Porphyrin derivatives as water-soluble receptors for peptides†

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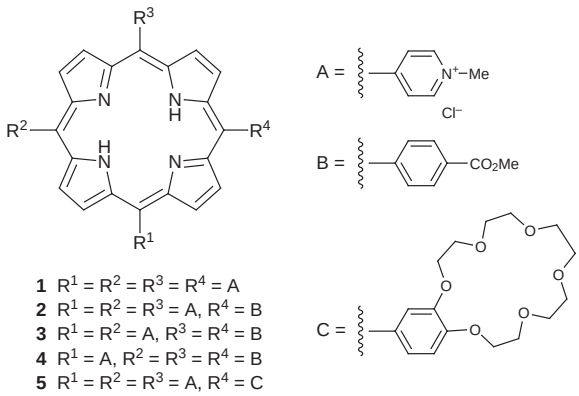
The porphyrin–crown ether derivatives described here exhibit affinities to unprotected peptides of hitherto unknown magnitude in aqueous solution; the optical signal built-in to porphyrin receptors makes these applicable also to sensor techniques; the results can also form the basis of new catalytic systems, *e.g.* for the catalytic oxidation of peptides (such as tyrosinase models).

Porphyrins and their non-covalent interactions are of interest in view of their role in biological systems. They also can provide new artificial host compounds with a built-in optical sensor by their Soret bands, which are sensitive towards binding of many ligands. Porphyrin derivatives with positively charged substituents in the *meso*-position were shown earlier to interact with nucleotides and with DNA[1] as well as with many aromatic substrates[2] primarily *via* stacking between the aromatic units. We wanted to explore the use of modified porphyrins as peptide receptors, which unlike most artificial host compounds in this important class of compounds[3] work in protic solvents. The goal of selective peptide complexation in aqueous solution was approached only recently,[4] and still needs considerable progress until artificial receptors come close to the efficiency of biological systems. The present paper reports a new step in this direction, with binding constants of hitherto unkown magnitude for natural, unprotected peptides.[5]

We first carried out UV-visible titrations using the commercially available 5,10,15,20-tetrakis(4-methylpyridinium)porphyrin **1** with several *N*-benzyloxycarbonyl-protected amino acids and peptides, as unprotected amino acids and peptides showed no detectable binding. Addition of the Z derivatives (Z = benzyloxycarbonyl) resulted in a intensity reduction of the Soret band with a concomitant red shift of the band maximum. The change in absorbance values (ΔA), varied from 0.2 to 0.4, and the change in wavelength ($\Delta\lambda$) from 5 to 10 nm. Non-linear least-squares fitting of the observed absorbance changes obtained at several different wavelengths in the region 400–430 nm showed satisfactory fitting with a 1 : 1 association model. Similar behaviour was observed with a new porphyrin derivative **2** with one methyl benzoate and three 4-pyridinium substituents in the *meso* positions. This as well as two other derivatives with only two or one 4-pyridinium substituents (**3** and **4**) were synthesized analogous to literature procedures,[6] as it was hoped that partial displacement of the charged units might lead to more selective binding. Dilution experiments, however, made evident the fact that **3** and **4** aggregate severely in water in spite of the charges even at 10^{-6} M concentration, and were therefore unsuitable for binding studies.



1 $R^1 = R^2 = R^3 = R^4 = A$
2 $R^1 = R^2 = R^3 = A$, $R^4 = B$
3 $R^1 = R^2 = A$, $R^3 = R^4 = B$
4 $R^1 = A$, $R^2 = R^3 = R^4 = B$
5 $R^1 = R^2 = R^3 = A$, $R^4 = C$

With host compounds **1** and **2** the log*K* values (2.9–3.7 M^{-1}, Table 1) show rather high affinities, in particular if a geometric fit is to be expected from the models. It is obvious that the binding strength with the protected peptides is generally larger for aromatic ligands, except in the case of Z-Asp, in line with previous findings.[1,2]

Considering the aqueous medium the affinities observed with **1** and **2** are promising, but unprotected peptides did not show any binding. The positive charge at the peptide NH_3 terminus will always destabilize complexes with **1** or **2**, which suggests the introduction of an additional binding site at the porphyrin ring in order to complex natural peptides. This was achieved by inserting a crown ether unit, which is known to complex primary ammonium groups. The synthesis of this porphyrin derivative **5** was carried out in acid medium using the classical Adler–Longo method.[7] Reaction of 4-formylbenzo-18-crown-6, pyridine-4-carbaldehyde and pyrrole in the presence of Ac_2O and refluxing propionic acid for 1.5 h gave a mixture of porphyrins which after purification on an alumina column and subsequent methylation yielded **5** in an overall yield of about 3%.

