Molecular Recognition of Anionic Species by Silica Gel Bound Sapphyrin

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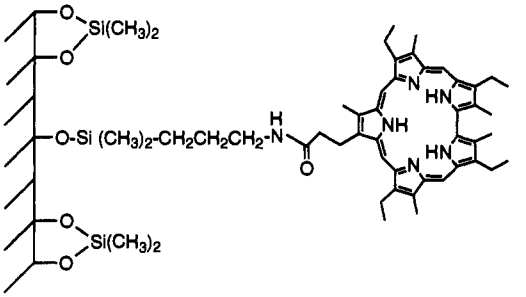
Previous work by Cram[1] and others[2-5] has shown that covalent attachment of a molecular receptor to a solid support can provide valuable insight into the understanding of receptor–substrate interactions. For instance, this approach allows binding interactions involving large numbers of substrates to be analyzed readily under identical experimental conditions. This same approach also allows quick evaluation of whether a given class of receptors can be used to construct improved solid phases for use in various chromatographic separations.

Recently, we reported a new and selective interaction between the monoprotonated form of sapphyrin,[6,7] a pentapyrrolic expanded porphyrin, and certain anionic species including phosphate.[8-10] A variety of spectroscopic studies as well as X-ray crystallography[9,11] have shown that phosphate species bind to the sapphyrin macrocycle via close contacts between a phosphate oxyanion and the pyrrolic hydrogens of the protonated macrocycle. We have called the interaction between sapphyrin and phosphate anions "phosphate chelation", and it results in surprisingly high affinities for simple phosphate compounds as well as nucleic acids in solution.[12] In this paper, we describe the synthesis of a new, sapphyrin-functionalized silica gel, a solid support that is highly selective for molecules with sterically accessible oxyanions such as phosphonates, phosphates, and arsonates. The sapphyrin-modified silica gels thus provide a method of separating various anions that complements the separations achieved by metalated tetraphenylporphyrin–silica gels.[13,14] Our sapphyrin-functionalized silica gel is also capable of separating polyphosphorylated species under standard, isochratic HPLC conditions at neutral pH. As expected for polyphosphates, the retention time correlates quantitatively with the total phosphate number. As a result, the sapphyrin-modified silica gel columns provide a new, nonelectrophoretic HPLC method for oligonucleotide separations that extends the types of separations that may be achieved by using diethylaminoethyl (DEAE) systems.[15]

The sapphyrin-substituted silica gel, shown as structure **1**, was prepared by treating aminopropyl silica gel[16] with the acid chloride of 3,8,17,22-tetraethyl-12-(carboxyethyl)-2,7,13,18,23-pentamethylsapphyrin in a starting sapphyrin-to-silica weight ratio of 1:10, and a starting sapphyrin-to-aminopropyl mole ratio of 1:6. (Dichlorodimethyl)silane was used to block all remaining free silanol groups.[17] The sapphyrin stationary phase coverage was calculated from elemental analysis of the modified silica gels[18] and found to be 0.12 μmol/m². A similar silyl-capped aminopropyl silica gel containing no sapphyrin was prepared as a control. Both modified silica gels were commercially packed into 4.6 mm × 100 mm HPLC columns (Alltech).[19]



The anion binding selectivies of the solid phases were tested by HPLC under conditions of isochratic elution with 100 mM ammonium phosphate pH 6.0, and the results are recorded in Table 1. For comparison, the same samples and elution conditions were used with a commercially available (Rainin) DEAE column.

