Soluble Silica with High Affinity for Aluminum under Physiological and Natural Conditions

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Abstract: Soluble silica reduces aluminum availability and ameliorates toxicity in several biological systems. It has therefore been suggested that these two species strongly interact in solution. However, there is only weak affinity between monomeric silicic acid and aluminum with reported log K_{eff} of between 4 and 6 at pH 7.2. We now show the existence of a soluble low molecular weight form of silica that is nonmonomeric but has an affinity for aluminum at least 1 000 000 times greater than the monomeric form (log $K_{eff} = 11.70 \pm 0.30$ at pH 7.2). This was established by competition, for binding of aluminum, between different preparations of soluble silica and the powerful M^{3+} chelator, 1,2-dimethyl-3-hydroxy-4-pyridinone (DMHP). At pH 7.2, this nonmonomeric silica quantitatively displaced one equiv of DMHP for every 34.42 ± 0.77 equiv of total silica, suggesting that the soluble silica that so avidly binds aluminum is an oligomer containing fewer than 35 silicon atoms. The presence of oligomeric or monomeric silica in solution depends on how the solution is prepared. The oligomer is formed when alkali stock solutions of silica (42 mM in this work) are pH neutralized *prior* to dilution, to physiological and natural levels of soluble silica (<2.5 mM). The diluted oligomeric silica is at least transiently stable (<1 day or >17 days; absence or presence of aluminum) and would compete effectively with endogenous physiological chelators for aluminum, such as citrate (log K_{eff} aluminum citrate = 7.15 at pH 7.2). Oligomeric silica is probably responsible for the many experimental observations on the amelioration of aluminum availability and toxicity, but whether this silica species occurs naturally in the environment or is formed *in vivo*, remains to be established.

Introduction

A unique affinity exists between aluminum, oxygen, and silicon,[1,2] both in the environment where highly stable solid phase aluminosilicates make up a major portion of the earth's crust[3] and in biological systems where silica, as soluble silicic acid, reduces aluminum availability[4] and ameliorates toxicity.[5−7] In the abundant clay minerals, units of octahedral $[AlO_6]^{3-}$ form layers with tetrahedral $[SiO_4]$, and a diversity of structures is gained from ion substitutions with the latter.[8] Notably aluminum will readily exchange for silicon yielding the lowest potential energy of the substituted clays.[8]

Such strong interactions, limiting the availability of aluminum, are mirrored in the biosphere. In experiments with isolated pancreatic acinar cells,[6] the toxicity of aluminum was prevented when silica was added intracellularly at concentrations 10-fold higher than the added aluminum. Similarly, in experiments with Atlantic salmon fry,[7] the ratio of 13:1 (silica to aluminum) prevented all fish death, although a ratio of 3.7:1 had no effect

at all. This high dependence on silica concentration is unexpected if monomeric silicic acid appears in a rate or equilibrium equation to describe the aluminum ameliorating effect. Indeed, monomeric silicic acid has only a relatively weak affinity for aluminum, with a theoretical log K_{eff} of 4.3 (ionic strength = 0.1 M)[9] and a measured log K_{eff} of 4.70 ± 0.05 (ionic strength = 0.1 M)[10] or 6.13 ± 0.06 (ionic strength = 0.01 M),[11] all at 25 °C and pH 7.2.

Below pH 9, the boundary concentration that separates monomeric and oligomeric silica is almost constant at 2−3 mM total SiO_2.[12] At silica concentrations above this, solutions contain mixtures of oligomers including linear and cyclic trimers and tetramers.[12] Upon dilution of such solutions, these oligomers persist, at least transiently, even below the boundary concentration, and both river and tap water contain some soluble nonmonomeric silica.[13]

Here the affinity of oligomeric silica for aluminum, was compared to monomeric silica for aluminum, by competition with the powerful M^{3+} chelator, 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone (DMHP) **1**.

The UV absorbance spectrum of the DMHP:aluminum (1:1) complex peaks at 289 nm and is distinct from that of the unbound chelator at 274 nm (Figure 1). This therefore allows

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(3) Klein, C. In *Reviews in Mineralogy vol. 28. Health effects of mineral dust;* Guthrie, G. D., Jr., Mossman, B. T., Eds.; Mineralogical Society of America, Washington, D.C.; Bookcrafters Inc.: Chelsea, MI, 1993; p 8.

