Nanomolar determination of copper(II) and zinc(II) using supramolecular complexes of *meso*-tetrakis(4-*N*-methylpyridyl)porphine on polyglutamate

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The remarkable acceleration of Cu^{II} and Zn^{II} insertion in cationic porphyrins monodispersed on a polyglutamate anionic surface allows determination of the two metal ions down to nanomolar concentrations.

In the last few years a large number of fluorescent sensors for pH or metal ions have been designed and synthesized by means of a supramolecular 'covalent' approach.[1] Most of them are two-component systems in which a 'receptor' unit is covalently linked to a 'sensing' one.

Owing to their high absorption coefficients and tunable fluorescence emission, porphyrins are excellent sensing units.[2] The introduction of polar groups at the periphery of the porphine ring makes them water-soluble and allows the formation of 'non-covalent' supramolecular species on oppositely charged templates.[3] Using this alternative supramolecular approach, it has been recently shown that the complexes of the tetraanionic *meso*-tetrakis(4-sulfonatophenyl)porphine on polylysine can be effective as pH-sensors.[3a] This 'non-covalent' strategy therefore allows one to achieve specific photochemical sensors‡ by mixing commercially available chemicals. On the other hand, the formation of these supramolecular complexes depends upon several factors (such as pH, ionic strength, *etc*.) and consequently they are less 'robust' than those obtained by the classical 'covalent' approach.

This report deals with supramolecular complexes formed by the tetracationic *meso*-tetrakis(4-*N*-methylpyridyl)porphine (H_2T4) and polyglutamate. As a result of a remarkable increase of the metallation reaction rate, this complex behaves as sensitive and specific fluorescent sensor for the determination of nanomolar concentrations of biologically relevant metal ions such as Zn^{II} and Cu^{II}.

Indeed, porphyrin metallation could be used, in principle, as a simple tool for analytical determination of trace amount of metal ions because, in most cases, the absorption and/or the emission properties of the metallo- and the free-porphyrins are quite different from each other, so that the two species can be clearly distinguished.§ Despite this, these reactions have been rarely used for analytical applications,[5] a major limitation being the slow rate of metal insertion.

Under the experimental conditions used in this work (succinate buffer 2 mM, pH = 5.6, [H_2T4] = 1 μM, [poly-L-glutamate] = 200 μM in residue) H_2T4 is mainly monodispersed on the anionic polypeptide.¶ In fact, only a small red shift ($\Delta\lambda$ = 4 nm) and hypochromicity (15%) of the Soret band was observed, while any characteristic feature of porphyrin assembly formation (such as resonance light scattering[6] or induced circular dichroism signals in the Soret region) was absent. Absorption experiments show that the addition of Zn^{II} to such H_2T4–polyglutamate supramolecular complexes leads to a shift of the Soret band at 440 nm (Fig. 1). Also, the main Q-band of H_2T4 (518 nm) is progressively replaced with that of ZnT4 (562 nm) (Fig. 1). These spectroscopic features are diagnostic

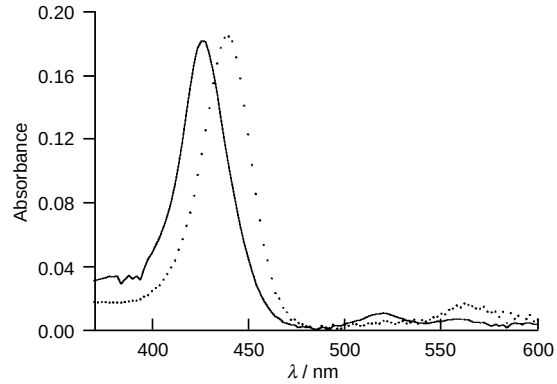

Fig. 1 Absorption spectra (pathlength = 1 cm) of H_2T4 (1 μM) in the presence of polyglutamate (200 μM) at pH 5.6 (succinate buffer 2 mM), before (—) and after (···) the addition of $ZnSO_4$ (0.05 μM)

of the formation of ZnT4. Note that the addition of successive aliquots, each containing a few pmol of Zn^{II}, leads to a linear increase of the fluorescence intensity at 622 nm‖ (ZnT4 emission maximum), as shown in Fig. 2.

The presence of polyglutamate turns out to be essential to observe a reasonable time response of the method. In the presence of the anionic polypeptide, Zn^{II} insertion was completed in about 10 min whereas in its absence, even after 24 h no evidence of metallation was observed *i.e.* the formation of the polyglutamate–H_2T4 supramolecular complex 'catalyzes' the insertion of Zn^{II}. Preliminary kinetic data show that the metallation rate under such conditions is about 1000 times higher compared to the 'uncatalyzed' reaction.**

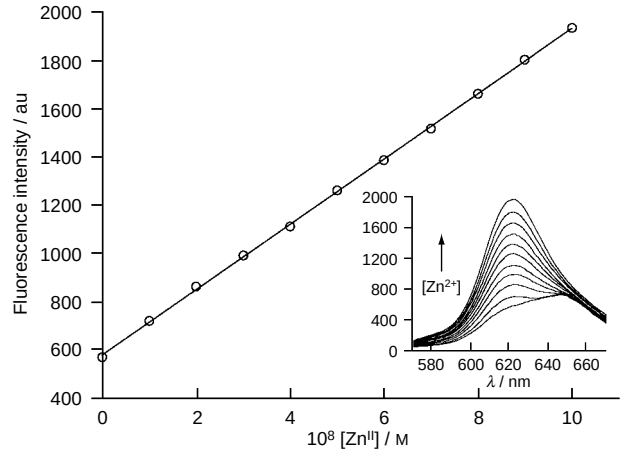

Fig. 2 Plot of fluorescence intensity at 622 nm (λ_{ex} = 445 nm) *vs.* Zn^{II} concentration, for a solution of H_2T4 (1 μM) in the presence of polyglutamate (200 μM) at pH 5.6 (succinate buffer 2 mM)

