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RESEARCH COMMUNICATIONS

Interaction of porphyrins with concanavalin A and pea lectin

Kancheenadham Bhanu, Sneha Sudha Komath, Bhaskar G. Maiya and Musti J. Swamy

School of Chemistry, University of Hyderabad, Hyderabad 500 046, India

Currently porphyrins are used as photosensitizers in photodynamic therapy for the treatment of cancer. However, this approach suffers due to the inability of many porphyrin-based drugs to accumulate preferentially in tumours. In view of this, we considered if the carbohydrate-binding proteins, lectins, which preferentially recognize malignant cells, could be used for the targeting of porphyrins to tumour cells. In the present study, we have investigated the interaction of a free base porphyrin, *meso*-tetrasulphonatophenylporphyrin and the corresponding metal derivative, *meso*-zinc-tetrasulphonatophenylporphyrin with two legume lectins, concanavalin A and pea (*Pisum sativum*) lectin. Each lectin subunit was found to bind one porphyrin molecule and the association constant, K_a, estimated from absorption and fluorescence titrations at room temperature (28 ± 1°C) was in the range of 1.2×10^4 M^{-1} to $6.3 \times 10^4 4$ M^{-1}. Both free lectin and lectin saturated with the specific saccharide were found to bind the porphyrin with comparable binding strength, indicating that porphyrin binding takes place at a site different from the sugar-binding site. These results indicate that lectins may potentially serve as drug-delivery agents for porphyrin sensitizers in photodynamic therapy.

LECTINS are ubiquitous carbohydrate-binding proteins of non-immune origin, which agglutinate cells or precipitate polysaccharides or glycoconjugates[1]. In recent years lectins have become increasingly important as tools for the study of carbohydrates, both in solution and on cell surfaces[2]. The sugar-binding and haemagglutinating properties of lectins are used for preparative and analytical purposes in biochemistry, cell biology and immunology. Some lectins have the ability to interact preferentially with transformed cells and hence they have been suggested for use as carriers for targeted delivery of chemotherapeutic agents. For example, conjugates of concanavalin A (Con A) and the α-chain of diphtheria toxin[3] or ricin[4] have been prepared and tested for targeting the toxin to tumour cells. In addition to binding of carbohydrates, lectins are also known to bind other small molecules such as 1,8-anilinonaphthalenesulfonate (ANS), 2,6-toluidinylnaphthalenesulfonate (TNS), adenine and cytokinin[5–7].

Photodynamic therapy (PDT), a new modality for cancer treatment that utilizes porphyrins and metalloporphyrins as sensitizers[8], has attracted a great deal of attention in recent years. When irradiated with light of the appropriate wavelength, these sensitizers go into the excited state and interact with molecular oxygen, converting it into the singlet state, ultimately resulting in tumour necrosis[9]. However, the selectivity of these sensitizers towards tumour cells is not always sufficient for PDT to be specific for the tumour tissue alone[10]. One possible way to improve the ability of the photosensitizers to target tumour tissues specifically is to couple them with another agent which can preferentially interact with the malignant cells.

