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Bioorganic & Medicinal Chemistry 13 (2005) 5159–5163

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Bioorganic & Medicinal Chemistry

Induction and memory of chirality in porphyrin hetero-aggregates: The role of the central metal ion

Angela Mammana,[a] Massimo De Napoli,[a] Rosaria Lauceri[b] and Roberto Purrello[a,*]

[a] *Dipartimento di Scienze Chimiche, Viale A. Doria 6, Catania 95125, Italy*
[b] *IBB-CNR Sezione di Catania, Viale A. Doria 6, Catania 95125, Italy*

Received 13 April 2005; revised 10 May 2005; accepted 10 May 2005
Available online 12 July 2005

Abstract—The anionic H_2TPPS porphyrin and its copper derivative, CuTPPS, form in aqueous solution hetero-aggregates with the cationic H_2T4 porphyrin and its copper derivative, CuT4. In the presence of poly-L-glutamate, at pH 4.0, a CD signal appears in the Soret region of the spectrum, indicating that the polypeptide has induced chirality into the structure of the aggregates. These species exhibit remarkable inertness due to the strength and number of the coulombic interactions between the anionic and the cationic porphyrins. This property allows them to preserve the chiral structure, even when the matrix changes or loses its chiral conformation, demonstrating that these aggregates are capable of memorizing the chiral information. The remarkable properties of the title systems may find various applications (chiral amplification, discrimination, and separation) that, on the other hand, require a more strict control of the aggregate dimension. Here, we show that the central copper of these macrocycles is crucial for determining the aggregate dimension.

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1. Introduction

Porphyrins are excellent building blocks to assemble supramolecular architectures. Their excellent tuntable spectroscopic properties have been, in fact, exploited to design and develop a paramount number of porphyrin arrays potentially useful as sensors, opto-electronic devices, antenna systems, models of metallo enzymes, etc.[1] Basically, two approaches have been adopted to synthesize these species: the covalent and non-covalent methods. The latter is based on molecular recognition processes leading to self-assembly of the single molecular components in a supramolecular complex species. In particular, the hydrophobic nature of the water-soluble porphyrin molecular surface allows it to easily drive their homo- and hetero-aggregation in aqueous solution. In fact, the presence of cationic or anionic peripheral substituents renders these macrocycles water-soluble but not hydrophilic. Therefore, partial or whole 'neutralization' of peripheral charges leads to their self-aggregation. Different factors (such as pH, number, relative position, nature of the meso-substituents, etc.) can help porphyrin self-assembly adjust in an aqueous solution.[1h,1p,1s,1z]

Owing to the intrinsic 'weakness' of the non-covalent interactions, the accuracy of this approach in terms of sequence and stoichiometry of the final species is poorer than that achieved by covalent syntheses and could even lead to a mixture of species derived from the coexistence of different chemical equilibria. The approach commonly used to bypass these limitations exploits a network of weak interactions whose cooperativity increases both the thermodynamic and kinetic stability of the designed non-covalent species. In most cases, the structure (sequence, stoichiometry, chirality, etc.) of the molecules designed and synthesized in this way is under hierarchic control: that is, the structure of the final product is regulated by a time-dependent specific sequence of chemical events (addition of reagents, pH changes, time evolution of the species)[1b] that leads to a major or a unique final product. If, during one of these synthetic steps, a chiral reagent is introduced, the final complex could then display chirality at a supramolecular level. In some cases, kinetic inertia of the chiral supramolecular complex could lead to the so-called 'memory' phenomenon.[1j]

We have recently published that, in the presence of 'polymeric'[2] chiral templates, aggregation of the

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Keywords: Porphyrin; Aggregation; Chirality; Scattering; Dichroism.

* Corresponding author. Tel.: +39 095 738 5095; fax: +39 095 580138; e-mail: rpurrello@unict.it

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doi:10.1016/j.bmc.2005.05.035