UV titrations with the new crown ether receptor **5** indeed showed with *unprotected* peptides binding constants (Table 2) which are even higher than those observed with the protected peptides necessary for the use of **1** and **2**. The fit to a 1 : 1 model was again satisfactory (Fig. 1), as long as the molar ratio of [peptide]/[**5**] did not exceed 200. At higher concentrations of the

Table 1 Logarithm of association constants of **1** and **2** with Z-amino acids and Z-peptides[a,b]

		1		**2**	
Entry	Amino acid/ peptide	log*K*	$\Delta\varepsilon/10^3$ dm^3 mol^{-1} cm^{-1}	log*K*	$\Delta\varepsilon/10^3$ dm^3 mol^{-1} cm^{-1}
1	Z-Ala	3.06	56.2	2.94	31.4
2	Z-Phe	2.91	75.0	3.29	44.8
3	Z-Tyr	2.93	80.4	3.39	47.5
4	Z-Trp	3.43	94.9	3.58	49.2
5	Z-Asp	3.73	63.9	3.69	48.2
6	Z-Ile	3.40	64.7	2.94	35.4
7	Z-Gly–Phe	3.17	44.8	3.38	50.6
8	Z-Ala–Trp	3.20	92.4	3.60	69.6

[a]Measured by UV-visible titration of **1** and **2** with Z-amino acids or Z-peptides at 25 °C. Titrations were carried out in 5 mM phosphate buffer, pH 6.9 ± 0.2 by adding concentrated stock solutions of Z-amino acid or Z-peptide ([Z-amino acid] or [Z-peptide] = 10 mM) containing *ca.* 5 μM of **1** or **2** to an equally concentrated solution of **1** or **2** in a 10 mm cuevette. Error limits: log*K* ±5%. [b] $\Delta\varepsilon$ changes at [ligand]/[host] = 200, the values agree within ±5% with the $\Delta\varepsilon$ from non-linear fit [ε (410 nm): **1**, 149300; **2**, 128 800, Error limits: ±5%).

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Table 2 Logarithm of association constants of **5**, with peptides[a,b]

Entry	Peptide	log*K*	$\Delta\varepsilon/10^3$ dm^3 mol^{-1} cm^{-1}
1	Gly–Gly	2.93	15.7
2	Gly–Phe	4.71	22.0
3	Phe–Gly	4.36	18.0
4	Ala–Phe	4.60	10.2
5	Asp–Phe	4.05	14.0
6	Phe–Phe	4.52	13.1
7	Gly–Gly–Gly	3.41	7.8
8	Gly–Gly–Phe	4.39	20.7
9	Gly–Phe–Gly	4.35	12.5
10	Phe–Gly–Gly	4.48	12.3
11	Gly–Gly–Trp	3.52	15.4
12	Trp–Gly–Gly	4.48	16.9
13	Gly–Gly–Gly–Gly	5.02	18.9

[a] See footnotes to Table 1. [b] ε (410 nm): **5**, 91200, Error limits: ±5%.

added peptide one observes a biphasic behaviour of the isotherm, indicating other binding modes. The complexation-induced absorption and wavelength changes were smaller than those with **1** and **2**, but in most cases sufficient for evaluation of *K*. Computer aided molecular modelling of the complex between **5** and Gly-Gly-Phe using energy minimization with the CHARMm force field established an essentially strain-free fit between the crown and $^+NH_3$ at one end, and between an adjacent 4-pyridinium unit and the COO^- groups at the other end, as well as stacking between the terminal phenyl ring and the porphyrin, with retention of most stable extended conformation of the peptide (Fig. 2). The two carboxylate oxygens of Gly–Gly–Phe are in van der Waals contact with the neighbouring pyridyl *ortho*-Hs [PheCO(1)···HC(pyridyl-*ortho*) = 2.52 Å] and the β-pyrrole carbon atom [PheCO(2)···C(β-pyrrole) 3.03 Å].