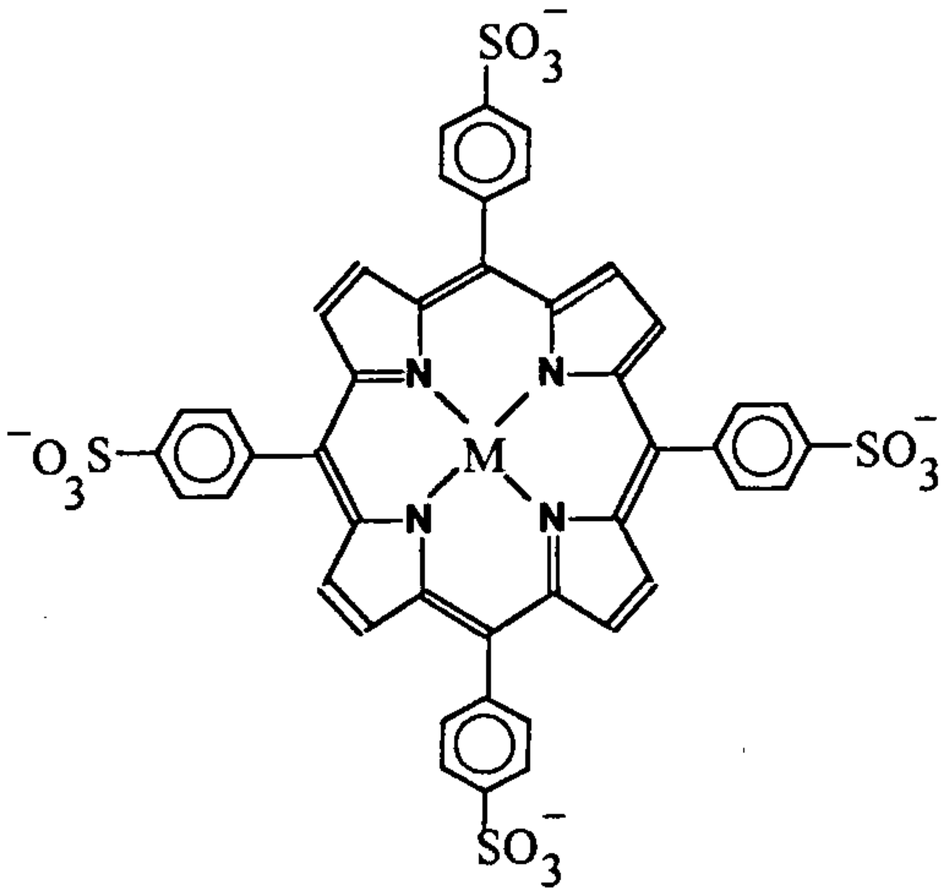
The above considerations prompted us to investigate if lectins could be used as carriers for the targeted delivery of porphyrins to tumour cells so as to increase their therapeutic potential. We have, therefore, undertaken

systematic studies on the interaction of porphyrins with plant lectins and, herein report on the binding of a free base porphyrin, *meso*-tetrasulphonatophenylporphyrin (H_2TPPS) and the corresponding metal derivative, *meso*-zinc-tetrasulphonatophenylporphyrin (ZnTPPS) (Figure 1) with two legume lectins, viz. Con A and pea (*Pisum sativum*) lectin (PSL). These two lectins have been chosen because (i) they bind various hydrophobic ligands[5,7,11] and (ii) Con A recognizes preferentially malignant cells[12] and shares extensive sequence homology and structural similarity with pea lectin[13].

Con A and pea lectin were purified from jack beans and peas by affinity chromatography on Sephadex G-50 and Sephadex G-100, respectively[14,15]. After elution, the lectins were dialysed exhaustively against 10 mM Tris-HCl buffer containing 0.15 M NaCl, 1 mM $MnCl_2$ and 1 mM $CaCl_2$ and concentrated by lyophilization. The purified lectins were found to be homogeneous by polyacrylamide gel electrophoresis under non-denaturing conditions[16] and their activity was checked by agglutination assay. The concentrations of the purified lectins were estimated spectrophotometrically by using $A^{1\%,\,1\,cm}_{280\,nm}$ values of 12.4 for tetrameric Con A and 15.5 for PSL[17,18]. Methyl α-mannopyranoside (MeαMan) was a product of Sigma Chemical Company, USA. H_2TPPS (tetra sodium salt) was obtained from Alfa Inorganics, USA. ZnTPPS (tetra sodium salt) was prepared according to a reported procedure[19].

The absorption spectra were measured on a JASCO 7800 double beam spectrophotometer with the overlay mode for porphyrin solutions having optical density < 1.0. The fluorescence measurements were carried out on a JASCO FP777 spectrofluorometer for porphyrin solutions whose optical density at the excitation wavelength was < 0.1. Right angle detection was employed and the bandwidth was 5 nm for both excitation and emission monochromators. Dilution effects were appropriately taken care of in all the titrations.

