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Interactions between expanded porphyrins and nucleic acids

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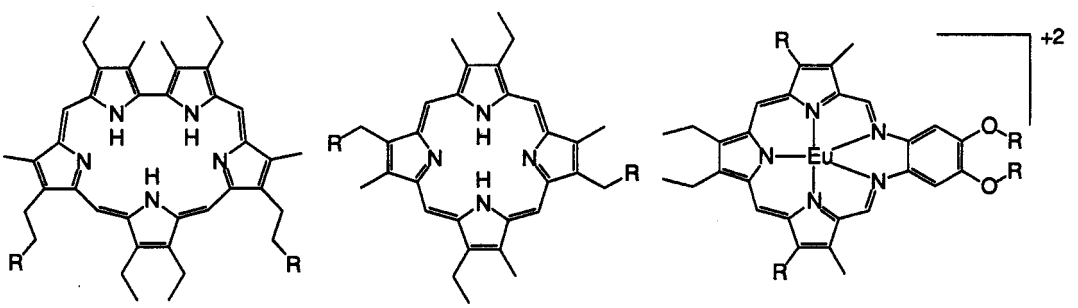
Two types of interaction of expanded porphyrins and nucleic acids are discussed. The first involves the specific chelation of the anionic phosphate diester backbone of DNA by the monoprotonated form of the water soluble sapphyrin **2**. Support for the proposed binding mode derives from a variety of spectroscopic and biochemical studies, including visible absorption spectroscopy, circular dichroism spectroscopy, and supercoiled DNA unwinding experiments. The second interaction described is that of the water soluble europium texaphyrin complex Eu(T2B2 txph)$(NO_3)_2$ and RNA. This complex acts as a hydrolytic cleaving agent for uridyl uridine monophosphate, UpU, with a pseudo-first order rate constant of 0.057 ± 2 h^{-1} at 37° C and pH = 7.0 with 0.49 mM UpU and 0.3 mM Eu(T2B2 txph)$(NO_3)_2$. Under identical conditions a 0.26 mM solution of $Eu(NO_3)_3$ hydrolyses UpU with a rate constant of 0.007 ± 3 h^{-1}.

Molecular recognition of DNA is one of the most fundamental processes in nature, and analyzing the interactions of small molecules with DNA continues to be an important area of research. In broad terms, three main types of noncovalent small molecule-DNA interactions have been described: Intercalation,[1] groove binding,[2] and simple electrostatic attraction.[3,4] In this paper we wish to present evidence for a new type of small molecule-DNA interaction in which the monoprotonated form of sapphyrin **2**, an aromatic pentapyrrolic macrocycle, "chelates" the anionic phosphate backbone of DNA in a precise, rigid fashion. We also present evidence for the hydrolysis of RNA by a second type of expanded porphyrin, the europium(III) chelate of texaphyrin, that must necessarily interact with this and other nucleotides, including uridylyl (3'→5') uridine, via an electrostatic type interaction. These two types of expanded porphyrin-to-nucleic acid interactions are now discussed in turn.



(**1**) R = CH_2OH
(**2**) R = $CON(CH_2CH_2OH)_2$

(**3**) R = $CON(CH_2CH_2OH)_2$

(**4**) R = $CH_2CH_2CH_2OH$

Fig. 1. Structures of sapphyrin, porphyrin, and texaphyrin derivatives used in this work.

Sapphryin-DNA

Sapphyrins (e.g., **1** and **2**; Fig. 1) are a class of pentapyrrolic macrocycles that are ideally suited for DNA binding. This is because, unlike porphyrins (e.g. **3**; Fig. 1),[5] the inner cavity is large and basic.[6] Thus sapphyrin derivatives such as **2** are protonated and positively charged at neutral pH.[7] This unique feature provides for some novel molecular-level properties. For example, we have been able to show that the protonated forms of sapphyrin bind *anions* rather than *cations* both in solution and the solid state.[8,9] In particular, the monoprotonated form of sapphyrin was observed to be an effective carrier for monoanionic phosphate esters,[10] while the diprotonated form was observed to form stable crystalline complexes with both fluoride anion[8] and monobasic phosphoric acid[9,11] (Fig. 2).

