METALLATION OF PROTOPORPHYRINS USED AS FLUORESCENT CHEMOSENSOR FOR IMIDAZOLE RECOGNITION

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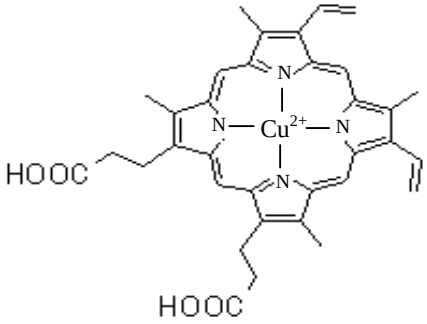
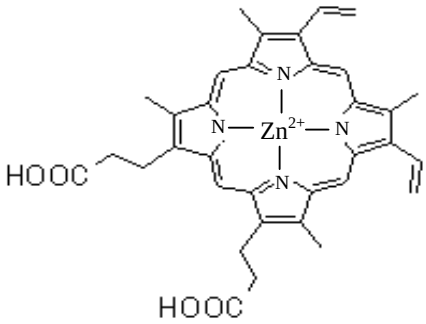
Abstract

Metalloporphyrin Complexes play significant roles in many biological and catalytic systems. The diversity of their functions is due in part to the variety of metals that bind in the "pocket" of the porphyrin ring system. Two kinds of metalloporphyrin derivatives, Cu (II) and Zn (II) protoporphyrins (PP), were microscale synthesized ((1) and (2)) and characterized by spectroscopic methods and magnetic measurements. A PP ligand bound to each metal center in a tetradentate fashion including four amine nitrogen atoms in the equatorial planes. These complexes were found to recognize imidazolyl groups of histidine and histamine derivatives as guest molecules by coordination and additional non-covalent interactions. These added analytes displace the selective fluorescent indicator, which when released to the solution displays its full fluorescence. Thus, analyte recognition is signaled by the sharp appearance of the fluorescence of the indicators. The binding affinities of (1) and (2) to histidine and histamine were investigated and accounted for different complexation properties. Moreover, we demonstrated that careful choice of a fluorescent indicator with tuned affinity toward the receptor can provide discrimination in sensing of a desired substrate and the role that the metal coordination plays on the hypsochromic shift and loss of fluorescence distincted characteristics of hypsoporphyrins were also discussed.



(1)



(2)

Keywords : Metallation, Imidazole recognition, Histidine, Histamine, Protoporphyrin, Fluorescent chemosensor.

Introduction

Considerable research has been recently focused on the specific detection of amino acids because of their important roles in biological, industrial, and environmental process (Enriz and Jauregui, 1990; Morel *et al*., 1990; Hortalá *et al*., 2002; Tong *et al*., 2002; Chow *et al*., 2003; Ho and Lecterc, 2003; Zheng *et al*., 2003; Hanaoka *et al*., 2004; Kojima *et al*., 2005). Many systems capable of recognizing, sensing, and transporting various amino acid species are based on supramolecular chemistry (Ho and Leclerc, 2003), which allows one to design amino sensing receptors with new selectivities. Fluorescent organic chemosensors for the detection of imidazole derivatives with high selectivity and sensitivity has always been of particular interest (Zheng *et al*., 2001; Hortalá *et al*., 2002; Tong *et al*., 2002; Chow *et al*., 2003; Hanaoka *et al*., 2004). Structurally, ionophore

and fluorophore are two essential parts determining the resultant performance of the chemosensor. One strategy employed in the design of chemosensors for the imidazole based amino acid is to link a fluorophore unit with a metal binding unit (ionophore). The two units are linked to each other in such a way that the binding of an imidazolate anion to the ionophore causes considerable changes in the fluorescence of the fluorophore. Such changes can be intensity, intensity ratio, anisotropy, time-domain lifetime or phase- modulation lifetime, etc (Zheng *et al*., 2001). The high sensitivity and abundance of fluorophores makes fluorescence technique among one of the most promising tools for chemo-and biosensor development. However, the selectivity of fluorescent chemosensors for imidazole resnains a significant challenge.

