Phosphate Recognition by Sapphyrin. A New Approach to DNA Binding

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Molecular recognition of DNA is one of the most important processes in nature, and the interactions of small molecules with DNA continues to be an area of active interest. In broad terms, three main types of noncovalent small molecule–DNA interactions have been described: intercalation, groove binding, and simple electrostatic attraction.[1-4] In this paper we present a new type of small molecule–DNA interaction in which the monoprotonated form of sapphyrin **2**,[5] a non-naturally occurring aromatic pentapyrollic macrocycle,[6] "chelates" the anionic phosphate backbone of DNA in a precise, rigid fashion.

Sapphyrins (e.g., **1** and **2**; Figure 1), unlike porphyrins (e.g. **3**),[5] have an inner cavity that is large and basic.[6] Thus sapphyrin derivatives such as **2** are protonated and positively charged at neutral pH.[6,7] This unique feature provides for some novel properties, and we have been able to show that the protonated forms of sapphyrin bind *anions* rather than *cations* both in solution and in the solid-state.[8-11]

Initial evidence for a strong sapphyrin–DNA interaction came from a simple mixing experiment: adding an excess of the water-soluble sapphyrin **2**, which is green, to double-stranded DNA (dsDNA) at neutral pH led to an immediate precipitation of the DNA as visible green fibers.[12]

When this coprecipitate was mixed with silica as a bulking agent and subjected to solid-state ^{31}P NMR analysis, we observed a 3.6 ppm upfield shift in the ^{31}P signal of this coprecipitate compared to the ^{31}P signal of DNA alone.[13] Under the same conditions, a control experiment with porphyrin **3** yielded a 1.6 ppm upfield chemical shift in this same ^{31}P signal. By comparison, the complex formed between phosphoric acid and sapphyrin **2** at pH 6.0 (but not that from porphyrin **3**) yielded a similar upfield shift of 3.8 ppm in its solid-state ^{31}P NMR spectrum. These results are consistent with the ring current effects expected for a DNA phosphate–sapphyrin complex analogous to the solid-state structure in Figure 2.

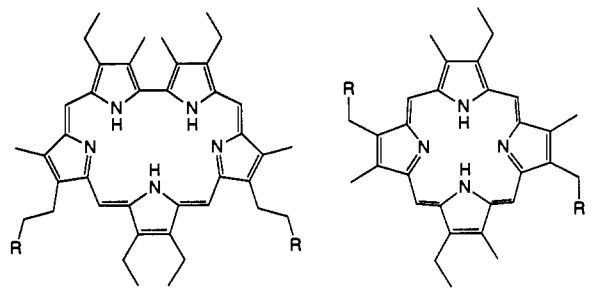

Figure 1. Structures of sapphyrins (**1** and **2**) and porphyrin (**3**).

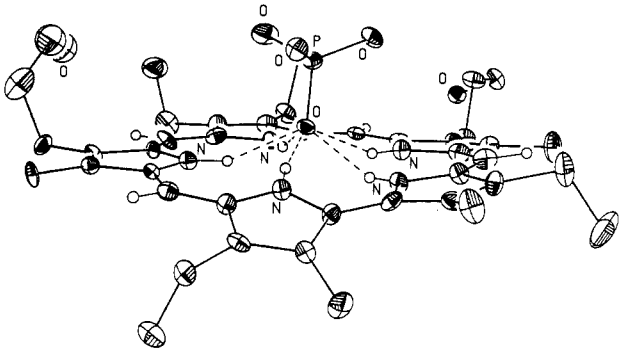

Figure 2. X-ray structure of the 1:1 complex formed between monobasic phosphoric acid and diprotonated sapphyrin **1**. The bound oxygen atom is found 0.83 Å above the root mean square plane of the nitrogen atoms and is chelated by five hydrogen bonds. Further details of this structure will be presented elsewhere.[10,11]

More quantitative evidence for the proposed sapphyrin–DNA interaction comes from UV–visible spectroscopic studies. Adding an excess of calf thymus dsDNA (ca. 200 phosphate anion equiv) to a solution of sapphyrin **2** produced an 11-nm bathochromic shift in the sapphyrin Soret band (from λ_{max} = 409 to 420 nm). Similar bathochromic shifts, both in magnitude and in direction, were also observed with single-stranded DNA (ssDNA) at roughly the same phosphate anion to sapphyrin ratios. Interestingly, bathochromic shifts in this same Soret band could be induced upon adding relatively high concentrations of diethyl phosphate (≥10 000 equiv), indicating that this cationic sapphyrin can indeed bind a small, anionic phosphodiester under conditions identical to those under which we conducted the DNA studies.

Using the observed spectroscopic changes, standard curve-fitting analysis[14] gave an apparent binding constant of ≤10 M^{-1} for sapphyrin binding to diethyl phosphate in aqueous solution at neutral pH.[15] In the case of ssDNA, a similar analysis yielded an apparent binding constant of 25 000 M^{-1}.[16] dsDNA, on the other hand, showed spectral shifts that could not be interpreted in a straightforward, quantitative manner.[17]

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(16) The difference in the association constants for diethyl phosphate and ssDNA could reflect a favorable hydrophobic interaction between sapphyrin and ssDNA. Alternatively, it could reflect a polyanion effect.