A similar behaviour was observed for the same system when Cu^{II} was employed in place of Zn^{II}. Here, the formation of the metalloporphyrin was confirmed by the gradual replacement of the H_2T4 Q-band (518 nm) with that of CuT4 (550 nm), as the Cu^{II} concentration was increased. However, since the CuT4 formed is non-fluorescent, we monitored the disappearance of H_2T4 monodispersed on polyglutamate. Here, as well, a plot of H_2T4 emission fluorescence intensity (λ_{ex} = 422 nm, λ_{em} = 655 nm) *vs*. Cu^{II} concentration (Fig. 3) shows a linear behavior.

Interestingly, the pH dependence of the metallation rate is also influenced by the presence of polyglutamate. For example, it has been reported that, in the presence of nitrate, the metallation rate is almost unaffected in the pH range 4.4–5.5 and accelerated at higher pH.[7] On the contrary, in the presence of polyglutamate the metallation rate increases with pH (*i.e.* with the number of negative charges on the polypeptide) in the range *ca*. 4.5–5.5 and then does not increase further at higher pHs. Also, at pHs lower than *ca*. 4.5 the insertion reaction is not catalyzed by polyglutamate. This observation suggests that, unlike other ligands,** the 'catalytic' role of the anionic template must be related to its electrostatic field by (i) increasing the 'local' concentration of metal ions,[8] and (ii) partially shielding the positive charges of the H_2T4 monodispersed on it (thus facilitating the approach of the cationic metal ions to the cationic porphyrins). As expected according to this hypothesis, no appreciable increase of metallation rate was observed when Zn^{II} or Cu^{II} were added to the solution containing the tetraanionic *meso*-tetrakis(4-sulfonatophenyl)-porphine monodispersed on the protonated polylysine. Most likely, the state of the porphyrins also plays a role in the reaction rate trend observed at pH lower than *ca*. 4.5. Decreasing the pH, H_2T4 tends to aggregate on polyglutamic acid.¶ In this case, the porphyrins face-to-face arrangement[9] should reduce the accessibility of the metal ions to the porphine 'core'. This hypothesis is in good agreement with previous experiments by Pasternack *et al.*[10] which show that aggregation and/or intercalation of porphyrins onto or into synthetic DNA have a negative influence on porphyrin metallation rate. Therefore, the nature of the matrix and of the supramolecular complex (*i.e.* of the molecular recognition processes) is crucial to allow the catalysis of the metal insertion by an anionic template.

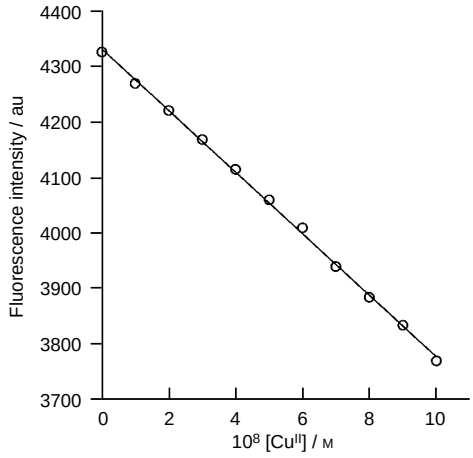

Fig. 3 Plot of fluorescence intensity at 655 nm (λ_{ex} = 422 nm) *vs.* Cu^{II} concentration, for a solution of H_2T4 (1 μM) in the presence of polyglutamate (200 μM) at pH 5.6 (succinate buffer 2 mM)

Finally, the addition of other metal ions, such as Co^{II}, Fe^{II}, Mg^{II}, Mn^{II} and Ni^{II} does not cause any of the spectroscopic variations described above in a time interval comparable to that observed for Zn^{II} and Cu^{II}. A reasonable explanation for this behavior is that the 'uncatalyzed' rate of insertion for Co^{II}, Fe^{II}, Mg^{II}, Mn^{II} and Ni^{II} in H_2T4 is much lower than that of Zn^{II} and Cu^{II}.

In conclusion, a selective and very sensitive supramolecular sensor for metal ions has been obtained by means of extremely simple routes. To date, the non-covalent nature of these species, and therefore their relatively low robustness, has hindered their application for the development of chemical devices. Further studies are currently in progress in our laboratory to investigate possible technological applications.

Notes and References

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‡ The term sensor in the following context is not used to indicate a device, but rather a chemical system able to recognize a species and to report its recognition (*e.g.* by spectroscopic variations).

§ For example, absorption and emission maxima for ZnT4 and H_2T4 are λ_{ex} = 436 nm, λ_{em} = 622 nm, and λ_{ex} = 422 nm, λ_{em} = 655 nm, respectively. On the other hand, the insertion of Cu^{II} causes a complete quenching of the fluorescence of H_2T4 (a weak emission, centered at *ca.* 770 nm, has been found in water, but only when CuT4 is intercalated into natural or synthetic DNAs).[4]

¶ H_2T4 is aggregated on polyglutamate in the pH range 3.4–4.5, and is monodispersed on the polypeptide both at higher and lower pHs (E. Bellacchio, S. Gurrieri, L. Monsù Scolaro and R. Purrello, in progress).

‖ In order to minimize the contribution of H_2T4 fluorescence, the excitation wavelength used in these experiments is 445 nm.

** Acceleration of porphyrins metallation has been also observed in the presence of simple ligands such as acetate, pyridine, ammonia, nitrate and ascribed to labilization of the water molecules bound to the metal ion.[7]

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