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SYNTHESIS AND DNA-BINDING STUDIES OF NEW CATIONIC GUANIDINE AND BETAINEAMIDE TETRAPHENYLPORPHYRINS

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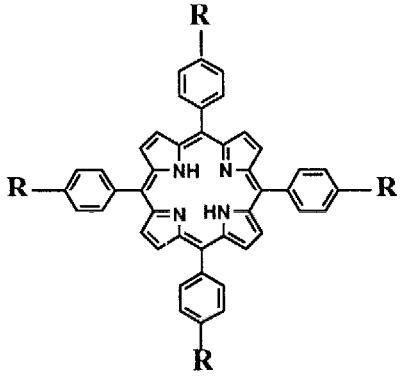
Summary: *Cationic water-soluble tetraphenylporphyrins with guanidine or betaine groups on the phenyl rings are prepared by the coupling reaction of hydrogen cyanamide and N-chlorobetainylchloride, respectively, with tetraaminophenylporphyrins and the preliminary report of their affinities for ct-DNA is described.*

Cationic water-soluble porphyrins and their metal complexes have been a subject of interest due to their strong affinities for DNA and potential nuclease activity.[1,2] Under physiological conditions, interactions of the positively-charged porphyrins with DNA have been characterized as either intercalation preferentially at the G-C sequences or outside groove binding at the minor groove of the A-T sites. Hydrogen bonding interaction, which is commonly observed in protein-DNA recognition, has not, however, been exploited in the porphyrin-DNA interaction. We here report two examples of cationic porphyrins incorporated with H-bond donating and/or accepting functional groups in both the *para*- and *meta*-positions of tetraphenylporphyrin; namely, tetrakis(4-guanidinophenyl)porphyrin (*p*-**1**) and its meta isomer (*m*-**1**), and tetrakis(4-betaineamidophenyl) porphyrin (*p*-**2**) and its meta isomer (*m*-**2**). Synthesis and the preliminary binding results with DNA are presented.

p-**1** and *p*-**2** were synthesized, respectively, by reaction of hydrogen cyanamide and the acid chloride of betaine hydrochloride with tetrakis (4-aminophenyl)porphyrin **3** (Figure 1).[3] The template **3** was prepared from the corresponding nitro derivative **4** according to the published method.[4] Like procedures were used to prepare *m*-**1** and *m*-**2**.[5]

Binding of *m*- and *p*-**1** and as well as *m*- and *p*-**2** with calf thymus DNA have been examined spectroscopically. Preliminary results are presented in Table 1. Absorption titration studies show that binding to DNA is accompanied by red shifts of 0 to 10 nm and varying hyperchromicity, 2 to 39%, at the porphyrin Soret bands. Among them, *m*-**1** shows the greatest spectral shift in the Soret region but the least hyperchromicity. Apparent binding constants were obtained based upon the ability of these porphyrins to compete for the binding sites along the DNA duplex with ethidium bromide. The DNA binding affinities were found in the range of 10^5 to 10^6 M^{-1}, following the order of *m*-**1** >*p*-**1** > *m*-**2** > *p*-**2**. Equilibrium binding constants are similar to those reported previously for tetracationic porphyrins.[2] The UV/vis spectral results seem to suggest that the binding of these porphyrins to DNA are predominantly in an external (groove) binding mode rather than intercalation.



p-**1**: R=NHC(=$H_2N^+Cl^-$)—NH_2

p-**2**: R=$NHCOCH_2(CH_3)_3N^+Cl^-$

3: R=NH_2

4 : R=NO_2

1: **3**, HCl, NH_2CN, EtOH, 100°C, 48h, 60-70%;
2: **3**, $Cl^-\overset{+}{N}(CH_3)_3CH_2COCl$, Et_3N, DMF, RT, 24h, 85-90%.

Figure 1

The results of Table 1 suggest that the guanidino substituent has an advantage over the betaineamido substituent of ~5-fold and that *meta*-substitution has an advantage of ~2-fold over *para*-substitution. The

advantage of the guanidino substituent may relate to its ability to hydrogen bond to the phosphate linkages of the DNA. Further investigation of the binding mechanism and plausible cleavage characteristics of these derivatives is currently under way.

