Highly Efficient Complexations of a Porphyrin Dimer with Remarkably Small Differences between Nucleosides and Nucleotides/The Predominance of Stacking Interactions for Nucleic Acid Components

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Received January 19, 2000

The complexation of nucleic acid components by synthetic receptors is an important target of supramolecular chemistry.[1] In most cases the complexation is predominantly based on ion pairing with phosphate groups;[2] complexes with neutral nucleosides or with nucleobases[3] rely on stacking interactions on hydrogen bonds, or on both, and are usually weaker as well as most often restricted to the use of lipophilic solvents. We wish to report on a new host compound, which allows for the first time to complex **both** natural nucleosides and nucleotides and does so in water with hitherto unknown large affinities with both biologically important substrate classes.

Porphyrins, which have the advantage to provide a sensitive optical signal also for sensors, have been used before also for nucleotide complexation[4,5] however with usually rather low affinities; furthermore, they were not suitable for an effective complexation of electroneutral nucleosides. The new receptor **1** is based on porphyrin units, which are made watersoluble by six pyridinium units, and are held together in cleft-like manner by an *o*-dioxymethylphenyl unit, thus allowing stacking with nucleobases and Coulombic interactions with phosphate residues. Preliminary gas-phase simulations with the CHARMm[6] force field helped to design the host with a rather optimal fit to nulceic acid derivatives (Figure 1). The *o*-dioxymethylphenyl spacer holds the two porphyrin units apart and prevents self-stacking (collapse) of the cleft. This is evident from force field energy minimizations of **1** in absence of the host (not shown) and by the very similar UV spectra of **1** in comparison to monomeric model compounds, showing no hypochromicity effects. The dimer was synthesized by the base-catalyzed coupling reaction of meso[5-(3-hydroxyphenyl)-10,15,20-tris(4-pyridyl)porphyrin] and the α,α′-dibromomethyl-*o*-xylene.

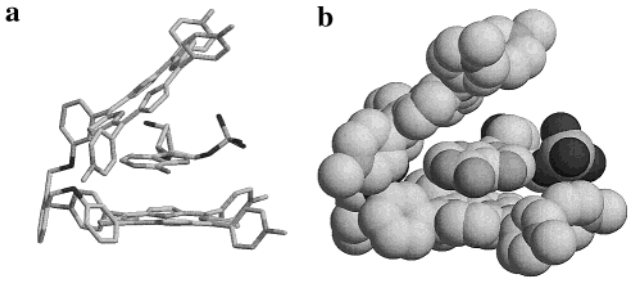

Figure 1. (a) CHARMm optimized structure of bis-prophyrin **1** complex with AMP. Hydrogens are omitted for clarity. The nucleobase is wedged parallel between the two porphyrin units and is making van der Waals contacts with the one of the porphyrin unit of **1** (d_{av} = 3.65 Å). (b) CPK model).