We studied here fluorescent chemosensor based on the metallations of proto-porphyrins by Cu(II) and Zn(II) ions to form metalloporphyrin derivatives. Metallo-porphyrin complexes play significant roles in many biological and catalytic systems (Marsh and Mink, 1996; Tong *et al.*, 2002; Saucedo and Ming, 2005; Kojima *et al*., 2005). The diversity of their functions is due in part to the variety of metals that bind in the "pocket" of the porphyrin ring system. Specifically, our systheses involve a ragne of microscale preparation, characterization and fluorescent reactivity. We chose a market production, unmetallated protoporphyrin-IX (PP) shown in Figure 1 and synthesized Cu(II)-PP and Zn(II)-PP complexes. In this paper, we describe synthesis and characterization of those complexes and their application to molecular recognition toward functionalized imidazole group.

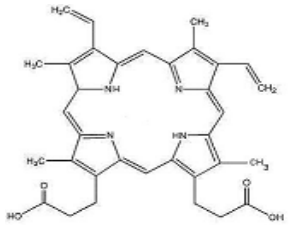


Fig. 1: Unmetallated protoporphyrin (PP)

Materials and Methods

Materials and Instrumentation

Protoporphyrin IX (8,13 Bis-(vinyl)-3,7,12,17-tetramethyl-21 H, 23 H-porphine-2, 18-dipropionic acid), PP, zinc acetate dihydrate (AR grade), histidine (AR grade) and histamine (AR grade) were purchased from Aldrich-Fluka. Copper nitrate (AR grade) was bought from AS FineChem Company. N,N-dimethyl formamide and deuterated dimethyl sulfoxide were the products of Fisher Scientific Co. Ltd. Other chemicals were of analytical grade, obtained from Ajex Chemical and Merck Co. Ltd., and used as received without further purification. ^{1}HNMR spectra were obtained in D_2O, $CDCl_3$, CD_2Cl_2 and deuterated DMSO used as purchased. Spectra were recorded on a Bruker ADVANCE 300 (300 MHz). FTIR spectra were recorded on Bio-Rad FTS 175 spectrometer in the 4000 – 400 cm^{-1} range with KBr pellet. UV-Vis spectra were obtained on a Perkin-Elmer LAMBDA35 UV-Vis spectrophotometer at room temperature. Fluorescence spectra were recorded with a Hitachi f-2500 fluorescence spectrophotometer.

Microscale synthesis of Cu(II)-PP

To a solution of 8,13-Bis (vinyl)-3,7,12,17-tetramethyl-21H, 23H-porphine-2, 18-dipropionic acid (0.080 g, 0.142 mmol) in DMF (72 mL) was added with $Cu(NO_3)_2.2.5\ H_2O$ (0.100 mg, 0.43 x 10^{-3} mmol) as solids. The mixture was refluxed at 80^{o}C for 30 min and allowed to warm up to room temperature to form greenish precipitate imidiately. The mixture was dried up by rotatory evaporator to afford blue-green powder, which was washed with small volume of cold water. Work-up procedure and purification method were similar to those of Saucedo and Mink, 2005. The suspension was then filtered to obtain green solid of Cu(II)-PP (53.42 % yield).

Microscale synthesis of Zn(II)-PP

This complex was prepared by the same method as that for Cu(II)-PP in 69.77 % yield.

Spectroscopic characterization of Cu(II)-PP and Zn(II)-PP

Metalloporphynin complexes can be characterized by UV-Vis absorption ^{1}HNMR spectroscopy and FTIR spectroscopy without further purification. The visible spectra (400 – 700 nm) or all compounds in DMF (0.1 mg/mL) can be obtained including the unmetallated protoporphyrins(PP). The NMR samples were prepared by dissolving 5.0 mg of each compound in 0.7 mL DMSO solvent. The infrared spectra of both complexes, PP and any further products were measured and any readily attributable intense bands were assigned. The three solutions (CU(II)-PP, Zn(II)-PP and PP) in DMF can be tested for fluorescence with a fluorescence spectrophotometer. The spectra were interpreted and discussed.

