Allosteric Receptors

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Simple Heme Dimers with Strongly Cooperative Ligand Binding**

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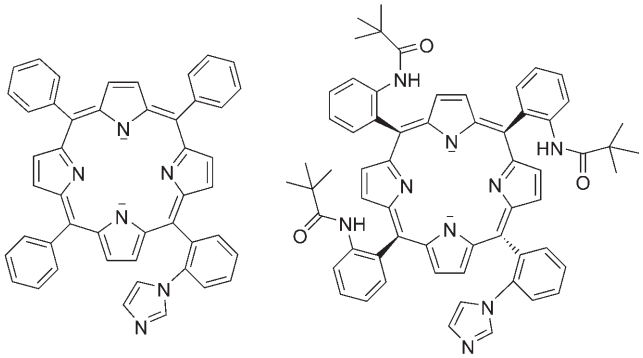
We report that the simple stable heme dimers (**1**-Fe)$_2$ and (**2**-Fe)$_2$ display strongly cooperative ligand binding. For example, (**1**-Fe)$_2$ binds a second isocyanide molecule with an approximately 50-fold higher affinity than the first, or with an allosteric interaction energy of $\Delta G_a = 13 \pm 3$ kJ mol^{-1}. The degree of cooperativity is comparable to that of hemoglobin, a prototypical cooperative receptor for small molecules, whereas the mechanism appears to be distinct.



$\mathbf{1}^{2-}$ $\mathbf{2}^{2-}$

With these results, we aim to help address the unmet need for simple, robust, and easily accessible synthetic receptors that bind small molecules (O_2, NO, CO) cooperatively. Such receptors would be of great value for a number of applications (noncryogenic air separation,[1] chemical O_2 delivery/storage,[2] chemical sensors[3]) and fundamental studies[3–5] (for example, kinetics of multisite catalysis with interacting sites, conformational information transfer). The heme molecule is unmatched as a receptor for small molecules and its exploitation in synthetic cooperative receptors seems natural. Herein we report a simple structural motif within which heme ligation is strongly cooperative. We are not the first to achieve cooperative heme ligation in a synthetic receptor: one of the best known examples was reported by Collman et al. over 25 years ago.[6] However, our compounds stand out in their simplicity: prior to our work, only hemes in crystals, in polymers, or in the presence of a large excess of a competing ligand displayed cooperativity.[7] Our receptors are cooperative in solution with the affector as the only ligand present. This simplicity necessarily limits their structural resemblance with allosteric proteins, such as hemoglobin, but could open up new opportunities for the applications discussed above.

The synthesis of (**1**-Fe)$_2$ and (**2**-Fe)$_2$ is straightforward.[8] UV/Vis spectra of (**1**-Fe)$_2$ in hexanes, toluene, or $CHCl_3$ at concentrations of 0.1–50 μM manifest a strong sharp Soret band at 434 nm ($\varepsilon = 2.3 \times 10^5$ M^{-1} cm^{-1}) with a weaker companion at 371 nm ($\varepsilon = 4.1 \times 10^4$ M^{-1} cm^{-1}) and two well-defined Q bands at 538 and 568 nm; the spectra of (**2**-Fe)$_2$ are similar.[8] These spectra are highly characteristic of a ferroheme coordinated axially to a single N-heterocyclic base,[9] which in our case is only possible if the hemes dimerize. The lower limit of the dimerization constant ($K_a > 5 \times 10^8$ M^{-1}) is determined by the absence of the spectroscopic signature of the monomeric (four-coordinate) heme in 0.1 μM solutions.

Exposure of paramagnetic (**1**-Fe)$_2$ or (**2**-Fe)$_2$ to CO, $Me_2C_6H_3NC$, or PhNO generates diamagnetic species with NMR spectra that are highly indicative of dimers with one exogenous ligand per heme.[8] In these adducts, imidazole hydrogen atoms resonate at frequencies 2–7 ppm upfield from those in the free bases, at positions typical for imidazole protons coordinated to a diamagnetic ferroheme.[8,9] Similarly, the resonances of the β-pyrrolic H atoms and those of the imidazole-bearing phenyl group shift upfield by as much as 3 ppm.[8] The pattern of shifts, which result from shielding by the porphyrin ring current, unambiguously reveals the dimeric heme core. The adduct formation is reversible: evacuation of the samples at < 10 μbar regenerates the five-coordinate hemes. (**1**-Fe)$_2$ reacts rapidly with O_2 to yield the (por)Fe(μO)Fe(por) chromophore (por: porphyrin); oxygenation of (**2**-Fe)$_2$ yields a product with a UV/Vis spectrum identical to that of the O_2 adduct of picket-fence porphyrin,[9] which persists at room temperature. It remains to be confirmed that the resulting species is (**2**-FeO$_2$)$_2$. Under no conditions did we observe the oligomers (**1**-Fe)$_n$ or (**2**-Fe)$_n$ ($n \geq 3$), which are easily distinguishable from the dimers by UV/Vis (by the presence of six-coordinate and four-coordinate hemes) and NMR spectroscopy.