Initial studies on the binding of porphyrins to Con A and pea lectin were performed using absorption spectroscopy by monitoring the changes in the absorption properties of the porphyrin when a 2.0 ml solution of the porphyrin was titrated with small aliquots of a concentrated solution of the lectin. The absorption spectrum of H_2TPPS is shown in Figure 2. In order to investigate the binding of H_2TPPS to the lectins, changes occurring in the absorption intensity at the peak of the Soret band (410–430 nm, $\varepsilon \approx 10^6\ M^{-1}\,cm^{-1}$) were monitored. Upon addition of Con A or pea lectin, the absorbance of H_2TPPS decreased by about 5–7% of the initial value. Absorption titration curve for H_2TPPS-binding to Con A is presented in Figure 3. It can be seen from this figure that binding of the porphyrin to the lectin leads to a sharp decrease in the absorbance initially, whereas, at higher lectin concentrations the change in absorbance decreases in a more gradual manner, i.e. binding of the porphyrin to the lectin displays a saturation behaviour. A plot of $(A_0/\Delta A)$ vs $(1/[P]_T)$, where ΔA refers to the change in absorbance of the sample at the total protein concentration (in subunits) in the sample, $[P]_T$, and A_0 corresponds to the absorbance of the sample in the absence of protein, yielded a straight line (not shown). From the ordinate intercept of the plot, A_∞, the absorbance of the sample at infinite protein concentration was obtained. Subsequently, log $[P]_F$, where $[P]_F$ is the free protein concentration, was plotted against log $\{(\Delta A)/(A_c - A_\infty)\}$ (Figure 3, inset). The abscissa intercept of this plot yielded the pK_a value of the Con A–H_2TPPS interaction according to the relationship[20]:



M = 2H H_2TPPS

M = Zn (II) ZnTPPS

Figure 1. Structures of the porphyrins H_2TPPS and ZnTPPS.

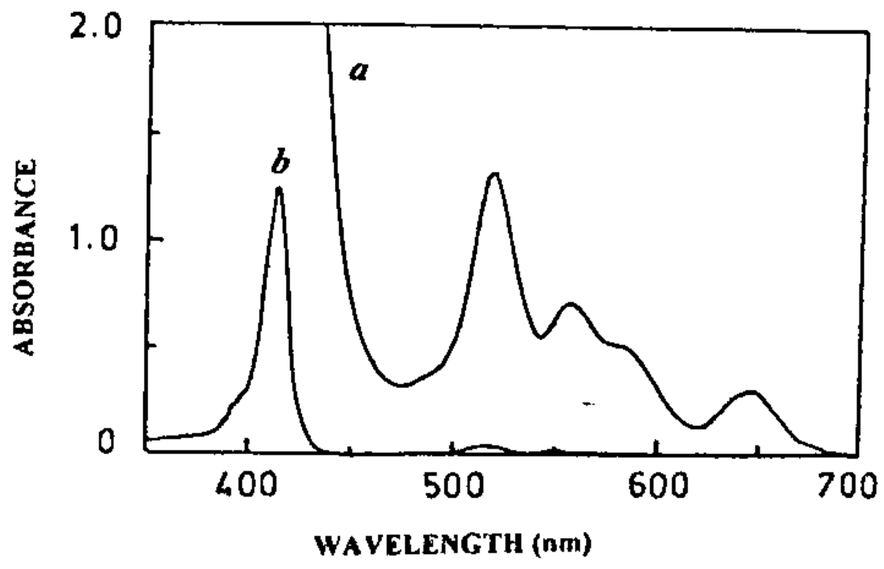


Figure 2. Absorption spectra of H_2TPPS. Spectrum recorded at a concentration of (*a*), 2.2×10^{-5} M and (*b*), 3.4×10^{-6} M.