As shown in Table 1, the neutral species benzophenone is not significantly retained on the sapphyrin-functionalized silica gel column. On the other hand, monoanionic entities such as diphenyl phosphate, benzenesulfonate, phenylphosphonate, and benzoate are all retained to varying extents. Together, these data lead us to suggest that hydrophobic or π-stacking modes are not the dominant forces responsible for the selective interactions of the sapphyrin-functionalized silica gel supports. This is in contrast to the results with the DEAE column in which hydrophobic or π-stacking interactions may be important for hydrophobic species.[15]

Furthermore, it does not appear as if purely electrostatic effects are responsible for the observed selectivity since different retention times are recorded for similar anions with the same net charge. Rather, we propose, the key mode of interaction involves a specific anion chelation between the positively charged sapphyrin core and oxyanions such as those found in phosphates and arsonates. Such an interaction has been observed in the solid state.[9,20]

The reason for the high selectivity for phosphate or arsonate-type anions is not yet entirely clear. However, there appears to be a strong correlation between oxyanion accessibility and retention times. If we compare, for instance, benzenesulfonate, phenylphosphonate, and phenylarsonate, for which retention times of 8.3 ± 0.2, 12.0 ± 0.2, and 16.0 ± 0.2 min are recorded, respectively, we see that these similar oxyanions have central

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(16) The aminopropyl silica gel (Aldrich) used to prepare the sapphyrin-modified silica gels consisted of spherical particles with a mean diameter of 52 μm, pore diamter of 60 Å, pore volume of 0.75 cm³/kg, and surface area of 480 m²/g.
(17) Control experiments indicate that sapphyrin is not derivatized by the (dichlorodimethyl)silane.
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(19) Column efficiency was found to be 584 theoretical plates.[13] The average column efficiency (n = 4) was determined from elution of a benzene band using 50% methanol/50% water (v/v).
(20) The ability of phosphates to bind sapphyrin-substituted silica gels was confirmed by solid-state ^{31}P NMR spectroscopy. Briefly, samples of both the sapphyrin-functionalized and simple silyl-capped silica gels were prepared by incubating with 5′-AMP. Relative to the control, the sapphyrin-containing sample showed a 4 ppm shift in the ^{31}P phosphate resonance. Such a shift is consistent with the proposed "phosphate chelation" interaction.

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Table 1. Retention Times for Chromatographic Separations of Various Species[a]

solute	pK_1[b,c]	pK_2[b,c]	pH	charge	retention time: control col (min)	retention time: DEAE col (min)	retention time: sapphyrin col (min)
benzophenone			6		6.8	23.4	6.4
benzenesulfonic acid	2.55		6	−1	5.9	8.8	8.3
benzoic acid	4.20		6	−1	5.0	10.1	10.4
phenylphosphonic acid	1.83	7.07	6	−1	5.9	7.4	12.0
diphenylacetic acid	3.94		6	−1	8.2	15.4	14.3
phenylarsonic acid	4.53	9.52	6	−1	6.1	6.9	16.0
diphenylphosphoric acid	1.85		6	−1	5.9	14.1	20.5

[a] pK_a's are for 25 °C in water. For each species 15 μL of a 5 mM solution was injected using an isochratic buffer of 100 mM ammonium phosphate at pH 6.0, at a flow rate of 0.2 mL/min. All separations were carried out at room temperature. [b] Dean, J. A. *Lange's Handbook of Chemistry*; McGraw-Hill Inc.: New York, 1992; pp 8.19–8.71. [c] *Dictionary of Organophosphorous Compounds*; Edmundson, R. S., Ed.; Chapman and Hall: New York, 1987.

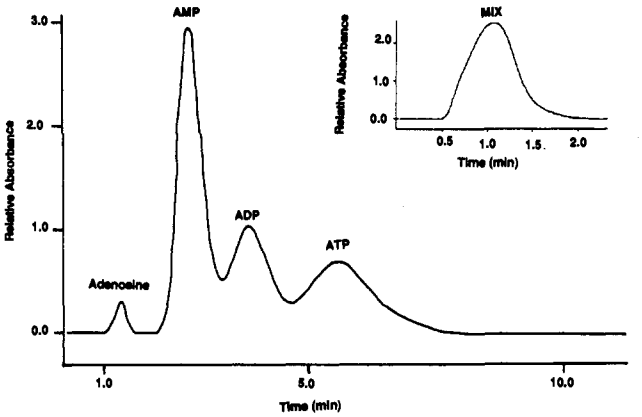

Figure 1. HPLC chromatogram showing the separation of adenosine, AMP, ADP, and ATP on the sapphyrin-modified silica gel column. Insert shows the same mixture on the silyl-capped, aminopropyl silica gel control column. Conditions: isochratic buffer of 0.5 M ammonium phosphate dibasic, at 1.0 mL/min, pH = 7.0, monitored at 260 nm.

