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Transfer, Memory and Amplification of Chirality in Porphyrin Aggregates

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The aggregation process of the tetraanionic H_2TPPS and the tetracationic CuT4 porphyrins leads, in aqueous solution and in the presence of a suitable chiral template, to the formation of remarkably stable and kinetically inert chiral porphyrin heteroassemblies. These properties enable the aggregates to survive template chirality disruption and its complete removal from the solution, maintaining unaltered the imprinted chiral structure. The free-template "imprinted" porphyrin aggregate is an excellent mold for the amplification of its own structure.

Keywords: Porphyrins; Self-assembly; Supramolecular chirality; Chiral memory

INTRODUCTION

Chirality is a central theme in Nature: from the molecular level (e.g. a single amino acid molecule) up to nanoscale and mesoscale supramolecular levels (e.g. double-stranded DNA and sea-shells, respectively). Increasing interest in chirality is relevant not only to understanding the chiral bias inherent in life but also to the possible technological applications (e.g. chiral membranes, separation of optical isomers relevant to pharmacological or alimentary industries, etc.). Chirality generates optical activity, which is commonly related to molecular dissymmetry. Symmetric molecules, however, may also present supramolecular chirality by forming intrinsically chiral assemblies [1–8], or by aggregating onto chiral polymeric templates (extrinsic chirality) [9–15]. The latter leads to induced circular dichroism (ICD) signals that, having a conformational origin [9] (i.e. arising from self-organization of the achiral guest by chiral templates), modify or disappear [9,10,12] following matrix conformational transition. Unlike molecular chirality, which originates from the local nonsymmetric environment of atomic centers in a covalent frame and therefore not prone to dissociation, supramolecular chirality derives from the complex nonsymmetric arrangement of various molecular components in a noncovalent ensemble. This kind of asymmetry is governed by "weak", noncovalent bonds and consequently leads to mixtures of optically active isomers. A possible way to overcome this limitation is by imprinting, from the onset, the desired chirality on to a supramolecular complex. This noncovalent modular synthesis allows us to synthesize quite complex chiral architectures by inducing handedness in assemblies built with achiral structural elements using chiral templates. If the resulting supramolecular species is under thermodynamic control, substitution or removal of the chiral mold results in loss of supramolecular chirality. However, if the species formed are kinetically inert, then chirality persists for hours or longer even when they are deprived of the chiral template [13–19]. This phenomenon is referred to as a "memory" effect (Fig. 1).

The chiral memory phenomenon is of particular interest because, in principle, using this synthetic approach allows the choice of the desired molecular (monomeric) moieties with specific functions (binding, catalytic or others) and (with the aid of the appropriate chiral mold) noncovalently assembling them in a permanent chiral fashion. The resulting supramolecular complex can exert predetermined functions accompanied by chiral discrimination. Understanding the forces and mechanisms accompanying induction, tunability and memory of

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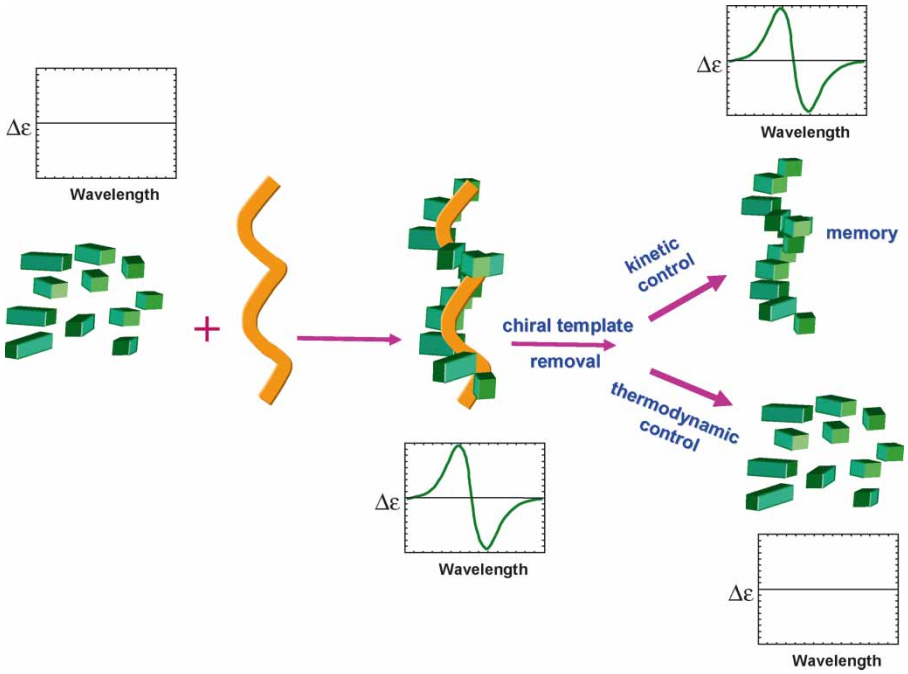

