A tetraphenylporphyrin–peptide hybrid with high affinity for single-stranded DNA

Rishi K. Jain, David A. Sarracino and Clemens Richert*†

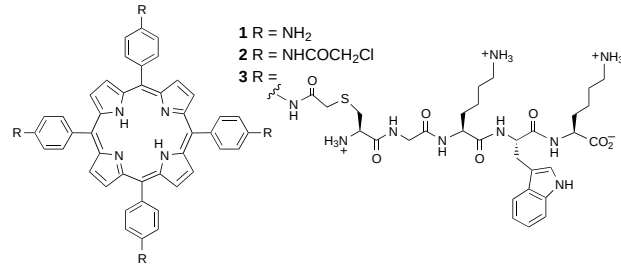
Department of Chemistry, Tufts University, Medford, MA 02155, USA

A tetraphenylporphyrin bearing four peptide chains, synthesized in two steps from *meso*-tetrakis(*p*-aminophenyl)porphyrin and the peptide CGKWK, binds single-stranded DNA oligomers with high affinity and inhibits degradation by nuclease S1.

Small ligands that bind single-stranded DNA (ssDNA) with high affinity are scarce. Non-protein ligands, such as cationic peptides with an intercalating residue,[1,2] cationic tetraarylporphyrins and sapphyrins[3] and naphthalene diimide intercalators,[4] give dissociation constants at or above the micromolar level. Lower dissociation constants are found for ssDNA binding proteins, but their crystal structures[5] do not suggest a simple way of designing small ligands with similar affinity. Further, increasing the length of a peptidic binder does not necessarily increase its affinity for ssDNA, as enthalpy gains are usually matched by entropic cost.[6] Since recent studies have shown that binding to ssDNA can inhibit polymerases, including HIV reverse transcriptase,[7,8] the search for tight ssDNA binders poses not only an intellectual challenge, but also promises to lead to drug candidates.

Presented here is the lead compound in our search for ssDNA binders. This is a tetraphenylporphyrin core molecule bearing short peptide chains. The design of this compound was based on the notions that (i) single stranded nucleic acids are highly flexible, favoring large rigid ligands to reduce the entropic penalty for complex formation, and (ii) ssDNA presents more lipophilic surface area than double-stranded DNA, where the nucleobases are buried, and its complexes may therefore gain substantial stabilization from hydrophobic interactions. Tetraphenylporphyrins are not only large, rigid and lipophilic macrocycles, they are also chromophores, allowing for sensitive monitoring of binding events. Further, tetraphenylporphyrins are straightforward to synthesize and easily detected by quantitative laser desorption mass spectrometry, a technique that has allowed us to perform monitored *in vitro* selection experiments.[9] The peptide sequence Lys-Trp-Lys (KWK), a known ssDNA-binder,[1] was chosen as substituent to provide additional binding affinity and solubility in aqueous media. A cysteinyl-glycine linker was designed to span the distance between KWK and the 'termini' of the phenylporphyrin and to provide a reactive thiol group.

Assembly of the peptide–porphyrin hybrid started from *meso*-tetrakis(*p*-aminophenyl)porphyrin **1**,[10] which was converted to the chloroacetamido derivative **2** under the conditions reported by Collman *et al.*[11] The subsequent coupling of **2** with CGKWK‡ was monitored by MALDI-TOF mass spectrometry. Thioether formation to the desired tetrapeptide **3** proceeded slowly under conditions optimized for peptide–DNA hybrids[12] (aqueous buffer, pH 7.0, 40 °C) and DMF as a co-solvent. Even with a 150-fold excess of the peptide and several days reaction time, less than 20% tetrasubstituted porphyrin **3** was found. Rigorous exclusion of air to prevent disulfide formation and stepwise addition of the peptide did not improve the yield. Further, purification of **3** from the crude product was complicated by the presence of many side products of similar molecular weight. Interestingly, coupling in dry DMF with Cs_2CO_3 as base,[13] conditions less typical for bioconjugations, proceeded smoothly with 2 equiv. peptide per chloroacetamide group.§ Despite the limited solubility of both the caesium salt and the highly charged peptide in the organic solvent, the reaction was more than 90% complete after 20 min at room temperature. Tetrasubstituted porphyrin hybrid **3** made up more than 65% of the crude product and could be obtained in satisfactory purity in a single HPLC step.¶