$$\log \{(\Delta A)/(A_c - A_\infty)\} = \log K_a + \log \{[P]_T - [M]_T\ (\Delta A/\Delta A_\infty)\}, \quad (1)$$

where A_c is the absorption intensity of the sample at any point during the titration, $[M]_T$ is the total concentration of the ligand, and $[P]_F$, the free protein concentration, is given by:

$$[P]_F = \{[P]_T - [M]_T\ (\Delta A/\Delta A_\infty)\}. \quad (2)$$

The slope of the linear least square fit of the plot in Figure 3 (inset) was found to be 1.3, indicating that there is one porphyrin-binding site on each lectin subunit, whereas from the X-intercept of the plot, the association constant, K_a, was estimated to be $1.1 \times 10^4\ M^{-1}$. Similarly, the association constant for the interaction of H_2TPPS with pea lectin was found to be $3.16 \times 10^4\ M^{-1}$ by the absorption titrations.

Fluorescence spectroscopic studies on the binding of H_2TPPS and ZnTPPS to Con A were carried out in a manner similar to the absorption spectroscopic studies described above. Experiments were conducted both in the presence of 0.1 M MeαMan and in the absence of any sugar. Upon binding to the lectin, the fluorescence intensity of H_2TPPS was found to decrease by about 42% in the absence of sugar whereas in the presence of MeαMan the average quenching was about 50%. The emission spectra of H_2TPPS with increasing concentrations of Con A, recorded in the absence of sugar at 28 ± 1°C are shown in Figure 4 and the corresponding fluorescence titration curve is displayed in Figure 5. Changes in the fluorescence intensity at infinite protein concentration, F_∞, were obtained from the Y-intercepts of plots of $(F_0/\Delta F)$ versus $1/[P]_T$ (not shown) and the association constants, K_a, were determined according to the method of Chipman *et al.*[20], described above for the absorption titrations. Equation (2) can be modified as shown below to treat the fluorescence titration data by substituting the appropriate fluorescence parameters

