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Conformations of a model protein revealed by an aggregating Cu^{II} porphyrin: sensing the difference†‡

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Aggregated t-CuP binds to poly-L-glutamate through supra-molecular interactions, revealing itself to be an extremely sensitive probe for the major conformations of the polymeric scaffold.

Structural motifs play a central role in the proper functioning of biomolecules and considerable effort has been focused on the search for molecular probes capable of reporting on specific conformations of nucleic acids and proteins.¹ Porphyrins are appealing candidates for this purpose because of their spectroscopic features which are markedly affected by self-aggregation and by the specific microenvironment.² Here we report on the chiroptical properties of an aggregating cationic metalloporphyrin, Cu^(II) 5,15-bis(*N*-methylpyridinium-4-yl)-10,15-bis(diphenylporphine) (t-CuP, Fig. 1a), on polyglutamate, as a model scaffold, and demonstrate that this porphyrin is a promising candidate as a structural probe of protein conformation. The copper(II) derivative was selected because of its stability over an extended pH range, its lack of pH-dependent and redox behavior (which would complicate the interpretation of experimental results), and its tendency to form extended assemblies.^{3–5} The analogous tetracationic Cu-porphyrin, which shows little tendency to aggregate, does not display sensitive, specific spectroscopic features required of a structural probe, losing its induced CD signal long before polyglutamate completely switches from helix to coil.⁶

The spectral features of t-CuP in the absence of poly-L-Glu (PLG) at 5 μM, no buffer or salt added, are shown in Fig. 1 (solid lines). The extinction spectrum (Fig. 1a) presents a rather broad B-band at 418 nm, accompanied by two weaker Q-bands at lower energy. As expected for an achiral molecule the corresponding CD spectrum (Fig. 1b) is silent, and the low intensity of scattered light (Fig. 1c) rules out the presence of large aggregates in solution.⁷ However, increasing temperature leads to significant changes in the UV/Vis extinction spectrum (Fig. S1†) suggesting that this porphyrin is an aggregate (probably a dimer) at room temperature, even at low concentration. Such small aggregates (dimers or oligomers) have been reported in low ionic strength (IS) aqueous solutions of the parent metal-free porphyrin.⁸ Addition of PLG (FW ~ 17 kD, 100 μM Glu residues, 2 mM buffer)

strongly affects all the porphyrin spectroscopic features, the changes being pH dependent. At pH 4.7 (Fig. 1, dashed lines), where the peptide adopts an α -helical conformation,⁹ the t-CuP extinction spectrum (Fig. 1a) displays a hypsochromic and broadened B-band at 410 nm. An induced conservative signal having a positive Cotton effect appears in the CD spectrum under these conditions (Fig. 1b)^{3,6,10} and a modest increase in intensity is observed in the resonance light scattering (RLS) spectrum (Fig. 1c) of the bound dimeric porphyrin (Fig. 1d, left). The appearance of an RLS signal in these samples implies some electronic coupling among the bound t-CuP dimers.

In the presence of PLG (100 μM) at pH 7.2, where the protein is in a random coil conformation,⁹ t-CuP spectral features (Fig. 1, dotted lines) are very different from those observed at low pH. The porphyrin extinction spectrum (Fig. 1a) displays a split B-band having broad components