In toluene or hexanes, the dimers bind *N*-Me-imidazole, $Me_2C_6H_3NC$, and nitrosoaryls cooperatively (Figure 1). The isocyanide and nitrosoaryls were chosen as analogues of CO and O_2, respectively.[10] Spectrophotometric titrations proceeded with well-defined isosbestic points,[8] a result indicative of interconversion between only two chromophores: the five-coordinate and six-coordinate hemes. The binding constants and allosteric interaction energies are independent of

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

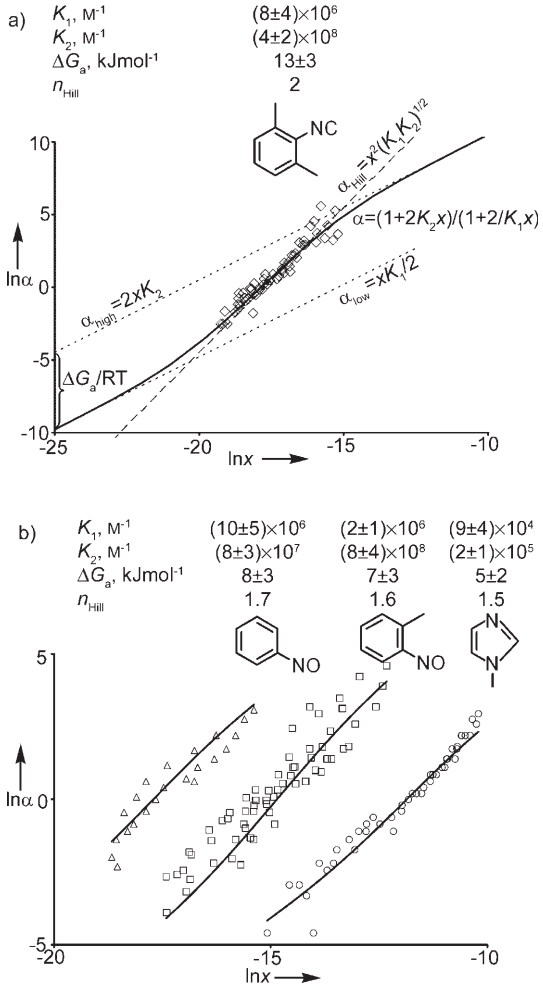

Figure 1. Thermodynamics of ligand binding to (**1**-Fe)$_2$ presented as Hill plots.[16] x: free-ligand concentration normalized to 1 M; α: fractional occupancy of the binding sites of the receptor. a) $Me_2C_6H_3NC$: ——, least-squares fit (LSF) of the binding equation for a ditopic cooperative receptor to the experimental data (◇); – – –, Hill equation binding curve for $n_{Hill}=2$; •••••, asymptotes of the binding curve at low and high ligand concentrations. As is typical in spectrophotometric titrations,[16] the limiting regimes are not accessible experimentally in our system. b) Nitrosoaryls and imidazole: for clarity, only LSF binding curves in the experimentally accessible regime are shown, along with the fitting parameters, K_1 and K_2. The data is from titrations of solutions in toluene (also in hexanes for $Me_2C_6H_3NC$ and TolNO) at 27±1 °C and dimer concentrations between 0.3 and 30 μM under strictly anaerobic and anhydrous conditions. (**2**-Fe)$_2$ behaves similarly.[8]

the solvent and the dimer concentration, a fact that rules out competition between dimerization and ligand binding as the origin of the cooperativity. We did not observe ligand-induced dissociation of the dimers. The geometric averages of the sequential binding constants are at the high end of reported values for Fe porphyrins.[11] By contrast, the affinity of 2-methylimidazole (2-MeIm) for (**1**-Fe)$_2$ is $< 10^{-5}$ times that for a four-coordinate heme.[7] We observed no evidence for cooperative ligation of (**1**-M)$_2$ and (**2**-M)$_2$ with M = Mg or Zn.

