattacharya and Volcani, 1980; Binder and Chisholm, 1980; Blank et al., 1986). The use of a silicate-organic starting compound would slow down the precipitation reaction considerably compared to precipitation from a silicic acid solution, because the silicate-organic compound must first be hydrolyzed before polymerization. The slower rate would, presumably, allow greater control on the shapes and sizes of the mesoporous silica precipitated. The same strategy is followed in sol-gel and biomimetic silica synthesis methods (e.g., Birchall, 1989; Morse, 1999; Tacke et al., 1999; Vrieling et al., 1999b). Furthermore, it has been known since the early to mid-1990s that hyper-coordinated silicate-organic complexes are highly reactive species occurring as transient intermediate species such as intermediates or as activated complexes, in both the mother-liquor and in the freshly precipitated solid (Belot et al., 1990; Laine et al., 1991; Herreros et al., 1994a,b).

Most modern experimental studies use ^{29}Si NMR to determine the chemical speciation of silicon in biologic systems (Perry and Mann, 1989; Kinrade et al., 2002). Recognizing the inherent challenges and difficulties in the experimental method, a novel approach has been developed based on ab initio molecular orbital theory to predict diagnostic ^{29}Si NMR shifts and relative stabilities of silicate-organic complexes potentially involved in silica biomineralization (Sahai and Tossell, 2001, 2002). Complexes with serine and polyalcohols have been studied previously. As part of this ongoing project, results are presented here for additional complexes of silicate with polyalcohols, sugar-acids, pyranose sugars, multicarboxylic acids and amino-acids. The effects of ligand size, dentition, chelate ring-size, silicon polymerization, and Si coordination number on ^{29}Si NMR spectra are examined. The likelihood for the existence of such compounds in diatom biochemical pathways is discussed based on energetic considerations. Excellent reviews of the biochemical pathways involved (Sullivan and Volcani, 1981; Volcani, 1981; Sullivan, 1986; Martin-Jezequel et al., 2002), and of silica morphogenesis and valve formation have appeared periodically in the literature. The processes considered here are transfer from the extracellular medium across the cell membrane, intracellular transport, storage in silicon pools, and polymerization within the silica deposition vesicle (Pickett-Heaps et al., 1990; Pickett-Heaps, 1991; Gordon and Drum, 1994).

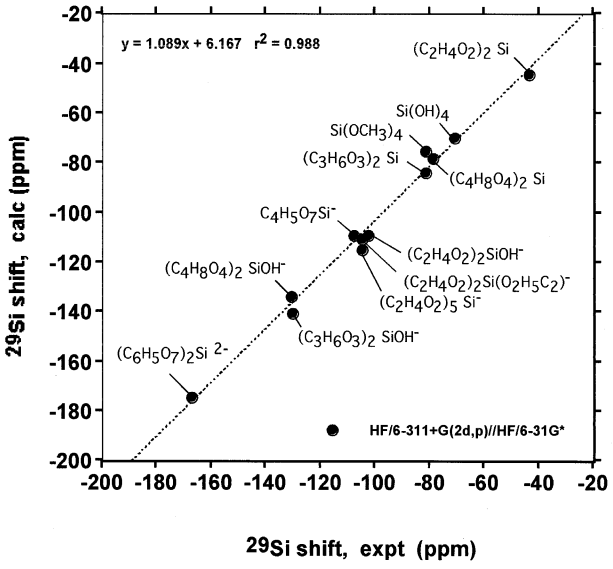

Fig. 1. Comparison of calculated ^{29}Si NMR shifts with experimental values. All calculated values from Sahai and Tossell (2002, 2001) except for $(C_6H_5O_7)_2Si^{2-}$ (this study). Experimental values from Gibby et al. (1972) in Duncan (1990), Kumara Swamy et al. (1990), Herreros et al., (1994), Kemmitt and Milestone (1995), Tacke et al. (2000, 2002), Benner et al. (2003).

2. COMPUTATIONAL METHOD

The same method as developed previously is used in the present study (Sahai and Tossell, 2001, 2002). Energies and optimized geometries were obtained at the HF/6-31G* level using the programs GAMESS, Gaussian 94 and Gaussian 98 (Hehr et al., 1986; Schmidt, 1993; Frisch et al., 1995, 1998). ^{29}Si NMR chemical shieldings are calculated for each molecule at the HF/6-311+G(2d,p) level using the GIAO method (Hehre et al., 1986; Wolinski et al., 1992) as implemented in Gaussian 94 and Gaussian 98. Isotropic shifts (δ) are obtained as the difference between the theoretical shielding for tetramethylsilane (TMS) and the shielding of the relevant molecule. The span (σ_{33}–σ_{11}) is the difference between the maximum and minimum values of the shielding tensor. Solvation considered via the Self-Consistent Reaction Field Model, and via the inclusion of explicit water molecules had little effect on the predicted shifts compared to gas-phase values (Sahai and Tossell, 2002). Therefore, all reported shifts are for gas-phase molecules. A linear least-squares fit of gas-phase calculated shifts to experimental shifts for twelve condensed-phase compounds with well-known structures, yields a regression ^{29}Si $\delta_{iso,calc} = 1.09\ ^{29}$Si $\delta_{iso,expt} + 6.2$ ppm, $r^2 = 0.988$ (Fig. 1). All molecules shown in Figures 2–5 were drawn using the software

