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Template-Imprinted Chiral Porphyrin Aggregates

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Interactions between cationic or anionic porphyrins and templates with opposite charges have been extensively investigated for their possible biomedical[1] and technological applications.[2] The formation of binary *heteroassemblies* of oppositely charged porphyrins has also been widely studied.[3] Very recently, we have shown[4] that it is possible to "build" chiral *heteroassemblies* of cationic and *anionic* porphyrins on *anionic* chiral matrixes. The formation of such supramolecular species is made possible by the shielding effect of the cationic component which minimizes the electrostatic repulsion between the anionic ones. We have pursued in studying these type of *ternary* species because their "second level" of coordination might allow a more refined design of the resulting supramolecular complexes, owing to the possibility of tuning the electronic and structural properties of *three* constituents.

Here we present a spectroscopic study in water solution of chiral ternary complexes of the protonated form of tetra-anionic *meso*-tetrakis(4-sulfonatophenyl)porphine (H$_4$TPPS, $\mathrm{p}K_\mathrm{a} \approx 4.8$, Figure 1)[5a,b] on a preformed complex between α-helical polyglutamate[6] and the copper(II) derivative of the tetracationic *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphine (CuT4, Figure 1). We anticipate that these ternary supramolecular species show a remarkable stability, 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.

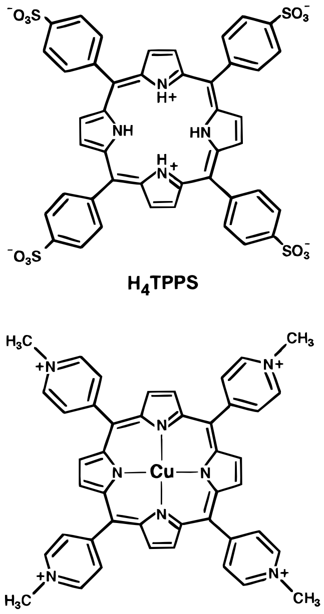

Figure 1. Schematic structure of protonated form of *meso*-tetrakis(4-sulfonatophenyl)porphine (H$_4$TPPS), and the copper derivative of *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphine (CuT4).

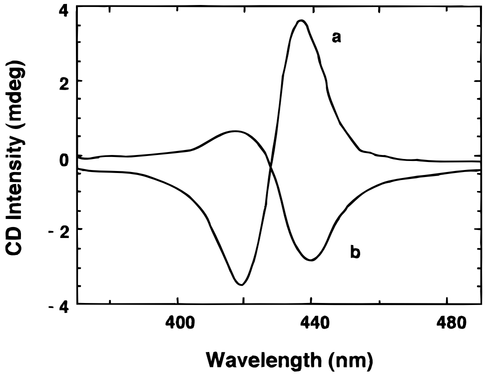

Figure 2. CD spectra of: (a) a solution of CuT4 (4 μM) in the presence of poly-L-glutamate (200 μM) at pH 3.6 and (b) the same solution, after the addition of a 4-fold excess of poly-D-glutamate.

In the pH range 3.2−4.8, 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 circular dichroism (CD) spectra (Figure 2, curve a)[7] and (ii) the inversion (in about 10 minutes) of the same band upon the addition of a 4-fold excess of the D-form of the polymer (Figure 2, curve b).[8] The addition of H$_2$TPPS (in the pH range 3.2−4.0) to such species induces drastic changes in the absorption, fluorescence, and CD spectra, indicating the formation of *chiral ternary* complexes. In particular, the Soret bands of both porphyrins experience quite strong hypochromic effects ($\sim$50%), and the fluorescence emission of H$_4$TPPS is quenched by $\sim$50%.[9] Also,

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(1) Pasternack, R. F.; Gibbs, E. J. *Metal Ions in Biological Systems*; Siegel, A., Siegel, H., Eds.; Marcell Dekker: New York, 1996; Vol. 3. and references therein.

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(6) Polyglutamic acid is α-helical at pHs lower than about 6, and "random coil" at higher pHs; see, Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; W. H. Freeman and Company: New York, 1980.

(7) The porhyrins used in this study are not CD active; therefore, a CD signal can only be *induced* by interaction of these dyes with chiral molecules.

(8) This is in agreement with previous findings where it was shown that the phase of the induced CD signal in the Soret region reflects the helical sense of the matrix; *i.e.*, the complex species formed on the L or D forms are characterized by mirror image CD spectra; see Pasternack, R. F.; Giannetto, A.; Pagano, P.; Gibbs, E. J. *J. Am. Chem. Soc.* **1991**, *113*, 7799−7800. Also, curve b of Figure 2 is not the perfect mirror image of curve a because both L- and D- supramolecular isomers coexist in solution, with the aggregate on poly-D-glutamate in excess.

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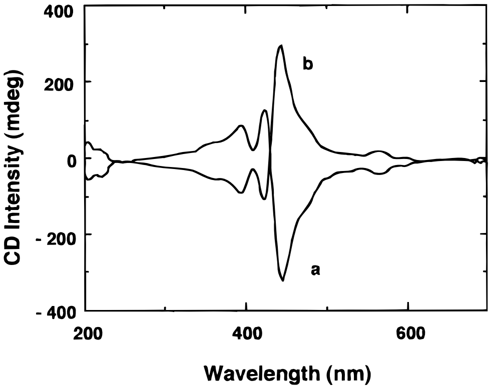

Figure 3. CD spectra of a solution of CuT4 (4 μM) in the presence of (a) poly-L-glutamate (200 μM) and (b) poly-D-glutamate (200 μM) after the addition of H_2TPPS (4 μM).

