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

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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. 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, 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. 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. Several artificial allosteric systems have been described, 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.

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 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 CH₃CN, 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), which came as two isomers. ¹H NMR identified these isomers as 6a and 6b, in a 10:1 ratio, respectively. 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 C₂ 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 > 10⁶ M⁻¹), the binding of the methyl derivative (V) and the ethanol derivative (etV) to 6a was investigated by a combination of UV—vis, fluorescence, and ¹H NMR titrations. The UV—vis and 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. 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. 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).

Figure 1. Spectroscopic titrations of host 6a in CHCl₃/CH₃CN (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 (λₜₐₜ = 422 nm, λₘᵦ = 645 nm (●), λₘᵦ = 527 nm (○)) of 6a with V.

Scheme 1
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. The results were compared with the binding of guests V and etV to 4 (Table 1).

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The complementarity between the host and guests is highlighted 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 figures of the complexes formed indicating the mechanism of binding as observed by 1H NMR.

Table 1. Association Constants $K$ (M$^{-1}$) and the Microscopic Stepwise Free Energies $\Delta G$ (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

<table>
<thead>
<tr>
<th>complex</th>
<th>$K_1$</th>
<th>$K_2$</th>
<th>$\Delta G_1$</th>
<th>$\Delta G_2$</th>
<th>$\Delta G_{\text{etV}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>etV:6a</td>
<td>$4 \times 10^4$</td>
<td>$3 \times 10^4$</td>
<td>$-42$</td>
<td>$-33$</td>
<td>$9 \pm 4$</td>
</tr>
<tr>
<td>V:6a</td>
<td>$7 \times 10^4$</td>
<td>$5 \times 10^4$</td>
<td>$-43$</td>
<td>$-28$</td>
<td>$15 \pm 5$</td>
</tr>
<tr>
<td>etV:4</td>
<td>$1 \times 10^4$</td>
<td>$5 \times 10^4$</td>
<td>$-42$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V:4</td>
<td>$3 \times 10^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

$\Delta G_{\text{etV}}$ clearly indicate that the latter process is responsible for the allostery 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 a 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. Upon the addition of guest, binding occurs first in the larger cavity, effectively pinching the second cavity as confirmed by 1H NMR (schematically shown in Figure 2B). The second binding of V (or etV) therefore becomes more difficult, resulting in the observed allostery 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 allostery interactions by Koshland, Némethy, and Filmer than for the rival concerted change model of Monod, Wyman, and Changeux.

Given the regulatory role of allostery interactions in nature and their possible application in signaling and sensing devices, 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.

Acknowledgment. This research was supported through a EC Marie Curie Fellowship to P.T. (Contract No. HPMF-CT-2000-01053) and the EC Socrates program to P.K.

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

(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$_2$-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 1H 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.
(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: Bernardino, A. R.; Stoddart, J. F.; Kaifer, A. E. J. Am. Chem. Soc. 1992, 114, 10624.

JA028463N

J. AM. CHEM. SOC. 5 VOL. 125, NO. 5, 2003 1187