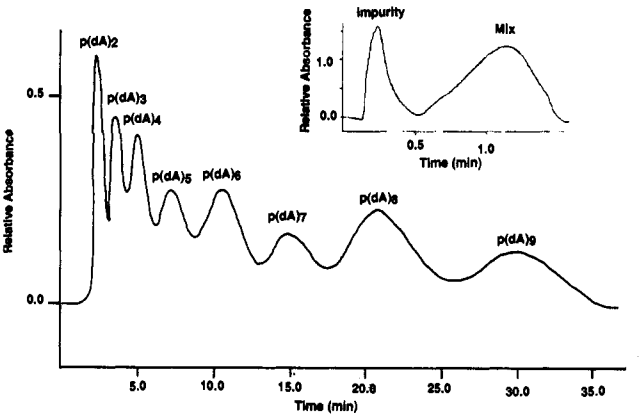

Figure 2. HPLC chromatogram showing the separation of 2–9 mers of polydeoxyadenylic acids. Insert shows the same mixture on the silyl-capped, aminopropyl silica gel control column. Conditions: isochratic buffer of 1.0 M ammonium phosphate dibasic, at 1.5 mL/min, pH = 7.0, monitored at 260 nm.

atom-to-oxygen distances of 1.47, 1.52, and 1.69 Å, respectively.[21] Furthermore, diphenyl phosphate is retained significantly longer than the analogous diphenylacetate (20.5 ± 0.2 versus 14.3 ± 0.2 min, respectively). Thus, as we interpret it, a longer heteroatom-to-oxygen bond results in greater oxyanion accessibility, and thus a stronger interaction with the silica supported sapphyrin; hence the longer retention times.

The sapphyrin-containing solid supports were also used to separate mixtures of simple phosphorylated nucleotides, such as AMP, ADP, and ATP, and more complex polyphosphorylated materials, such as oligonucleotides, as shown in Figures 1 and 2. AMP, ADP, and ATP are separated easily from each other and from the nucleoside adenosine on the sapphyrin column, but not on the silyl-capped control (Figure 1 and insert). Interestingly, this mixture is also not separated on the DEAE column at neutral pH, but rather requires a pH of 2–3.[15] Similarly, the sapphyrin-modified silica gel columns can be considered to be a new HPLC support for the nonelectrophoretic separation of oligonucleotides, since 2–9 mers of polydeoxyadenylic acid (Figure 2) and 3–5 mers of polydeoxycytidylic acid (data not shown) are cleanly separated using the sapphyrin-bearing column. In all instances, as expected, the order of elution correlated directly with the number of phosphate groups present. Not surprisingly, then, plots of the logs of the observed retention times versus the numbers of phosphates in the molecules yielded straight lines.[22] This confirms that the energetic effect of each additional phosphate group is additive, consistent with phosphate oxyanion chelation as being the major mode of interaction. Research is currently underway that attempts to understand further details of the interaction between silica-bound sapphyrin and simple anions such as nitrates, phosphates, phosphonates, and arsonates.

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Supplementary Material Available: A description of the calculation of the energetics of binding and plots of retention time versus number of phosphates and free energy change versus number of phosphates for the 2–9 mers of polydeoxyadenylic acid (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(22) From these plots, free energy values of 233, 200, and 270 cal/mol were derived for the poly-A, poly-C, and monoadenosine phosphate series, respectively; cf. supplementary material.