Addition of DNA octamers 5′-TGGTTGAC-3′ **4** and $(dC)_8$ **5** to solutions of **3** led to > 50% hypochromicity of the Sôret band but little shift of the maximum. Comparable hypochromicity has been found for a macrocyclic porphyrin-containing cryptand bound to poly(dT).[14] Unlike this single-strand specific cryptand, which is too bulky for intercalation, and cationic tetraarylporphyrins that intercalate, or, more probably, hemi-intercalate[15] into double-stranded DNA, **3** appeared to bind quite tightly to the short, single-stranded oligomers. Unconjugated KWK (2 equiv.) in the solution did not influence the binding curve measurably. Further evidence for tight binding came from the observation that the complex **3·4** did not show hyperchromicity when the NaCl concentration was raised to 3 M. In the plot of the titration data (Fig. 1) hypochromicity levels off at 1 equiv. of DNA, both at 900 and 400 nM **3**. This suggests that a 1 : 1 complex between **3** and the DNA strands is preferred when a molar excess of DNA is available, and that above 1 equiv. DNA very little unbound **3** exists at these concentrations. The latter makes it difficult to determine the dissociation constant accurately‖ but allows an estimation of $K_d \leqslant 5$ nM. This

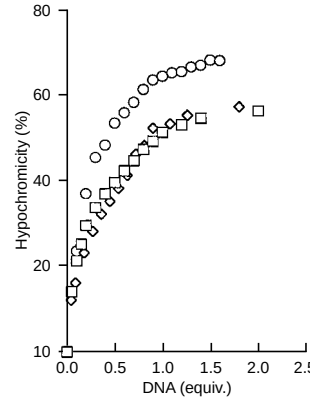


Fig. 1 Hypochromicity of the Sôret and absorption of **3** (415 nm) upon addition of DNA octamers **4** and **5**, 10 mM ammonium acetate solution, pH 6.0: (□) **3** (900 mM) + **5**, (◇) **3** (900 mM) + **4** and (○) **3** (400 nM) + **4**

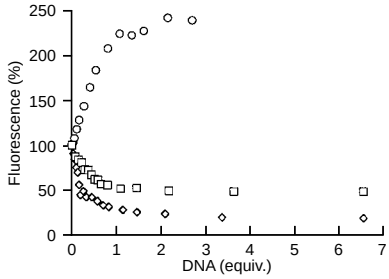


Fig. 2 Changes in fluorescence intensity (λ_{ex} = 415 nm, λ_{det} = 660 nm) upon addition of DNA octamer **4** to a solution of **3** (55 ± 10 nM) at (□) pH 6.0, (◇) 4.6 and (○) 2.5 (10 mM ammonium acetate buffers)

estimate was corroborated by fluorescence titrations performed at 55 nM **3** and increasing amounts of **4**, **5**, $(dA)_8$ **6** and 5′-GTCAAA-3′ **7** (Figs. 2 and 3). The fluorescence of **3** changed with the concentration when up to 1 equiv. DNA was added, but remained mostly unchanged at higher DNA : hybrid ratios. Only for the oligopyrimidine **5**, and the shorter oligomer **7** was a fraction of unbound porphyrin detected at 1 equiv.

When titrations with **4** were performed at pH 2.5, where the porphyrin core is at least partially protonated,[16] a fluorescence increase rather than decrease accompanied addition of DNA (Fig. 2). Apparently, **3** can reverse its fluorescence reporter properties. At a pH where the porphyrin ring is uncharged, DNA induces quenching, just like in other neutral chromophors. At low pH, however, DNA induces the fluorescence enhancement typical for cationic aromatic intercalators, such as the ethidium, acridine orange and proflavin ions. In both states, tight 1 : 1 complexes appear to form with the DNA octamer.