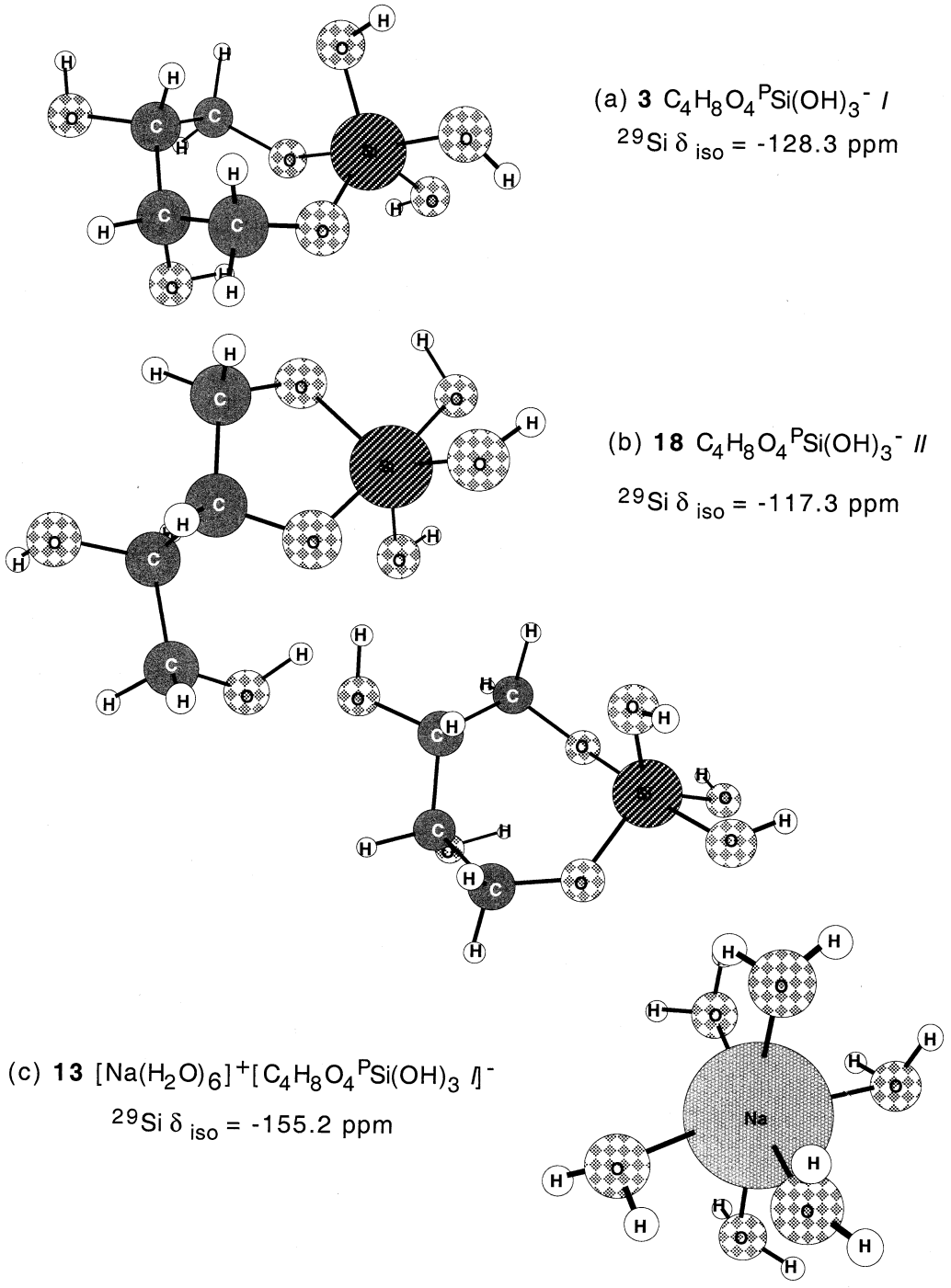

Fig 2. Monocyclic threitol polyalcohol complexes showing the effect of ring-size and ion-pair formation on calculated ^{29}Si NMR isotropic shifts (δ_{iso}) (a) $C_4H_8O_4{}^PSi(OH)_3{}^-$ *I*, (b) $C_4H_8O_4{}^PSi(OH)_3{}^-$ *II*, and (c) $[Na(H_2O)_6]^+$ $[C_4H_8O_4{}^PSi(OH)_3]^-$ *I*. All structures in this and subsequent figures were obtained at the HF/6–31G* level. Key to spheres representing atoms for this and subsequent figures: unfilled-H; solid-C; large checks-O; stripes-Si; white spots-Na.

MacMolPlt (Bode and Gordon, 1998). Quadra-, penta- and hexa-coordinated Si are denoted ^{Q}Si, ^{P}Si and ^{H}Si.

3. RESULTS

Theoretical ^{29}Si NMR shifts are reported in Tables 1–3 for polyalcohol, sugar-acid, glucopyranose, carboxylic acid and amino-acid complexes. Unless otherwise noted, the structures contain Si attached to bidentate ligands (L) in seven-membered rings (Table 1), and five-membered rings (Table 2). The complexes with amino-acids are monodentate and monomeric. Monocyclic compounds have Si:L ratios equal to 1, and spirocyclic compounds have Si:L ratios < 1. Effects of changes in

