Highly Negative Homotropic Allosteric Binding of Viologens in a Double-Cavity Porphyrin

Pall Thordarson, Edward J. A. Bijsterveld, Johannes A. A. W. Elemans, Peter Kasák, Roeland J. M. Nolte, and Alan E. Rowan*

Department of Organic Chemistry, NSRIM, University of Nijmegen, Toernooiveld 1, 6525 ED, Nijmegen, The Netherlands

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The synthesis and study of well-defined synthetic host systems that are capable of mimicking the various complicated binding processes in nature have long been significant goals in chemistry. One of the natural processes that is very important in many enzymatic systems is allosteric binding that allows them to respond to external influences.[1] In allosteric systems, the information associated with a binding event is transmitted to another binding site by structural changes in the host molecule. Allosteric systems by definition are cooperative,[2] negative, or positive. However, not all cooperative systems are allosteric; in allosteric systems, the binding events have to cause a discrete reversible alteration of the host.[3] In this paper, we report on the synthesis of a double-cavity porphyrin **6** that shows very strong allosteric binding behavior upon binding of viologens.

The best-known case of allosteric behavior is the one of oxygen binding to the four binding sites of hemoglobin, which is classified as being of the positive homotropic type, although more recent studies have indicated that this is somewhat an oversimplification.[4] Several artificial allosteric systems have been described,[2,5] the vast majority of which are of the heterotropic type; that is, two or more different ligands interact with a multiple-binding site host. The homotropic type has been less studied, and, until now, only one negatively homotropic allosteric system has been reported: the "sugar tweezers" by the group of Shinkai.[5b]

We developed the synthesis of the double-cavity porphyrin **6** as a part of our efforts to optimize the synthesis of **4**. These studies showed that the reaction of the simple porphyrin **1** and tosylate[6] **3** under basic conditions yielded **4** in over 30% yield. Likewise, the analogous reaction of porphyrin **2** with tosylate **3** was carried out to give **5** and **6**. The choice of solvent was critical; in CH_3CN, the intermediate cavity-appended porphyrin **5** was the main product (14% yield, route A in Scheme 1), while in DMF the main identified product was the double-cavity porphyrin **6** (1% yield, route B in Scheme 1),[7a] which came as two isomers. ¹H NMR identified these isomers as **6a** and **6b**, in a 10:1 ratio, respectively.[7b] Alternatively, intermediate **5** could be isolated and reacted further with tosylate **3** to give **6a** in an overall 2% yield. The intermediate porphyrin **5** is interesting in its own right because it can easily be functionalized on the "top face" to give a variety of new cavity compounds.

The low yields in the formation of **6** as compared to both **4** and **5** suggest that **6** is a significantly more strained molecule. The unequal product ratio of **6a** and **6b** has its origin in the C_2-symmetry of the cavity in **5**, which makes the reaction of the second tosylate molecule **3** more favorable in one orientation than in the other.

Given the fact that host **4** had been shown to be an excellent receptor for viologens (N,N'-disubstituted 4,4′-bipyridines, $K > 10^6$ M^{-1}),[6,8] the binding of the methyl derivative (**V**) and the ethanol derivative[6] (**etV**) to **6a**[7c] was investigated by a combination of UV−vis, fluorescence, and ¹H NMR titrations. The UV−vis and
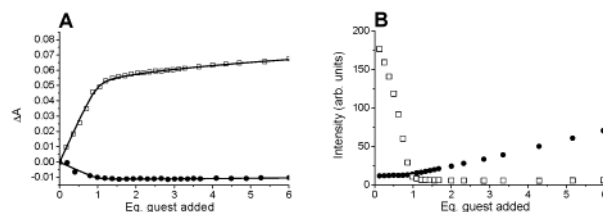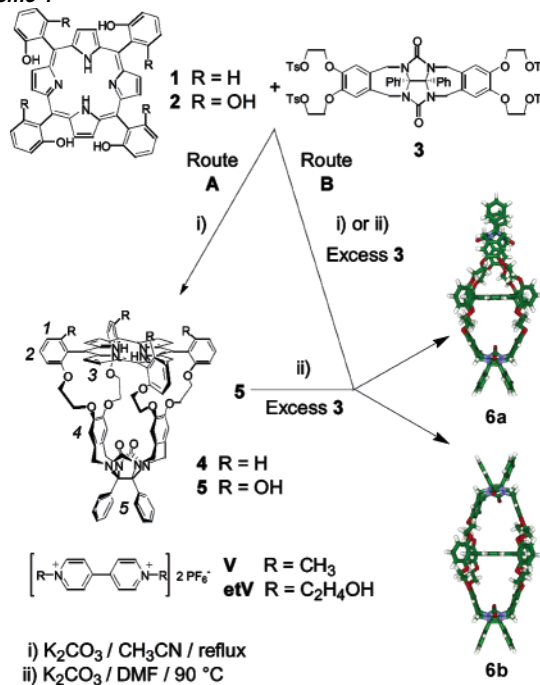

Figure 1. Spectroscopic titrations of host **6a** in $CHCl_3/CH_3CN$ (4:1, v/v). (A) UV−vis titration of **6a** with **V** (● at 428 nm) and **etV** (□ at 431 nm). Also shown are the calculated binding isotherms (—) obtained by nonlinear regression assuming 1:2 allosteric binding. (B) Fluorescence titration ($\lambda_{ex} = 422$ nm, $\lambda_{em} = 645$ nm (□), $\lambda_{em} = 527$ nm (●)) of **6a** with **V**.

