Hierarchical Porphyrin Self-Assembly in Aqueous Solution

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Hierarchical self-organization is a time-dependent process in which progressively more complex structures are formed upon sequential self-organization of simpler elements.[1] Nature extensively uses hierarchical processes (the so-called "sergeant–soldier principle" vide infra) to build structures with distinct functions from the individual components, thus inspiring scientists to gather new insights into this synthetic strategy.[2] Porphyrin self-assembly has been well-characterized,[3] and examples of hierarchical heteroassembly have been reported.[4] However, few studies on hierarchical homo-self-aggregation processes have been conducted, especially in aqueous solution. Hierarchy, in such systems, is illustrated in Figure 1 and finds its origin in one of the central topics of supramolecular chemistry: (homo- or hetero-) aggregates are chemical entities distinct from their monomeric constituents from a chemico-physical viewpoint.

Herein we report that the *meso*-tetrakis(4-phosphonatophenyl)-porphyrin, H_2TPPP (Figure 2a), shows pH-dependent hierarchical homo-self-assembly in aqueous solutions. The unique behavior of this molecule is due to its polyprotic character. This porphyrin was synthesized[5] and studied because: (i) it possesses three types of protonation sites and (ii) each protonation step can, in principle, lead to species prone to self-aggregation. These properties open, for each protonation step, interesting new vistas, since it should be possible to direct the system toward two different pathways: (a) the normal thermodynamic protonation pathway (Figure 2a, left) or (b) alternative, parallel, time-dependent kinetic aggregation routes (Figure 2a, right). From an experimental viewpoint, the differentiation between the two pathways is feasible because protonation is a much faster process (diffusion-controlled) than aggregation, which can take minutes or hours.

On the contrary, an anionic porphyrin such as *meso*-tetrakis(4-sulfonatophenyl)porphyrin (H_2TPPS, Figure 2b)[6] is not expected to show self-aggregation phenomena controlled by the "sergeant–soldier principle" (Figure 1). In this case, the thermodynamic and kinetic pathways are not parallel but consecutive. After protonation, the positive charges on the macrocycle core interact with the negative charges on the sulfonate peripheral groups. These interactions result in aggregation, leading to the formation of the thermodynamic products. No alternative pathway exists.[7]

In the case of H_2TPPP (Figure 2a), three protonation steps are possible: (1) first protonation of the phosphonate groups ($pK_a \cong$ 7.8), (2) protonation of the core nitrogen atoms ($pK_a \cong 5.4$), and (3) second protonation of the phosphonate groups ($pK_a \cong 2.4$). By comparing H_2TPPP with H_2TPPS, it is predictable that the second protonation step, leading to a zwitterionic form, might induce
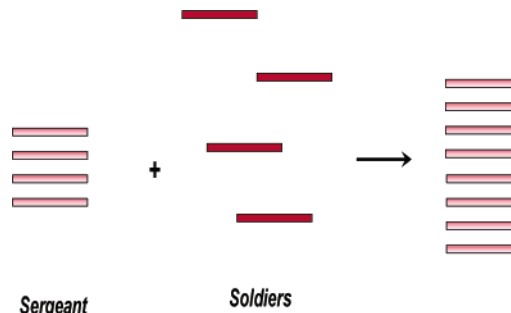

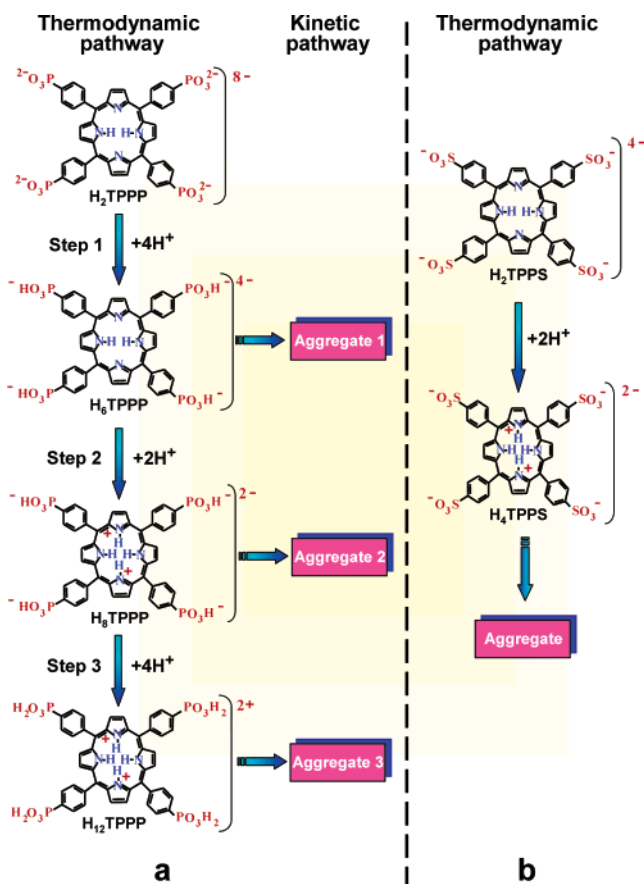***Figure 1.*** Sergeant–soldier principle in homo-self-aggregation.

Figure 2. H_2TPPP and H_2TPPS protonation and potential aggregation pathways.

aggregation. On the other hand, the first protonation step produces a nonzwitterionic species that is able to aggregate. In the latter case, in addition to $\pi-\pi$ interactions hydrogen bonds involving the phosphonic groups might also foster self-assembly.