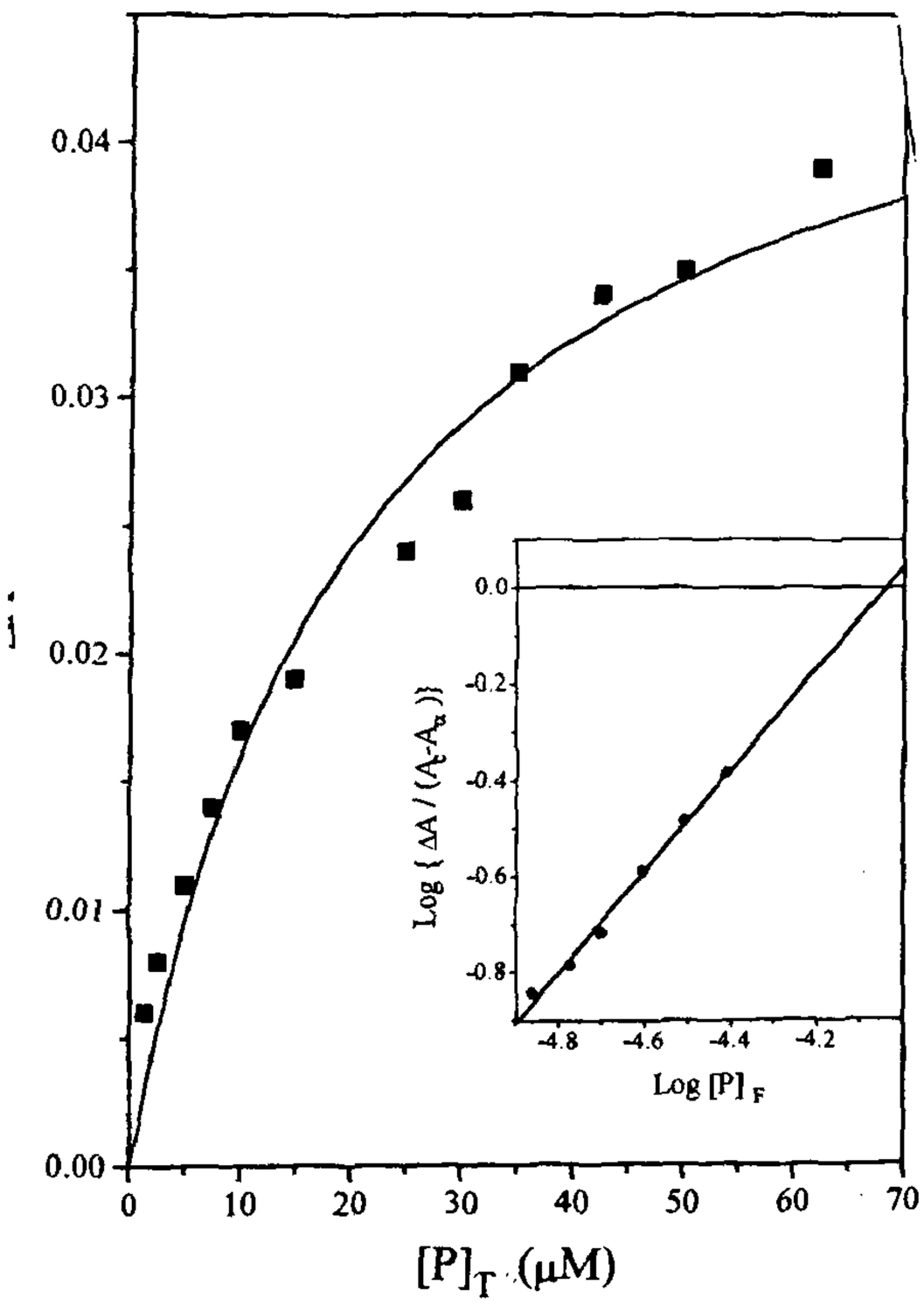


Figure 3. Plot of change in absorption intensity at 414 nm of H_2TPPS s a function of added Con A concentration. *Inset*: Determination of ıe K_a value for the binding of H_2TPPS to Con A by absorption trations. The X-intercept yields the pK_a of the binding equilibrium.