Exploratory experiments show that 2 equiv. of **3** inhibit the degradation of **4** by nuclease S1 by more than 50% at 37 °C [28 μM **4** in 83 mM $(NH_4)_2SO_4$–5 μM ZnSO4, pH 5.6, 0.8 u μl^{-1} nuclease, monitored by quantitative MALDI-TOF MS[17]]. Footprinting analysis[17] indicates that nuclease cleavage after the third, fourth and fifth nucleotide is similarly inhibited in the presence of **3**, with a slightly decreased protection at the latter site (not shown). This suggests that hybrid **3** covers more than a small fraction of DNA octamer **4**.

In conclusion, we report a facile synthesis of a porphyrin-peptide hybrid. The synthetic methodology requires only unmodified deprotected peptides, which are accessible *via* standard automated peptide synthesis and biotechnological processes. Hybrids prepared *via* the route presented here may become valuable bioorganic model compounds, *e.g.* as heme protein analogs[18] or photosynthetic reaction centre maquettes.[19] Further, the finding that porphyrin–tetrapeptide hybrid **3** binds ssDNA corroborates assumptions (i) and (ii) about the nature of tight ssDNA binders (*vide supra*). Initial results from a study on the binding of **3** and related compounds to double-stranded DNA, presently under way in these laboratories, shows that such hybrids can bind to short ssDNA oligomers without preventing duplex formation. Therefore, **3** may also be a lead compound for the development of protective 'coats' for antisense oligonucleotides, as, unlike complementary oligonucleotide strands, the only other known high affinity ligands to ssDNA, **3** does not seem to block Watson–Crick base pairing.

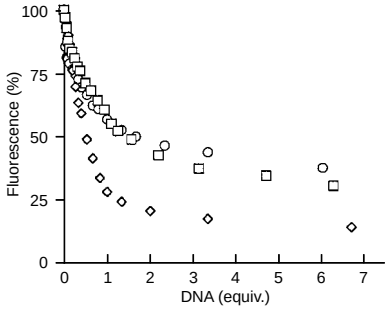


Fig. 3 Fluorescence quenching upon addition of DNA oligomers (□) $(dC)_8$ **5**, (◇) $(dA)_8$ **6** and (○) GTCAAA **7** to a solution of **3** at pH 4.6; experimental conditions are the same as in Fig. 2

The authors thank M. Simon for the preparation of synthetic intermediates and C. Tetzlaff for the acquisition of NMR spectra. Support by the NIH (grant GM54783 to C. R.) is gratefully acknowledged.

Notes and References

† E-mail: crichert@emerald.tufts.edu

‡ Synthesized *via* a standard Fmoc/Bu^t protocol. Homogeneous by RP_{18}-HPLC and single peak in MALDI-TOF MS.

§ All steps were performed under exclusion of light to prevent photosensitized oxidation. Typically, a mixture of porphyrin **3** (0.15 mg, 0.15 μmol) CGKWK (0.74 mg, 1.2 μmol), and Cs_2CO_3 (3.9 mg, 12 μmol) was dried *in vacuo*, and stirred under argon for 15 min, followed by addition of dry DMF (120 μl). The reaction was monitored by MALDI-TOF MS, and quenched after 0.7–20 h by addition of 0.2% TFA. Extended coupling (> 20 h) led to noticeable cleavage of the linking acetamide, most likely *via* transamidation to the free *N*-terminus of the peptide. Experiments with acetoxy-linked hybrids show that acyl transfer cleavage can be suppressed by acetylation of the *N*-terminus.

¶ HPLC: Vydac RP_{18} column, A = 0.1% TFA, B = MeCN, linear gradient of 0–33% B in 90 min, elution after 71 min. UV–VIS (10 mM ammonium acetate, pH 6.0) λ_{max}/nm 657, 598 (sh), 569, 527, 414, 282; δ_H([$^2H_6$]DMSO, 300 MHz, selected resonances) 8.86 (s, 8 H, pyrrole H), 8.73 (br s, 4 H, amide NH), 8.33 (br s, 4 H, amide NH), 8.19–8.03 (m, 20 H, phenyl H and amide NH), 7.83 (br s, 4 H, amide NH), 7.55, 7.31, 7.02, 6.94 (4 m, 4 × 4 H, six-membered ring H Trp), 7.14 (s, 4 H, H^{δ} Trp), −2.92 (br s, 2 H, porphyrin NH); MALDI-TOF MS (α-cyano-4-hydroxycinnamic acid matrix, positive mode, delayed extraction): calc. for $C_{164}H_{211}N_{40}O_{28}S_4$ [M + H]$^+$, 3316.5; found 3315.6. Hybrid **3** does adsorb onto glass surfaces and extensive handling and repetitive lyophilizations should be avoided. Titrations were performed from MS-checked HPLC fractions in H_2SO_4- or HNO_3-cleaned cuvettes.