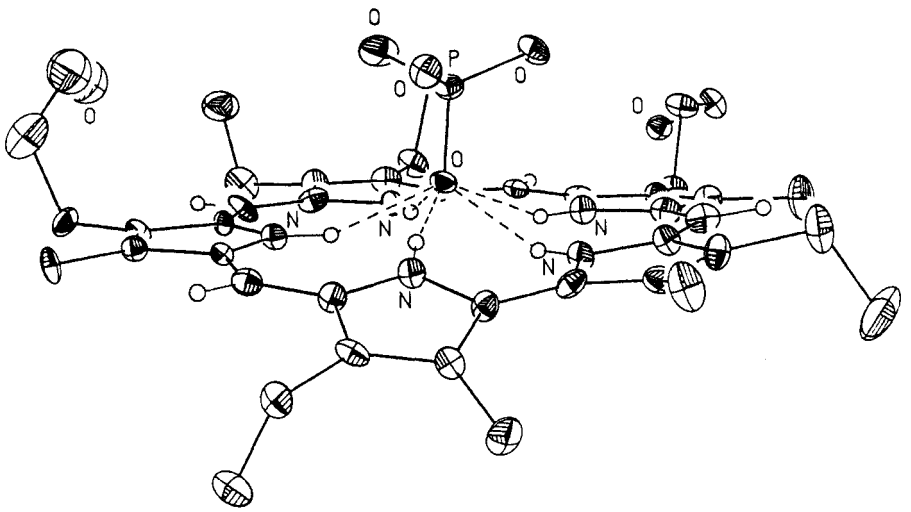


Fig. 2. X-ray structure of the 1:1 complex formed between monobasic phosphoric acid and diprotonated sapphyrin **1**. The bound oxygen atom is found 0.83 Å above the RMS plane of the nitrogen atoms and is chelated by five hydrogen bonds. Further details of this structure will be presented elsewhere.[10,11]

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. More quantitative evidence for the proposed sapphyrin-DNA interaction comes from spectroscopic studies. Adding an excess of calf thymus dsDNA (ca. 200 phosphate anion equivalents) to a solution of sapphyrin **2** produced a 12 nm redshift in the sapphyrin Soret band (from λ_{max} = 410 nm to 422 nm). Similar redshifts, both in magnitude and direction, were also observed with single-stranded DNA (ssDNA) at roughly the same DNA-phosphate-to-sapphyrin ratios (Fig. 3). We also observed redshifts in this same Soret band upon adding relatively high concentrations of diethyl phosphate ($\geq$ 10,000 equivalents), indicating that sapphyrin can indeed bind a small phosphodiester under conditions identical to those under which we conducted the DNA studies. Using the observed redshift as a quantitative measure of binding, standard curve fitting analysis[12] gave a binding constant of 20 ± 5 M^{-1} for sapphyrin binding to diethyl phosphate in aqueous solution at neutral pH.

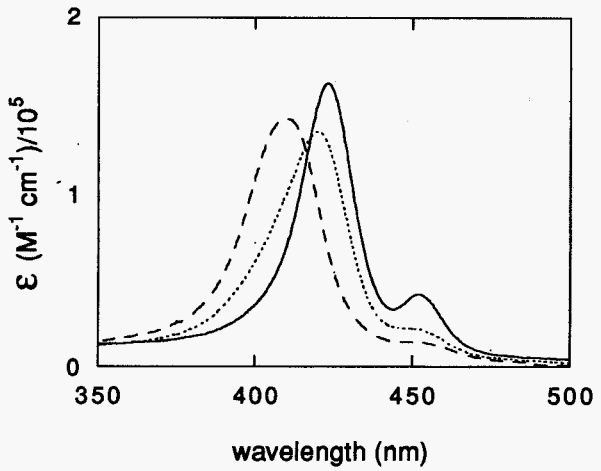


Fig. 3. UV-Visible absorption spectrum of sapphyrin **2** in the absence of DNA (----) and presence of 200 phosphate equivalents of dsDNA (·····) and ssDNA (——). All spectral were obtained in 5 mM PIPES pH 7.0.