Table1: Interactions of *m*- and *p*-Substituted Porphyrins with ct-DNA: Absorption Maxima Shifts, Hyperchromic Effects and Apparent Binding Constants

Porphyrin	Red shift[a]/Hyperchromicity[b] of Soret band	Apparent binding constant[c]
m - 1	10/2	2.0
p - 1	~0/11[d]	1.3
m - 2	5/39	0.4
p - 2	8/10	0.2

[a] in nanometers. 20 μM of each porphyrin solution was titrated with concentrated calf thymus DNA solution (6 mM), the final porphyrin to DNA ratio was 1:25. [b] in percentages. [c] $X10^6M^{-1}$,following the ethidium bromide displacement procedures reported previously,[2] fluorescence intensities of solutions containing 3.3 μM calf thymus DNA were measured after each titration with concentrated ethidium bromide solution in the presence of 0-2 μM of porphyrin. [d] See ref. 6. Fluorescence measurements were performed on a Perkin-Elmer LS-50 spectrofluoremeter with excitation at 546 nm and emission at 595 nm. All experiments were performed in a buffer containing 0.1 M Tris-HCl (pH 7.5), 0.1 M NaCl.

Acknowledgements:

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2. M.A.Sari, J.P.Battioni, D.Dupre, D.Mansuy, and J.B.Le Peq, *Biochemistry*, 1990, **29**, 4205.
3. J.L.Hughes, R.C.Liu, and T.Enkoji, *J. Med. Chem,* 1975, **18**, 1077. *p*-**1**: ^{1}H-NMR, DMSO-d_6 δ (ppm) : -2.90 (br s, 2H, pyrrole N-H); 7.69 (d, 8H phenyl-H); 7.71 (br s, 16H, guanid. NH_2); 8.26 (d, 8H, phenyl-H); 8.97(s, 8H, pyrrolic-H); 9.96 (br s, 4H, guanid. NH). UV-VIS, CH_3OH λ_{max} nm (ε x $10^{-3}M^{-1}cm^{-1}$): 414 (160), 514 (12.0), 548 (5.8), 590 (4.6), 646 (3.4). IR KBr disk ν (cm^{-1}): 3340, 3310, 3188, 3110, 3025(guanid.N-H stretching). FAB-MS: calcd for $C_{48}H_{42}N_{16}$ (M^+-5 HCl) m/e 842.95, found m/e 843.
p-**2**: ^{1}H-NMR, DMSO-d_6 δ (ppm) : -2.93 (br s, 2H, pyrrole N-H) ; 3.33 (s, 36 H, N-CH_3) ; 4.65 (s, 8H, betaine CH_2); 8.14 (d, 8H, phenyl-H) ; 8.22 (d, 8H, phenyl-H) ; 8.88 (s, 8H, b-pyrrolic H) ; 11.73 (br s, 4H, amide NH) . UV-VIS, CH_3OH, λ_{max} nm (ε x$10^{-3}M^{-1}Cm^{-1}$): 417 (139) , 515 (6.44) , 552 (4.59) , 597 (2.21) , 648 (1.68) . FAB-MS: calcd for $C_{64}H_{74}N_{12}O_4$ (M^+- 5 HCl) m/e 1075.63, found 1075.
4. A.Bettleheim,B.A.White,S.A.Raybuck,and R.W.Murray, *Inorg. Chem.* 1987, **26**, 1009.
5. With the exception of the positions of the ^{1}H-NMR signals, the spectral features of the *meta* and *para* isomers are much the same.
6. The Soret band of the free base and the porphyrin-DNA complex solution of *p*-**1** shows small degree of splitting which makes the exact determination of degree of red shift difficult.; this may have been caused by aggregation which is expected to be minimal at μM concentration and/or alternative tautomeric structures as has been shown for *p*-substituted phenyl guanidinium salts and tetraaminophenylporphyrin (i.e., R.E.Botto et. al., *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 23; and M.J.Gunter et. al., *Aust.J. Chem.*, 1989, **42**, 1897).

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