Scheme 1

fluorescence titrations revealed that the binding of **V** and **etV** in the two cavities of **6a** was not a statistical 1:2 process (Figure 1A) but a cooperative one, one of the criteria for allosterism.[2] The first binding step is considerably stronger than the second. The fluorescence emission from the porphyrin was fully quenched after the addition of only 1 equiv of guest (Figure 1B). Upon the addition of more guest, a second emission band appeared, attributed to the binding in the second cavity (λ_{ex} maximum at 466 nm).[9] Despite the different binding geometries of the two guests (vide infra), the fluorescence binding isotherms were very similar (Figure 1B, only guest **V** is shown for clarity).

Table 1. Association Constants K (M^{-1}) and the Microscopic Stepwise Free Energies ΔG (kJ mol^{-1}) for the Complexation of **etV** and **V** to Hosts **4** and **6a** in CHCl$_3$/CH$_3$CN (4:1, v/v) at 298 K

complex	$K_1{}^a$	$K_2{}^a$	ΔG_1	ΔG_2	$\Delta\Delta G_{allost.}{}^b$
etV:6a	4×10^7	3×10^5	-42	-33	9 ± 4
V:6a	7×10^7	5×10^4	-43	-28	15 ± 5
etV:4	1×10^9		-51		
V:4	3×10^7		-43		

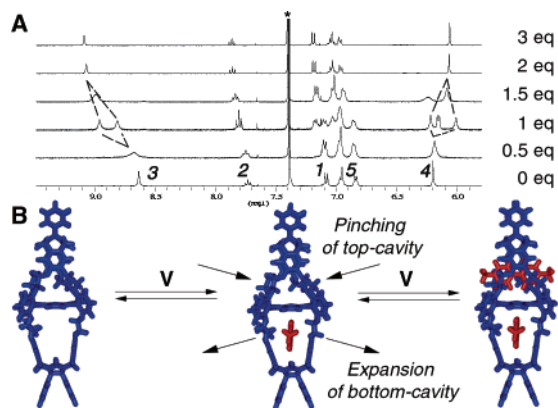^{a} Estimated errors for K_1 and K_2 = 50%. ^{b} Defined as $\Delta\Delta G_{allost.} = \Delta G_2 - \Delta G_1$.

Figure 2. Complexation of host **6a** with **V**. (A) ^{1}H NMR spectra (400 MHz, CDCl$_3$/CD$_3$CN (4:1, v/v)) from a titration experiment with equivalents of **V** added indicated on the right of the spectra. Labels correspond to those in Scheme 1. The splitting of the (*3*) and (*4*) resonances when 1 equiv of **V** is added is indicated with dashed (- -) lines (the doublet at 6.15 ppm is due to the α-proton of the bound **V**). (B) Molecular modeling[11] figures of the complexes formed indicating the mechanism of binding as observed by ^{1}H NMR.

The binding isotherms from both UV−vis and fluorescence titrations were fitted to 1:1 and 1:2 binding models. The best fit was always obtained for a nonstatistical 1:2 binding model involving strongly negative cooperativity.[2] The results were compared with the binding of guests **V** and **etV** to **4** (Table 1).

The complementarity between the host and guests is highlighted by the exceptionally high calculated binding energies > −40 kJ mol^{-1}. The difference between the binding of the first and the second guest in both systems is approximately 10 kJ mol^{-1}. The magnitude of the negative cooperativity observed here is to the best of our knowledge the strongest reported so far for an artificial allosteric system. Numerically, the free-energy penalty for the second binding as compared to the first is similar to the Wyman interaction energy in hemoglobin,[10] that is, the difference between the first and last binding of oxygen to hemoglobin ($\Delta\Delta G_{allost.} \approx -15$ kJ mol^{-1}).[4a]

The observed negative cooperativity is also evident from ^{1}H NMR titrations. Under the conditions used here, only the 1:1 complex is observed up to the point that 1 equiv of viologens **V** or **etV** has been added, as is evident by the breaking in the symmetry of the host resonances. When the second cavity is filled, the initial symmetry of **6a** is restored (Figure 2A). The negative allosteric effect is similar for both guests **V** and **etV**, despite **V** and **etV** binding to **6a** in different complexation geometries. The ^{1}H NMR and UV−vis titration data indicate that the guest **etV** is bound parallel to the porphyrin in both cavities and the methyl derivative **V** is bound orthogonal to the porphyrin.[6]