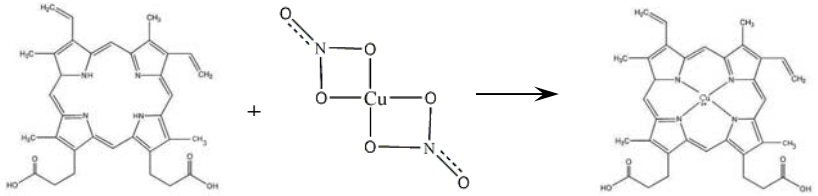
Imidazole recognition studies

Fresh standard solutions of Cu(II)-PP and Zn(II)-PP in various concentrations (16.0 – 64.0 μM) were prepared. Excitation and emission spectra of these complexes were recorded. The chemosensing ensemble solutions for competition assays were prepared by adding aliquots of a fresh solution of histidine or histamine to these chemosensing ensemble solutions**.** Concentrations of histidine and histamine aqueous solutions were varied in the range of 32.2 to 540.0 μM. Determination of molecular sensing of Cu(II)-PP and Zn(II)-PP receptors was performed by mixing a series of histidine (or histamine) solutions to a standard solution of Cu(II)-PP or Zn(II)-PP in a 5 mL test tube at room temperature. The mixture was transferred into a 1 cm cuvette, the mixture was stirred 30 s before the fluorescence emission spectrum was recorded. A stable reading was thus obtained with the standard deviation less than 0.5 %. Three repeated measurements of the suspension were performed and the average maximum intensity at 494 and 467 nm for Cu(II)-PP and Zn(II)-PP, respectively were recorded as *I* (the fluorescence intensity in the presence of histidine (or histamine) and I_o (the fluorescence intensity in the absence of histidine (or histamine)).

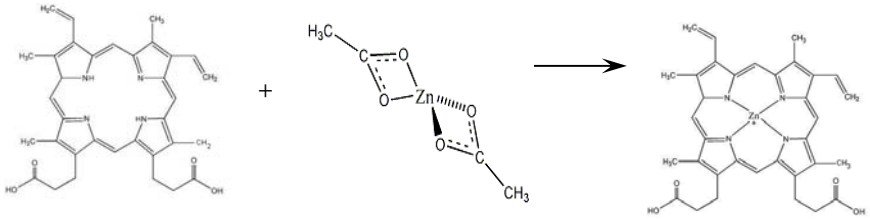
Results And Discussion

Characterization of Cu(II)-PP and Zn(II)-PP

A Cu(II) and a Zn(II) complex having PP as a tetradentate ligand, was assembled via the reaction depicted in *Scheme 1* and *Scheme 2*, respectively.



Scheme 1



Scheme 2

The full ^{1}HNMR spectra of PP and Zn(II)-PP in deuterated DMSO Figure 1 and Figure 2, respectively with solvent peak at 2.49 ppm.

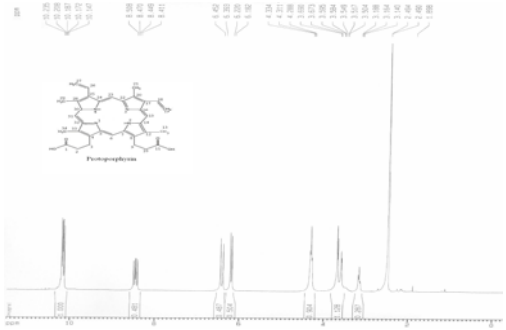


Fig. 1: ^{1}H NMR of protoporphyrin in deuterated DMSO. Solvent peak of deuterated DMSO at 2.49 ppm.

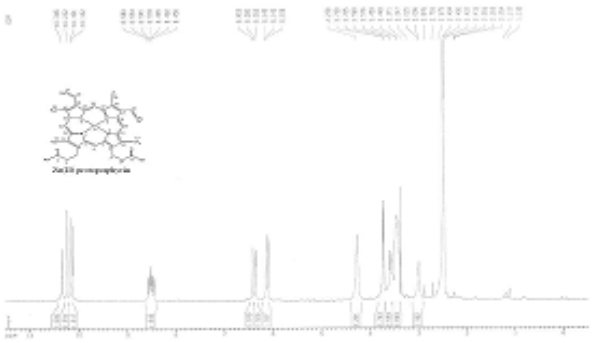


Fig. 2: ^{1}H NMR of Zn(II) protoporphyrin indeuterated DMSO. Solvent peak of deuterated DMSO at 2.49 ppm.

