Kinetic Investigation of Porphyrin Interaction with Chiral Templates Reveals Unexpected Features of the Induction and Self-Propagation Mechanism of Chiral Memory

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In recent years it has been shown that it is possible to transfer chirality from polymeric covalent or noncovalent chiral templates (e.g., aggregates of phenylalanine(Phe), PheA) to aggregates of achiral porphyrins (e.g., CuT4 and TPPS, Figure 1).[1–3] Phenylalanine (Phe) self-assembles at concentrations of approximately 8 mM, giving rise to PheA having concentrations of about 10^{-13} M. At this femtomolar concentration, PheA is able to induce chirality in porphyrin aggregates.[3] A network of noncovalent interactions among porphyrins allows for memorizing chirality which remains after the template removal (Figure 1).[3]

One of the most intriguing properties of these supramolecular species is the ability to self-amplify their own chirality.[3] The interaction of monomeric cationic and anionic porphyrins with chiral templates leads to a chiral self-growth rate much faster than porphyrin assembling as achiral species when they are further away from the chiral template. To understand the discrimination mechanism we have undertaken a kinetic investigation of (1) the self-assembly of CuT4 with TPPS, (2) the interactions of each porphyrin with PheA and (3) the formation of CuT4-TPPS-PheA, a ternary system. These studies have been performed by rapid mixing of the reactants, following transient absorption and CD spectroscopy.[4] This approach allows for detecting the formation of eventual intermediates along the pathway leading to the various porphyrin aggregates.

Interaction between CuT4 and TPPS Figure 2 shows the progress curve corresponding to the kinetics of interaction between TPPS and CuT4, as monitored at the Soret maxima at 411 and at 424 nm, respectively (see Supporting Information).[5] Apart from the amplitudes of the various processes, both wavelengths report the same events and therefore only the 424 nm data will be shown.

In this work the analysis of the kinetic progress curves has been carried out according to the following equation:

$$OD_{obs} = OD_{t=\infty} + \sum_{i=1}^{i=r} \triangle OD_i \exp(-k_i t)^{\beta_i} \tag{1}$$

where OD_{obs} is the observed optical density at a given time, $OD_{t=\infty}$ is the optical density at infinite time (i.e., the end of the reaction), r is the total number of exponentials, ΔOD_i is the optical density change corresponding to the exponential i, k_i is the rate constant associated to the exponential i, t is time, and β_i is the stretching factor, that is the exponent related to the exponential i. A stretching factor $\beta \neq 1$ is generally referable to either diffusion-limited phenomena (with $\beta <$ 1) or to cooperative phenomena (with $\beta > 1$).[6] Interaction between the two porphyrins shows a small and fast absorption increase (curve a in Figure 3), followed by a large very slow absorption decrease (Figure 2), which can be described by two exponentials.
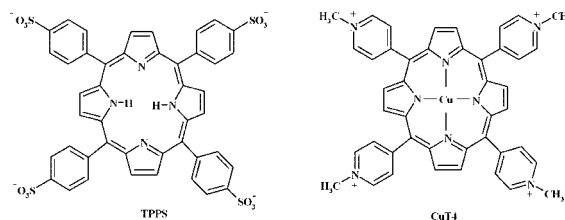

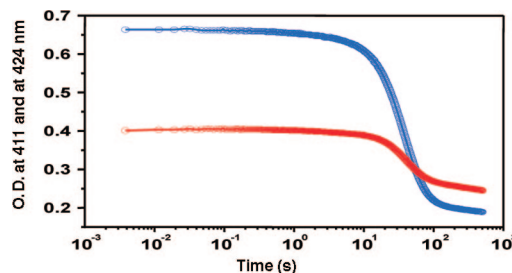**Figure 1.** Schematic structure of porphyrins.