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(6) Petersen, O. H.; Wakui, M.; Petersen, C. C. H. In *Aluminum in Biology and Medicine;* Chadwick, D. J., Whelan, J., Eds.; Ciba Foundation Symposium 169; John Wiley and Sons Ltd.: Chichester, 1992; pp 237−253.

(7) Birchall, J. D.; Exley, C.; Chappell, J. S.; Phillips, M. J. *Nature* **1989,** 338, 146−148.

(8) Laszlo, P. *Science* **1987,** 235, 1473−1477.

(9) Martin, R. B. *Polyhedron* **1990,** 9, 193−197.

(10) Farmer, V. C.; Lumsden, D. G. *Geochim. Cosmochim. Acta* **1994,** 58, 3331−3334.

(11) Browne, B. A.; Driscoll, C. T. *Science* **1992,** 256, 1667−1670. This high value compared with the calculated (ref 9) and other experimental (ref 10) values is likely to be due to contamination of the solution with low levels of oligomeric silicic acid, rather than differences in the ionic strengths (ref 17).

(12) Iler, R. K. *The Chemistry of Silica;* John Wiley: New York, 1979. Glasser, L. S. D.; Lachowski, E. E. *J. Chem. Soc., Dalton Trans.* **1980,** 393−402. Glasser, L. S. D. *Chem. Bri.* **1982,** 36−39. Sjoberg, S. *J. Noncryst. Solids* **1996,** 196, 51−57.

(13) Bogdanova, V. I. *Eksp. Issled. Mineral.* **1972−1973,** 1974, 183; [*Chem. Abstr. 82,* 160059x].
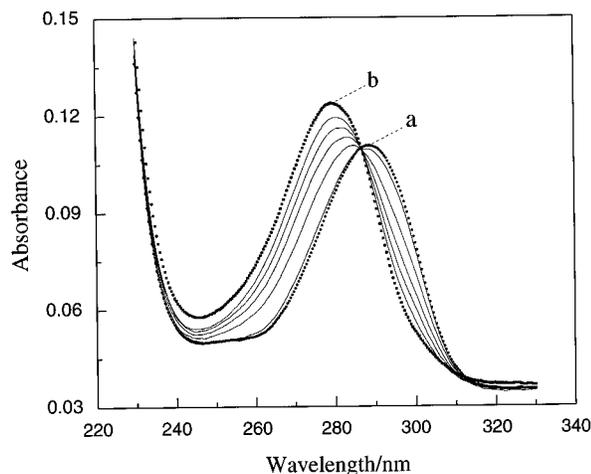
Figure 1. (a) The UV spectrum of 8 μM DMHP:Al (1:1) complex has a λ_{max} at 289.2 nm at pH 7.2 (285.2 nm at pH 4.6). Free DMHP (i.e., unbound) has a λ_{max} at 274.4 nm. (a→b) Titration of the 8 μM DMHP:Al complex with increasing concentrations of oligomeric silica leads to an increase in the free DMHP that may thus be monitored (Figure 3, pH 7.2 curve).

Scheme 1

1
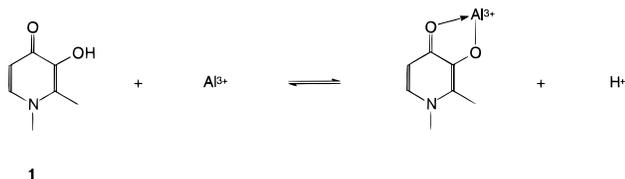
the distribution of aluminum between DMHP and a competing ligand, such as silica, to be monitored, and hence the affinity of silica for aluminum was calculated using this competition assay. In this work, 8 μM DMHP and 8 μM Al were used where the 1:1 complex is the only significant species.[14] Although this 1:1 complex is certainly hydrolyzed, especially toward neutral pHs, to [AlLOH]$^+$ or even [AlL(OH)$_2$], we quote only pH specific equilibrium constants with no implication of hydrolysis, as this should make little difference to *relative* affinities.[15]