FIGURE 1 Schematic representation of the "memory" effect phenomenon. After the chiral template removal, if the process is under thermodynamic control, it resolves in disruption of the achiral molecular aggregate (or racemization, not represented) and loss of the CD signal; if the process is under kinetic control, the supramolecular chiral structure persists as well as the CD signal.

supramolecular chirality could allow for the rational synthesis of designed chiral supramolecular systems.

Here we describe some recent results concerning the chiral memory phenomenon in porphyrin assemblies [13,14]. In addition, we show that our systems are able to self-replicate in solution, "re-proposing" their chirality with almost complete enantiospecificity [14]. These systems are good models for understanding the transfer of information in biologically relevant aggregation processes (i.e. prion-mediated diseases, diabetes, cataracts, etc.) and also for technological applications.

EXPERIMENTAL

meso-tetrakis(*N*-Methylpyridium-4-yl)porphinato-copper(II) (CuT4) (Fig. 2a) and *meso*-tetrakis-(4-sulfonatophenyl)porphyrin (H_2TPPS) (Fig. 2b) were purchased from Mid-Century and used without further purification. Amino acids were purchased from Sigma-Aldrich and used without further purification. Amino acids were removed by ultrafiltration, using Centricon filters (cutoff at 10 kDa). About 95% of amino acid was removed after the first filtration. Residual amino acid concentration after ultrafiltration was checked both in the aggregate solution and in the filtrate by fluorescence: the lower detection limit was *ca.* 1×10^{-8} M. Absorption measurements were performed on a Varian Cary 500 spectrophotometer, fluorescence measurements on a Jobin–Yvon Fluorolog3-111-VUV spectrophotometer, and circular dichroism (CD) measurements were carried out on a Jasco 810 instrument. Ultrapure water was used throughout.

DISCUSSION

The first step toward chiral memory in the porphyrin aggregates was the synthesis of the chiral ternary heteroassembly built by the achiral tetracationic CuT4 (Fig. 2) and tetraanionic H_2TPPS (Fig. 2) porphyrins self-aggregating on to the helical anionic poly-L- or D-glutamate [12,13,15]. This complex is the first example of a ternary porphyrin assembly where two components (one of the porphyrins and the template) bear the same charge. Its formation is made possible by the shielding effect of the cationic unit, which minimizes the electrostatic repulsion between the two anionic components. We have shown that these ternary supramolecular species are remarkably (thermodynamically and kinetically) stable, which allows them to "memorize" the chiral information imprinted by the template and to retain it even if the matrix chirality is "disrupted" by pH-induced conformational transitions. Polyglutamic acid is a very versatile matrix: it adopts a helical conformation when the carboxylic side groups are partially protonated [20] and undergoes a helix-to-coil transition following deprotonation of the carboxylic moieties. The latter transition is probably