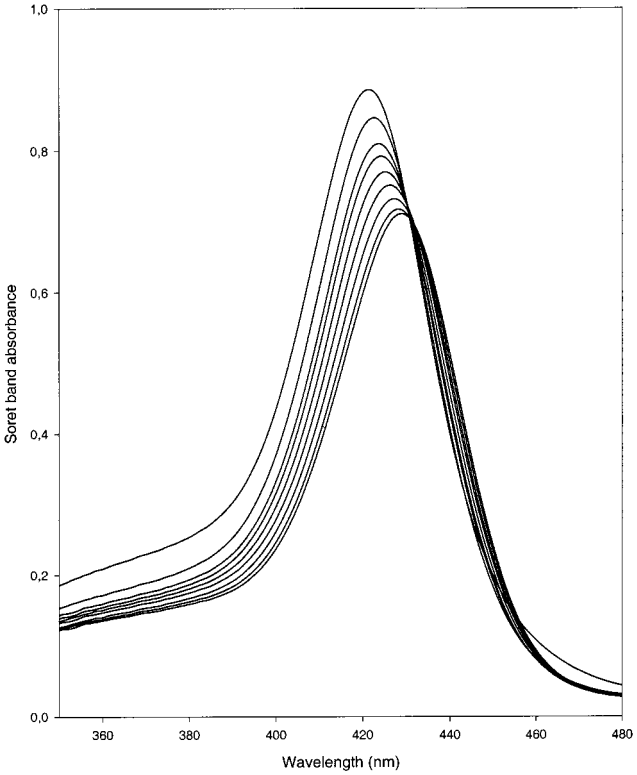

Figure 2. UV/vis-titration curves for the complex between host **1** (4 μM) and **AMP** (**AMP** added from 0.0 to 1.0 mM concentration).

Addition of various nucleosides and nucleotides to host **1** resulted in distinct changes of the Soret band wavelength, $\Delta\lambda$ by 3 to 8 nm and the extinction coefficients $\Delta\epsilon$ (25×10^{-3} to 65×10^{-3} dm^3 mol^{-1} cm^{-1}). Figure 2 shows a representative titration curve with an isosbestic point, in line with formation of a 1:1 equilibrium. Nonlinear least-squares fit of the curves to 1:1 calculational model yielded excellent agreement (Figure 3). Self-association of the host does not occur under the very low concentration (2−5 μM) needed for the UV/vis measurements; this is borne out also by the absence of spectrocopic changes of the ligand upon dilution between 1 and 50 μM.

(1) For reviews see: (a) *Supramolecular Chemistry of Anions*; Bianchi, A., Bowman-James, K., Garcia-Espana, E., Eds.; Wiley-VCH: New York, NY, 1997; pp 355−419. (b) Diederich, F. *Cyclophanes*; Royal Society of Chemistry: Cambridge, 1991. (c) Rebek, J., Jr. *Acc. Chem. Res*. **1990**, *23*, 399. (d) Hamilton, A. D. *Bioorganic Chemistry Frontiers*; Dugas, H., Ed.; Springer: Berlin, Heidelberg, 1991; Vol. 2, p 115.

(2) For recent references on nucleotide receptors, usually dominated by ion-pairing, see: (a) Shi, Y.; Schneider, H.-J. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1797. (b) Schwinte, P.; Darcy, R.; Okeeffe, F. *J. Chem. Soc., Perkin Trans. 2* **1998**, 805. (c) Nation, D. A.; Lu, Q.; Martell, A. E. *Inorg. Chim. Acta* **1997**, *263*, 209. (d) Aguil, J. A.; Garcia-Espana, E.; Guerrero, J. A.; Luis, S. V. *Inorg. Chim. Acta* **1996**, *246*, 287. (e) Ragunathan, K. G.; Schneider, H.-J. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2597. (f) Eliseev, A. V.; Schneider, H.-J. *J. Am. Chem. Soc.* **1994**, *116*, 6081. (g) Dhaenens, M.; Lehn, J. M.; Vigneron, J. P. *Perkin Trans. 2* **1993**, 1379.

(3) For recent references on complexation with electroneutral nucleosides or nucleobase derivatives see: (a) Yu, L.; Schneider, H.-J. *Eur. J. Org. Chem.* **1999**, 1619. (b) Conn, M. M.; Deslongchamps, G.; Mendoza, J. de; Rebek, J., Jr. *J. Am. Chem. Soc*. **1993**, *115*, 3548. (c) Rotello, V. M.; Viani, E. A.; Deslongchamps, G.; Murray, B. A.; Rebek, J., Jr. *J. Am. Chem. Soc*. **1993**, *115*, 797. (d) Jeong, K. S.; Tjivikua, T.; Muehldorf, A.; Deslongchamps, G.; Famulok, M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 201. (e) Hamilton, A. D.; Little, D. *J. Chem. Soc., Chem. Commun*. **1990**, 297. (f) Inouye, M.; Itoh, M. S.; Nakazumi, H. *J. Org. Chem.* **1999,** *64*, 9393.