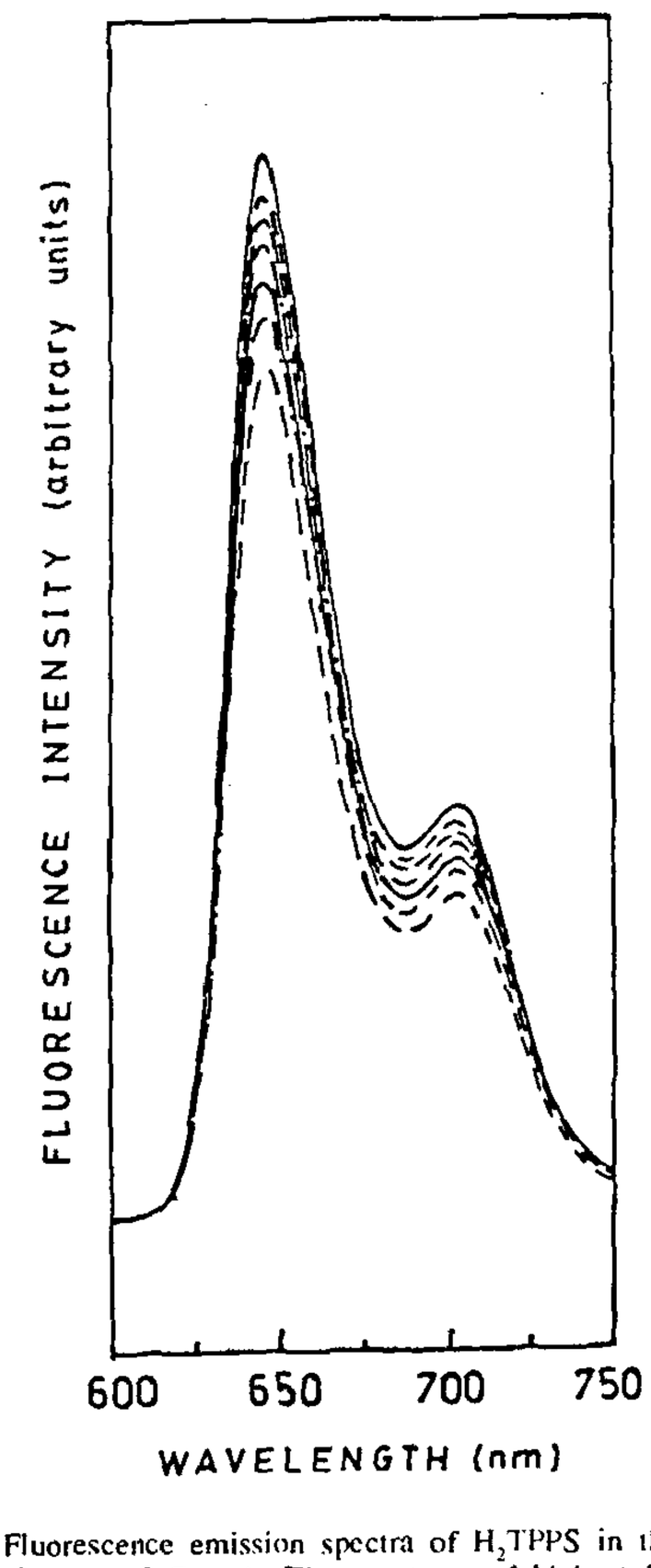


Figure 4. Fluorescence emission spectra of H_2TPPS in the presence and in the absence of Con A. The spectrum of highest fluorescence intensity is that of H_2TPPS in buffer alone and the remaining spectra of decreasing intensities correspond to spectra recorded after the addition of 5, 20, 60, 120, 170, and 250 μl of 392 μM Con A (protomer).

in place of the absorption parameters [equation (3)]:

$$\log\{(\Delta F)/(F_c - F_\infty)\} = \log K_A + \log\{[P]_T - [M]_T(\Delta F/\Delta F_\infty)\}, \quad (3)$$

where F_0, F_c and F_∞ are the fluorescence intensities of the porphyrins in the absence, in the presence, and at infinite concentration of Con A, respectively, and $[P]_F$ is the free protein concentration. A plot of log $\{(\Delta F)/(F_c - F_\infty)\}$ vs log $[P]_F$ for H_2TPPS–Con A interaction is shown in Figure 5 (inset).

In an analogous set of experiments, the fluorescence intensity of ZnTPPS was seen to be decreased by about 78% upon binding to Con A in the absence of sugar while, in the presence of 0.1 M MeαMan, the porphyrin fluorescence was totally quenched. Plots similar to those shown for H_2TTPS in Figure 5 were also obtained for interaction of Con A with ZnTPPS (not shown). The slopes of these plots are in the range of 0.9–1.3 and indicate that the stoichiometry of porphyrin–Con A (subunit) complex is unity. The association constants determined for the interaction of these two porphyrins with Con A in the absence and in the presence of MeαMan are presented in Table 1.