FTIR spectra for PP compound with Cu(II), (1), and Zn(II), (2), are shown in Figure 3, 4 and 5, respectively and are summarized as the followings : PP 3856 – 3314 [ν (NH, CH_3 stretching)], 1696, 1628 [ν (C=C, C=O stretching)], 1431, 1379 [ν (C=N, C=C)]; (1) 3417 [ν (O-H stretching)], 1651, 1559, 1386 [ν (C=O, C=N, C=C stretching)]; (2) 3010, 2914 [ν]C-H stretching)], 2857 [ν (O-H)], 1565, 1534, 1444 [ν (C=O, C=N, C=C)].

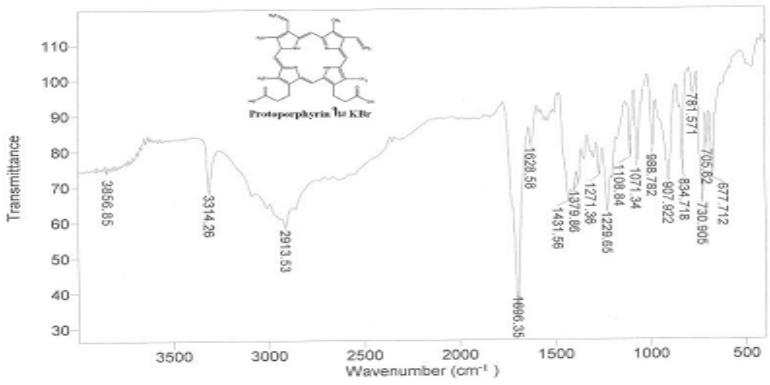


Fig. 3: IR spectrum of PP at 300 K.

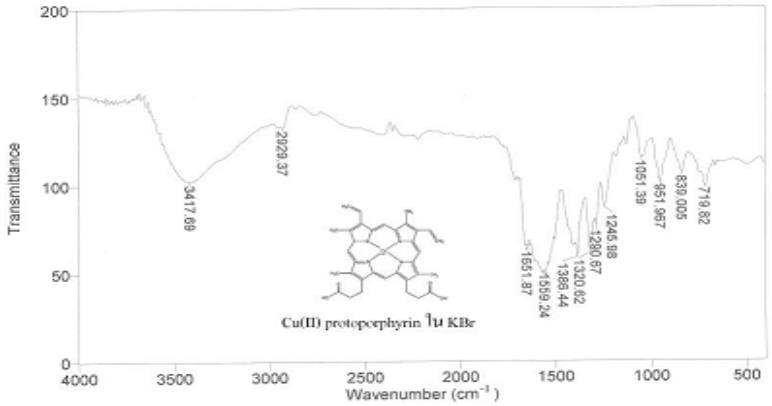


Fig. 4: IR spectrum of Cu(II)-PP at 300 K.

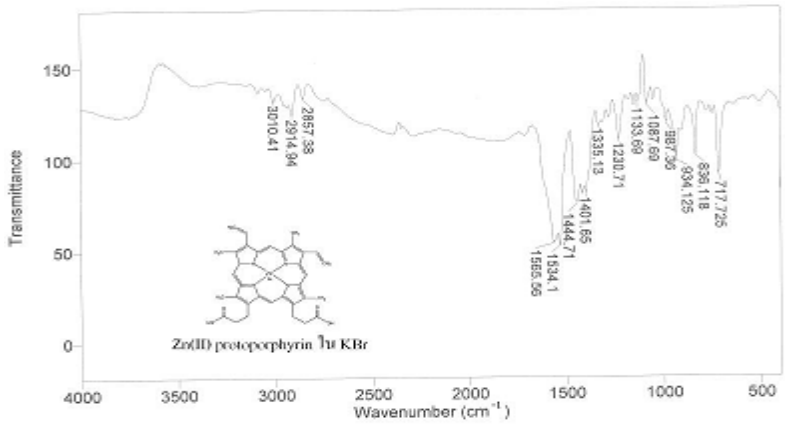


Fig. 5: IR spectrum of Zn(II)-PP at 300 K.

Spectroscopic properties of Cu(II)-PP and Zn(II)-PP

The absorption spectra for the unmetallated protoporphyrin exhibit intense bands between 422 – 630 nm (Figure 6). Upon metallation the absorption spectra is simplified. An intense bond for each metalloprotoporphyrin is observed (Figure 7).

