6-7-2011

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Supramolecular chirality transfer to large random aggregates of porphyrins†‡

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Received 26th February 2011, Accepted 1st April 2011
DOI: 10.1039/c1cc11165e

Chirality is rapidly induced in a fractal aggregate of the porphyrin t-CuPagg by addition of α-helical poly-glutamate. These results demonstrate a facile transfer of chirality via noncovalent interactions to preformed supramolecular assemblies grown in the absence of a chiral template.

Chirality transfer, amplification and memory1 are attracting considerable interest in the field of supramolecular chemistry, in part because of their close correspondence to various biological processes. The role of achiral building blocks able to self-assemble onto homochiral templates has received particular attention.2 Many examples are now available of the formation of organized supramolecular assemblies of chromophores in which different types of chiral templates are exploited to achieve control of their size and morphology.3 Porphyrins are rather appealing candidates for studies of these phenomena because of their rich spectroscopic features which are markedly affected by self-aggregation and the local microenvironment.4 A few studies have dealt with the use of random porphyrin aggregates that are capable of detecting even tiny amounts of chiral substrates, when these are already present as templating reagents during the growth of the assemblies.5

Here we describe a new phenomenon involving the interaction between preformed fractal clusters of a cationic porphyrin, the Cu(II) derivative of trans-bis(N-methylpyridinium-4-yl)diphenylporphine (t-CuPagg), and poly-glutamate (PGA) in its α-helical conformation. We demonstrate that these large, random porphyrin aggregates show an unanticipated rapid response to the chirality of PGA as detected by large enhancements of the induced circular dichroism (ICD). While recent research has made rather commonplace the impact of the initial presence of a chiral templating agent, this chiral transfer effect is unprecedented for pre-organized assemblies, and emphasizes once again how the protocol used to mix components may strongly affect the nature of supramolecular assemblies.6

Previous investigations have demonstrated that the parent free base porphyrin, trans-bis(N-methylpyridinium-4-yl)diphenylporphine (t-H2Pagg), self-aggregates in aqueous solution upon salt addition yielding micro-sized fractal clusters.7 Insertion of copper(II) into the centre of the macrocycle accomplishes two simplifying features: (i) it avoids protonation of the core, suppressing pH effects on the porphyrin charge under acidic conditions, and (ii) it slightly increases the self-aggregation tendency of this porphyrin, so that it exists as a dimer even at micro-molar concentration.8

In the presence of NaCl (0.05–0.1 M), the extinction spectrum of t-CuPagg displays a split B-band with two components at 440 and 381 nm (Fig. 1a, black).9 The presence of large assemblies in solution is proved by an intense resonance light scattering (RLS) signal but, as expected for homoaggregates of achiral species, the corresponding CD spectrum displays a hypsochromic and broadened extinction spectrum (Fig. 1a, red). An induced conservative extinction and RLS spectra. See DOI: 10.1039/c1cc11165e

The interaction of the Cu(II) porphyrin with α-helical PGA at pH 4.2 and at high ionic strength (IS) strongly depends on the order of mixing the various components. If L-PGA (500 μM) is added to a 5 μM t-CuPagg solution and the IS is increased to 0.1 M only afterwards (protocol: protein first), the extinction spectrum displays a hypsochromic and broadened B-band at 410 nm (Fig. 1a, red). An induced conservative signal having a positive Cotton effect appears in the CD spectrum under these conditions, accompanied by a moderate intensity in the corresponding RLS spectrum (Fig. 1b, c, red). These spectral features remain the same, except for the mirror CD spectrum, when D-PGA is used as templating reagent.

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In agreement with recently reported results, this evidence points to t-CuPagg dimers bound to the helical polypeptide (Fig. 1d). If, on the other hand, the porphyrin is completely pre-aggregated by salting a 5 mM t-CuPagg solution (IS = 0.1 M, NaCl), and PGA is then added to this solution (protocol: protein last), dramatically different spectral features occur. The extinction spectra (Fig. 1a, green and cyan) are very similar to those of DLA aggregates (see Fig. 1a, black), and the RLS spectra (Fig. 1c, green and cyan) point to the presence of large clusters. The ICD bands reflect the handedness of the PGA (Fig. 1b, green: L-PGA, cyan: D-PGA), and may be described as a pair of bisignate features centered on the two maxima of the split B-band and having opposite phasing. These signals are an order of magnitude larger than those obtained by using the protein first protocol that characterizes porphyrin dimers on the α-helices. Our experimental findings suggest that, upon interacting with the preformed metalloporphyrin assemblies, the helical biopolymer transfers chiral information to the DLA fractal clusters (Fig. 1e). A preliminary attempt to measure the rate of this chiral transfer has been done by mixing the porphyrin clusters with PGA using a stopped-flow apparatus and detecting the ICD at 453 nm. This experiment showed that the process is biphasic; some 90% of the ICD signal is generated within 250 ms, while the remaining 10% intensity increases with very slow kinetics of the order of minutes (ESI†, Fig. S3).