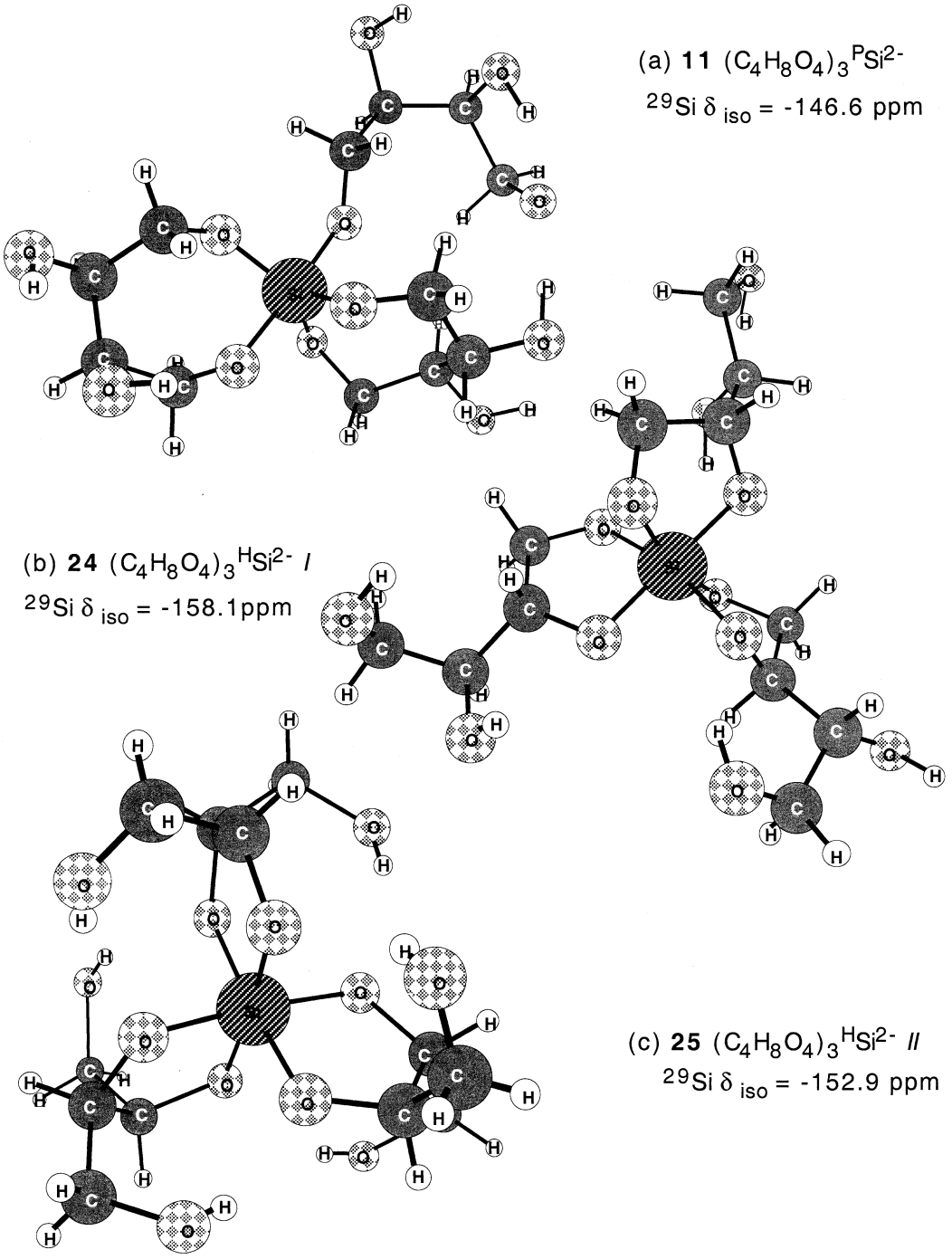

Fig. 3. Spirocyclic threitol polyalcohol complexes showing the effect of ring-size and the specific oxygen bonding-site for a given ring-size on calculated ^{29}Si NMR isotropic shifts (a) $(C_4H_8O_4)_3{}^{P}Si^{2-}$, (b) $(C_4H_8O_4)_3{}^{H}Si^{2-}$*I*, and (c) $(C_4H_8O_4)_3{}^{H}Si^{2-}$ *II*. Key to spheres as in Figure 2.

ligand-size, functional group, ring-size, chelation, and coordination number on ^{29}Si NMR shifts are compared to the ^{P}Si- and ^{H}Si-bidentate complexes **3**, **14** which have a seven-membered ring structure. The seven-membered ring was taken as the reference because it was the basic structure proposed in the original experimental NMR studies of silicate-polyalcohol solutions (Kinrade et al., 1999).

Figure 2 demonstrates the effect of ring-size and ion-pairing on calculated shifts of monocyclic complexes. In terms of energy at the HF/6-31G* level, the five-membered ring structure (Fig. 2b) is ~91 kJ mol^{-1} more stable than the seven-membered ring structure (Fig. 2a), as also found for similar sorbitol complexes by Kubicki and Heaney (2003). The effects of ring-size and specific oxygen binding sites are illustrated in