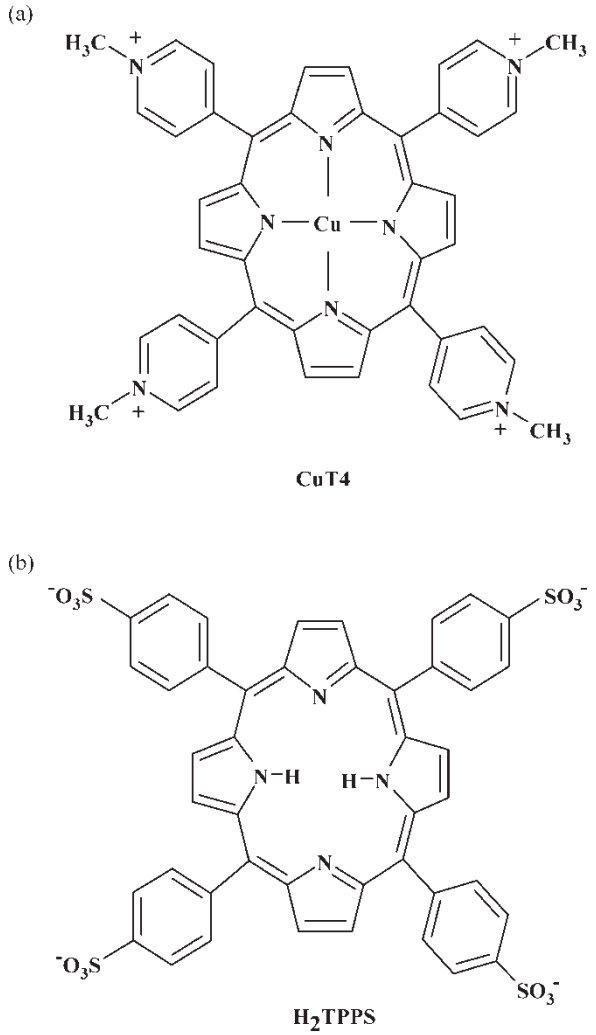

FIGURE 2 Schematic structures of (a) CuT4 and (b) H_2TPPS.

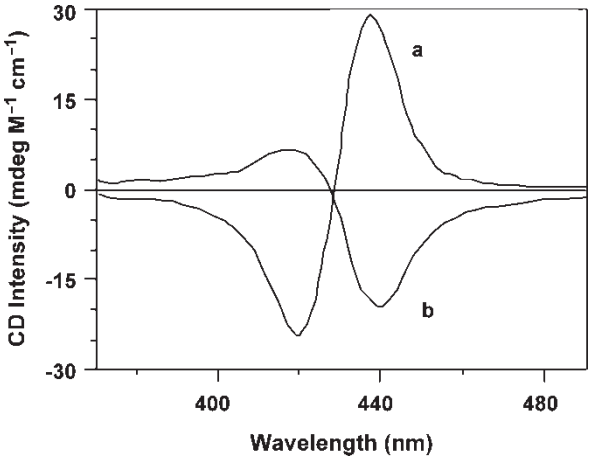

FIGURE 3 (a) CD spectrum of CuT4 (4×10^{-6} M) in the presence of poly-L-glutamate (2×10^{-4} M) at pH 3.6 (citrate buffer 5 mM). (b) Solution (a) after the addition of poly-D-glutamate (8×10^{-4} M).

driven by electrostatic repulsions between the carboxylates, which disfavor a right-handed helical arrangement.

The first step in the synthesis is the formation of the cationic porphyrin-polyglutamate binary complex. In the pH range 3.2–4.8 [13,15], CuT4 interacts with poly-L-glutamate forming a kinetically labile chiral binary complex. This is shown by (i) the presence of an induced band in the Soret region[†] of the CD spectrum (Fig. 3, curve a), (ii) the inversion (in about 10 min) of this band upon the addition of a fourfold excess of the D-form of the polymer (Fig. 3, curve b), and (iii) the disappearance of the signal when the pH is raised to about 12 due to the pH-induced helix-to-coil transition [20].