Equilibrium was demonstrated by the independence of the final spectrum upon the order of addition of the DMHP and silica (Figure 2). The logarithm of the conditional stability constant of the complex formed between aluminum and oligomeric silica was obtained by reference to the log K_{eff} for the complex formed between aluminum and DMHP, which has a value of 9.70 $\pm$ 0.03 at pH 7.2 (0.1 M KCl, 25°C).[16] Throughout, conditional stability constants (K_{eff}), defined as [complex]/([unbound ligand] [Al^{3+}]), take into account the differing protonation of the ligands (DMHP, citrate, Si(OH)$_4$, or oligomeric silica), at pH 7.2, but not hydroxy−aluminum species since these contribute an equal but ill defined correction to all K_{eff} values.[15] As above, aluminum−hydroxy−ligand complexes were also excluded since these are poorly characterized and, although their inclusion would strongly affect the

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(14) Under the conditions of the assay used in this work, the [AlL]$^{2+}$ complex was present, while the [AlL$_2$]$^+$ and [AlL$_3$] complexes only appear at significantly higher ligand concentrations. We have performed the most comprehensive simultaneous automated spectrophotometric/potentiometric titrations of the DMHP:Al system at extremely slow rates of titration, under a range of metal−ligand ratios. We have then analyzed the single wavelength titrations as well as the principle components of the spectral data matrix after factor analysis, using global nonlinear regression of the entire data set. We have yet to publish these results although they indicate the presence of species [AlLOH]$^+$, [AlL(OH)$_2$], and [AlL$_2$(OH)] with stability constants that we have now determined.
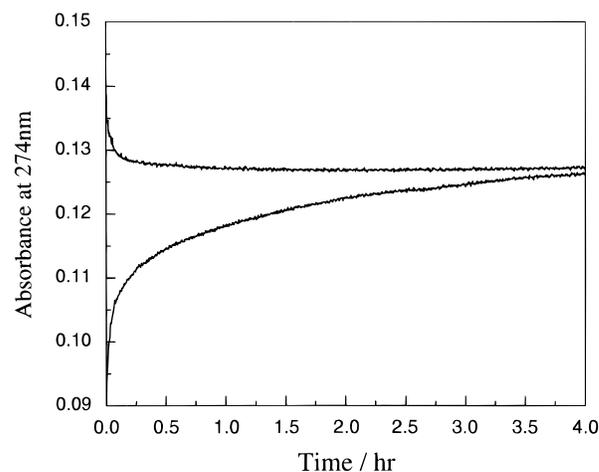
Figure 2. (bottom) Kinetics of the displacement of DMHP from aluminum by oligomeric silica corresponding to Figure 3 at pH 7.2, at two-thirds saturation. (top) The reverse reaction. Displacement of oligomeric silica from aluminum under the same conditions. Thus final absorbance is not significantly dependent on order of addition of reagents. Oligomeric silica was prepared as in Figure 3. Similar converging absorbance curves were observed for a range of oligomeric silica concentrations (0−1.4 mM), at pH 7.2 (DMHP and Al at 8 μM). The full spectral analysis (230−330 nm, as in Figure 1) further confirmed these observations, as the individual spectra of the forward and reverse reactions were superimposed.

absolute affinities, the relative affinities would change little.[15] Ionic conditions are indicated, but no preferred equation for small corrections in the stability constants for variation in ionic strength is applied.[17]

Experimental Section

Spectra were recorded on a Perkin-Elmer Lambda 5 UV/vis spectrophotometer interfaced to an IBM computer. The molybdate assay for monomeric silica was according to the method of Alexander.[18] Ammonium molybdate (Analar; BDH Ltd., Poole, UK) was found to contain only trace amounts of Si and Al by inductively coupled plasma optical emission spectroscopy (ICPOES). Total elemental analyses by ICPOES (Jobin-Yvon JY24 instrument S.A., Longumeau, France) were with a v-groove nebulizer and conventional Scott type double pass spray

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(15) The hydrolytic behavior of aluminum complexes is not well understood, but there is no suggestion in existing work that it significantly alters *relative* aluminum-ligand affinities. For aluminum-ligand complexes of equal charge, the affinity of the hydroxide ion for aluminum is relatively insensitive to the identity of the ligand. Furthermore, the cooperativity of aluminum hydrolysis lessens the dependence on charge. Hence, until the hydrolytic behavior of these complexes is understood, relative affinities are best handled using stability constants obtained at low pH with correction for the different pK_a values of the ligands. Thus: log K_{eff} = log K − log(1 + [H$^+$]/Ka_1 + [H$^+$]^2/$Ka_1 Ka_2$ + ...).