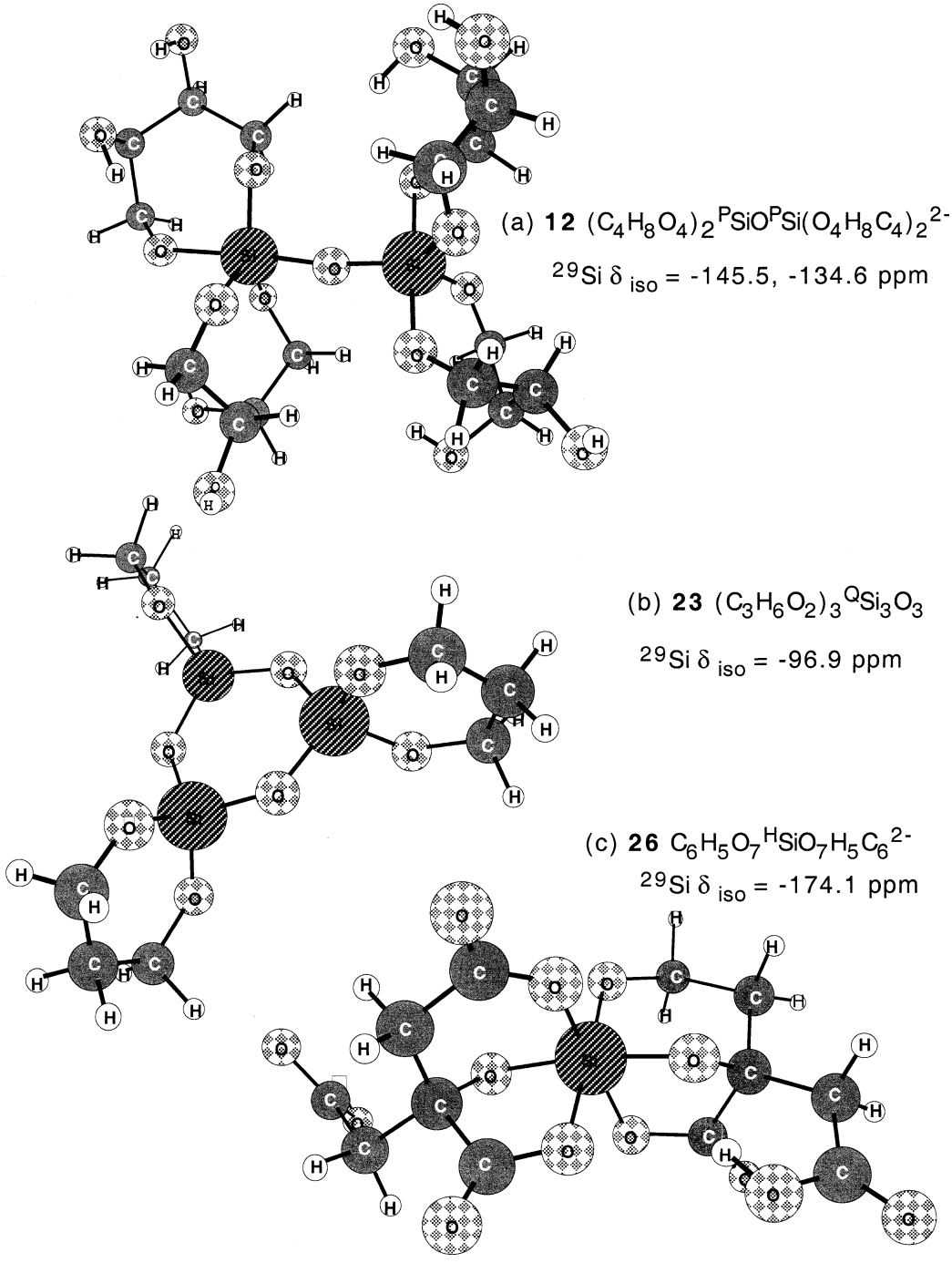

Fig. 4. Spirocyclic threitol polyalcohol complexes showing the effect of branched Si dimerization, cyclic dimerization and denticity on calculated ^{29}Si NMR isotropic shifts (a) $(C_4H_8O_4)_2{}^{P}SiO^{P}Si$ (O $O_4H_8C_4)_2{}^{2-}$, (b) $(C_4H_8O_4)_3{}^{Q}Si_3O_3$, and (c) $(C_6H_5O_7)_2{}^{H}Si^{2-}$. Key to spheres as in Figure 2.

Figure 3. The 1:3 spirocyclic seven-membered ring with ^{H}Si (**11**) turns out to be unstable. One Si-O bond is broken during geometry optimization to yield the ^{P}Si analog (Fig. 3a). The five-membered H Si analogs **24–25** are stable (Fig. 3b,c). Branched and cyclic polymers are shown in Figure 4a,b and the effect of increased denticity is represented in Figure 4c.

Referring to results in Tables 1–3, it can be seen that increasing ligand size and changing functional group does not affect the ^{29}Si shift in tetrahedral ^{Q}Si complexes (**1, 2**; Table 3 in Sahai and Tossell, 2002). For hyper-coordinated ^{P}Si, increasing ligand size from two to four carbons results in a ~15–20 ppm decrease (more negative) in shift but further increase in size has no major effect (**3–5**; Tables 2 and 3 in Sahai and Tossell, 2002). Also, changing the functional group on the