For dsDNA and ssDNA, spectral shifts were observed that cannot be understood using a simple binding model such as that used for the diethyl phosphate case.[13] For both species, spectral shifts were observed that were *linearly* dependent on nucleic acid concentration when one or fewer equivalents of DNA phosphate were added. A lower limit for the apparent binding constant of 1 x 10^5 M^{-1} can still be estimated for sapphyrin binding to both double and single stranded DNA. This is because essentially quantitative binding between sapphyrin **2** and these nucleic acids is observed with both species at 3 μM.

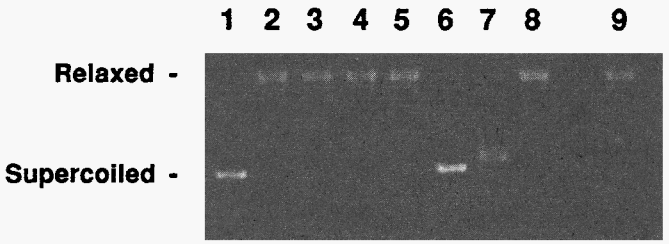


Fig. 4. Photograph of a 0.8% agarose gel stained with ethidium bromide showing the results of calf thymus DNA topoisomerase I unwinding studies carried out with supercoiled pBR322 DNA. The reactions were run in accord with the general procedure of reference;[14] The following specific reagent concentrations 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.

Topoisomerase I from calf thymus was used to probe further the nature of the interaction between sapphyrin **2** and dsDNA.[14] This enzyme, when used as a test, can detect the unwinding of the dsDNA helix induced by a small molecule, a classic signature of DNA intercalation.[15] Under conditions in which sapphyrin is known to be bound to the DNA, no DNA unwinding was detected by this topoisomerase I assay (Fig. 4). This serves as an indication that sapphyrin does not intercalate into dsDNA.

Circular dichroism (CD) spectroscopic studies were used to probe the stereogenic environment around the bound sapphyrin molecules.[16] Fig. 5 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.[17] In the case of ssDNA, a different shape and lower intensity is 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.

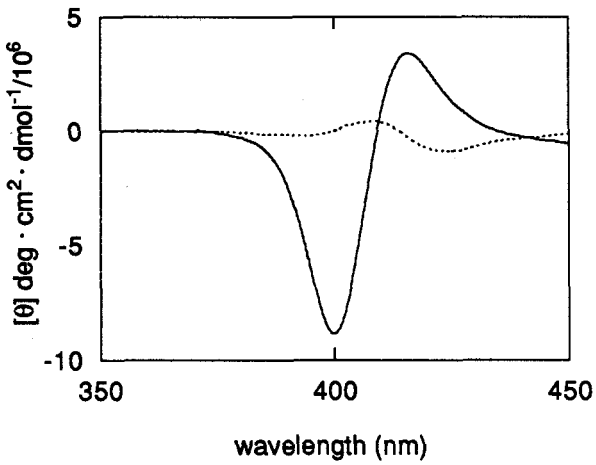


Fig. 5. Circular dichroism (CD) spectrum of sapphyrin **2** in the presence of 10 phosphate eqv. of dsDNA (——) and 10 phosphate eqv. of ssDNA (·····). Both spectra were obtained in 5 mM PIPES buffer pH 7.0.

Interestingly, the induced CD resembles the DNA-induced CD spectra observed with certain cationic porphyrins[18] that are known to interact with DNA via the ordered outside stacking binding mode originally proposed by Fiel.[19] In the case of sapphyrin, however, we feel that such an outside stacking mode does not represent the dominant interaction. There are two reasons for this. First, using steady state fluorescence, we observe no significant difference in the emission intensity when r = 1 or r = 100 (where r is defined as the ratio of DNA phosphate equivalents to sapphyrin). Aggregation in the manner proposed for outside binding porphyrins, on other hand, would be expected to contribute to decreased emission intensity for sapphyrin when r is low.[20] Second, for cationic porphyrins, optimal electrostatic contacts between the positively charged *periphery* of the porphyrin macrocycle and the negatively charged backbone would be expected to stabilize the stacked helical arrangement of porphyrins along the DNA helix. No such contacts exist on the periphery of sapphyrin; for sapphyrin the positive charge is located in the *center* of the macrocycle. Thus, these findings, along with those discussed above, are considered by us to provide support for what we believe is a new type of small molecule-DNA interaction. This proposed

interaction, which involves the specific chelation of the DNA phosphate diester oxyanion by the protonated sapphyrin core, in analogy to the structure of Fig. 2, differs substantially from other, previously reported DNA binding motifs. At present, we are studying further the consequences and opportunities afforded by this new type of DNA recognition.