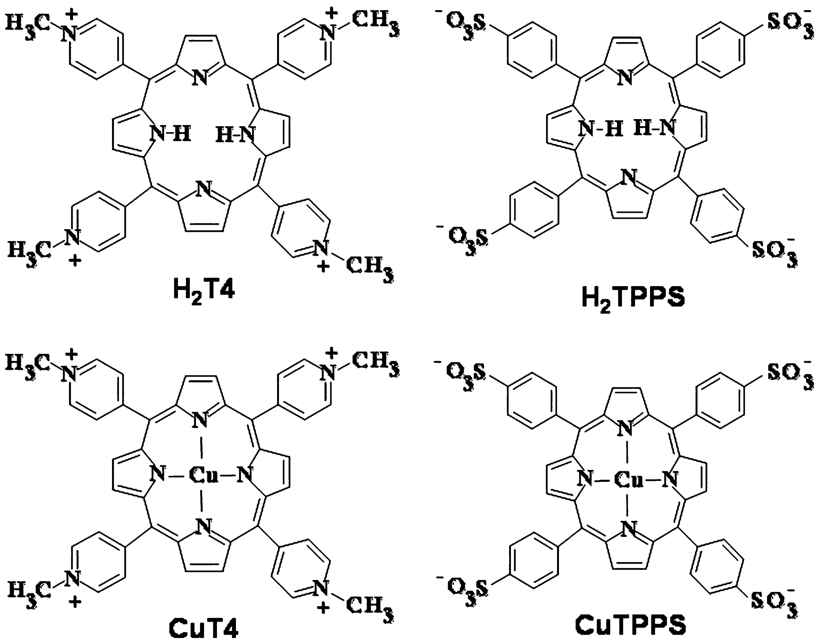

Figure 1. Schematic structures of H_2T4, CuT4, H_2TPPS, and CuTPPS.

tetra-anionic *meso*-tetrakis(4-sulfonatophenyl)porphine (H_2TPPS, Fig. 1) with the copper(II) derivative of the tetra-cationic *meso*-tetrakis(4-*N*-methylpyridyl)porphine (CuT4, Fig. 1) leads to chiral porphyrin assemblies that are capable of memorizing the template chirality. This phenomenon is mainly on account of kinetic inertia of the supramolecular porphyrin assemblies, which are stabilized by a network of net electrostatic contacts along with other non-covalent interactions.

We present here a study on the aggregation of cationic and anionic free bases with cationic or anionic copper derivatives (Fig. 1), both in the presence and absence of poly-L-glutamate. The aim of this study is to understand the role played by non-covalent interactions other than the electrostatic ones. We chose to employ only the free-base and copper derivatives because, as already published,[3] penta- or hexa-coordinated porphyrins or planar metallo-derivatives bearing a positive charge in the inner core (as the gold(III) derivatives) lead to the formation of supramolecular species for which the chiral memory phenomenon does not take place.

Comprehension of the forces involved in this phenomenon is of importance because it helps in designing the best species for the various possible technological applications that range from resolution of racemates to amplification of nanomolar concentrations of chiral analytes.[1j] The latter two applications require, in fact, different properties. Incorporation of the aggregates into a membrane that is able to undergo chiral discrimination requires, in fact, small aggregates to effect their uniform dispersion into the membrane. On the other hand, chiral amplification is effected by the formation of big aggregates, as because the exciton coupling between a high number of chromophores should give rise to more intense signals.

2. Experimental

Poly-L-glutamate sodium salt (mean degree of polymerization 95) was purchased from Sigma–Aldrich. *meso*-Tetrakis(4-*N*-methylpyridyl)porphine, H_2T4, and *meso*-tetrakis(4-sulfonatophenyl)porphine, H_2TPPS, were obtained from Mid-Century Chemicals as chloride and sodium salts, respectively. Porphyrin metallation was performed by following literature methods. Absorbance and circular dichroism (CD) data on monomeric porphyrins and their aggregates are given in Table 1. The forma-

Table 1. Spectroscopic properties of monomeric porphyrins and their aggregates in water at pH 4 in the absence and in the presence of poly-L-glutamate (Glu)

Porphyrins	Absorption (nm) (λ_{max}; ε ($M^{-1}\,cm^{-1}$))[a]	CD (λ_{max}; $\Delta\varepsilon$ ($M^{-1}\,cm^{-1}$))[b]
H_2TPPS	434 (4.86×10^5)	No CD
H_2T4	422 (2.26×10^5)	No CD
CuT4	424 (2.31×10^5)	No CD
CuTPPS	412 (4.16×10^5)	No CD
H_2T4-H_2TPPS	434 (1.06×10^5)	No CD
CuT4-H_2TPPS	433 (1.58×10^5)	No CD
H_2T4-CuTPPS	413 (1.67×10^5)	No CD
CuT4-CuTPPS	410 (2.10×10^5)	No CD
Glu-H_2T4-H_2TPPS	434 (1.72×10^5)	445 nm (+148) 496 nm (−97)
Glu-CuT4-H_2TPPS	432 (2.27×10^5)	422 nm (+98) 442 nm (−320)
Glu-H_2T4-CuTPPS	413 (2.26×10^5)	416 nm (+24) 429 nm (−143)
Glu-CuT4-CuTPPS	411 (2.22×10^5)	406 nm (+14) 424 nm (−18)

[a] For the various porphyrin couples ε has been calculated considering the couple itself as chemical unit.