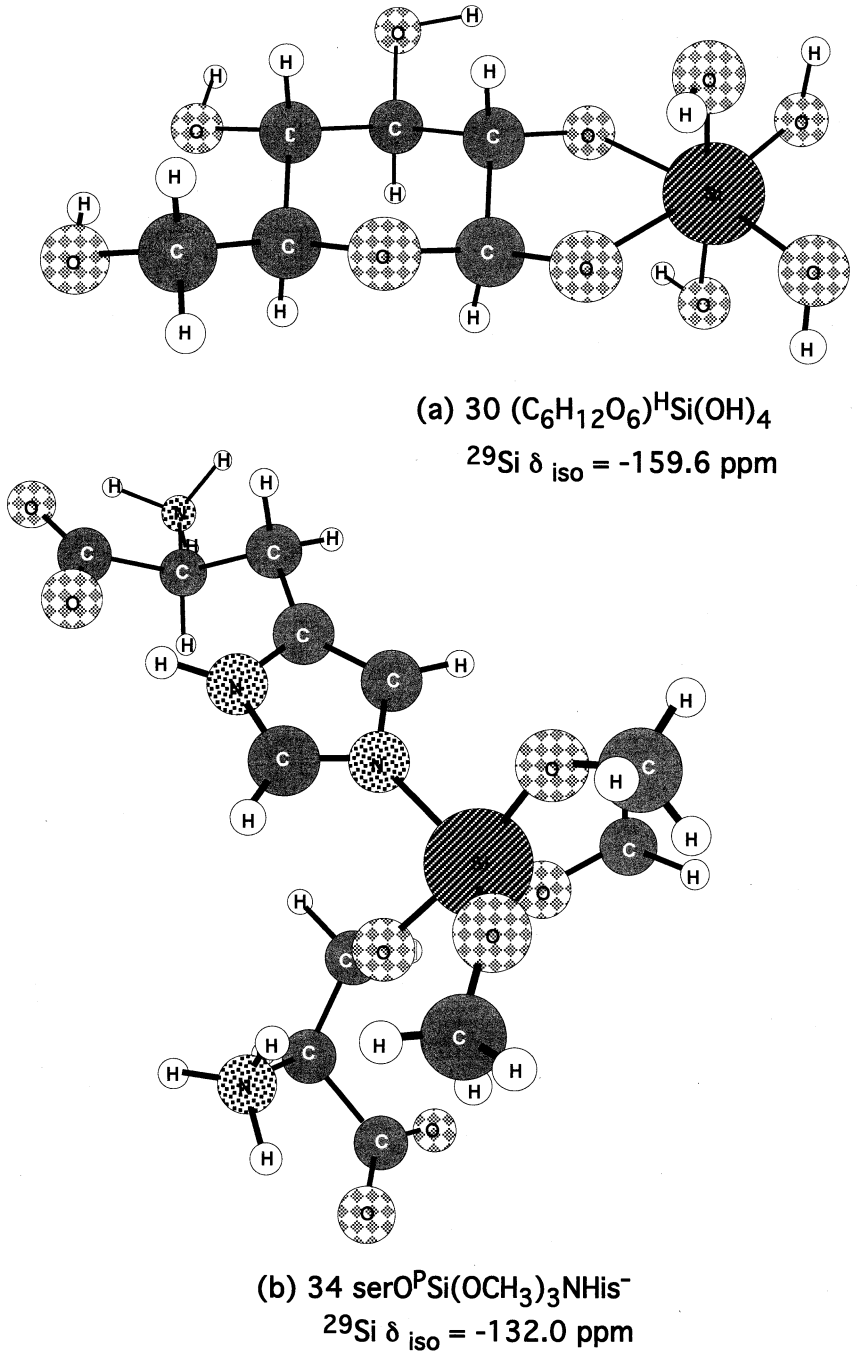

Fig. 5. Glucopyranose complex showing the effect of the pyranose oxygen, and serine-trimethoxysilicate-histidine complex showing the effect of the nitrogen on the calculated ^{29}Si NMR isotropic shifts (a) $C_6H_{12}O_6{}^{H}Si(OH)_4{}^{2-}$ and (b) $serO^{P}Si(OCH_3)_3NHis^-$. Key to spheres as in Figure 2, and small checks-N.

carbohydrate, for example, from alcohol to acid has no effect on the isotropic shift (**5** *vs.* **6**; **7** *vs.* **8**; **18** *vs.* **19**) compared to reference structure **3**. Greater denticity of the ligand in **7–9** has no effect. The formation of an ion-pair **13** with a hexaquo Na^+ ion increases shielding significantly compared to structure **1**.

Increasing the number of ligands in **10–11** increasingly shields the nucleus (more negative shift) in seven-membered rings but not in five-membered rings (**17** *vs.* **20–21**). The ^{P}Si branched dimer **12** is similar in shift to the spirocyclic structures **10–11** but is significantly more shielded than the reference structure **3**. In contrast, the shift of the five-membered ring branched dimer **22** is similar to the monocyclic five-membered ring structure **17**. The shift calculated for the six-membered ring **27** is similar to that for the seven-membered rings **5–6**.

Hexa-coordinated Si in seven- and mixed five- and six-membered rings **14–15** and **26** yield extremely deshielded

Table 1. Theoretical ^{29}Si NMR shifts (ppm) of silicate-carbohydrate complexes at HF/6-311+G(2d,p)//HF/6-31G* level. All structures are bidentate ligand complexes in seven-membered rings, unless otherwise specified.

Structure	Complex, description	Formula	δ	$\sigma_{33} - \sigma_{11}$
1	^{Q}Si, monocyclic threitol	$C_4H_8O_4{}^{Q}Si(OH)_2$	−75.2[a]	35.6
2	^{Q}Si gluconic acid	$C_6H_{10}O_7{}^{Q}Si(OH)_3{}^-$	−74.6	49.1
3	^{P}Si bonded to O1, O4, monocyclic, C4 polyol	$C_4H_8O_4{}^{P}Si(OH)_3{}^-$/	−128.3[a]	140.8
4	C5 polyol	$C_5H_{10}O_5{}^{P}Si(OH)_3{}^-$	−128.0	137.5
5	C6 polyol	$C_6H_{12}O_6{}^{P}Si(OH)_3{}^-$	−128.5	138.7
6	gluconic acid	$C_6H_{10}O_7{}^{P}Si(OH)_3{}^-$	−129.7	161.0
7	Tridentate threitol	$C_4H_7O_4{}^{P}Si(OH)_2{}^-$	−122.4[a]	101.7
8	Tridentate tartaric acid analogue[b]	$C_4H_3O_5{}^{P}Si(OH)_2{}^-$	−122.7	46.3
9	Tridentate citric acid	$C_6H_5O_7{}^{P}Si(OH)_2{}^-$	−120.1	83.2
10	1:2 spirocyclic threitol	$(C_4H_8O_4)_2{}^{P}SiOH^-$	−133.3[a]	127.9
11	1:3 spirocyclic threitol	$(C_4H_8O_4)_3{}^{P}Si^{2-}$	−146.6[c]	87.9
12	Branched dimer threitol	$(C_4H_8O_4)_2{}^{P}SiO{}^{P}Si(O_4H_8C_4)_2{}^{2-}$	−145.5, −134.6, mean = −141.1[c]	145.4, 103.0
13	Ion pair, threitol	$[Na(H_2O)_6{}^+][C_4H_8O_4{}^{P}Si(OH)_3{}^-/]$	−155.2[c]	142.7
14	^{H}Si, monocyclic, threitol	$C_4H_8O_4{}^{H}Si(OH)_4{}^{2-}$	−179.9[a]	
15	^{H}Si, 1:2 spirocyclic, threitol	$(C_4H_8O_4)_2{}^{H}Si(OH)_2{}^{2-}$	−186.5[a]	11.2