Eu(texaphyrin)-RNA

The synthesis of efficient highly selective RNA cleaving reagents would be useful in biotechnology. Indeed, a great deal of interest has centered on catalyzing the selective cleavage of RNA using metal complexes. For instance, Morrow has recently demonstrated that hexaamine Schiff-base (HAM) complexes of the lanthanide metals are effective in cleaving RNA.[21] For example, a 490 μM solution of an europium complex (EuHAM) has a pseudo-first order rate constant of 0.14 h^{-1} at 37° C.[21] These complexes cleave RNA hydrolytically, rather than oxidatively, and thus have several advantages. These advantages include selectivity for RNA over DNA, and the possibility of obtaining RNA fragments that are suitable for subsequent recombination. These same complexes, however, are also plagued by several deficiencies, including hydrolytic instability and intrinsic toxicity. This suggests that other, better behaved lanthanide complexes (e.g. **4**; Fig. 1) might prove superior in these types of RNA hydrolysis applications. Thus, we have recently become interested in exploring their possible utility as RNA hydrolysis catalysts and wish to report here some initial results arising from these explorations.

Water soluble lanthanide complexes of texaphyrin, an expanded porphyrin macrocycle, have recently been synthesized and demonstrated to be remarkably stable towards demetallation and hydrolysis.[22] In our opinion these lanthanide texaphyrins could offer several advantages over Morrow's HAM complexes. Texaphyrins have a very high binding affinity for lanthanide metals, are far less toxic than the HAM ligands, and can be modified easily to include a variety of functional groups on their periphery. Finally, they possess a strong porphyrin-like chromophore that might facilitate their detection in a concentration range useful for *in vivo* or *in vitro* applications.

Treating an aqueous solution of uridylyl (3'→5') uridine, UpU, (0.49 mM) buffered at pH 7.0 using 5 mM HEPES with a 0.30 mM aqueous solution of Eu(T2B2 txph)$^{2+}$ (**4**) at 37° C hydrolytically cleaves UpU with a pseudo-first order rate constant of 0.057 ± 2 h^{-1}. Control experiments, conducted simultaneously, in which no europium complex was present, gave no evidence of UpU cleavage. The reaction, and controls, were followed by HPLC,[23] monitoring the formation of uridine as compared to the internal standard, cytosine. Uridine-2'-monophosphate (2UMP), uridine-3'-monophosphate (3UMP), and uridine-2':3'-cyclic monophosphate (cUMP) are also observed by HPLC. Taken together, these results indicate a hydrolytic rather than an oxidative mechanism for the cleavage reaction.

Under conditions identical to those discussed above, a 0.26 mM solution of $Eu(NO_3)_3$ hydrolytically cleaves UpU with a pseudo-first order rate constant of 0.007 ± 3 h^{-1}. The rate of UpU cleavage by the uncomplexed salt is, therefore, 8 times slower than the rate of cleavage by (EuT2B2 txph)$^{2+}$. This particular finding thus eliminates the possibility that free europium salts alone account for the observed UpU cleavage in the (EuT2B2 txph)$^{2+}$ studies. Consistent with this conclusion is the preliminary finding that a variety of other lanthanide T2B2 texaphyrin complexes are also able to cleave UpU hydrolytically and that the rate of hydrolysis is a strong function of the complexed cation. These latter findings, however, will be reported at a later date.

Conclusion

Taken together, the results presented herein indicate that expanded porphyrins are capable of interacting with nucleic acids in at least several interesting ways. Certain expanded porphyrins, such as for instance, the prototypical sapphyrins, are capable of interacting via phosphate anion chelation. Others, such as the metal-binding texaphyrins, are able in appropriate cases to bind and react via metal-centered phosphate anion hydrolysis. This leads us to suggest that the further study of these and other expanded porphyrin-to-nucleic acid interactions is likely to be both rich and rewarding. Such studies, therefore, are currently in progress. They will be reported on in due course.

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