The formation of the ternary complex is achieved by adding the tetraanionic H_2TPPS to this parent binary supramolecular complex. In addition to a 50% decrease in both the Soret band intensity and the H_2TPPS emission (CuT4 does not emit), the formation of the ternary chiral complex also induces an ICD signal much more intense than that observed for the binary CuT4-polyglutamate complex (from 60 to 3500, see Fig. 4, curve a). When H_2TPPS is added to a preformed CuT4-poly-D-glutamate binary complex the CD mirror image is obtained (Fig. 4, curve b). It is noteworthy that self-aggregation of the title porphyrins in ultrapure water leads to achiral 1:1 complexes. No dichroic signal is observed in the Soret region if polyglutamate is added after porphyrin self-aggregation. This behavior testifies to the kinetic inertia of these porphyrin supramolecular species.

These supramolecular chiral ternary complexes behave very differently with respect to the parent CuT4-polyglutamate binary species in that they are remarkably inert. For example, the addition of a fourfold excess of poly-L-glutamate to a ternary complex "built" on the poly-D-glutamate does not lead to inversion of the ICD signal in the Soret region (even many days after the addition; data not shown). The only evidence of the L-form excess is the inversion of the helix marker bands at 222 and 208 nm[‡]. Other proof of this notable inertia is given by the persistence of the ICD after the polyglutamate pH-induced helix-to-coil conformational transition. As shown previously (Figs. 3c and 4a,b), the chirality of the supramolecular complex is transferred from polyglutamate to porphyrins. Therefore, like the binary CuT4-polyglutamate complex, the pH-induced helix-to-coil transition should cause

[†]The Soret band is the main feature in the visible region of the absorption spectrum. The porphyrin assembly causes broadening and hypochromicity of this band.

[‡]The same behavior has been observed for the ternary "L"-supramolecular species upon addition of an excess of poly-D-glutamate.

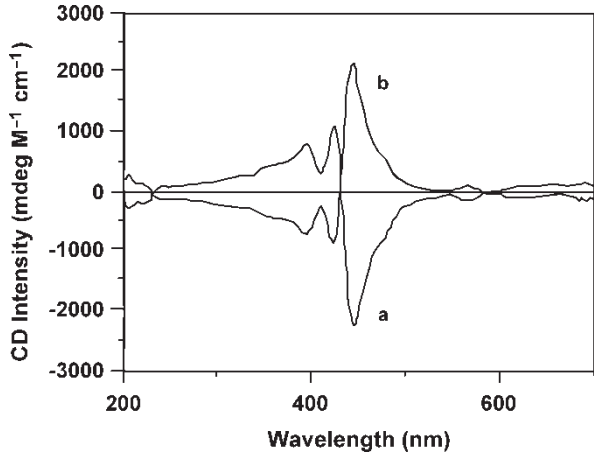

FIGURE 4 CD spectrum of CuT4 and H_2TPPS (4×10^{-6} M each) in the presence of (a) poly-L-glutamate (2×10^{-4} M) and (b) poly-D-glutamate (2×10^{-4} M). Citrate buffer 5 mM, pH 3.6.

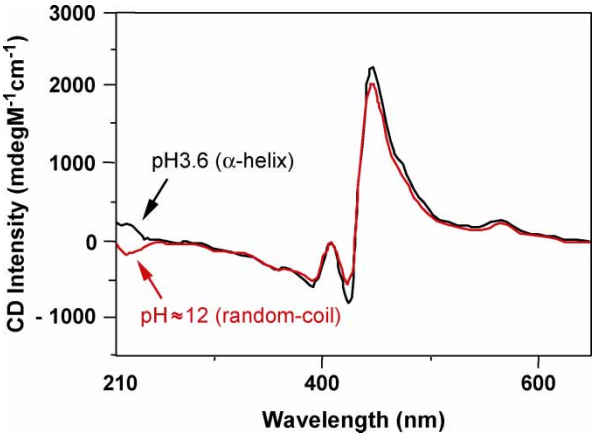

FIGURE 5 CD spectrum of CuT4 and H_2TPPS (4×10^{-6} M each) in the presence of poly-D-glutamate (2×10^{-4} M) in the α-helix conformation (black curve, pH 3.6) and in the random-coil conformation (red curve, pH ≈ 12).

the disappearance of the ICD of the ternary species. Surprisingly, the increase in pH to 12 does not perturb the ICD, strongly indicating that these porphyrin assemblies retain their "original" chirality even when the matrix loses its chirality (Fig. 5). We have also examined the time stability under these critical experimental conditions (pH 12), and found that the complexes remain stable for several days, as indicated by the CD intensity in the Soret region, which decreases by only 30% in about 2 weeks [15].