(4) General references on the use of porphyrin and analogues for supramolecular complexes see: (a) Sessler, J. L.; Sansom, P. I.; Andrievsky, A.; Kral, V. in ref 1a, p 355ff. For porphyrin dimers see: (b) Elemans, J. A. A. *J. Org. Chem.* **1999**, *64*, 7009. (c) Twyman, L. J. *Tetrahedron Lett.* **1999**, *40*, 6681. (d) Baudin, O. *J. Org. Chem.* **1997**, *62*, 5458. (e) Graça, M. *Chem. Commun.* **1999**, 1771.

(5) Nucleotide complexation with porphyrin and related host compounds: (a) Pasternack, R. F.; Gibbs, E. J.; Gaudemer, A.; Antebi, A.; Bassner, S.; De Poy, L.; Turner, D. H.; Williams, A.; Laplace, F.; Lansard, M. H.; Merienne, C.; Perree-Fauvet, M. *J. Am. Chem. Soc.* **1985**, *107*, 8179. (b) Kral, V.; Andrievsky, A.; Sessler, J. L. *Chem. Com.* **1995**, 2349.

(6) Brooks, C. L.; Karplus, M. *Methods Enzymol*. **1986**, *127*, 369; Brünger, A. T.; Karplus, M. *Acc. Chem. Res.* **1991**, *24*, 54.

10.1021/ja000208k CCC: $19.00
Published on Web 06/01/2000

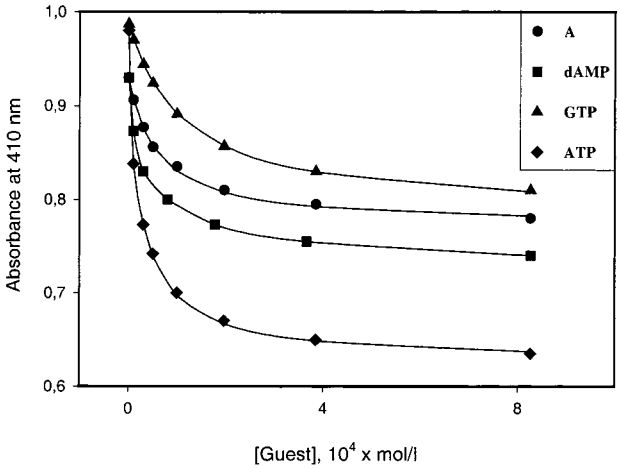

Figure 3. UV/vis titration of differerent nucleosides and nucleotides with the porphyrin dimer **1**: nonlinear least-squares fit with adenosine **A** and the nucleotides **dAMP**, **ATP**, and **GTP**.

Table 1. Logarithm of Association Constants for the Dimeric Porphyrin **1** with Nucleotides[a]

	without buffer			with 0.3 M buffer		
ligands	logK	$\Delta\lambda$ (nm)	ΔA	logK	$\Delta\lambda$ (nm)	ΔA
adenosine	4.59	3	0.10	4.25	3	0.16
2′-dA				4.96	2	0.06
AMP^{2-}	4.80	8	0.30	4.82	8	0.15
$dAMP^{2-}$	4.70	6	0.38	5.09	5	0.14
ADP^{3-}	5.25	8	0.49	4.77	3	0.12
ATP^{4-}	5.30	7	0.39	4.47	4	0.26
$dGMP^{2-}$	4.83	6	0.32	4.58	2	0.15
GTP^{4-}	5.63	6	0.44	3.17	2	0.20
thymidine	3.35	1	0.1	4.69	2	0.12
$dTMP^{2-}$	4.31	3	0.23	4.82	1	0.11
cytidine	5.42	4	0.09	4.69	1	0.10
$dCMP^{2-}$	3.73	3	0.27	4.77	1	0.10
(2′-5′) CpG	4.13	5	0.41			
$dUMP^{2-}$	4.49	3	0.17	4.78	1	0.17
TpT				4.10	3	0.14

[a] Measured by UV−visible titration of **1** and **2** with nucleotides in water in the absence and in the presence of 0.3 M phosphate buffer (pH 7.9 ± 0.2) at 25 °C. Titrations were carried out by adding concentrated stock solutions of nucleotides ([nucleotide] = 10 mM) containing also 2 μM **1** to 2 μM solutions of porphyrin **1** in a 10 mm cuvette. Error limits (from repetitions): log K ± 5%.