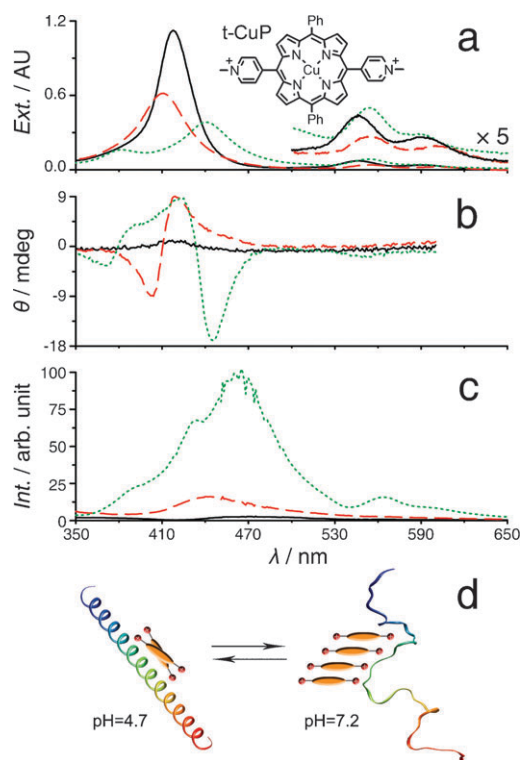


Fig. 1 Extinction (a), CD (b) and RLS (c) spectra of t-CuP in the presence of PLG as α -helix (pH = 4.7; dashed lines) or random coil (pH = 7.2; dotted lines). ([t-CuP] = 5 μM; [Glu] = 100 μM, FW ~ 17 kD; [buffer] = 2 mM). Solid curves: spectra of t-CuP (no protein). (d) Model for the interaction between PLG and t-CuP: (left) dimers on an α -helix; (right) aggregates on a random coil.

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† This paper is dedicated in memory of Prof. Raffaello Romeo.

‡ Electronic supplementary information (ESI) available: Experimental details; spectral changes on increasing *T* for t-CuP; spectra of t-CuP in MilliQ water or buffers; CD spectra for t-CuP/PGA on increasing pH; RLS spectra corresponding to Fig. 4. See DOI: 10.1039/b918433c

centered at 381 nm and 440 nm (minor and major, respectively) which, together with a strong signal in the RLS spectrum centered near the longer wavelength (Fig. 1c), point to the presence of large J-aggregates.¹¹ The CD spectrum (Fig. 1b) shows a rather complex induced signal, with a pair of bisignate features centered at the two extinction maxima and having opposite phasing. The formation of large t-CuP assemblies, normally observed under high IS conditions,¹² is here seen at 2 mM buffer, no salt added, by the interaction of the porphyrin with the completely deprotonated PLG strands (Fig. 1d, right).

The presence of a large ICD signal in the region of t-CuP extinction when the dye interacts with PLG at pH 7.2 may at first seem surprising. The degree of chiral information of PLG in a random coil conformation is much less than that of the α -helices, and for systems thus far studied, dyes interacting with this protein at pH \sim 7 have shown markedly reduced ICD signals as a consequence of the transition from helix to coil^{6,13} (unless other species act as stabilizers and allow a previously induced dye organization to retain its chiral memory).^{6,10,14} Only a few reports^{3,15} deal with a different signal phasing in the presence of the two different polypeptide conformations, and these usually employ high dye loads that might influence the structure of the final adducts. Here, the major CD feature (centered around 440 nm) displayed by the porphyrin aggregates upon interaction with the random-coiled protein presents an opposite (negative) Cotton effect as compared to that of t-CuP dimers bound to α -helices. This observation may reflect the different orientation of the porphyrin with respect to the protein scaffold and also agrees with recent results¹⁶ showing that at alkaline pH PLG folds as a poly-proline II (PPII) left-handed helix. Very likely, this ability of t-CuP to sense the chirality of the less organized random coil is afforded by the dye binding to the biopolymer strands in a highly aggregated state, leading to an amplification of the chiral information of the template ("antenna effect").¹⁷

The transition between α -helix and random coil has been studied through changes in the CD spectrum of the protein in the UV region.¹⁸ Fig. 2a shows that in a batch titration the trend of ellipticity (at 222 nm) vs. pH for neat PLG (100 μ M; data shown in black) is not modified markedly by the presence of the porphyrin (data shown in red; [dye]/[Glu] = 1/20); the presence of t-CuP does not perturb the conformational transition of the peptide. The helix to coil transition in the presence of the dye can also be monitored through changes in the spectral features of t-CuP. On increasing the pH, all spectroscopic techniques consistently reveal the conversion from t-CuP dimers on an α -helix to aggregates on a random coil (Fig. 2b). However, the inflection points displayed by the signals belonging to the porphyrin are shifted to a somewhat higher pH than that exhibited by the protein (indicated by the dashed vertical line in Fig. 2). This difference suggests that the two binding modes of t-CuP to PLG are not energetically equivalent, with porphyrin dimers bound to α -helices as the favored situation. As a consequence, the sensitivity to the presence of α -helical protein increases if the signal chosen as a probe belongs to the porphyrin: when the curve of the protein CD signal vs. pH has already reached the plateau in the high pH region, the signals of the t-CuP have achieved only half of