In order to evaluate the ability of the porphyrin clusters to respond to the presence of the chiral polymer, increasing amounts of L-PGA (up to 1 mM) have been added to different batches of pre-aggregated t-CuPagg (5 mM, IS = 0.1 M). Fig. 2 shows that the intensity of ICD spectra steeply increases on adding up to 20 μM L-PGA, leveling off for concentrations higher than 100 μM. As neither the corresponding extinction spectra, nor the RLS profiles exhibit relevant differences upon increasing the amount of protein (ESI†, Fig. S4), the dissymmetry g-factor (ΔA/A) follows the same saturation profile displayed by the ICD (Fig. 2, inset). It is interesting to note that, in as much as t-CuPagg is a dicationic porphyrin and PGA is semi-protonated at the experimental pH, the initial rapid increase of ICD intensity corresponds to an apparent stoichiometric charge neutralization between the two interacting components. These considerations suggest that electrostatic interaction plays a major role at lower polymer loads, whereas hydrophobic ones may account for the subsequent gradual increase in the chiral transfer beyond the stoichiometric ratio.

In order to investigate the impact of the cluster size on the chirality transfer, the batch kinetic experiment shown in Fig. 3 has been performed; the overall time evolution can be conveniently monitored by UV/Vis extinction. The kinetics of this process exhibit a typical sigmoidal shape in which an induction period is observed corresponding to nucleation onset. In this experiment, the same amount of L-PGA (200 μM) is injected into various aggregating solutions of t-CuPagg at different time delays (marked by arrows in Fig. 3a) from the initial addition of NaCl, i.e. from the beginning of the aggregation process. The IS (60 mM) was chosen for convenience; complete aggregation of t-CuPagg occurs within two minutes under these conditions. On comparing the various spectral features of the solutions in the batch after

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**Fig. 1** UV/vis extinction (a), CD (b) and RLS (c) spectra of t-CuPagg in the absence (black) of protein and in the presence of D- (blue, cyan) or L- (red, green) PGA in their α-helical conformation, as a function of the mixing protocol: protein first (red, blue); protein last (green, cyan). Cartoon representations of dimers (d) and fractal aggregates (e) of t-CuPagg interacting with PGA helices. Experimental conditions: [t-CuPagg] = 5 μM; [Glu] = 500 μM; IS = 0.1 M; [buffer] = 5 mM, pH 4.2.

**Fig. 2** CD spectra of a batch titration experiment, where increasing amounts of L-PGA (as indicated by the arrows) have been added to fully-aggregated solutions of t-CuPagg. Inset: dissymmetry g-factor vs. protein concentration. Experimental conditions: [t-CuPagg] = 5 μM; IS = 0.1 M; [L-PGA] = 0–1000 μM; [buffer] = 5 mM, pH 4.2; mixing protocol: protein last.
of L-PGA, as chosen for the batch kinetic experiment. (b) Comparison addition of salt (triggering the aggregation processes) and the injection of 60 mM NaCl; the arrows indicate the time delays between the injecting L-PGA at the different time delays. (c) Dissymmetry between the CD spectra of the t-CuPagg aggregating solutions after \([\text{buffer}] = 5 \text{ mM}, \ pH = 4.2; \) mixing protocol: protein last.

equilibration, it appears that addition of the biopolymer quenches the aggregation kinetics of t-CuPagg. The UV/Vis extinction and RLS spectra (ESI, Fig. S5) show that longer time delays allow a more extended aggregation of the samples. The ICD spectra (Fig. 3b) corresponding to the smaller time delays point to the occurrence of a bisignate component centered at 410 nm together with the conservative feature at 440 nm, suggesting that the porphyrin interacts as a distribution of dimers and clusters with the \(\alpha\)-helices. After a time delay longer than 10 s, the ICD band of the DLA aggregates is the predominant component. The dissymmetry g-factor of these samples displays a fairly linear correlation with the corresponding intensity of RLS (Fig. 3c). This result is rather fascinating since it implies that the quantity of the chirality transferred from the biopolymer to the porphyrin clusters is related to the size of these clusters,\(^1\) pointing to an effective amplification effect.

Our experimental findings were unanticipated since a large fractal aggregate would be expected to be stabilized in solution by the screening effect of the surrounding ionic cloud. This in turn should inhibit any further interaction. However, recent papers by Avnir and coworkers show that even in the absence of chiral inducers fractal clusters are inherently chiral, since the virtual mirror image is not superimposable to the cluster itself.\(^2\) The chaotic nature of the aggregation process leads to a statistical mixture containing a large distribution of quasi-enantiomeric forms i.e., a quasi-racemic mixture, opening different possible scenarios. A propagation mechanism could be proposed, considering that the interaction with an optically active conformation of PGA biases the mixture toward a more specific form. The high porosity, a residual positive charge and the flexibility of the structure allow for a fast and efficient interaction with the much smaller and negatively charged biopolymer. Still, chiral recognition and selection of PGA with a specific handedness by a population of chiral clusters cannot be excluded. The exact nature of chirality transfer and amplification reported here is intriguing and its comprehension may well open the way to many potential chiroptical sensing applications, e.g. to investigate the evolution of non-inert, complex systems.

**Notes and references**

10. Even if an estimate of the clusters size has been obtained through dynamic light scattering measurements, pointing to hydrodynamic radii larger than 1 \(\mu m\), the polydispersity of the samples has prevented an accurate determination. See ESI materials.
13. RLS intensity is related both to the number and size of the aggregates in solution (see P. J. Collings, *et al.*, *J. Phys. Chem. B*, 1999, 103, 8474–8481). Nonetheless, although a trivial relationship between the ICD intensity and the cluster concentration is expected, the g-factor should not depend on this concentration, since it is derived as the ICD value normalized to the extinction value at the maximum of Soret band.