(16) Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta.* **1992**, 191, 57−63.

(17) Because of the problems with trace aluminum contamination in high concentrations of salts, we avoided using high ionic strengths, of say 0.6 M NaCl. We therefore considered the corrections that should be made to make comparisons of K_{eff}s from our work with those in the literature at higher ionic strengths. However the Davies modification of the Debye−Huckel formula (IUPAC recommended) gives much larger corrections than are found experimentally. For example, at ionic strength 0.6 M (NaCl) Ohman gives log $\beta_{-1,1,1}$ for [Al cit]^0 as −4.9, while at 0.25 M ionic strength (NaClO$_4$) the value is −4.7 (*Stability constants of metal-ion complexes*; Sillen, L. G., Martell, A. E., compilers; The Chemical Society Special Publications, no. 25 (supplement no. 1); Chemical Society: London, 1971). The Davies correction calculates these to differ by 1.93 log units and is clearly not applicable at these high ionic strengths and with the large changes in charge on complexation. This is a known problem with the equation under such conditions, and thus we feel that a mathematical correction would introduce a larger error than using stability constants with uncorrected ionic strength.

(18) Alexander, G. B. *J. Am. Chem. Soc.* **1975**, 75, 5655−5657. The molybdate method measures labile silica that is chiefly monomeric but may include dimers and perhaps linear trimers.

chamber. Analytical wavelengths were 251.611 nm for silicon (252.411 nm if molybdenum (Mo) was present) and 396.152 nm for aluminum as previously described.[19] Silicon (1010 mg/L in 5.3% wt NaOH) and aluminum (1001 mg/L in 0.5% HNO₃) ICP/DCP standard solutions were purchased from Aldrich Chemical Co. (Gillingham, UK) and BDH Ltd., respectively.

Water was purified to 18 MΩcm⁻¹ using a Branstead E-pure ion-exchange system. Aluminum and DMHP were prepared from dilution of 20 mM stock solutions. The aluminum stock solution was prepared from aluminum nitrate nonahydrate (Analar, BDH Ltd.), potassium chloride (Aristar, BDH Ltd.) and hydrochloric acid (1 N, Aldrich Chemical Co.) to give 20 mM aluminum, 0.1 M potassium chloride, and 0.04 M hydrogen chloride (pH <2). A stock solution of DMHP was prepared from the hydrochloride salt (gift; Dr. G. Tilbrook, Kings College, London), also in 0.1 M potassium chloride. MOPS buffer (50 mM, pH 7.2) was prepared from 4-morpholinepropanesulphonic acid (Aldrich Chemical Co.) and sodium hydroxide (1 N volumetric standard, Aldrich Chemical Co.). Acetate buffer (50 mM, pH 4.6) was prepared from sodium acetate trihydrate (Analar, BDH Ltd.) and hydrochloric acid.

All silica solutions were prepared from a basic (14% NaOH) concentrated (7 M Si by ICPOES) sodium silicate solution (Aldrich Chemical Co.). Throughout the experiments, final silica concentrations were 0−2.8 mM, at pH 7.2. However, *monomeric* silica solutions were prepared by appropriate dilution of the concentrated silicate solution *and then* pH neutralization, whereas, *oligomeric* silica solutions were prepared by preparation of a 42 mM stock solution at pH 7.2 *and then* (24 h later) appropriate dilution. The contaminant aluminum in the concentrated silicate solution was determined by ICPOES (1.59 μmol Al per mmol Si) and was accounted for in any calculations that used total Al concentrations (total Al = added Al (8 μM) + contaminant Al). In fact, including or ignoring this contaminant Al had little effect on values of K_{eff} and p (see Results and Discussion).