[b] For the various porphyrin couples Δε has been calculated considering the couple itself as chemical unit.

tion of porphyrin assemblies is obtained by the addition of equimolar amounts of the two macrocycles because stoichiometry of aggregates has already been determined to be 1:1 (cationic:anionic porphyrin).[3] Resonance light scattering (RLS) measurements were recorded on a Fluorolog3, absorption measurements on a Cary 500, and circular dichroism measurements on a Jasco J-810. All the experiments were carried out using 1 cm optic path quartz cuvettes and ultrapure water (Milli Q-RG system) was used throughout the experiments. No buffer was used; the pH of the solution was adjusted with H_2SO_4 and NaOH. The various ternary complexes were formed (if not differently specified) by adding to a 200 μM polyglutamate solution at pH 4.0 the cationic (4 μM) and the anionic (4 μM) porphyrin, respectively.

3. Results and discussion

Before presenting and discussing the experimental data, it is necessary to discuss some of the chemico-physical properties of the single components and of the aggregates.

3.1. Porphyrins and polyglutamic acid

Under the experimental conditions used in this work (pH 4.0), all porphyrins are monomeric.[4] The reason to work at this pH value is related to the different conformations that polyglutamate adopts at acid or basic pH values. Polyglutamic ($pK_a \approx 4.5$) is, in fact, known to adopt an α-helical conformation only when partially protonated, that is, for pH values lower than about 5.0. Deprotonation of the side carboxylic groups drives the conformational transition to random coil.

3.2. Porphyrin assemblies

The formation of porphyrin assemblies is reported by variation of the absorption and emission spectral properties. In particular, aggregation leads to quite an extensive hypochromism of the Soret band (Table 1) and to a relevant or, often, almost complete quenching of the porphyrin emission. Porphyrin aggregation is also accompanied by the appearance of a signal in the resonance light scattering (RLS) spectrum. This technique has been proposed by Pasternack et al. as a powerful tool to detect the formation of aggregates and is particularly useful to investigate the formation of homo- and hetero-aggregates of porphyrins.[5] In particular, porphyrin aggregation leads to the appearance of intense RLS signals when the transition dipole moments are coupled to give a macroscopic dipole.

The formation of porphyrin assemblies in solution already containing polyglutamic acid at pH 4.0 leads to quite an intense induced circular dichroism (ICD) signal in the Soret region (Table 1). Only in those experimental conditions ($pH \leqslant pK_a$) polyglutamic is able to induce chirality onto the porphyrin aggregates. The title porphyrins, in fact, are not chiral, and induction of chirality can be observed only on their interaction with a chiral template. The data reported in Figure 2 show the impact of polypeptide conformation on the ICD in the Soret region. When H_2T4 (4 μM) and H_2TPPS (4 μM) are added to an aqueous solution of polyglutamic acid (200 μM) at

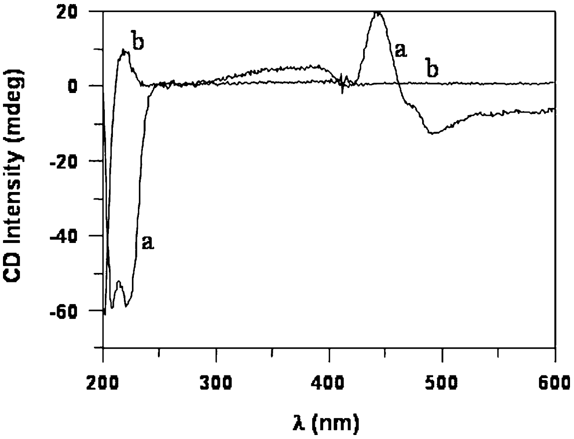

Figure 2. CD spectrum of the porphyrin aggregate formed on adding H2T4 and H_2TPPS to a polyglutamate aqueous solution (curve a) at pH 4.0 (α-helical conformation) and (curve b) at pH 10.0 (random-coil conformation).

