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Protein Surface Recognition by Synthetic Receptors Based on a Tetraphenylporphyrin Scaffold

Rishi K. Jain and Andrew D. Hamilton*

Department of Chemistry, P.O. Box 208107, 225 Prospect Street, Yale University, New Haven, Connecticut 06520-8107

andrew.hamilton@yale.edu

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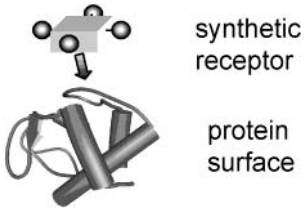
ABSTRACT



Receptors based on a tetraphenylporphyrin scaffold bearing different charged and hydrophobic groups have been synthesized. The interactions of these with horse heart cytochrome *c* were studied by fluorescence spectroscopy. Receptor 4 was identified to be the strongest synthetic receptor (K_d = 20 nM) for cytochrome *c*. The differences in affinity among the receptors reflected a dependence on the number of anionic and hydrophobic groups.

The past two decades have seen enormous progress in the design of synthetic molecules targeted to disrupt protein−ligand interactions.[1] The majority of these medicinally important molecules disrupt interactions occurring inside well-defined cavities on the proteins. In contrast, synthetic molecules that mediate protein function through binding to the solvent-exposed *exterior surface* are largely unexplored.[2] This is surprising since such molecules may provide a fundamentally different mechanism for modulating protein function, by sterically preventing protein−ligand and protein−protein interactions. This strategy may not only result in the discovery of novel drug candidates but may also provide opportunities to systematically understand the role of protein exterior surfaces in molecular recognition.

Recently, we have reported a novel family of synthetic receptors that target protein exteriors.[3] The initial design, involving four peptide loops arrayed around a central calix[4]arene core, was shown to inhibit the approach of small molecule substrates to cytochrome *c* and chymotrypsin at submicromolar concentrations. However, the calixarene unit suffers several complications, including difficulty of synthesis and tendency to aggregation. We sought to improve this synthetic approach by studying different organic scaffolds onto which recognition sites can be attached. Porphyrins, owing to their large size, rigidity, and photophysical properties, have been used in numerous artificial receptors and model systems in bioorganic and bioinorganic chemistry.[4] In this Letter, we report on the design, synthesis, and recognition properties of a series of tetraphenylporphyrins that bind, in certain cases with high affinity, to the surface of cytochrome *c*.

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Cytochrome *c* is a particularly attractive target since it plays key roles in electron transfer and apoptosis, which are mediated by complex formation to other proteins (cytochrome *c* oxidase, Apaf1, etc.). One critical recognition region involves an array of lysine and arginine residues surrounding the exposed heme edge surface.[5] The tetraphenylporphyrin scaffold closely matches the arrangement of hydrophobic and charged domains present in this region (Figure 1). In pioneering work, Fisher recognized this

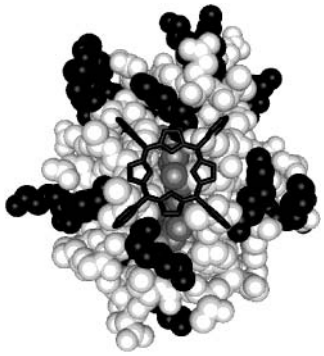
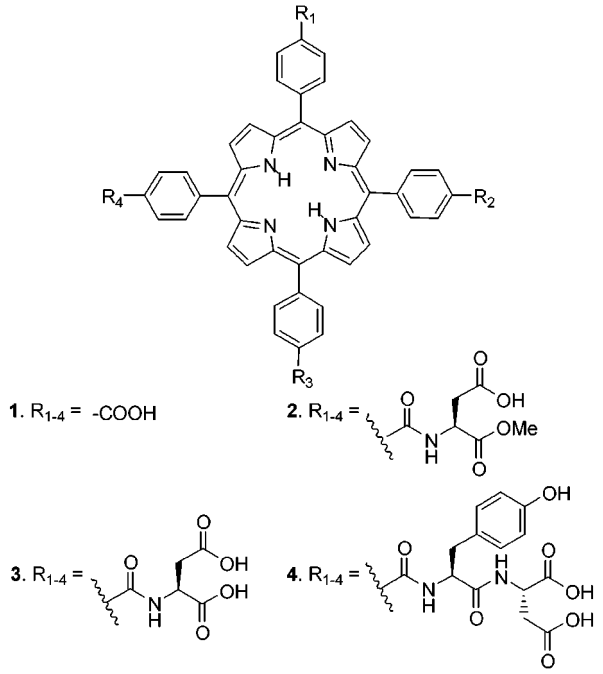