Results and Discussion

Competition Study. At the same *total* concentration of SiO₂, the affinity for aluminum depended dramatically on the method by which the silica solution was prepared. Thus, at 0−2 mM total SiO₂, which is below the polymerization boundary, a solution of oligomeric silica prepared by pH neutralization (7.2) at 42 mM[20] *and then* diluted, clearly bound aluminum more strongly than that diluted and then pH neutralized (Figure 3).

At pH values much above 7.2, and particularly > 9, the oligomeric silica would rapidly favor formation of the low aluminum-affinity monomeric silica[12] (Figure 3). At pH values below 7.2, the affinity of oligodentate silica for aluminum decreased relative to DMHP (Figure 3), indicating that displacement of DMHP by oligomeric silica was accompanied by a net release of protons:

$$(-SiOH)_m + AlL \rightleftharpoons$$
$$(-SiOH)_{m-n}(-SiO)_nAl + (n-1)H^+ + LH \qquad (n > 1)$$
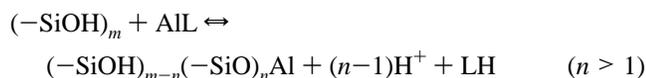
Hence the oligomeric-silica/aluminum interaction is particularly favored around the neutral pH's of most natural and physiological systems.

From the competition data (i.e., Figure 3), log K_{eff} of the oligomeric-silica/aluminum interaction and a maximum estimate of the molecular size of the oligomeric silica were obtained using the following competitive binding model:

(19) Burden, T. J.; Powell, J. J.; Taylor, P. D.; Thompson, R. P. H. *JAAS.* **1995**, 10, 259−266.
(20) As shown by competition with the DMHP:Al (1:1) complex, the oligomers that show high affinity for Al are formed within minutes of neutralization of the sodium silicate solution (42 mM), prior to its subsequent dilution (0−2.8 mM). This 42 mM oligomeric silica reaches maximum affinity for aluminum at 3 h and maintains this for 48 h, following which significant polymerization starts and affinity lessens. In parallel to this DMHP affinity assay, the molybdate assay (ref 18), allowed the oligomerization/polymerization of silica to be followed.
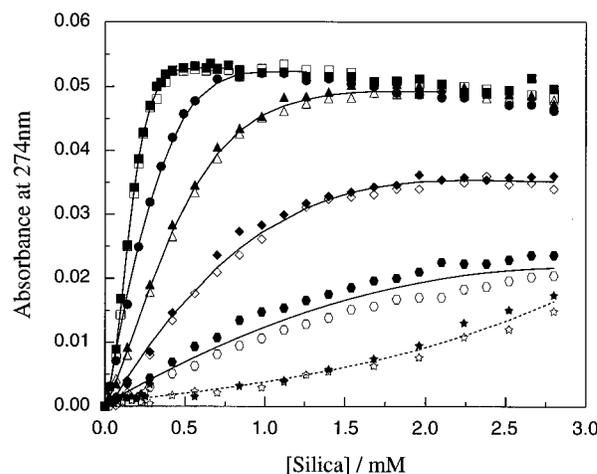
Figure 3. (solid lines) Displacement, monitored at 274 nm (shown as the change in absorbance), of DMHP from 8.0 μM (1:1 ratio) DMHP: aluminum complex as competing oligomeric silica is added at pH 7.2 (squares), pH 6.56 (circle), pH 5.32 (triangles), pH 4.63 (diamonds) or pH 4.16 (hexagons), all at 25 °C. Open and solid symbols show duplicate data sets. Stock solutions of *oligomeric silica* were freshly prepared by addition of appropriately pH adjusted buffer to a basic sodium silicate solution (originally Na₂O:SiO₂ = 1:2.87; 53 mM SiO₂, prepared from the concentrated (7 M Si) sodium silicate solution) to give final stock concentrations of 42 mM SiO₂ and 10 mM buffer (MOPS or acetate) at the required pH. Twenty-four h after preparation of the 42 mM silica solution, differing amounts of this solution were then added to a similarly pH buffered solution of the DMHP/aluminum complex and equilibrated for 48 h, which ensured completeness of reaction (Figure 2) before analysis by UV spectrophotometry. The solid lines are simulated from the competitive binding model (see text). In the case of $q = 1$, for the top five curves the values of log R and p, determined by nonlinear regression are as follows. At pH 7.2 (log R = 2.00 ± 0.27, p = 34.42 ± 0.77); pH 6.56 (1.40 ± 0.11, 51.72 ± 0.95); pH 5.32 (1.29 ± 0.07, 83.10 ± 1.03); pH 4.63 (1.40 ± 0.14, 113.94 ± 2.46); pH 4.16 (0.54 ± 0.10, 185.80 ± 7.15). (broken line) Addition of monomeric silicic acid (stars) (diluted to below polymerization boundary *before* pH adjustment) to 8.0 μM DMHP:aluminum (1:1) complex at pH 7.2 (25 °C). Open and solid symbols show duplicate data sets. The slight competition toward 2−3 mM silica shows the onset of oligomerization.