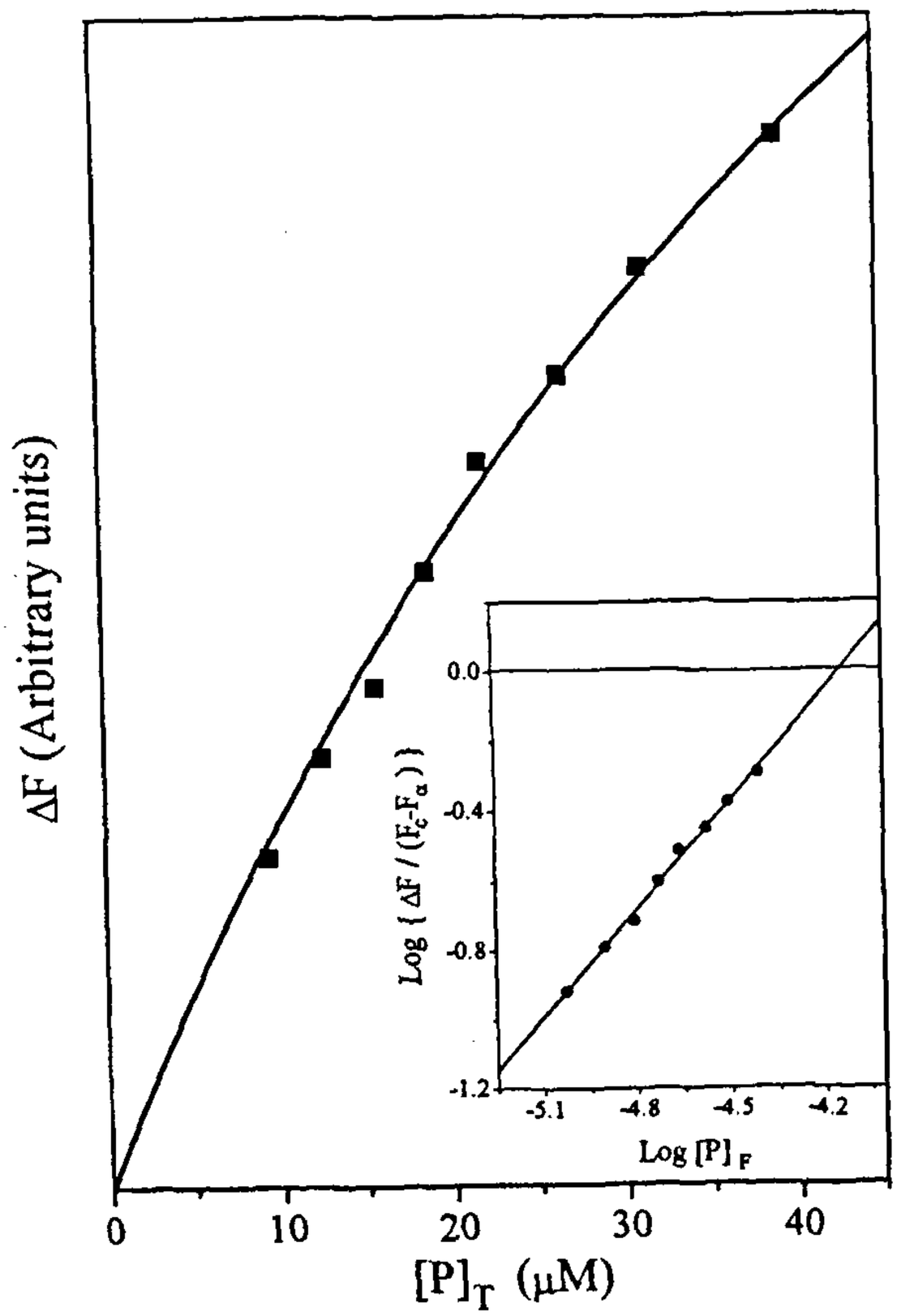


Figure 5. Plot of change in fluorescence intensity of H_2TPPS as a function of added Con A concentration. *Inset*: Determination of the K_a value for the binding of H_2TPPS to Con A by fluorescence titrations. The X-intercept yields the pK_a of the binding equilibrium.

Fluorescence titrations for the interaction of H_2TPPS and ZnTPPS with pea lectin both in the presence and in the absence of MeαMan have been carried out. Upon binding to this lectin, the fluorescence intensities of H_2TPPS and ZnTPPS were found to decrease by about 27% and 57%, respectively, both in the presence of 0.1 M MeαMan and in the absence of sugar. From the titration data, the association constants were calculated as described above for Con A–H_2TPPS interaction. The slopes of plots of log $\{(\Delta F)/(F_c - F_\infty)\}$ vs log $[P]_F$ were found to be in the range of 0.8–1.3, which indicates that there is a single porphyrin-binding site on each lectin subunit. The association constants for the binding of H_2TPPS and ZnTPPS to pea lectin, both in the presence and in the absence of MeαMan, determined from the X-intercepts of these plots are listed in Table 1.