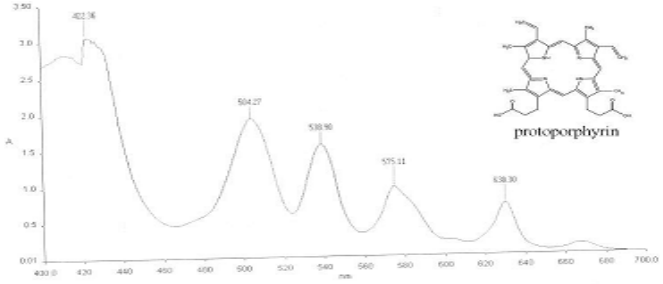
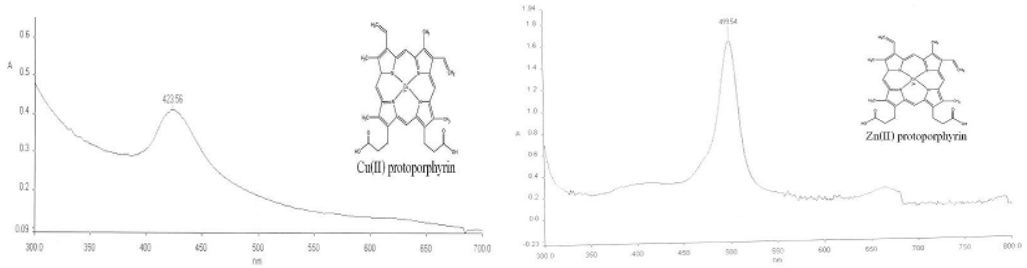


Fig. 6: UV-Vis absorption spectrum of PP (50 ppm in DMF, pH 7.4) at 300 K.



(a) (b)

Fig. 7: UV-Vis absorption spectra of (a) Cu(II)-PP. (b) Zn(II)-PP.

The fluorescence spectra for unmetallated and metallated protoporphyrins are shown in Table 1.

Table 1: Flurrescence intensities PP, Cu(II)-PP and Zn(II)-PP

Receptor	Excitation		Emission	
	Wavelength (nm)	Intensity	Wavelength (nm)	Intensity
PP	441	82	634	116.3
	495	118		
	506	118		
	539	114		
	580	101		
	633	91		
Cu(II)-PP	494.5	7024	492	7000
Zn(II)-PP	466.5	7564	467.5	7235

Table 1 compares the fluorescence intensity of protoporphyrin when covalent binding with different metals. It is clear that fluorescence intensity of pure PP disappeared with the metallation of Cu(II) and Zn(II) ions. A typical metalloprotoporphyrin emission spectrum with a maximum emission band contered at 492 and 468 was observed for Cu(II) and Zn(II), respectively.

Binding mode studies and fluorescence sensing toward imidazolyl group

Subsequently, we tested the sensing capability of these receptors for imidazolyl groups of histidine and histamine. In previous works (Hortalá, L. *et al*., 2002; Tong, A. *et al*., 2002; Panpae *et al*., 2007), it was preliminarily reported that the various receptors could preferentially sense a series of amino acids with and without imidazolyl groups. To understand such recognition of our receptors in detail, we prepared Cu(II)-PP and Zn(II)-PP and evaluated the binding affinity by determination of fluorescence intensity change. Such quenching effect occurred when Cu(II)-PP and Zn(II)-PP were bound with hisdidine or histamine. Fluorescence intensity change of the Cu(II)-PP with histidine as well as the Cu(II)-PP with histamine (Figure 8 and 9). This suggests that the carboxylate or ethylamine residues in target molecules are not responsible in guest binding and the selectivity. It is clear that the fluorescence intensity change of the Cu(II)-PP with imidazolyl derivatives was little larger than that of the Zn(II)-PP (the results of histamine recognition was not shown here).