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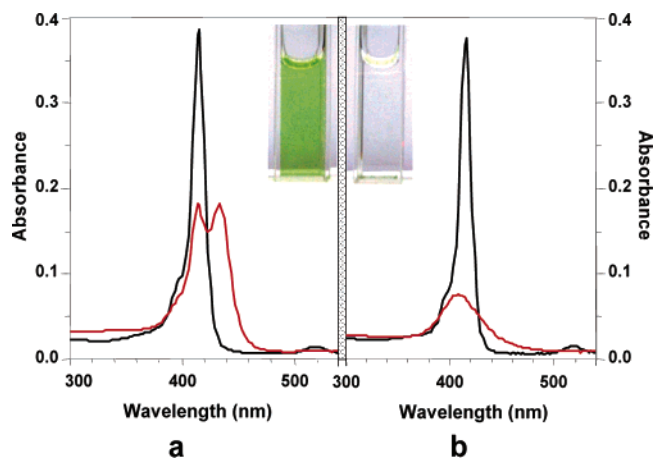
Figure 3. H$_2$TPPP absorption spectrum at pH 10.4 (black lines) and 5.0 (red lines), (a) rapidly decreasing the pH of the porphyrin solution and (b) slowly decreasing the pH. Inset: Photo of the 2×10^{-6} M H$_2$TPPP water solutions brought from pH 10.4 to pH 5.0 (a) over a few minutes and (b) during a few hours.

To study whether such processes follow the "sergeant–soldier principle", the pH of the porphyrin solution was varied from 10.4 to 5 according to two different procedures: (1) rapidly, over a few minutes and (2) slowly, during about 3 h. In the first case, the system is expected to follow the thermodynamic pathway and, in fact, at pH 5.0 the absorption band at 438 nm (characteristic of the inner core protonated form) is clearly detectable (Figure 3a, red curve), and the expected visual color change to green occurs (Figure 3a, inset). In the second case the system has time to follow the kinetic route, and at pH 5.0 the absorption band of the protonated form at 438 nm is not detected (Figure 3b, red curve). As a consequence, no visual color change is observable at pH 5.0 (Figure 3b, inset). Also, unlike the former solution, resonance light scattering (RLS) measurements indicate aggregation phenomena (Supporting Information).

The absence of the Soret band of the protonated form[8] and the pH variation of the scattering (RLS, Supporting Information) strongly suggest that the aggregate presiding over the hierarchy is that formed at pH close to 8, before inner nitrogen protonation (Figure 2). The hierarchical role of the (initially) small aggregates forming at pH 8 (RLS, Supporting Information), is underlined by the absence of aggregation also when H$_2$TPPP is directly dissolved at pH 5.0. The green solution obtained in these conditions does not show any sign of aggregation even after several hours. The phosphonic groups are protonated, but the concomitant inner nitrogen protonation (inducing two central positive charges) hinders aggregation.

In summary, it is possible to control the system final state (i) as a function of the time spent lowering the pH from 10.4 to 5.0 or (ii) by "skipping" the crucial protonation step (the first of the phosphonates, in this case).

The two systems (Figure 3, insets) have different chemico-physical properties and therefore can exert different functions. For example, the fluorescence of the porphyrin solution at pH 5.0, prepared following the first procedure, is quenched by subnanomolar concentrations of Mg^{2+} (Supporting Information).[9] On the other hand, no variation is detectable for the porphyrin solution at pH 5.0 prepared following the second procedure, even when greater amounts of Mg^{2+} are added (data not shown).

These results stimulate further studies and insight into the role and the balance among the interactions (both attractive and repulsive) that determine the above-reported behavior.[3c] In fact, the strategy used in this work might provide a general approach to the design of systems bearing different properties, using the same building blocks and the same chemico-physical conditions. Finally, this type of study could also be of interest in the biomedical field, where some pathologies, such as prion or Alzheimer disease, are related to abnormal protein aggregation processes similarly subjected to hierarchical control.

Supporting Information Available: RLS titrations following the two procedures. Mg^{2+} fluorescence titration. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) H$_2$TPPP was prepared following the Adler–Longo procedure, by reaction of diethyl(4-formylphenyl)phosphonate and pyrrole in refluxing propionic acid. The intermediate phosphonate diethyl ester was separated and then subjected to hydrolysis catalyzed by bromotrimethylsilane to give H$_2$-TPPP in a total 15% yield.
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(7) *meso*-Tetrakis(4-carboxyphenyl)porphyrin (H$_2$TPPC) and *meso*-tetrakis-(4-pyridyl)porphyrin (H$_2$TPyP) have two different protonation sites: (i) the inner core nitrogen atoms and (ii) the carboxylic and pyridyl groups. However, no hierarchical self-aggregation has been observed in these cases. The deprotonation of the carboxylic acid groups of H$_2$TPPC and the protonation of the pyridyl groups of H$_2$TPyP only have the role to increase the solubility of these porphyrins in water. Thus, as with H$_2$TPPS, H$_2$-TPPC and H$_2$TPyP have only one "useful" protonation step.
(8) The "lack" of the Soret band of the protonated form can be attributed to two different phenomena: (i) a strong hypochromicity related to aggregation or (ii) the shift of the nitrogen protonation to very low pH. We are currently investigating the system to clarify this point.
(9) Fluorescence quenching is most likely related to H$_2$TPPP aggregation induced by "bridging" complexation of the Mg^{2+} ions. This mechanism would also explain the lack of sensitivity observed for the other system in which porphyrins are already aggregated.

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