The negative cooperativity can be attributed to either electrostatic repulsion of the second guest by the fully bound first guest or a change in the shape (allosteric effect) of the second cavity upon binding of the first guest. ^{1}H NMR data and computational

modeling[11] clearly indicate that the latter process is responsible for the allosteric effect observed and the electrostatic repulsion plays no significant role at all. The modeling studies calculate that the two cavities in the empty host **6a** are not equivalent due to C_2-symmetry of the cavity part of the molecule. One cavity is calculated to be wider (6.2 Å) than the other (5.6 Å). On the NMR time scale, the shapes of the two cavities are rapidly interconverting.[7d] Upon the addition of guest, binding occurs first in the larger cavity, effectively pinching the second cavity as confirmed by ^{1}H NMR (schematically shown in Figure 2B). The second binding of **V** (or **etV**) therefore becomes more difficult, resulting in the observed allosteric effect. In addition, the chemical shifts observed upon going from the 1:1 to the 1:2 complex clearly indicate a structural change in the host and highlight the allosteric behavior of this system. The mechanism described fits much better for the sequential (or induced-fit) theory of allosteric interactions by Koshland, Némethy, and Filmer[12] than for the rival concerted change model of Monod, Wyman, and Changeux.[3b]

Given the regulatory role of allosteric interactions in nature and their possible application in signaling and sensing devices,[1] we are more closely investigating the binding properties of the double-cavity porphyrin **6** and its derivatives. We also expect this molecule to be an excellent catalyst, as the active center is fully protected by the two cavities, thus allowing the selective binding of an axial ligand and a variety of substrate molecules. These studies are currently in progress in our laboratory.

Supporting Information Available: Selected experimental data for the synthesis of **6a** and binding studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Shinkai, S.; Ikeda, M.; Sugasaki, A.; Takeuchi, M. *Acc. Chem. Res.* **2001**, *34*, 494. (b) Takeuchi, M.; Ikeda, M.; Sugasaki, A.; Shinkai, S. *Acc. Chem. Res.* **2001**, *34*, 865.
(2) (a) Perlmutter-Hayman, B. *Acc. Chem. Res.* **1986**, *19*, 90. (b) Connors, K. A. *Binding Constants*; John Wiley & Sons: New York, 1987.
(3) (a) Monod, J.; Changeux, J.-P.; Jacob, F. *J. Mol. Biol.* **1963**, *6*, 306. (b) Monod, J.; Wyman, J.; Changeux, J.-P. *J. Mol. Biol.* **1965**, *12*, 88.
(4) (a) Ackers, G. K.; Doyle, M. L.; Myers, D.; Daugherty, M. A. *Science* **1992**, *255*, 54. (b) Huang, Y.; Doyle, M. L.; Ackers, G. K. *Biophys. J.* **1996**, *71*, 2094. (c) Johnson, M. L. *Methods Enzymol.* **2000**, *323*, 124.
(5) (a) Tabushi, I. *Pure Appl. Chem.* **1988**, *60*, 581. (b) Takeuchi, M.; Imada, T.; Shinkai, S. *J. Am. Chem. Soc.* **1996**, *118*, 10658.
(6) Elemans, J. A. A. W.; Claase, M. B.; Aarts, P. P. M.; Rowan, A. E.; Schenning, A. P. H. J.; Nolte, R. J. M. *J. Org. Chem.* **1999**, *64*, 7009.
(7) (a) Selected experimental data for the synthesis of **6a** are given in the Supporting Information; details for all of the compounds described here will be reported in a forthcoming paper. (b) Molecular modelling suggests that **6a** has a C_s-symmetry and **6b** a D_{2h}-symmetry. (c) Because of the low yield of **6b**, no binding studies have been carried out to date. (d) Variable temperature ^{1}H NMR spectra (down to 220 K in CDCl$_3$) showed that the symmetry of the host **6a** breaks down as expected, although it should be noted that this could also be due to symmetry breaking that normally occurs when the inner imine-proton (NH) tautomerism is frozen out on the NMR time scale.
(8) Rowan, A. E.; Aarts, P. P. M.; Koutstaal, K. W. M. *Chem. Commun.* **1998**, 611.
(9) This emission band is a result of excitation of the charge-transfer complex formed between the catechol-like cavity walls and the viologen guest. See also: Bernardo, A. R.; Stoddart, J. F.; Kaifer, A. E. *J. Am. Chem. Soc.* **1992**, *114*, 10624.
(10) Wyman, J. *Adv. Protein Chem.* **1964**, *18*, 223.
(11) Hyperchem 6.01, Hypercube Inc., Gainesville, FL, 2000.
(12) Koshland, D. E., Jr.; Némethy, G.; Filmer, D. *Biochemistry* **1966**, *5*, 365.

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