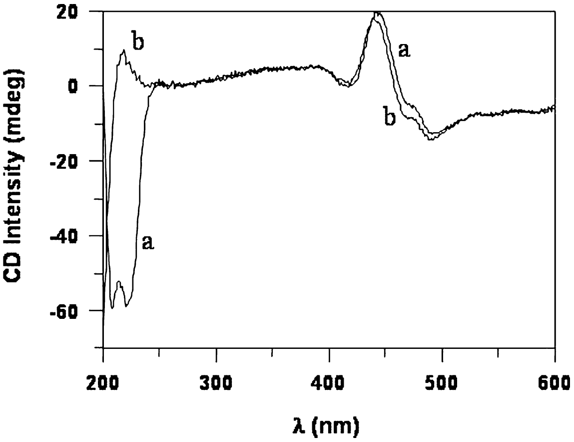

Figure 3. CD spectrum of the porphyrin aggregate formed on adding H_2T4 and H_2TPPS to a polyglutamate aqueous solution at pH 4.0 (curve a) and after a pH-jump to pH 10.0 (curve b).

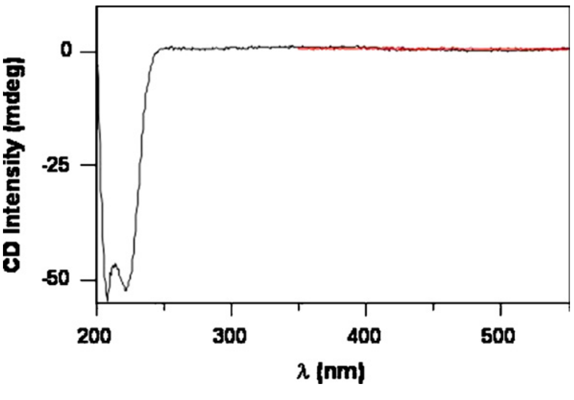

Figure 4. CD spectrum of the H_2T4–H_2TPPS aggregate at pH 4 (red curve) and of the same solution after the addition of polyglutamate (black curve).

pH 4.0 (α-helical conformation, spectrum a), the CD spectrum shows quite an intense and bisignate signal in the Soret region that reports the formation of chiral porphyrin aggregates. On the contrary, addition of the two porphyrins to a polyglutamate solution at pH 10.0 (random coil, spectrum b) does not lead to any ICD in the Soret region. Also, porphyrin interaction with poly-D-glutamate induces a CD spectrum, which is a mirror image of that shown in Figure 2 (not shown).[6] These observations demonstrate the fact that aggregates borrow their chirality from the template. It is worth noting that the helical-coil conformational transition of the polypeptide is underlined by the disappearance of the helical marker bands at 208 and 222 nm, and the appearance of a positive band at about 217 nm.

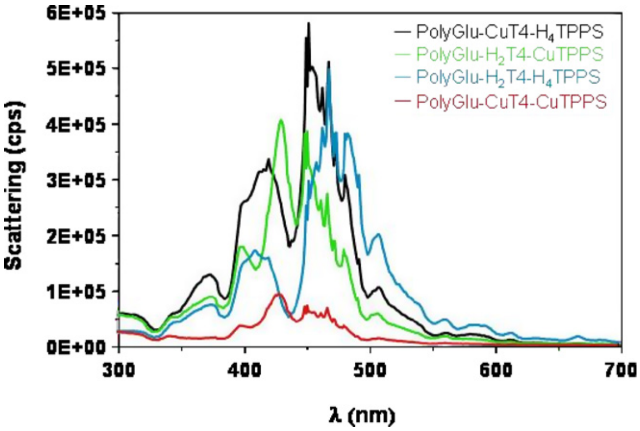

Figure 5. RLS spectra of the various porphyrin aggregates.

Another remarkable feature of this system is shown in Figure 3, showing the CD spectra of chiral H_2T4–H_2TPPS aggregates built on polyglutamic before (curve a) and after (curve b) a pH variation from 4.0 to 10.0. Following the pH-jump, polyglutamate α-helix is disrupted to give a random-coil conformation (as underlined in the UV region of the CD spectrum). On the contrary, the ICD of porphyrin aggregates remains mostly unaffected, showing that the matrix structure plays only a templating role. Once the aggregate is formed, its inertness (mainly due to the extended network of columbic interactions between the anionic and cationic macrocycles) permits it to retain (*memorize*) the chiral information. The crucial importance of the inertness of the aggregate in determining the memory process is also confirmed by the incapability of the chiral matrix to affect the porphyrin aggregate structure after its formation. That is, the addition of polyglutamate to an 'amorphous' porphyrin aggregate solution at pH 4.0 does not determine the appearance of any ICD signal even after several days (Fig. 4).