[a] Sahai and Tossell (2002).
[b] This molecule has a -CH group where tartaric acid has a -COH group.
[c] Matches experimentally observed peak at −141 to −145 ppm.

values at ~ −174 to −187 ppm. The spirocyclic ^{H}Si five-membered ring analogs **24–25** have a more shielded nucleus at −158.1 and −152.9 ppm. The effect of bonding to different oxygens for a given ring-size can be seen in structures **24** and **25**.

The presence of the oxygen atom in the glucopyranose structures **29–30** (Fig. 5a) results in a more negative shift similar to **24**. The silicate-serine complexes **31–33** show trends similar to the ^{Q}Si and ^{P}Si analogs with the other organics. A ^{P}Si species in an organic quadraoxo-azo complex **34**, where Si is bonded to a serine molecule, three methoxy groups and the N atom of a histidine molecule yields a calculated shift of −132 ppm (Fig. 5b).

4. DISCUSSION

4.1. Origin of the Observed Peaks at −101 and −141 ppm, and the Heptet at −141 ppm in ^{1}H-^{29}Si Coupled Spectra

Experiments conducted at alkaline pHs showed peaks at −101 and −141 ppm. With ^{1}H-^{29}Si coupling, no multiplet was

Table 2. Theoretical ^{29}Si NMR shifts (ppm) of silicate-carbohydrate complexes at HF/6-311+G(2d,p)//HF/6-31G* level. All structures are bidentate ligand complexes in five-membered rings, unless otherwise specified.

Structure	Complex and description	Formula	δ	$\sigma_{33} - \sigma_{11}$
17	^{P}Si monocyclic ethylene glycol	$C_2H_4O_2{}^{P}Si(OH)_3{}^-$	−116.9[a,b]	130.6
18	threitol, Si bonded to O1, O2	$C_4H_8O_4{}^{P}Si(OH)_3{}^-$//	−117.3[a,b]	124.4
19	citrate	$(C_6H_6O_7){}^{P}Si(OH)_3{}^-$	−112.6[b]	112.4
20	1:2 spirocyclic ethylene glycol	$(C_2H_4O_2)_2{}^{P}SiOH^-$	−108.9[a,b]	102.7
21	1:3 spirocyclic ethylene glycol	$(C_2H_4O_2)_2{}^{P}Si(OC_2H_4OH)^-$	−110.2	81.7
22	Branched dimer, ethylene glycol	$(C_2H_4O_2)_2{}^{P}SiOC_2H_4O{}^{P}Si(O_2H_4C_2)_2{}^2$	−114.4[a,b] (expt. −103 to −105)[c]	
23	^{Q}Si, six-membered rings, cyclic trimer	$(C_3H_6O_2)_3{}^{Q}Si_3O_3$	−96.9[a,b]	32.8
24	^{H}Si bonded to O1 and O2, 1:3 spirocyclic threitol	$(C_4H_8O_4)_3{}^{H}Si^{2-}$/	−158.1[d] −151.0[f]	16.5
25	^{H}Si bonded to O2 and O3, 1:3 spirocyclic threitol	$(C_4H_8O_4)_3{}^{H}Si^{2-}$//	−152.9[d,e] −151.0[f]	2.1
26	^{H}Si, five- and six-membered rings, tridentate citrate	$(C_6H_5O_7){}^{H}Si(O_7H_5C_6)^{2-}$	−174.1[f] (expt = −167.4[g])	54.6
27	^{P}Si, six-membered ring citrate	$(C_6H_6O_7){}^{P}Si(OH)_3{}^-$	−123.4	127.1
28	^{H}Si, six-membered ring glycerol	$(C_3H_6O_2)_3{}^{H}Si_3O_3$	−96.9[b]	
29	^{P}Si, glucopyranose	$(C_6H_{12}O_6){}^{P}Si(OH)_3{}^-$	−115.4[b]	113.4
30	^{H}Si, glucopyranose	$(C_6H_{12}O_6){}^{H}Si(OH)_4{}^-$	−159.6[d]	28.5

[a] Sahai and Tossell (2002).
[b] Matches experimentally observed peaks at −101 ppm.
[c] Herreros et al. (1994a).
[d] Matches experimentally observed peaks at −141 to −145 ppm.
[e] XRD structure from Benner et al. (2003) used directly without optimization.
[f] Starting structure from Benner et al. (2003) reoptimized in gas phase at HF/6-316* level.
[g] XRD structure from Tacke et al. (2002) used directly without optimization geometry.
[h] Tacke et al. (2002).