Figure 1. A space filling representation of horse heart cytochrome *c* based on its X-ray crystal structure.[7] The heme group which directly faces the reader is drawn in gray spheres. Black spheres represent positively charged lysine and arginine residues. White spheres correspond to all other residues. A tetraphenylporphyrin scaffold (black cylindrical bonds) is drawn in the center.

geometrical relationship and showed that tetracarboxyphenyl porphyrin **1** binds to cytochrome *c* with a K_d of ~5 μM.[6]

To determine the optimum combination of recognition features in synthetic receptors of this type, we designed a series of tetraphenylporphyrins with various amino acid derivatives attached. The resulting receptors contain a large, flat, and semirigid molecular surface of approximately 300−400 Å^2 in area. The receptors were prepared by the generation of the tetra-acid chloride ($(COCl)_2$, DMF) starting from *m*-tetrakis(4-carboxyphenyl) porphyrin **1** and subsequent coupling with the corresponding *tert*-butyl-protected amino acid or peptide amines. Deprotection of the *tert*-butyl groups with trifluoroacetic acid provided receptors **2**−**4**. Acidic and hydrophobic attachments were chosen to complement the cationic and hydrophobic surface surrounding the heme edge of cytochrome *c*.

Compounds **1**−**4**, and coproporphyrin I, a naturally occurring tetraanionic porphyrin, were initially screened for binding to horse heart ferricytochrome *c* using fluorescence spectroscopy. Addition of cytochrome *c* to solutions containing **1**−**4** resulted in quenching of porphyrin fluorescence emission (ex = 420 nm, em = 650 nm) due to the enforced proximity of the Fe(III) heme that results from complex formation between the protein and the receptors.



In contrast, titrations with tetracationic *m*-tetrakis(4-trimethylaminophenyl) porphyrin (TTMAPP), showed no quenching even at high concentrations, indicating the absence of nonspecific binding. Typical titrations of **3**, **4**, and TTMAPP with cytochrome *c* are shown in Figure 2 (see Supporting Information for others).

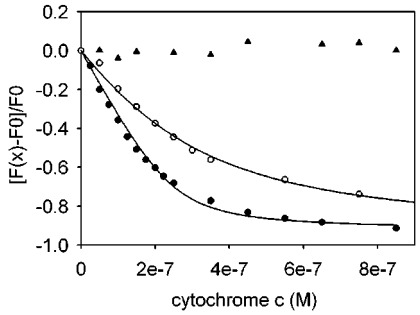


Figure 2. Fluorescence quenching of **4** (●), **3** (○), and TTMAPP (▲) upon addition of cytochrome *c*. The curve fit indicates a K_d of 20 ±5 nM for **4** and 160 ±20 nM for **3**. Titrations were carried out under 250 nM initial receptor concentration in 5 mM sodium phosphate buffer, pH = 7.4, at 298 K.

Dissociation constants were derived by curve fitting to a 1:1 binding equation[8] with stoichiometries being fixed at $n = 1$ for all compounds. Preference for 1:1 binding was confirmed for receptors **3** and **4** by Job's method[9] (see Supporting Information). K_d values for all compounds are summarized in Table 1.

