Journal of Inorganic Biochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Aggregates of a cationic porphyrin as supramolecular probes for biopolymers

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ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form 11 September 2015
Accepted 30 September 2015
Available online xxxx

Keywords:
Porphyrin
Supramolecular aggregate
Chiroptical sensor
Chirality
Circular dichroism

ABSTRACT

The copper(II) derivative of the dicationic *trans*-bis(N-methylpyridinium-4-yl)diphenylporphyrin (t-CuPagg) forms large fractal aggregates in aqueous solution under moderate ionic strength conditions. A kinetic investigation of the aggregation process allows for a choice of experimental conditions to quickly obtain stable assemblies in solution. These positively charged aggregates are able to interact efficiently with negatively charged chiral species, (including bacterial spores) leading to induced circular dichroism signals in the Soret region of the porphyrin, now acting as a sensitive chiroptical probe.

1. Introduction

The design and synthesis of supramolecular optical sensors for the detection of chiral molecules/biomolecules is actively investigated due to their implications both in fundamental and applicative fields [1]. In particular, it is important to find systems able to quickly report information on very diluted samples. Porphyrins have been extensively used to this purpose, due to their rich spectroscopic features, e.g., high extinction coefficients and resonant light scattering effects [2] that allow their use as sensitive probes. Hetero-aggregates based on water soluble porphyrins have been successfully exploited to detect and amplify traces of aggregated amino acids [3] and polypeptides [4–6]. The large circular dichroism (CD) signals observed for J-aggregates of the anionic *tetrakis*(4-sulfonatophenyl)porphyrin has been ascribed to the presence of optically active species (i.e. bacterial membranes) present as traces in ultrapure water [7], or to added chiral templates [8–12]. *Trans*-bis(N-methylpyridinium-4-yl)diphenylporphyrin and its copper(II) derivative (t-CuPagg, Fig. 1) are dicationic water soluble porphyrins that have been shown to form extended supramolecular aggregates on nucleic acids [13,14].

This species as well as its parent unmetalated porphyrin (t-H_2Pagg) form fractal assemblies whose structural features are modulated by the ionic strength [15–18]. These aggregates are quite large (sizes ranging from 100 nm up to 1–2 μm), flexible and porous. The strong electronic coupling among the neighboring chromophores, along with the extended dimensions of the aggregates, is responsible for large resonant light scattering signals in their aqueous solutions [2]. Quite recently, t-CuPagg porphyrin in its dimeric [19] and largely aggregated form [20] has been proposed as chiroptical sensors for conformations and chirality of poly(glutamate). In particular, t-CuPagg studies have shown the presence of a left handed helix (poly-proline-II) and the coexistence of different conformations for this simple polypeptide [19]. Here we report an investigation on the kinetics of formation of aggregates of t-CuPagg and their use as chiroptical probes for a variety of chiral negatively charged biopolymers, i.e. calf thymus DNA, polyglutamate and human serum albumin (HSA), as well as bacterial spores.

2. Experimental

2.1. Materials and methods

5,15-bis(N-methylpyridinium-4-yl)-10,15-bis-diphenylporphine (t-H_2Pagg, chloride salt) was purchased from Frontier Scientific and used as received. Its copper(II) derivative (t-CuPagg) was prepared by literature procedures [21]. Porphyrins stock solutions were prepared dissolving the solids in dust-free water (Galenica Senese) and stored in the dark. Solution concentrations were determined from known molar extinction coefficient at the Soret maximum (t-CuPagg: 2.34×10^5 M^{-1} cm^{-1}) [13].

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http://dx.doi.org/10.1016/j.jinorgbio.2015.09.013
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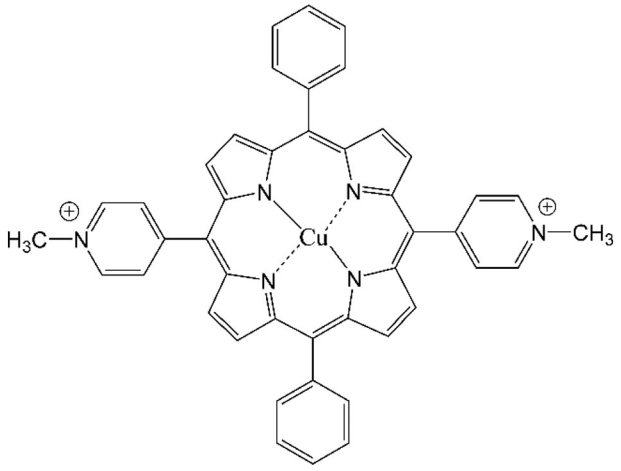

Fig. 1. Structure of the copper(II) derivative of *trans*-bis(N-methylpyridinium-4-yl)diphenylporphyrin (t-CuPagg, chloride salt).

Calf thymus DNA (ct-DNA, type I) was obtained from Sigma and was purified according to literature methods [22]. The concentration of a ct-DNA stock solution in 1 mM phosphate buffer, pH 7, containing 10 mM NaCl, was determined in molar base-pairs using ε_{260} = 13,200 M^{-1} cm^{-1} [23]. Sodium salts of poly-L-glutamic acid (FW ~17 kD and ~1.5 kD) were obtained from Aldrich Chemicals Co. Stock solution of the polypeptides were prepared by dissolving the solids in acetate buffer at pH 4.2 (ionic strength 5 mM). Concentrations of the peptide solutions were determined spectrophotometrically, using ε_{205} = 2150 M^{-1} cm^{-1} [24]. Human Serum Albumin (HSA) was purchased from Sigma and stock solutions were prepared by dust-free Millipore water in phosphate buffer 0.01 M pH = 7.4. Aqueous suspensions of spores of *Bacillus clausii* were prepared from commercial samples (8×10^8 units/mL, Sanofi), removing the excipients through dialysis against Millipore water. All other reagents were obtained from Aldrich Chemicals Co. and used as received without further purification.