This evidence strongly indicates that the porphyrin heteroaggregates initially borrow their chirality from the template and then, because of their remarkable kinetic inertness, "memorize" the mold handedness, becoming themselves intrinsically chiral.

However, the presence of the porphyrin cationic N-methyl groups close to the carboxylate side groups of the polyglutamate could shield the electrostatic repulsions, preserving small region of the polypeptides from the helix-to-coil transition (partially) and explaining the persistence of the CD signal of the porphyrin aggregate after the pH jump experiment. A definitive and unambiguous demonstration of the memory phenomenon requires the survival of the ICD in the Soret region after the chiral mold removal (e.g. after ultrafiltration).

The latter experiment is not feasible when the mold is a (covalent) polymer, because it can remain trapped by the fairly large porphyrin aggregates (heteroaggregate solutions are cloudy). As clarified below, the "ideal" template ($\mathcal{A}$) for transferring the chirality to the porphyrin assembly and for the following removal experiment is a fluorescent monomeric chiral molecule (characteristic 1) that, above a given concentration threshold, self-assembles in a chiral fashion (characteristic 2), therefore establishing the equilibrium: n monomers $\leftrightarrow$ $(\text{monomer})_n$ (characteristic 3). The first condition allows us to monitor the residual concentration of the template much more efficiently compared with both the absorption and CD techniques. The second condition is indispensable for transferring chirality from the template to the porphyrin aggregates: induction of chirality (from a template to a supramolecular assembly) has a conformational origin; that is, monomeric $\mathcal{A}$ cannot induce chirality onto the achiral porphyrin aggregates, but $\mathcal{A}$ aggregates can transfer their handedness. Finally, the third condition helps to reduce the concentration of the template: as soon as the monomeric form of the "template" is removed (e.g. by ultrafiltration), the equilibrium n monomers $\leftrightarrow$ $(\text{monomer})_n$ shifts toward the left and the aggregated form disappears even if traces of the monomers (below the threshold) remain in solution.

We chose phenylalanine (Phe) as the first candidate for the following reasons: (1) it has a good fluorescence yield in a region where porphyrins only slightly absorb, and (2) its self-aggregation should be favored by the hydrophobic character of a large portion of the molecule. Indeed, addition of the cationic and anionic porphyrins to an aqueous solution of L-Phe led to an ICD in the Soret region[†] (Fig. 6a)[¶]. With the above considerations, the capability of aromatic amino acids to induce chiral aggregates is clear evidence of the presence in solution of self-organized Phe chiral structures. The threshold concentration of Phe to induce the ICD is about 1×10^{-3} M[§]. Therefore, unlike all

[¶]The mirror image ICD is induced by D-Phe. The same behavior is observed for tryptophan and tyrosine but the threshold concentration to observe the CD is different for the three amino acids, being ca. 1×10^{-4} M for tyrosine and tryptophan.

[§]Other experimental proof of the formation of chiral clusters of Phe has been reported in Ref. [14] and is not discussed here.

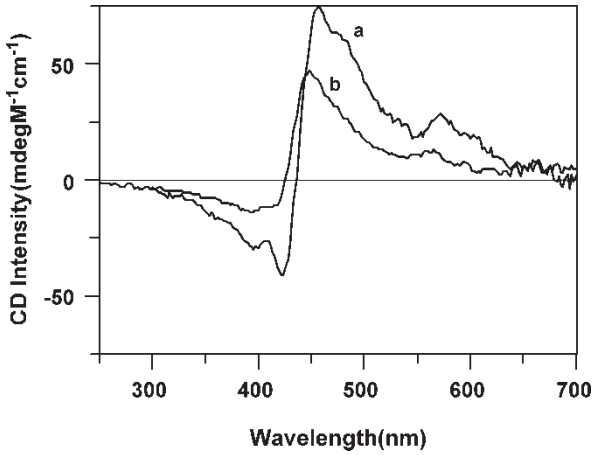

FIGURE 6 CD spectrum of CuT4 and H_2TPPS (2×10^{-6} M each) in Milli-Q water (a) in the presence of L-Phe (8×10^{-3} M) and (b) after amino acid removal by ultrafiltration.