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Table 1. Dissociation Constants[a] and Structural Properties of Synthetic Receptors and Other Water-Soluble Porphyrins

compound	K_d (nM)	charge	aryl groups
1	950 ± 250	−4	4
2	860 ± 90	−4	4
3	160 ± 20	−8	4
4	20 ± 5	−8	8
coproporphyrin I	7700 ± 270	−4	0
uroporphyrin I	1000[b]	−8	0

[a] Determined at 5 mM sodium phosphate, pH 7.4, 298 K. [b] From ref 10, at m = 4 mM sodium phosphate, pH 7.26, 298 K.

Significant changes in affinity for cytochrome *c* were observed by altering the relative proportions of acidic and aromatic functionalities in the receptors (Table 1). Receptors **2** and **3** differ only by the substitution of four methyl esters by carboxylic acids, respectively, providing controls for probing the charge requirements for cytochrome *c* recognition. A ~5-fold increase in affinity was seen, on going from receptor **2** to receptor **3**, indicating a preference for octaanionic receptors over their tetraanionic counterparts. Receptors **1** and **2**, with the same number and type of charged groups, show little difference in their binding affinities. Similar trends were observed when aromatic groups were incorporated into the receptor while keeping the number of charged groups constant. Rodgers had earlier shown that uroporphyrin I, containing eight carboxylate groups, binds cytochrome *c* with μM affinity.[10] However, receptor **3** which has eight anionic groups and 4 phenyl groups binds to cytochrome *c* approximately 6-fold stronger than uroporphyrin. In a similar analysis, receptor **2** binds cytochrome *c* 9-fold tighter than tetraanionic coproporphyrin I. These results suggested that an appropriate combination of charged and hydrophobic groups on the porphyrin periphery would give a molecule with exceptionally high affinity for cytochrome *c*. To confirm this, we designed receptor **4** to contain eight negatively charged groups and eight phenyl groups. The titration curve (Figure 1) shows a sharper achievement of saturation with **4** compared to **3**. This corresponds to a K_d of 20 ±5 nM for **4** binding to cytochrome *c* and represents an 8-fold increase in affinity compared to **3** (Table 1).To our knowledge, **4** is one of the strongest synthetic receptors for cytochrome *c*, under these experimental conditions. The binding experiments between **4** and cytochrome *c* were carried out in 5 mM phosphate buffer at pH 7.4. Remarkably, receptor **4** with a molecular weight of ~1900 Da can bind to cytochrome *c* 100 fold stronger than its natural protein partners such as cytochrome *c* peroxidase, which has a K_d of 2.4 μM measured at 5 mM phosphate buffer, pH = 7.[11] The combination of many electrostatic and hydrophobic interactions over a large contact surface is primarily responsible for the formation of high-affinity protein−protein complexes in nature.[12] The synthetic receptors reported here behave similarly, attaining large enhancements in affinity through the incorporation of both anionic and aromatic groups. The tetraphenylporphyrin scaffold appears to provide a template in which the peripheral anionic and aromatic groups take up a good geometrical relationship to the cationic and hydrophobic side chains of cytochrome *c*. On the basis of these findings, it should be possible to extend this approach to identify new receptors for protein targets that are known to interact with simple porphyrin derivatives.[13]

In summary, we have designed and synthesized synthetic receptors that recognize a protein surface with high affinity in aqueous medium. Analogues of receptor **4** with less symmetrical substitution patterns may provide a clearer understanding of the detailed recognition properties of the cytochrome *c* protein surface. In addition to providing a new strategy for protein surface recognition, these findings may have important medicinal consequences. Cytochrome *c* has been shown to interact with Apaf1, leading to the activation of programmed cell death or apoptosis.[14] Receptor **4** may serve as a valuable lead in the search for efficient disruptors of Apaf1−cytochrome *c* interaction.

Acknowledgment. This work was supported by the National Institute of Health (GM35208) and the U.S. Army (DAMD17-99-1-9458).

Supporting Information Available: Experimental procedure and characterization for compounds **2**−**4**. Fluorescence titration plots for **1**−**2** and coproporphyrin I and Job's plots for **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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