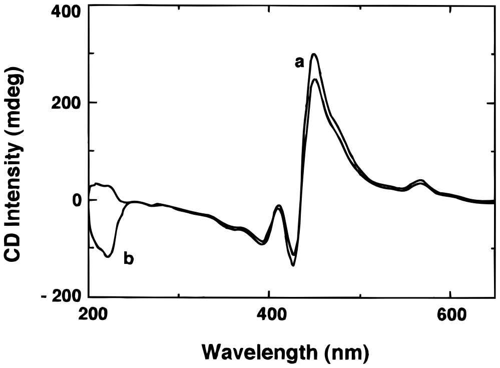

Figure 4. CD spectra of: (a) a solution of CuT4 (4 μM), H_2TPPS (4 μM) in the presence of poly-D-glutamate (200 μM) and (b) the same solution 1 day after the addition of a 4-fold excess of poly-L-glutamate.

both the shape (bisignate) and the unusually high intensity of the induced CD features (Figure 3, curve a) strongly indicate that both porphyrins are extensively aggregated onto the α-helical polyglutamate.[10] When H_2TPPS is added to a preformed CuT4-poly-D-glutamate binary complex, the mirror images of the CD signals observed with the L-isomer are obtained (Figure 3, curve b), indicating that the chirality of these assemblies follows the matrix chirality.

However, these supramolecular ternary complexes behave quite differently compared to the parent CuT4-polyglutamate *binary* species. In fact, the addition of a 4-fold excess of poly-L-glutamate to a *ternary complexes* “built” on the poly-D-glutamate does *not* lead to the inversion of the induced CD signal in the Soret region (even after *5 days* following the addition). The only indication of the L-form excess is the inversion of the helix marker bands at 222 and 208 nm[6] (Figure 4). The same behavior has been observed for the ternary “L”-supramolecular species upon addition of an excess of poly-D-glutamate. The lack of inversion of the *induced* CD bands shows that, in contrast to their *binary* precursors, the polyglutamate-CuT4-H_4TPPS aggregates are kinetically *inert*. Another evidence of this remarkable inertia is given by the stability vs. pH as well. In fact, once the ternary complexes are formed, no significant variations of the *induced* CD spectra are monitored by raising to ~12 the pH of the solution (Figure 5). Recalling that at this pH polyglutamate is in a “random-coil” conformation,[6] this finding strongly indicates that these porphyrin assemblies retain their “original” chirality even when the matrix loses it. We have also checked the time stability under such critical experimental conditions (pH ≈ 12). It turns out that these complexes remain stable for several days, as indicated by the CD intensity in the Soret region which decreases only by 30% in about two weeks. To the best of our knowledge this is the first example of porphyrin assemblies which do not follow the “chirality” of the template they were grown on. A possible explanation for this behavior is that aggregation could lead to the formation of clusters which have a fractal structure[11] and which are tightly assembled and stabilized by an extended network of electrostatic interactions (*i.e.*, those between the oppositely charged peripheral groups and the “$\pi-\pi$” between the porphine rings).[12]

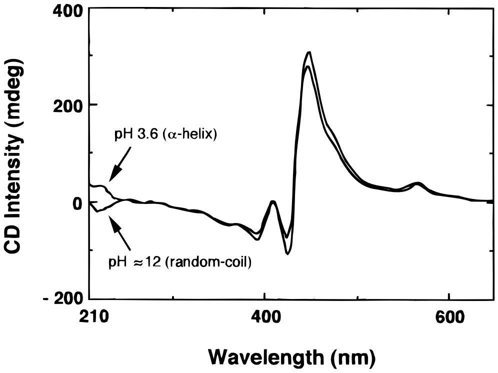

Figure 5. CD spectra of: (a) a solution of CuT4 (4 μM), H_2TPPS (4 μM) in the presence of poly-D-glutamate (200 μM) at pH 3.6 and (b) the same solution at pH ≈ 12.

Interestingly, also the interactions between the cationic and anionic free porphyrins leads to aggregates which show the same kind of “memory” effect. However, the intensity of the CD signals is about 10 times weaker. On the other hand, neither ZnT4 nor MnT4 form this type of ternary complex under the same experimental conditions.[13] Most likely, this is due to the presence of, respectively, one or two water molecules axially coordinated to the central metal ions which hinders a close “contact” among porphyrin molecules.

In conclusion, our data suggest that the role of the matrix is crucial only in the very first step of the formation of these ternary chiral species. In fact, once formed, these aggregates seem to have a “life” independent from the template but retain “memory” of the shape of the “mold” used for their formation.

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[9] Only the fluorescence emission of H_4TPPS could be monitored because CuT4 is not fluorescent.

[10] Gibbs, E. J.; Tinoco, I.; Maestre, M.; Ellinas, P.; Pasternack, R. F. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 350−358.

[11] The fractal nature of some porphyrin aggregates has been already demonstrated; see Mallamace, F.; Micali, N.; Trusso, S.; Monsù Scolaro, L.; Romeo, A.; Terracina, A.; Pasternack, R. F. *Phys. Rev. Lett.* **1996**, *17*, 4741−4744.

[12] Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525−5534.

[13] They form in about 20 h the chiral nonstoichiometric J- and H-type aggregates discussed in ref 4.