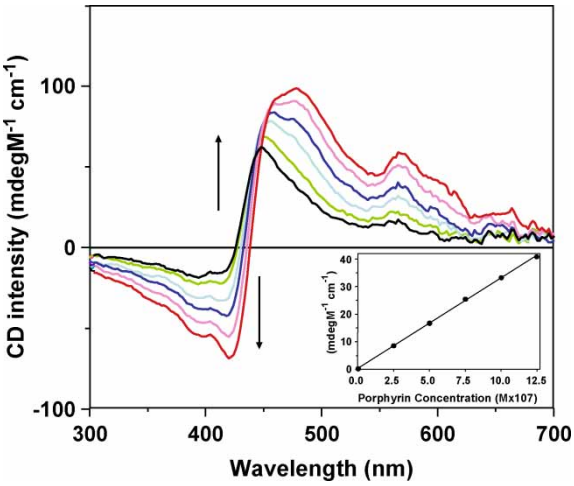

FIGURE 7 CD spectra of the L-Phe imprinted aggregates before (black curve) and after (others curves) five additions of the individual porphyrin aliquots (2.5×10^{-7} M each). Inset: CD increase versus added porphyrin concentration, considering the initial black curve intensity as reference.

the previous examples reported in the literature, in this case the preferential conformation of the porphyrin aggregates is borrowed by chiral noncovalent polymers.

According to our previous results [13] the title aggregates are inert enough to memorize the chirality of polymeric helical templates even after helix disruption. A similar result was also expected upon Phe removal. Ultrafiltration of the solution does in fact leave the CD signal almost unaltered (Fig. 6b), showing that the imprinted aggregates are now intrinsically chiral. The residual concentration of Phe was measured by fluorescence, which allowed detection of up to 1×10^{-8} M of Phe. This concentration is far below the concentration threshold necessary to transfer chirality to the porphyrin aggregates (about 10^{-3} M). DLS measurements [14] have shown that, under these conditions, the concentration of aggregated Phe is very small, at about 10^{-7} M. Therefore, as most of the monomeric Phe was removed, the equilibrium $n\,\text{Phe} \leftrightarrow (\text{Phe})_n$ can be considered completely shifted toward the monomeric form.

A direct consequence of the "memory" phenomenon is that the title aggregates are inherently chiral and are, in principle, excellent templates for the amplification of their own structure. The issue is intriguing and worthy of investigation. The scenario that opens up foresees the possibility of indefinitely building in solution exact copies of an initial molecule. Figure 7 shows, indeed, that when equimolar amounts of CuT4 and H_2TPPS were individually added to solutions containing about 6×10^{-13} M of imprinted assemblies (each formed by about 2×10^6 porphyrin molecules, see [14]), the ICD of the imprinted aggregates increased and doubled on doubling the concentration of the porphyrins (inset of Fig. 7). Interestingly, the linear increase in CD with porphyrin concentration shows that the chiral growth process is substantially completely enantiospecific.

Altogether our data provide evidence of a correlated sequence of induction, memory and amplification of chirality in mesoscopic assemblies. They also suggest that the role of the matrix is crucial only in the very first step of the formation of these ternary chiral species. Once formed, these aggregates have a "life" independent of the template and not only retain "memory" of the shape of the mold but are also able to self-propagate.

The simplicity of this "mix and shake" synthetic approach, the robustness and the remarkable ability to build exact duplicates of themselves suggest that these imprinted noncovalent aggregates (obtained with inexpensive and commercially available chemicals) are candidates for a wide range of possible technological applications as enantioselective catalysts, resolution agents and very sensitive amplifiers of chirality.

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