Figure 2. Kinetic progress curves at 411 (blue points) and at 424 nm (red points) for the interaction of CuT4 with TPPS. Continuous lines represent the nonlinear least-squares.
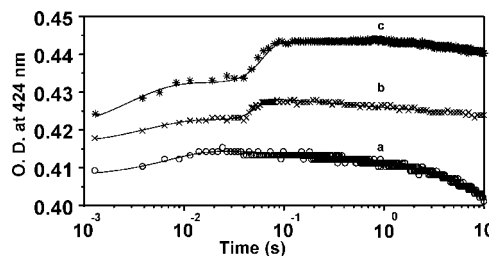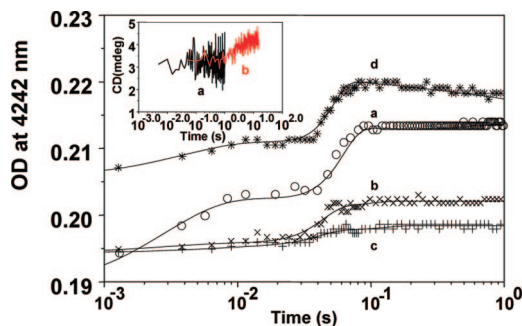

Figure 3. Kinetic progress curves for interaction of CuT4 with TPPS (curve a), TPPS with PheA (curve b), and CuT4 with PheA (curve c). Continuous lines represent the nonlinear least-squares fitting of data. In all the experiments the porphyrin concentration is 2 μM and Phe is 8 mM to ensure amino acid aggregation.

Interaction between One Porphyrin and PheA: The Binary Species. Interaction of CuT4 or TPPS with PheA[7] brings about smaller absorption changes (Figure 3), since we never observed the large absorption decrease typical of CuT4 with TPPS aggregation (Figure 2).[8] More importantly, the kinetic profiles are quite different: thus, in this system, three processes are observed (Figure 3): (i) a very rapid absorption increase with a normal exponential behavior ($\beta = 1$), which is completed in about 10 ms (and similar to what observed for the interaction between CuT4
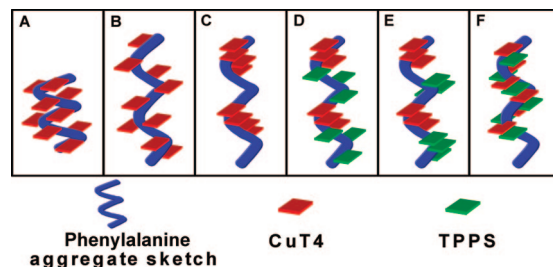
Figure 4. Kinetic progress curves for the interaction of CuT4 with (curve a) PheA by simple mixing, (curve b) PheA after 15 ms of exposure to TPPS, (curve c) PheA after 150 ms of exposure to TPPS, and (curve d) PheA after full equilibration (i.e., 20 min) with TPPS. Individual curves have been displaced for a better comparison.

Figure 5. Sketches of a possible sequence of events. The order of porphyrin addition does not affect the process.

and TPPS, curve a in Figure 3), (ii) a second slower process, completed in about 100 ms and characterized by a marked autocatalytic behavior ($\beta > 1$) and an absorption increase (not observed in the case of the interaction between CuT4 and TPPS, curve a in Figure 3), (iii) a third slower process (completed in about 10 s and characterized by an absorption decrease with a stretching factor $\beta \approx 0.3$) which is similar to the second process observed for the interaction of CuT4 with TPPS (curve a, Figure 3).[9]

We propose that the first event concerns a collision between monomeric porphyrins and PheA. This event paves the way to the interaction of other porphyrin molecules with PheA (likely through a conformational change of PheA) to form small aggregates (second event).[9,10] These aggregates might represent an activated intermediate to which additional porphyrin molecules aggregate according to a nucleation mechanism,[11] following a diffusion-limited behavior (as indicated by the $\beta \leq 1.0$). The most important and distinctive feature is represented by the second cooperative phase, which underlines the critical role of PheA templates in boosting the growth of (achiral or weakly chiral) aggregates.[8]