‖ Compare *e.g.* footnote 8 in: Y. Kuroda, A. Kawashima, Y. Hayashi and H. Ogoshi, *J. Am. Chem. Soc.*, 1997, **119**, 4929.

1 J.-J. Toulme and C. Hélène, *J. Mol. Biol.*, 1977, **252**, 244; C. Hélène and J.-L. Dimicoli, *FEBS Lett.*, 1972, **26**, 6.
2 D. P. Mascotti and T. M. Lohman, *Biochemistry*, 1997, **36**, 7272.
3 R. F. Pasternack, R. A. Brigandi, M. J. Abrams, A. P. Williams and E. J. Gibbs, *Inorg. Chem.*, 1990, **29**, 4483; B. L. Iverson, K. Shreder, V. Kral, P. Samson, V. Lynch and J. L. Sessler, *J. Am. Chem. Soc.*, 1996, **118**, 1608.
4 S. Takenaka, M. Manabe, M. Yokoyama, M. Nishi, J. Tanaka and H. Kondo, *Chem. Comm.*, 1996, 379.
5 A. Bochkarev, R. A. Pfuetzner, A. M. Edwards and L. Frappier, *Nature*, 1997, **385**, 176.
6 D. P. Mascotti and T. M. Lohman, *Biochemistry*, 1993, **32**, 10 568.
7 R. L. Rill and K. H. Hecker, *Biochemistry*, 1996, **35**, 3525.
8 R. M. Wadkins, E. A. Jares-Erijman, R. Klement, A. Rüdiger and T. M. Jovin, *J. Mol. Biol.*, 1996, **262**, 53.
9 K. Berlin, R. K. Jain, C. Tetzlaff, C. Steinbeck and C. Richert, *Chem. Biol.*, 1997, **4**, 63.
10 A. Bettelheim, B. A. White, S. A. Raybuck and R. W. Murray, *Inorg. Chem.* , 1987, **26**, 1009; B. C. Bookser and T. C. Bruice, *J. Am. Chem. Soc.*, 1991, 113, 4208.
11 J. P. Collman, B. Boitrel, L. Fu, J. Galanter, A. Strautmanis and M. Rapta, *J. Org. Chem.*, 1997, **62**, 2308.
12 K. Arar, A.-M. Aubertin, A.-C. Roche, M. Monsigny and R. Mayer, *Bioconjugate Chem.*, 1995, **6**, 573.
13 J. Buter and R. M. Kellogg, *J. Chem. Soc., Chem. Commun.*, 1980, 466; J. Buter and R. M. Kellogg, *J. Org. Chem.*, 1981, **46**, 4481.
14 A. Slama-Schwok and J.-M. Lehn, *Biochemistry*, 1990, **29**, 7895.
15 L. A. Lipscomb, F. X. Zhou, S. R. Presnell, R. J. Woo, M. E. Peek, R. R. Plaskon and L. D. Williams, *Biochemistry*, 1996, **35**, 2818.
16 R. Pottier and J. C. Kennedy, *J. Photochem. Photobiol. B: Biol.*, 1990, **8**, 1.
17 D. Sarracino and C. Richert, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2543.
18 J. P. Collman, L. Fu, P. C. Herrmann and X. Zhang, *Science*, 1997, **275**, 949.
19 F. Rabanal, W. F. DeGrado and P. L. Dutton, *J. Am. Chem. Soc.*, 1996, **118**, 473.

Received in Corvallis, OR, USA, 28th October 1997; 7/07803J