From Table 1 it is clear that Con A and pea lectin bind porphyrins with moderate to considerable affinity. The association constants, K_a, as obtained by both the fluorescence and absorption titration methods are comparable and are in the range of 1.2×10^4 $M^{-1} - 6.3 \times 10^4$ M^{-1}. In addition, these values are comparable to those observed generally for the binding of simple saccharides to lectins. The presence of the specific saccharide does not inhibit the porphyrin-binding, indicating that porphyrin-binding occurs at a site that is different from the saccharide-binding site. However, there are some differences in the association constants and the magnitude of change in fluorescence intensity determined in the presence and in the absence of the sugar. It is known that saccharide-binding can bring about conformational changes in the lectin structure. It is possible that such saccharide-induced conformational changes can result in alterations in the association constants and change in fluorescence intensity characterizing the porphyrin-binding.

Hydrophobic ligands such as ANS and TNS have earlier been shown to interact with a variety of plant

Table 1. Association constants estimated by fluorescence titrations for the binding of porphyrins to Con A and pea lectin

Lectin	Porphyrin	Association constant, K_a $(M^{-1})^a$	
		In the absence of sugar	In the presence of 0.1 M MeαMan
Con A	H_2TPPS	1.22×10^4	1.70×10^4
	ZnTPPS	5.64×10^4	6.07×10^4
Pea lectin	H_2TPPS	2.96×10^4	1.30×10^4
	ZnTPPS	2.58×10^4	6.35×10^4

[a]Error limits: ± 10%.

lectins and these interactions are characterized by association constants in the range of 1.0×10^3 to 1.0×10^5 M^{-1} (refs 6, 7). It is interesting to note that K_a values for the porphyrin–lectin interaction reported here are in the same range as those for lectin–hydrophobic ligand and porphyrin–serum protein interaction[21]. However, the interaction of porphyrins with lectins has more potential for their targeted delivery because of the preferential interaction of the lectins with tumour cells.

Con A could be completely freed of the porphyrin from a Con A–H_2TPPS mixture by gel permeation chromatography on a column of Sephadex G-50, clearly demonstrating that the association of porphyrin with the lectin is reversible. Therefore, in a given solution containing lectin and porphyrin, the porphyrin will exist in two forms: associated with the lectin, and free in solution. Though only partial association with the lectin is seen in the present study, the magnitude of K_a values is such that considerable improvement in the preferential localization of the porphyrin in tumour tissues may be expected when lectin–porphyrin mixtures are used as compared to using porphyrin alone in photodynamic therapy. In this regard, it can be noted that, with an association constant of *ca.* 5×10^4 M^{-1}, under typical *in vitro* PDT conditions, use of about 10^{-5} M of the porphyrin and a ten-fold molar excess of lectin would provide more than 80% of the lectin-bound porphyrin molecules that are capable of localizing in the tumour. Nonetheless, studies with cultured cells and animal models are required to evaluate the tumour selectivity that can be achieved by using lectin–porphyrin complexes in PDT. Additionally, further increase in the tumour localization of the porphyrins can be envisaged by covalently linking them to the lectins. Future studies should be aimed in this direction.

In summary, this communication reports the first demonstration of the interaction of porphyrins with lectins. In view of the current interest in porphyrins as photosensitizers in photodynamic therapy for the treatment of cancer, this suggests that lectins may be useful in tumour-specific targeted delivery of porphyrins.

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Bioleaching of copper from ferromanganese sea nodule of Indian ocean

Seema Khan, Rani Gupta and Rajendra Kumar Saxena

Department of Microbiology, University of Delhi South Campus, Benito Juarez Road, New Delhi 110 021, India

Ferromanganese sea nodule is an abundant low-grade metal reserve of Indian ocean. However, it can be a potential substrate for bioextraction of metals like copper. It has been found that 85% of copper could be leached in seven days in batch fermentation using *Aspergillus* spp. at 30°C. Bioleaching of copper was found to be organic-acid mediated. Scanning electron micrographs revealed eroded areas indicating biosolubilization of copper from the nodule.

BIOLEACHING of valuable metals like copper, gold, uranium has become a prime concern of biometallurgists due to