To understand the stereoelectronic changes in (**1**-Fe)$_2$ upon isocyanide binding in hexanes ($\Delta G_a = (13 \pm 3)$ kJ mol^{-1}), we performed computations for compounds **3**–**5** (Figure 2) at the B3LYP/6-31g level in vacuum.[8,12,13] The relevance of the calculated structures of **3**–**5** to the corresponding (**1**-Fe)$_2$ derivatives is supported by[8] 1) our calculations accurately reproducing the electronic ground states and metric parameters of relevant five- and six-coordinate hemes from the literature and 2) the weak sensitivity of these results to the presence of the peripheral phenyl groups, in accordance with the literature data.[14]

The computed structures (Figure 2) are consistent with the effective C_{2h} symmetry of the dimer/ligand adducts observed by NMR spectroscopy. The Fe–N_{Im} distances, the displacement of the Fe atoms from the porphyrin planes, and the residual doming of the hemes in **3**–**5** resemble those in corresponding monomeric hemes with a single 2-MeIm ligand.[8] In agreement with experiment, the ground electronic state of **3** is calculated to contain eight unpaired electrons

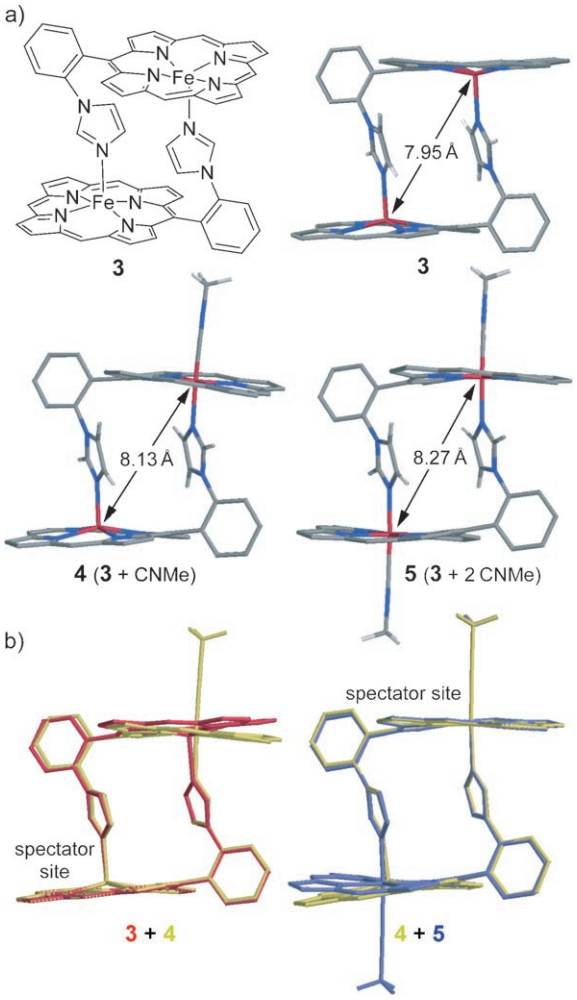

Figure 2. a) Minimum-energy conformations of compounds **3**–**5**: Fe (red), C (gray), N (blue). For clarity, only hydrogen atoms on the imidazoles and MeNC are shown (white). b) Structural changes upon binding of the first and second MeNC molecules (**3** red, **4** yellow, **5** blue). The structures were overlaid by using sets of four nitrogen atoms from the hemes in the same ligation state.

equally distributed among the two essentially noninteracting Fe sites. The nonet and antiferromagnetically coupled singlet states of the dimer are isostructural and isoenergetic, a fact indicating that the spins of the Fe sites are uncoupled. The triplet state of the five-coordinate sites in **3** is calculated to lie above the quintet, with the quintet–triplet gap close to that in the monomeric analogue, and invariant to the spin state of the other Fe center. This lack of an electronic interaction is consistent with the large separation of, and the absence of conjugation between, the Fe sites.

The binding of a ligand is calculated to induce significant but expected stereoelectronic changes at the binding site, that is, conversion of Fe to a low-spin diamagnetic state, Fe–N_{Im} bond contraction, and flattening of the macrocycle. The spectator site undergoes a minor rearrangement (Figure 2b). The calculations therefore suggest that the low- and high-affinity forms of the binding site (that is, the five-coordinate hemes in **3** and **4**, respectively) differ little in their minimum-energy conformations.

This seemingly surprising result is suggestive of a large contribution of the entropic term to the ΔG_a value for isocyanide binding. In receptors with entropic homoallostery,[3,15] the minimum-energy conformations of the low- and high-affinity forms of the binding sites are often too close in energy for DFT calculations to identify the global minimum. (Other conformations of **3**–**5** found computationally are discussed in the Supporting Information.) In future work, we will estimate the ΔS_a contribution to cooperativity in (**1**-Fe)$_2$ by measuring the temperature dependence of the ΔG_a value.

In summary, we have reported that Fe complexes of the new simple porphyrins **1**-H_2 and **2**-H_2 exist as stable dimers that, in solution, bind a variety of ligands cooperatively with an allosteric free energy of up to $(13 \pm 3)\ kJ\,mol^{-1}$. The reported dimers appear to comprise the simplest system in which cooperative ligation of a five-coordinate heme is realized. They illustrate a structural motif and verify a design strategy that can be exploited and elaborated upon to produce allosteric receptors tuned for practical applications.

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