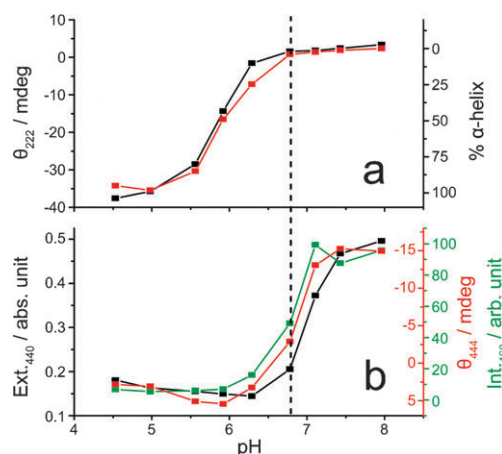


Fig. 2 (a) Changes of CD signal vs. pH for PLG solutions, with or without added t-CuP (black and red, respectively). (b) Changes in extinction, CD and RLS signals vs. pH for PLG/t-CuP. ([Glu] = 100 μ M, FW \sim 17 kD; [t-CuP] = 0–5 μ M; [buffer] = 2 mM.)

their dynamic range available to detect residual amounts of α -helices still present in solution.

Another important feature of the sensing capabilities of t-CuP becomes apparent in the presence of short PLG chains (FW \sim 1.5–3 kD) at around pH 4.5. It is known that the propensity of this peptide to fold as α -helices decreases dramatically for short strands, in line with the cooperativity of the coil to helix transition.^{18,19} For a short PLG, the coexistence of helix and coil conformations has been detected even at low pH by comparing CD signals with those displayed by longer biopolymers in the UV spectral region.¹⁹ When t-CuP (5 μ M) is added to a 20 μ M solution of short PLG at pH 4.7, the phase of the CD signal in the visible region (Fig. 3, solid line) is reminiscent of that observed in the presence of random-coiled peptides (Fig. 1b, dotted line). This observation is explained in terms both of the distribution of the peptide lengths and the face-on arrangement proposed for the interaction between the porphyrin dimers and the α -helical protein. Estimates of the distance between the two positive charges of t-CuP (\sim 15.5 Å) and the structural characteristics of the α -helix (pitch: 5.4 Å; residues per turn: 3.6) suggest that, for a dimer to bind to the helix with a parallel orientation of the dye planes to the helix axes, at least 10 contiguous Glu residues have to adopt this conformation. However, at pH 4.7 only a fraction of the short PLG is helical¹⁹ and when the length distribution of this peptide is also taken into account it becomes clear that the protein fraction suitable to allow the porphyrins to bind to helices as described above is even smaller. In agreement with this hypothesis, when 5 μ M t-CuP is added to a more concentrated (2 mM) solution of short PLG, at pH 4.7, the profile of the ICD signal in the visible spectral region (Fig. 3, dashed line) reverts to that attributed to dimers bound to helices: the overall increase in protein concentration causes a consequent increase of the fraction of strands having the appropriate length and helicity to allow the dimers to bind the protein in a parallel fashion.

The porphyrin response to the different conformations of PLG has also been tested under conditions usually leading to aggregated t-CuP,¹² *i.e.* in the presence of 0.15 M NaCl. The

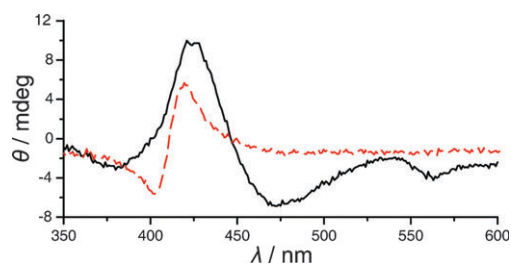


Fig. 3 CD spectrum of t-CuP in the presence of short PLG at pH = 4.7 and [Glu] = 20 μM , 2 mM (solid and dashed curve, respectively). (PLG FW \sim 1.5–3 kD; [t-CuP] = 5 μM ; [buffer] = 2 mM.)