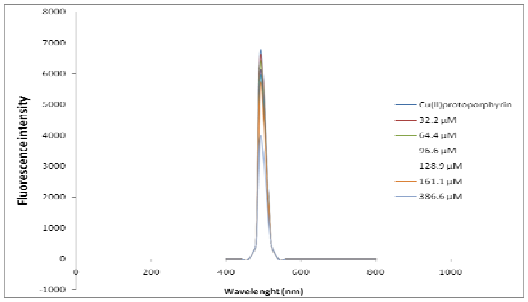


Fig. 8: Fluorescence spectral change of Zn(II)-PP (80.0 µM) upon addition of histidine: [histidine] = 32.2, 64.4 96.6, 128.9 and 386.6 µM in DMF, pH 7.4 at 20°C and λ_{ex} = 495 nm.

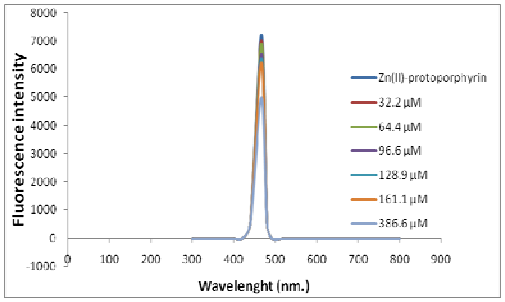


Fig. 9: Fluorescence spectral change of Cu(II)-PP (80.0 µM) upon addition of histidine: [histidine] = 32.2, 64.4 96.6, 128.9 and 386.6 µM in DMF, pH 7.4 at 20°C and λ_{ex} = 467 nm.

The recognition behaviors of Cu(II)-PP and Zn(II)-PP toward imidazolyl group was given further screenity in viewpoint of apparent binding constant. (Table 2).

Table 2: Apparent binding constant $(K_b)^*$ for the interactions of the receptors with histidine and histamine

K_b of histidine (M^{-1})			K_b of histamine (M^{-1})		
[histidine] µM	Cu(II)-PP x 10^3	Zn (II)-PP x 10^3	[histamine] µM	Cu(II)-PP x 10^3	Zn (II)-PP x 10^3
32.2	1.06	0.91	45	1.06	0.84
64.4	1.26	1.11	89.9	1.07	0.91
96.6	1.31	1.19	134.9	1.09	0.95
128.9	1.38	1.20	179.9	1.18	1.00
161.1	1.42	1.26	224.8	1.29	1.13
386.6	2.11	1.28	539.6	1.36	1.26

- Apparent binding constant defined by the equation $K_b = [(M(II)\text{-}PP)\ (G)_{tot}] / [M(II)\text{-}PP]_{tot}\ [G]_{tot}$, where $[(M(II)\text{-}PP)(G)]_{tot}$, $[M(II)\text{-}PP]_{tot}$ and $[G]_{tot}$ are the total concentrations of M(II)-PP(G) (metalloprotoporhyrin-guest complex); M(II)-PP (metalloprotoporphyrin) and G (guest : histidine or histamine) at a given pH (7.4). Measured in DMF solution.

The evaluated binding constants for imidazole are in good agreement with other fluorescent chemosensing ensembles (Hortalá, L. *et al*., 2002). Therefore, we can conclude that the fluorescence change in the receptors directly reflects the binding to the imidazolyl group. It is noteworthy that even the structurally simple receptors show such a dependent recognition toward the imidazole as a fundamental binding motif.

Conclusion

We present here the microscale preparation, characterization and chemosensing reactivities of a rigid square phanar Cu(II)-PP and Zn(II)-PP that has grown out of our research to recognize imidazolyl groups of histidine and histamine. Our experimental results clearly indicated that receptors (1) and (2) can effectively bind to an imidazolyl group on a histidine or histamine under neutral aqueous conditions by the cooperative use of the

metal-ligand interaction of the Cu(II) and Zn(II)-PP sites. Binding by the imidazolate residue of constant concentrations of histidine and histamine has been confirmed by the fact that plain imidazole shows higher binding affinity for Cu(II)-PP than Zn(II)-PP. Although the binding affinity of the receptors between the fluorescent based protoporphyrin ring and biological histidine derivatives (histidine amino acid and histamine) is not satisfactory, because of these K_{bind} are rather low binding constants of a synthetic receptor to an amino acid species in water reported so far, elaborated structural designation could provide artificial chemoreceptors with a high recognition toward an imidazolyl group. Furthermore, such artificial receptors may serve a potential regulator of metal-induced quenching of the bound of fluorescent probe and provide discrimination in sensing of a desired substrate.

Acknowledgements

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