$$K_{eff}/K'_{eff} = R = \frac{[Al(Si_n)_q]\{[L_t] - ([Al_t] - [Al(Si_n)_q])\}}{([Al_t] - [Al(Si_n)_q])([Si_t]/p - q[Al(Si_n)_q])^q}$$

K_{eff} and K'_{eff} are the aluminum-binding constants of silica and DMHP, respectively. [Al$_t$] is the total aluminum concentration, and [Si$_t$] and [L$_t$] are the concentrations of total silica and organic chelator. [Al(Si$_n$)$_q$] is the concentration of oligomeric-silica/aluminum complex, and q is the number of oligomers required to displace one molecule of DMHP. $1/p = m/n$ where m is the fraction of total silica in the high affinity form and n is the number of silicon atoms in the high affinity oligomer.

This competitive binding model was derived from the following simplified competitive equilibria

$$AlL + qSi_n \rightleftharpoons Al(Si_n)_q + L_{unbound}$$

The chemical equilibrium is expressed by

$$R = \frac{[Al(Si_n)_q][L_{unbound}]}{[AlL][Si_n]^q} \qquad (1)$$

The conservation equations are

$$[L_t] = [AlL] + [L_{unbound}]$$

$$[Al_t] = [AlL] + [Al(Si_n)_q] + [Al_{free}]; \quad [Al_{free}] \sim 0$$

$$[Si_t] = qn[Al(Si_n)_q] + n[Si_n] + [Si]$$

From which

$$[L_{unbound}] = [L_t] - [AlL] \tag{2}$$

$$[AlL] = [Al_t] - [Al(Si_n)_q] \tag{3}$$

$$[Si_n] = \{([Si_t] - [Si])/n\} - (q[Al(Si_n)_q])$$

By definition

$$m = ([Si_t] - [Si])/[Si_t]$$

hence

$$([Si_t] - [Si]) = [Si_t]m$$

and

$$[Si_n] = \{([Si_t]m)/n\} - (q[Al(Si_n)_q])$$

However m and n cannot be independently resolved from this experiment, so the ratio m/n was combined as the single parameter $1/p$.

$$[Si_n] = ([Si_t]/p) - (q[Al(Si_n)_q]) \tag{4}$$

Substitution of eq 2−4 into the chemical equilibrium eq 1 gave the competitive binding model.

A single isobestic point in the UV spectra (Figure 1) is consistent with a two component system suggesting that mixed complexes are not significant and that a single multidentate silica oligomer is the competitor. We therefore set $q = 1$. The expression for the competive binding model can then be rearranged to a simple quadratic equation in [Al(Si$_n$)], with coefficents determined by the known values of [Al$_t$], [L$_t$], and [Si$_t$] and the unknown parameters p and R.

$$[Al(Si_n)]^2(R - 1) + [Al(Si_n)]\{[Al_t] - [L_t] -$$
$$R([Si_t]/p + [Al_t])\} + R[Al_t]([Si_t]/p) = 0$$

A program written in Visual Basic was used to solve the quadratic for [Al(Si$_n$)] and calculate the concentrations of all the remaining species in the conservation equations as a function of [Si$_t$]. This was incorporated in the program STABOPT,[21−23] and the parameters p and R were optimized from the experimental data by nonlinear regression using the Gauss−Newton−Marquardt algorithm with the numerically calculated first derivatives.