Fractal aggregates of t-CuPagg were freshly prepared by adding a concentrated sodium chloride solution (0.06–0.1 M) to an aqueous solution of the porphyrin (5 μM). When needed, an appropriate buffer was added to set the pH. The chiral biopolymers were added as last reagents to these preformed aggregates.

UV–Vis absorption and extinction spectra were measured on an Agilent mod. 8453 diode-array spectrophotometer using 1 cm path length quartz cells (Hellma). Resonance light scattering (RLS) experiments were performed on a Jasco model FP-750 spectrofluorimeter using a synchronous scan protocol with right angle geometry [2]. The circular dichroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter, equipped with a 450 W xenon lamp. CD spectra were corrected both for the cell and solvent contributions. The dissymmetry g-factor has been calculated as (ΔA/A) [25].

The kinetic measurements (extinction) were followed at fixed wavelength by mixing the reagents in a silica cell in the thermostated compartment of the instrument, with a temperature accuracy of 0.1 K. The average aggregation time, τ_E, defined according to the equation $\tau_E = \int I(t)dt/I(0)$ was evaluated by numerical integration of the observable $I(t) = Ext(t) - Ext(\infty)$ versus time, divided by $I(0) = Ext(0) - Ext(\infty)$ (where Ext(t), Ext(0) and Ext(∞) are the extinction at time t, at t = 0 and after completion of the reaction, respectively) [18].

3. Results and discussion

3.1. t-CuPagg

The UV–Vis extinction spectra of t-CuPagg at micromolar concentration in neat water exhibit a B band at 418 nm, together with two Q bands at 545 and 590 nm. Under these conditions, the presence of extended aggregates can be ruled out by the low intensity in the resonance light scattering (RLS) spectra, that show only a minimum at 418 nm, due to photon loss by absorbance of the sample. Previous investigations have pointed out the dimeric nature of this porphyrin even at very low concentrations [19]. When the ionic strength of the solution is increased, large spectral changes are observed in the extinction and RLS spectra. Fig. 2 shows a typical UV–Vis spectral change observed in time after adding sodium chloride (0.1 M) to a 3 μM solution of t-CuPagg. The decrease of the B band is paralleled by an increase of a new band at 444 nm, that is ascribed to extended porphyrin aggregates, as confirmed by the large enhancement in the RLS spectra (strong peaks between 350 and 650 nm, Fig. 3).

In order to find a range of experimental conditions to form these species, we investigated the effect of added sodium chloride in the range of 2–150 mM on the kinetics of aggregation and the final distribution between non-aggregated and aggregated porphyrin for a 5 μM aqueous solution of t-CuPagg. When [NaCl] < 15 mM, the UV–Vis extinction spectra display only a slight decrease of the 418 nm band. Above this concentration, the band at 444 nm, corresponding to the aggregated porphyrin, begins to appear, first as a shoulder, then for [NaCl] > 50 mM as the main spectral feature in this region (Fig. 4a–b). The corresponding aggregation kinetics have been monitored and, as expected, they become faster on increasing the ionic strength. The extinction traces follow a typical sigmoidal profile with an initial lag time [26,27]. The mean aggregation times have been determined and the values are reported in Fig. 5.

On the bases of these experimental findings, in order to have a quite rapid (<60 s) and complete formation of aggregates in solution, we chose to use [NaCl] = 0.06–0.1 M. A higher concentration of salt is to be avoided due to the instability of the colloidal system, leading to precipitation of the assemblies. The behavior of this porphyrin is similar to that of the parent t-H_2Pagg, although the latter species requires higher ionic strength conditions to achieve a complete aggregated state [18]. This difference can be ascribed to the coordination of copper(II) ion into the macrocycle core, that makes it more rigid and less flexible [26,27]. An analogous pattern could be found in the case of *tetrakis*(N-methylpyridinium-4-yl)porphyrin and its copper(II) derivative, the latter being much more prone to aggregation than the metal free derivative [28].

3.2. t-CuPagg@ct-DNA

The supramolecular assembling of t-CuPagg onto nucleic acids has been reported in literature, using a completely different protocol [13]. Since porphyrins are able to intercalate into the double stranded helix

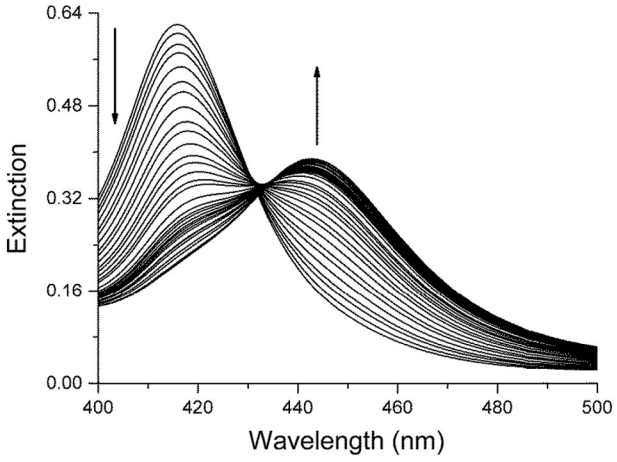

Fig. 2. UV/Vis extinction spectral changes after adding NaCl (final concentration: 0.1 M) to a solution containing 5 μM t-CuPagg (T = 298 K, scanning time 1 s).

Please cite this article as: I.G. Occhiuto, et al., Aggregates of a cationic porphyrin as supramolecular probes for biopolymers, J. Inorg. Biochem. (2015), http://dx.doi.org/10.1016/j.jinorgbio.2015.09.013