All the other systems follow the same trend but are not described here for the sake of simplicity (Table 1). The only significantly different behavior was found out for the CuT4–CuTPPS system. This species exhibits the

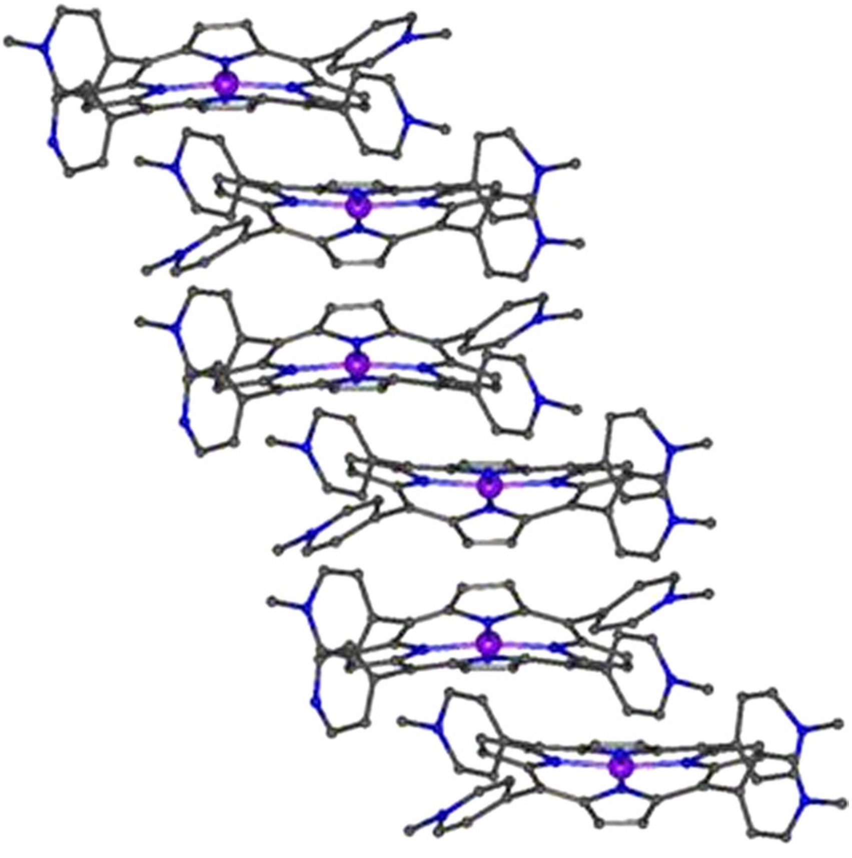

Figure 6. Structure of the CuT4 'polymeric' structure.

remarkable inertness and the chiral memory phenomenon presented by the other porphyrin aggregates, but its CD (Table 1) and RLS (Fig. 5) signals are considerably smaller then those exhibited by the other porphyrin couples (H_2T4–H_2TPPS, CuT4–H_2TPPS, and H_2T4–CuTPPS). These findings suggest to us that the title aggregate is sensibly smaller than the others.

We have recently shown that CuT4 forms, in the solid state, a polymeric arrangement that is characterized by a remarkable distortion of the pilled porphyrin macrocycles.[7] This distortion is caused by the π-like interactions between the peripheral pyrrole C_b–C_b 'double bond' and the metal center (Fig. 6). We hypothesize that similar relevant distortions are also present in the CuT4–CuTPPS aggregate, hindering the formation of extended porphyrin assemblies. Moreover, literature data[8] suggest that distortion caused by the central metal affects the attractive interactions between adjacent porphyrins. Aggregates in which only one (or no) copper porphyrin derivative is involved are less affected by the macrocycle distortions and, then, may form larger assemblies. Our hypothesis is corroborated by the RLS data (Fig. 5).

4. Conclusion

The study of the aggregation behavior of the reported systems confirms that net electrostatic interactions (i) constitute the driving force of the self-assembly process and (ii) lead, thanks to their large number and strength, to the formation of inert ensembles. Kinetic inertia, then, turns out to be crucial in determining the memory phenomenon. Finally, the aggregation process is affected by other non-covalent interactions as well. In particular, the central copper ion does not play a trivial role; in fact, it is able to affect the assembly dimension. This finding may turn out to be useful to modulate the aggregate size, whose control is crucial for the possible application of these systems.

Acknowledgments

We thank the Istituto Superiore della Sanita, MURST, and CNR for partial support.

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