The optimized value of log R at pH 7.2 was 2.00 ± 0.27, giving a log K_{eff} for the oligomeric-silica/aluminum interaction of 11.70 ± 0.30 at pH 7.2. The optimized value of p (n/m) at pH 7.2 was 34.42 ± 0.77. Similar values for p were obtained using, first, a crude precipitation assay,[24] and, secondly, by monitoring depolymerization of oligomeric silica in the presence and absence of aluminum.[25] Since the maximum possible value of m is 1, the upper limit of the oligomer size (n) is a 35-mer.[26]

(21) Taylor, P. D.; Hider, R. C.; Morrison, I. E. G. *Talanta* **1988**, 35, 507.

(22) Taylor, P. D. *Talanta* **1995**, 42, 243.

(23) Taylor, P. D. *Chem. Commun.* **1996**, 405−406.
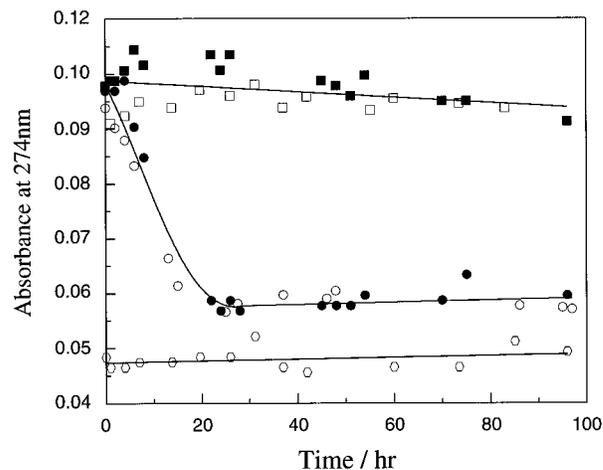
Figure 4. Affinity of silica for aluminum (pH 7.2, 25°C) as a function of time following dilution of oligomeric silica to a concentration below the polymerization boundary. Absorbance is corrected for background ($A_{274 \text{ nm}} - A_{330 \text{ nm}}$). Solid and open symbols show duplicate data sets. (circles) *Aluminum absent during incubation time.* Oligomeric silica was diluted to 560 μM SiO$_2$. At the times indicated in the graph, a sample was withdrawn and added to a small volume of the DMHP:Al (1:1) complex to give a final concentration of 8.0 μM complex. The release of DMHP was measured 160 min after addition of complex when equilibration was > 90% complete. (squares) *Aluminum present during incubation time.* Oligomeric silica was diluted to 560 μM SiO$_2$ as before, but, in this case, aluminum nitrate was immediately added before depolymerization occurred. At the times indicated in the graph, a sample was withdrawn and added to a small volume of DMHP to give final concentrations of 8.0 μM for both DMHP and aluminum. Nonaluminum-bound-DMHP was then measured as above. (hexagon) *Aluminum in the absence of added silica.* In control experiments, without silica, aluminum was fully bound to DMHP at all time points.

Stability of the Silica Oligomer. Dilution of oligomeric silica to below 2 mM led to depolymerization and loss of affinity for aluminum within 24 h (Figure 4). However when oligomeric silica was diluted well below the boundary region and then aluminum *immediately* introduced, depolymerization was inhibited, and high affinity for aluminum was maintained (Figure 4) for at least 17 days.[25,27] However under the conditions used here, we did not find evidence for template oligomerization of silica around aluminum, since preincubation of monomeric

(24) The extent of precipitation of Al, forming Al(OH)$_3$ at neutral pH, has been used to measure the Al binding capacity of physiological ligands (Powell, J. J.; Taylor, P. D.; Thompson, R. P. H. *J. Inorg. Biochem.* **1993**, 51, 188). In a similar manner the maximum aluminum binding capacity of an oligomeric silica solution was measured in 50 mM MOPS buffer at pH 7.2. A total silica concentration of 2.61 mM, prepared from the original 42 mM solution, maintained 72.18 μM Al in solution, giving a Si:Al ratio of 36.16, compared to 34.42 ± 0.77 from the text. Aluminum and silicon concentrations were measured by ICPOES.