The data in Table 2 illustrate that the binding strength increases as a function of the peptide length and with the number of aromatic units in the amino acid side chains, as expected from stacking contributions. A force field-minimised structure of the complex between **5** and Phe–Gly–Gly did show stacking interactions between the porphyrin pyridyl group and the peptide phenyl ring, in addition to other non-covalent interactions. However, the extremely large association constants, such as $K = 10^5$ M^{-1} with tetraglycine, are obviously due to additional binding effects. Preliminary measurements with aliphatic amides, hampered by very small spectral changes,

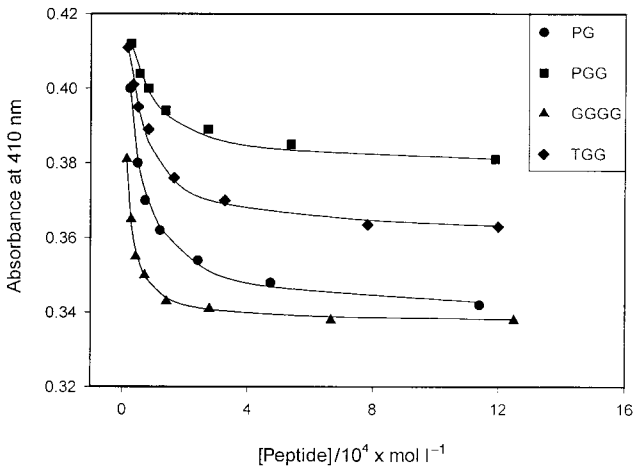

Fig. 1 UV-visible titration curves of porphyrin **5** with peptides (PG = Phe–Gly, PGG = Phe–Gly–Gly, GGGG = Gly–Gly–Gly–Gly, TGG = Trp–Gly–Gly).

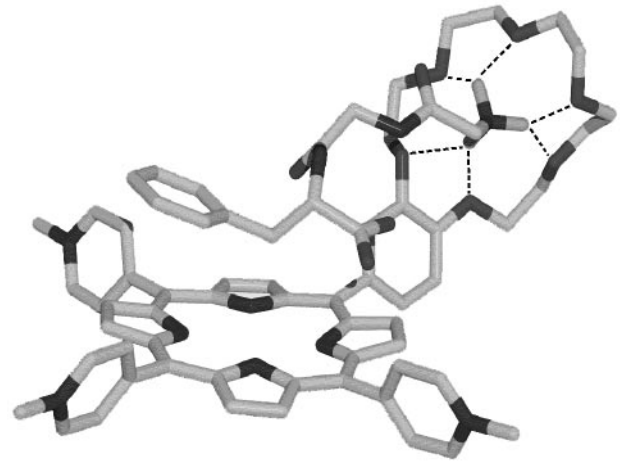

Fig. 2 A force field (CHARMm) optimized structure of porphyrin **5** with the peptide Gly–Gly–Phe. Hydrogens are omitted for clarity except those of peptide NH_3 . The phenyl group of Phe is not far from van der Waals contacts to the pyrrole (d_{av} = 3.95 Å); the NH_3 protons have distances of 1.90–2.05 Å to the crown ether oxygen; the carboxylate group comes close to neighbouring pyridyl protons with d_{av} = 3.0 Å.

suggest the amide function itself contributes significantly to the associations.

In conclusion, we have shown that porphyrins can associate with peptides with high affinities, if the charges are accommodated in a complementary fashion. The findings suggest new approaches to the design of peptide receptors and potentially also to supramolecular catalysts for peptide modification; they also shed light on possible interaction mechanisms in protein–porphyrin aggregations.[8]

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8 All new compounds showed satisfactory analytical data; the synthesis will be described elsewhere.

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