addition of salt to the porphyrin/protein assemblies formed at pH \sim 4.5 (5 μM t-CuP, 100 μM protein) does not lead to appreciable changes in the optical features (Fig. 4, S4 \ddagger , solid lines). In the UV/vis spectrum the B-band is still found at 410 nm (Fig. 4a), the corresponding ICD signal is centered at the same wavelength and exhibits a positive Cotton effect (Fig. 4b), and the RLS signal is as weak as under low IS conditions (Fig. S4 \ddagger). Two main comments can be made about these results: (i) the hypothesis that the interaction of t-CuP dimers with α -helical PLG consists not only of an electrostatic component, but also of a hydrophobic one, is substantiated by the fact that the porphyrins do not detach from the biopolymers upon salt addition, and (ii) the stability of t-CuP/protein adducts is such that the typical aggregation phenomena shown by this porphyrin upon addition of 0.15 M NaCl are suppressed in the presence of the helical biopolymer. Also at pH \sim 7 (Fig. 4, S4 \ddagger , dashed lines) the optical properties of t-CuP/PLG adducts are not affected significantly by the presence of 0.15 M NaCl: the UV/vis extinction spectrum (Fig. 4a) is almost unchanged and, together with a strong RLS signal (Fig. S4 \ddagger), point to largely aggregated dyes, as in the absence of added salt. However, different from the batch titrations carried out under low IS that displayed a smooth transition between the two binding modes of t-CuP onto PLG, in the presence of 0.15 M NaCl very unusual optical features appear over a narrow pH range near 5.5 (Fig. 4, S4 \ddagger , dotted lines). The very broad UV/vis extinction and RLS signals (Fig. 4a, S4 \ddagger , respectively), covering almost completely the screened spectral range, point to the formation of extremely large J-aggregates. More remarkable is the presence of the most intense, unusual ICD signals observed so far for this system (Fig. 4b), with profiles that seem largely affected by a differential scattering component. These results are interpreted as due to t-CuP aggregating at a completely different binding site, *e.g.* interface regions where both helical and random coil conformations are present on the same strand of PLG. This hypothesis is also supported by pH dependence studies on polymers of different lengths in which higher intensity ICD signals are obtained for shorter peptides with respect to longer ones at pH 4.5 (data not shown). On increasing the polymer length, the interface regions are expected to decrease in importance due to cooperativity effects.

In summary, t-CuP is able to bind to PLG as a supra-molecular aggregate, revealing it to be a sensitive reporter for the major conformational structures of this polymeric scaffold. Its sensitivity opens the way to a variety of potential applications as a non-covalent chiroptical sensor.

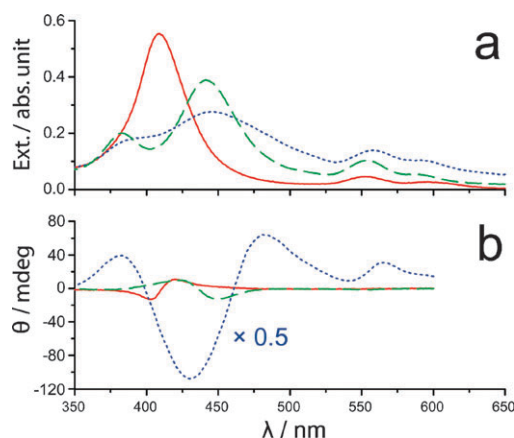


Fig. 4 Extinction (a) and CD (b) spectra of 5 μM t-CuP in the presence of PLG (pH = 4.58, solid line; pH = 5.5, dotted line; pH = 6.86, dashed line), at high IS. ([Glu] = 500 μM , FW \sim 17 kD; [buffer] = 2 mM; [NaCl] = 0.15 M.)

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