Interaction of Both Porphyrins with PheA: The Ternary System. If we allow TPPS to equilibrate with PheA and then we mix (in the stopped-flow) this mixture (**1**) with CuT4 (to form a ternary complex), we observe a kinetic pattern (Figure 4, curve d) similar to that displayed for the formation of the binary complex between CuT4 and PheA (Figure 3, curve c). This suggests that CuT4 (i) initially reacts with **1** at sites topologically distinct from those occupied by TPPS in the aggregates with PheA and (ii) forms small self-aggregates (achiral or weakly chiral). Finally, the small self-aggregates of TPPS and CuT4 (bound at topologically distinct sites) diffuse on the PheA surface to form chiral heteroaggregates (Figure 4, inset), this process corresponding to the third slower phase (curve d in Figure 4).[3] These data indicate that this is a multistep mechanism. Sequential stopped-flow mixing experiments where PheA was first reacted with TPPS for a different time intervals (i.e., either a 15 ms interval, corresponding to the completion of the first event, or a 150 ms, corresponding to the completion of the second event) and then mixed vs CuT4 show a progressive decrease of the amplitude of the second cooperative process (Figure 4, curves a−c). This behavior (which is observed also if we allow for the equilibration at different time intervals of the binary system between PheA and CuT4 and we follow the subsequent reaction with TPPS) suggests that during the nucleation process of TPPS onto PheA,[12] and even at the end of the second cooperative process, there is less probability for CuT4 to interact with the template complexed with TPPS.[13] However, during equilibration (corresponding to the third slow, hypochromic diffusion-limited process, Figure 4) a reorga-

nization of porphyrin distribution on the PheA surface seems to occur, such that the reaction of a second porphyrin may take place through a similar pathway (as observed for CuT4, Figure 4, curve d). Figure 5 sketches a possible sequence of events: (1) after the initial (within the first 10 ms) random distribution of the porphyrin along the template surface (panel A), a second process (within 0.2 s) may lead to a conformational change of the template (panel B) which then favors the formation of a more discrete distribution of porphyrins on the template (panel C). It is only after this event that the interaction of a second porphyrin at topologically distinct sites may occur (panel D). At this point, the interaction of the second porphyrin follows the same sequence of events (panel E), followed by a slow heteroaggregation of the two porphyrins, driven by electrostatic interactions and characterized by a strong chirality (panel F), as suggested by CD (see inset of Figure 4).

In conclusion, we have shown that femtomolar concentration of chiral noncovalent polymers plays an efficient catalytic role in the aggregation of opposite-charged porphyrins. The rate of heteroaggregation onto the chiral surface is in fact about 2 orders of magnitude faster than that measured in its absence, explaining the chiral amplification exerted by porphyrin aggregates.

Supporting Information Available: Complete ref 6; absorption changes of the reaction between CuT4 and TPPS, kinetic parameters for all reported curves. This material is available free of charge via the Internet at http://pubs.acs.org.

References

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(2) Purrello, R.; Raudino, A.; Monsù Scolaro, L.; Loisi, A.; Bellacchio, E.; Lauceri, R. *J. Phys. Chem. A* **2000**, *104*, 10900.
(3) Lauceri, R.; Raudino, A.; Monsù Scolaro, L.; Micali, N.; Purrello, R. *J. Am. Chem. Soc.* **2002**, *124*, 894.
(4) As already reported,[1–3] CuT4-TPPS interactions do not lead to CD signals because their assemblies are not chiral in the absence of a chiral matrix.
(5) In this experimental approach light scattering (due to porphyrin aggregation and causing baseline increases, see Supporting Information) is competing with absorption and must be carefully considered.
(6) Steinbach, P. J.; et al. *Biochemistry* **1991**, *30*, 3988.
(7) Kinetic profiles for L- or D-PheA are the same.
(8) Interactions of one porphyrin with PheA do not lead to an ICD. The absence of a CD signal can be due either to the absence of chiral species or (more likely) to the formation of species having very weak CD signals.
(9) This fast cooperative event is accompanied by an increase of light scattering (data not shown) which reflects porphyrin aggregation. This impairs the observation of the typical hypochromicity associated to aggregation.
(10) The observation that β value is always oscillating between 3.0 and 4.0 suggests structural organization of at least four molecules.
(11) Abraham, F. F. *Homogeneous Nucleation Theory*; Academic Press: New York, 1974.
(12) Likely occurring within the first second after the mixing.
(13) This is suggested by the decreasing intensity of the signal upon addition of the latter porphyrin.

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