Table 3. Theoretical ^{29}Si NMR shifts (ppm) of silicate-amino-acid complexes at HF/6-311+G(2d,p)//HF/6-31G* level. All structures are Si-monodentate ligand complexes.

Structure	Complex	Formula	δ	$\sigma_{33} - \sigma_{11}$
31	^{Q}Si, serine	$C_3H_6O_3{}^{Q}Si(OH)_3$	−69.7[a]	55.4
32	^{P}Si, serine	$C_3H_6O_3{}^{P}Si(OH)_4^-$	−121.7[a]	141.1
33	^{P}Si, serine	$C_3H_6O_3{}^{P}Si(OCH_3)_4^-$	−82.0[a]	22.4
34	Serine-^{P}Si(OCH$_3$)$_3$-histidine	$C_3H_6O_3$-$^{P}Si(OCH_3)_3$-$N_3O_2H_9C_6^-$ or serO-Si(OCH$_3$)$_3$-Nhis	−132.0[b]	

[a] Sahai and Tossell (2001).
[b] This study.

reported at −101 ppm and the peak at −141 ppm split into a heptet suggesting the existence of six equivalent H atoms in H-C-O-Si bonds (Kinrade et al., 1999, 2001a). Any proposed structures would, therefore, have to account for all these features.

Comparing the results in Tables 1 and 2, it is evident that five-membered rings containing ^{P}Si and ^{H}Si can explain the observed shifts at −101 and −140 to −145 ppm, but seven-membered rings cannot (Figs. 2–4). Five-membered ring structures proposed previously in the literature and seven-membered ring ion pairs (**13**) can also explain the ^{29}Si shifts at −101 and −141 to −145 ppm (fig. 2d in Sahai and Tossell, 2002; Sahai, 2003; Kubicki et al., 2003), but they cannot explain the heptet in the ^{1}H-^{29}Si coupled spectra. In addition, it was previously suggested that ring-shaped silicate trimers containing ^{Q}Si (**28**, Fig. 4b), and mixed coordination Si with rapid exchange between ^{P}Si and ^{H}Si sites (fig. 3i in Sahai and Tossell, 2002) could also explain the −101 ppm resonance. Structure **28** is consistent with observed ^{1}H-^{29}Si coupled and decoupled spectra. It is, however, difficult to justify the rapid exchange of a heavy atom like Si between ^{P}Si and ^{H}Si sites in the relatively rigid structure of the trimeric ring (fig. 3i in Sahai and Tossell, 2002). Likewise, the seven-membered ring structures (η^2-L$_3$)^{H}Si and (η^2-L)$_2{}^{H}$Si(-μ-L)$_2{}^{H}$Si(η^2-L)$_2$ proposed by Kinrade et al. (2001) do not satisfy all the observed spectral features. In this notation, L represents ligand, ^{H}Si represents hexa-coordinated Si, η and μ^{i} represent nonbridging and bridging ligands bonded to the metal through i atoms (i is the ligand's denticity). These structures contain six equivalent H atoms and can explain the heptet, but as shown by calculations they yield ^{29}Si resonances that are far too negative (Sahai and Tossell, 2002; Kubicki and Heaney, 2003; this study). Only structures containing five-membered rings and also containing six equivalent H positions can explain both features of the ^{29}Si spectrum, viz., the −141 ppm isotropic shift and the ^{1}H-coupled heptet (e.g., **25, 28**). This conclusion is consistent with a recent X-Ray Diffraction structure determination and NMR shift measurement for silicate-polyalcohol crystals (Benner et al., 2003). In summary, with the exception of **25** and **28**, the detailed structural assignments in previous theoretical and experimental studies were not correct, but the observation that hyper-coordinated Si-aliphatic compound complexes can form in aqueous solution is a significant discovery of its own merit. A separate question is whether quadra-coordinated and hyper-coordinated silicate complexes play a role in biologic silica utilization.

4.2. Silicon Utilization in Diatoms

To determine silicon speciation in diatoms, it is important to distinguish between the different metabolic processes ultimately leading to silica biomineralization. These processes include uptake of dissolved silicon from the ambient extracellular aqueous medium by transport across the cell membrane, intracellular transport to silicon "pools" where silicon is held until required for polymerization, transport to the silica deposition vesicle, and finally, silica precipitation (reviewed in Martin-Jezequel et al., 2002).

In the first instance, the organism needs to bring silicon into the cell, by transporting either inorganic silicon as silicic acid or silicate-organic complexes. Silicate-organic complexes may be formed by ligands exuded by the organism with the specific purpose of sequestering silicon, or by silicon complexing with organic ligands already present in the environment.

Experimental evidence indicates that transport of silicon from the extracellular medium across the diatom cell membrane is carrier-mediated, electrogenic, involves silicic acid and Na^+ in a 1:1 ratio, and requires metabolic energy (Azam et al., 1974; Bhattacharya and Volcani, 1980; Hildebrand et al., 1997). Different types of silicic acid transporter genes have been identified, and code for different types of transporters, probably of varying binding character and localization within the cell (Hildebrand et al., 1997, 1998). Using model compounds, we have attempted to determine whether it is more favorable for the transporter to bind silicic acid or a silicate-organic complex (Sahai and Tossell, 2001). The transporter was represented by serine, and the silicate-organic compound by Si(OCH$_3$)$_4$. We found that the formation of ^{Q}Si-serine complexes is relatively more favorable when the starting compound is Si(OH)$_4$ compared to Si(OCH$_3$)$_4$ (Sahai and Tossell, 2001). These facts suggest that the organism has no thermodynamic advantage in exuding extracellular organic ligands that would bind silicic acid from seawater into a silicate-organic compound before transporting the silicon across the cell membrane.