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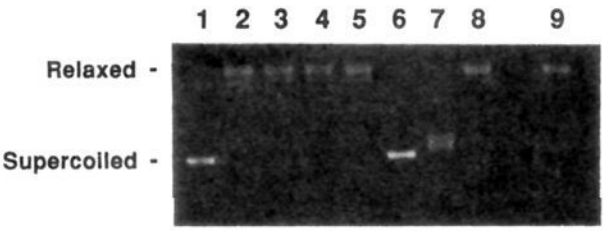

Figure 3. Photograph of a 0.8% agarose gel stained with ethidium bromide showing the results of calf thymus DNA topoisomerase I unwinding studies carried out with supercoiled pBR322 DNA. The reactions were run in accord with the general procedure of ref 18; The following specific reagent concentrations were employed: DNA, 18 ng/μL; topoisomerase I, 0.17 units/μL; and the specified amount of sapphyrin **2** or ethidium bromide. Lane 1: supercoiled DNA standard (provided for reference). Lane 2: reaction in the absence of sapphyrin. Lane 3: 25 μM sapphyrin. Lane 4: 12.5 μM sapphyrin. Lane 5: 6.3 μM sapphyrin. Lane 6: 10 μM ethidium bromide. Lane 7: 1 μM ethidium bromide. Lane 8: 0.1 μM ethidium bromide. Lane 9: control reaction in which the DNA is originally relaxed in the presence of 25 μM sapphyrin indicating that sapphyrin does not inherently inhibit topoisomerase I activity.

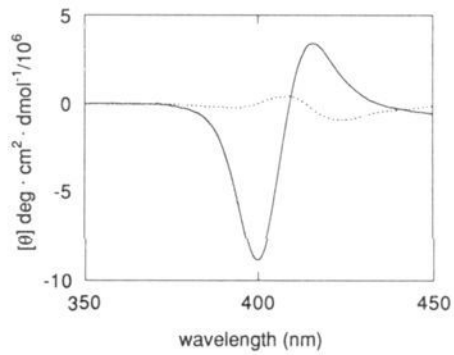

Figure 4. Circular dichroism (CD) spectrum of sapphyrin **2** in the presence of 10 phosphate equiv of dsDNA (—) and 10 phosphate equiv of ssDNA (···). Both spectra were obtained in 5 mM PIPES buffer pH 7.0.

Topoisomerase I from calf thymus was used to probe further the nature of the interaction between sapphyrin **2** and dsDNA.[18] Under conditions in which sapphyrin is known to be bound to the DNA, absolutely no DNA unwinding was detected by this topoisomerase I assay (see Figure 3), indicating that sapphyrin does not intercalate into dsDNA.

Circular dichroism (CD) spectroscopic studies were used to probe the stereogenic environment around the bound sapphyrin molecules.[19] Figure 4 shows the CD spectra of sapphyrin **2** in the presence of both double-stranded and single-stranded DNA. As can be seen in the figure, a strong sapphyrin-based signal is observed for the Soret-like transition at ca. 408 nm when sapphyrin **2** is mixed with dsDNA. This signal is taken as direct evidence for the sapphyrin being bound in a rigid fashion to the chiral dsDNA scaffold. For dsDNA, the CD of the DNA portion of the spectrum (220–300 nm) shows no evidence for a significant distortion of the DNA.[20] In the case of ssDNA, a different shape and lower intensity are observed for this same induced CD signal. However, in the case of porphyrin **3**, no induced CD signals were observed in the presence of either dsDNA or ssDNA.

Interestingly, the CD signal resembles the DNA-engendered CD spectra observed with acridine orange[21] and certain cationic porphyrins[22,23] that are known to interact with DNA via an ordered outside stacking binding mode. While some form of ordered aggregation may be involved in the interaction with dsDNA at high sapphyrin to phosphate ratios, we do not favor a stacking mode as being the dominant interaction. Rather, we feel that the observed binding behavior derives primarily from phosphate anion chelation.

We disfavor the outside stacking mode for two reasons. First, using steady-state fluorescence spectroscopy, we observe no significant difference in the emission intensity when $r = 1$ or $r = 0.01$ (where r is defined as the ratio of sapphyrin to dsDNA phosphate equivalents). DNA surface interactions in the manner proposed by Fiel,[24] for example, in the case of *meso*-tetra(*p*-*N*-trimethylanilinium)porphine (TMAP) would be expected to contribute to decreased emission intensity for sapphyrin at high values of r,[25,26] a phenomenon that is not, however, observed. Second, for both cationic porphryins such as TMAP[27] and acridine orange,[28] optimal, simple electrostatic interactions between the positively charged *periphery* of the aromatic ring and the negatively charged DNA backbone have been proposed to stabilize the stacked helical arrangement of these molecules alongside the dsDNA helix. No such possible contacts exist on the periphery of sapphyrin; for sapphyrin the positive charge is located in the *center* of the macrocycle.

We believe the experimental findings presented in this paper to be consistent with a new type of small molecule–DNA interaction. This interaction, which involves a specific chelation of the phosphate diester oxyanion by the protonated sapphyrin core, differs substantially from other, previously reported DNA binding motifs. The topoisomerase I experiment militates against intercalation being a major component of binding to nucleic acids. Likewise, the fact that sapphyrin interacts strongly with single-stranded DNA rules out groove binding as being a major stabilizing interaction. Finally, outside stacking modes are ruled out on the basis of fluorescence analyses. Thus, we conclude that sapphyrin is interacting with nucleic acids via a novel type of phosphate recognition in analogy to the solid-state structure of Figure 2. Current work is focused on further investigations of this new type of small molecule–DNA interaction.

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(17) This lack of simplicity could reflect the fact that sapphyrin, which is known to be aggregated in aqueous solution, could remain partially aggregated in the presence of dsDNA, especially at high relative sapphyrin to phosphate ratios. We believe this to be the result of a structure-dependent cooperativity and are currently investigating this phenomenon.

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