(25) The depolymerization of oligomeric silica upon dilution (42 → 0.56 mM) was also followed by measuring the increase in concentration of monomeric silicic acid (Si(OH)$_4$) with time, using the molybdic acid assay (ref 18), and this method similarly showed that, in the presence of 8 μM Al, oligomeric silica was stabilized in solution. Depolymerization was not completely inhibited, since silica was in great excess compared to Al, but the rate was dramatically reduced. Furthermore, the ratio of nonmonomeric silica atoms to Al atoms, 48 h after the dilution, was 44.35 ± 5.25 (mean $\pm$ SD of six determinations; range 36.85−48.5), compared to 34.42 ± 0.77 from the competition study.

(26) A better "maximum estimate" for m was determined by the following method. Total silicon was measured by ICPOES, while monomeric silicic acid was determined chemically with the molybdic acid assay of Alexander (ref 18). Twenty-four after neutralization of the 42 mM silicate solution (i.e., immediately prior to its use in the DMHP competition studies), $m = 0.927 \pm 0.001$ for the oligomeric preparation and 0.009 ± 0.007 for the monomeric preparation. Both values, mean $\pm$SD of four determinations. These m values, however, are the total fraction of nonmonomeric silica in solution, and the proportion of this that is the oligomeric form with high aluminum affinity cannot be calculated.

silicic acid with aluminum did not alter the extent of uptake of aluminum by DMHP, compared to control with no silicic acid.

Conclusions

Hence, our findings show that, firstly, the complex formed between aluminum and oligomeric silica has a log K_{eff} of 11.70 $\pm$ 0.30 at pH 7.2 in 10 mM MOPS buffer (an affinity for aluminum at least one million times greater than monomeric silica) and, secondly, that aluminum (and perhaps other "keeper ions") stabilize silica oligomers for many days under conditions in which depolymerization would otherwise be complete within 24 h.[27] Under physiological conditions this soluble oligomeric silica competes effectively for aluminum with the endogenous chelator citrate,[28] for example, that has a log K_{eff} for aluminum of 7.15 at pH 7.2 (0.6 M NaCl).[29]

Clearly the oligomeric-silica/aluminum interaction is of high affinity, and work demonstrating the biological activity of soluble silica should carefully distinguish between monomeric and oligomeric forms. Thus, *in vitro*, it is likely to be the powerfully chelating oligomeric silica that has been demonstrated to inhibit the aggregation of erythrocytes[30] and reverse the aluminum induced β-sheet conformations of β-amyloid-(1-42)-peptide[31] and modeled Alzheimer's tangles.[32] In other studies care has apparently been taken to ensure that silica is monomeric in the bulk solution, as with the well described experiments of fish survival in aluminum-rich waters.[7] However, it remains puzzling with such work that, firstly, there was a high order of dependence on silica concentration and, secondly, that the protecting concentration of silicic acid also increased the lability of aluminum in the bulk water, as determined by ion-exchange studies.[7] It may not, however, be safe to assume that monomeric silica in bulk solution remains monomeric at the site of its aluminum protecting action. Biomineralized silica is widespread in the plant kingdom,[33-35] and it is worth considering that similar processes may lead to oligomeric silica on certain biological surfaces.[35-38]

This work demonstrates the high affinity of soluble oligomeric silica for aluminum at physiological pH. Traces of oligomeric silica in biological systems would profoundly affect the availability of aluminum and would readily account for many of the existing results.

(27) Stabilization of the oligomeric silica with aluminum, was monitored for 50 days. After 17 days affinity of the oligomeric silica solution for aluminum gradually started to decrease, although, even after 50 days, significant oligomeric silica with high affinity for aluminum remained. In contrast oligomeric silica diluted in the absence of aluminum, fully deoligomerized by 24 h and lost its aluminum binding capacity.

(28) Similar studies to those in the text with citrate (0−2 mM) competing for DMHP-bound-aluminum (8 μM, 1:1 ratio of DMHP:Al) confirmed that citrate is a considerably weaker competitor than oligomeric silica for aluminum at pH 7.2.

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