Also, considering that pK$_a$ of silicic acid is 9.5 (Sjoberg et al., 1981), H_4SiO_4 is the dominant form of dissolved silicon in the circum-neutral to mildly alkaline pH of most natural waters. The preferential uptake of H_4SiO_4, relative to $H_3SiO_4^-$ by many, albeit not all, diatom species has been confirmed experimentally (Amo and Brzezinski, 1999). Thus, neutral silicic acid is the most likely form of silicon transported from the ambient aqueous medium across the cell membrane.

During transport, the silicon is bonded to the transporter

compounds, and stored subsequently in silicon pools. Silicon concentrations two to three orders of magnitude larger than that allowed by silica solubility have been measured in these pools, yet the silicon appears to be in monomeric or small oligomeric form. The existence of dissolved Si at concentrations exceeding SiO_2 solubility has lead to suggestions that the silicon must be complexed or bound in some sort of silicate-organic compound in the pools (Azam et al., 1974; Sullivan, 1979; Bhattacharya and Volcani, 1980; Binder and Chisholm, 1980; Blank et al., 1986). Ab initio MO calculations can be used to determine if such compounds are thermodynamically even feasible.

Results of MO calculations show that formation of silicate-organic compounds, such as ^{Q}Si-serine and ^{Q}Si-threitol, where silicon is quadra-coordinated and is linked to the organic by a direct Si-O-C bond, is energetically more favorable than the formation of H-bonded complexes between H_4SiO_4 and any -OH group on the organic compound (Sahai and Tossell, 2002). Thus, quadra-coordinated silicate-organic compounds are entirely possible in the silicon pools. It would be difficult to identify such compounds using simple ^{29}Si NMR spectroscopy because of their similar resonance frequency is similar to that of inorganic silicon species. For example, monomeric ^{Q}Si-organic aliphatic linear or branched compounds (**1–2**, **31**, **33**) and oligomeric ^{Q}Si-organic compounds (**23**) may overlap with Q^1, Q^2 and Q^3 inorganic silicon at -71 to -75, -82 and -96 to -100 ppm (Q^n indicates a quadra-coordinated silicon with n polymerized bonds). Techniques such as ^{1}H-coupling, ^{13}C and, probably most sensitively, ^{17}O NMR, would help to distinguish between these compounds (Sahai and Tossell, 2001).

The formation of hyper-coordinated silicate-organic complexes is highly endothermic compared to the formation of ^{Q}Si complexes (Sahai and Tossell, 2001, 2002). For example, formation of structure **3** from silicic acid and threitol is 175.7 kJ.mol^{-1} more endothermic than formation of structure **1**. Even structure **18,** which is more stable than **3** by 91.2 kJ. mol^{-1}, is more endothermic to form than **1**. Formation of ^{P}Si complexes becomes more favorable when the starting silicon compound is $H_3SiO_4^-$. Assuming that pH $<$ 9.5 in the silicon pools, $Si(OH)_3O^-$ concentrations should be low compared to H_4SiO_4. Thus, hyper-coordinated complexes are highly unlikely for silicon storage over the periods of hours corresponding to silica valve formation. These results are consistent with experimental observation that at neutral pH and dilute silicon concentrations, only the -71 and -101 ppm peaks were found, implying that structures responsible for the -144 ppm signal only become abundant under conditions of very high pH and high silicon concentration. In fact, the experiments were conducted at conditions of high pH and/or high silicon and high organic concentrations (Kinrade et al., 1999, 2001b; Benner et al., 2003).

The silicon is ultimately transported to the silica deposition vesicle where silica polymerization occurs. Two different schools of thought exist for the form in which silicon is present in the silica deposition vesicle. One possibility is that silicon starts out as silicic acid as assumed by the German group (Kroger et al., 1999, 2000). These scientists isolated proteins (silaffins) from diatoms and found that sections containing polyamines are capable of polymerizing silicic acid with maximum efficiency at the low pH typical of the silica deposition vesicle. The alternative assumption of a silicate-organic starting compound is implicit in the work of the Santa Barbara group (Shimizu et al., 1998; Cha et al., 1999; Zhou et al., 1999). An enzyme named silicatein was isolated from a sponge, and found to contain serine and histidine at the active sites. $Si(OC_2H_5)$ was used as the starting silicon compound. The enzyme catalyzed $Si(OC_2H_5)$ hydrolysis and polymerization. An activated complex or reaction intermediate consisting of ^{P}Si bonded to the side-chain of a serine, three ethoxy groups, and the N atom of a histidine molecule was proposed. The calculated ^{29}Si-NMR shift for the complex is -132 ppm, close to the value measured in a live diatom culture (Kinrade et al., 2002). The present mo calculations do not address which form of silicon is most likely in the silica deposition vesicle.

5. CONCLUSIONS

Ab initio calculations and experimental work together suggest that hyper-coordinated silicate-complexes in five-membered rings are stable in aqueous solution at high pHs and/or high silicon or organic concentrations. Hyper-coordinated complexes may exist as transient intermediates or activated complexes during hydrolysis and polymerization of silicate-organic compounds. This was first demonstrated in the mid-1990s by inorganic synthetic chemists studying new routes for the sol-gel synthesis of silica. When energetic considerations are combined with NMR spectral information, however, it appears that an organism has no thermodynamic advantage in transporting and storing silicon as hyper-coordinated silicate-organic complexes at the pHs of ambient seawater, freshwater and the solution in the silica deposition vesicle. Energetic considerations also suggest that quadra-coordinated complexes may be involved in silicon transport.

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