The new receptor **1** shows unprecedented large affinities even with electroneutral nucleosides, reaching K values of up to 270 000 M^{-1} with cytidine (Table 1), or 430 000 M^{-1} with **GTP**. Stacking interactions with the large porphyrin surfaces represent the dominant binding force[7] as is obvious from the relatively small affinity differences between nucleosides and nucleotides. Absence of the hydrophilic 2′-OH group as in 2-deoxyadenosine or **dAMP** also seems to enhance the affinity. The association constants measured in the presence of usual buffer concentrations were for nucleotides with more charges than two distinctly smaller than those measured without buffer (the pH in these cases were checked by and kept constant by adding very dilute acids or bases if necessary). The difference Δlog K (without−with buffer) increased, for example, from **AMP** (inverse, +0.02) to **ADP** (0.48) to **ATP** (0.83) and reaches 2.46 with **GTP**. Thus, traditionally used buffers can change association constants by orders of magnitudes, which can be explained by the competition of the buffer anions against the ligands to be bound, an effect which must increase with the contribution of charges and therefore Coulombic interactions in the complexes.[8] However, the size of this salt effect varies considerably, as seen in the Δlog K differences between **AMP**, **dAMP**, **dGMP**, **dTMP**, and **dUMP**, ranging from −0.52 units (**dTMP**) to +0.02 (**AMP**). Noticeably, deoxy mononucleotides tend to bind **better** in the presence of buffer, with the exception of **dGMP**. This is in line with the small Coulombic effects in these nevertheless doubly charged systems, and with the predominance of stacking effects in all these cases as borne out by the similarly large affinities with the electroneutral nucleosides. Dinucleotides are not complexed significantly better than mononucleotides, indicating that the essential binding contribution is the enclosure of one nucleoside moiety in the cleft of receptor **1**.

The results demonstrate, that open chain cleft compounds hold much promise for highly effective complexation of nucleic acid components. Such host units may by incorporated in polydentate artificial receptors, containing also hydrogen binding units,[9] thus achieving extremely high affinities and base selectivities by Watson−Crick base pairing. The strikingly small affinity differences observed between nucleosides and nucleotides emphasize the paramount importance of stacking forces for nucleic acid components, which recently has been also found to dominate formation of double-stranded nucleic acids.[10] As earlier investigations of porphyrin complexes have shown that non-aromatic moieties in the bound ligands show little, if any, binding contribution,[11] stacking as opposed to general hydrophobic interactions are believed to be the essential non-covalent forces in such associations.

Acknowledgment. Our work is supported by the Deutsche Forschungsgemeinschaft, Bonn, and the Fonds der Chemischen Industrie, Frankfurt. We also thank the A.-von Humboltd foundation for a stipend for M.S.

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(7) For other cases of dominating stacking interactions in nucleotide complexes see: (a) Constant, J. F.; Fahy, J.; Lhomme, J.; Anderson, J. E. *Tetrahedron Lett.* **1987,** *28*, 1777. (b) Zinic, M.; Tomišic, V.; Simeon, V.; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1995**, 1073. (c) Lorente, A.; Fernandez-Saiz, M.; Lehn, J.-M.; Vigneron, J.-P. *Tetrahedron Lett.* **1995**, *36*, 8279. (d) Baudoin, O.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Lehn, J.-M. *J. Org. Chem.* **1997**, *62*, 5458.

(8) Sirish, M.; Schneider, H.-J. *Chem. Commun.* **2000**, 23.

(9) For an early model system, see: ref 7a.

(10) Matray, T. J.; Kool, E. T. *J. Am. Chem. Soc.* **1998**, *120*, 6191.

(11) Schneider, H.-J.; Wang, M. *J. Org. Chem.* **1994**, *59*, 7464.