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Binding Of Meso-Tetra Kis (4-N-Methyl-Pyridinium) Porphyrin And Its Mn (III) And Co (III) Complexes With Calf Thymus DNA: A Thermodynamic Approach

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ABSTRACT

The binding of meso-tetra kis (4-N-methy-pyridinium) porphyrin, TMPyP and its Mn (III) (MnTMPyP) and Co(III) (CoTMPyP) derivatives to calf thymus DNA have been studied in thermodynamic viewpoint using Uv/Vis spectroscopy. The measurements were done in 1mM phosphate buffer, pH 7.0 and various temperatures. The optical absorption spectra of porphyrins were analyzed in order to obtain binding constants and stoichiometries using SQUAD soft ware .The results show that the best fitting corresponds to a 1:1 complex model between base pair of DNA and porphyrins. The estimation of binding constant at various temperatures enabled us to calculate all of the thermodynamic parameters of binding using Vant Hoff equation. The following order has been obtained for binding affinity and exothermicity of binding process, MnTMPyP > CoTMPyP > TMPyP.This results and the trend of variation of spectral features of porphyrin in titration experiments with DNA indicate the groove binding mode and special feature of central metal ion of porphyrin in the strength of binding.

KEYWORDS

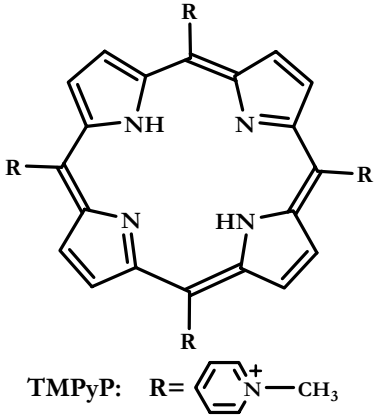
DNA;
Porphyrin;
SQUAD;
Thermodynamic parameters.

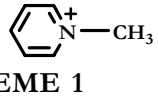
Regular Paper

INTRODUCTION

Cationic porphyrins have attracted considerable attention due to their remarkable ability to form complexes with DNA and cleave nucleic acids[1-4]. Since the molecular recognition of DNA is of fundamental importance to life, analyzing the interaction of small molecules with DNA continues to be an important area of research. Potential applications of these system include photodynamic therapy of cancer (PDT)[5-9] molecular biology applications such as DNA foot printing[10], design of telomerase inhibitors[11], stabilizing DNA /RNA hybrids[12], DNA triplexes[13] or quadruplexes[14], specific sensing of DNA quadruplexes[13], etc. Development in these area are predicated upon a detailed understanding of the porphyrin-nucleic acid binding mechanism .The cationic meso-tetrakis (N-methyl-pyridiniumyl) porphyrin and some of the metal derivatives such as Cu(II), Ni(II), Pd(II), Fe(III), Co(III) and Mn(III) complexes have been extensively studied[15-17]. It has been shown that this cationic porphyrin has a very high binding affinity to anionic DNA strands ,with association constants at 10^5-$10^7 M^{-1}$ level[18,19]. However, there is not any comprehensive thermodynamic study on such systems.

In the present study, the interaction of meso-tetrakis (4-N-methyl-pyridinumyl) porphyrin (SCHEME 1) and its Mn(III)and Co(III) derivatives with calf-thymus DNA have been studied at various temperatures using Uv/Vis absorption spectroscopy. The spectral data were analyzed using SQUAD software. The molecular interpretation of estimated thermodynamic parameters determined some new aspects of binding mechanism.



TMPyP: R=  CH_3

SCHEME 1

MATERIALS AND METHODS

Calf thymus DNA was obtained from Sigma, 2mg of DNA was dissolved in 1mM phosphate buffer at pH 7.0 by exhaustive stirring at 4°C for obtaining heterogeneous solution. The DNA concentrations were determined spectrophotometrically using the value of $1.32 \times 10^4 M^{-1}cm^{-1}$ for molar extinction coefficients at 260nm[20].

TMPyP was prepared, purified and converted to chloride form $[TMPyP]Cl_4$, by a modified published procedure[21]. TMPyP metallated according to the literature method[22]. These complexes were characterized by Uv/Vis spectroscopy and elemental analyses. The spectral characteristics of the isolated materials were compared to the literature values and found to be in excellent agreement. All of the chemicals, which have been used for these syntheses, were of analytical grade and purchased from Sigma. All solutions were prepared using double-distilled water. Porphyrin stock and working solution were stored at room temperature in the dark to avoid undesired photochemical reactions.

The concentration of porphyrins was determined from their optical absorption spectra using the molar absorption coefficients $\varepsilon_{425_{nm}} = 2.24 \times 10^5 M^{-1} \cdot cm^{-1}$ for TMPyP, $\varepsilon_{436_{nm}} = 2.20 \times 10^5 M^{-1} \cdot cm^{-1}$ for CoTMPyP and $\varepsilon_{462_{nm}} = 0.94 \times 10^5 M^{-1} \cdot cm^{-1}$ for MnTMPyP.

The absorption spectra were recorded on a Cary 100 double beam spectrophotometer using 1cm quartz cuvettes with thermostat cell compartment, that control the temperature around the cell within ±0.1°C.

Titration of porphyrin solution with DNA was performed at 20, 25, 30, 35, 40 and 45°C in a 1mM phosphate buffer pH 7.0.

A stock solution of calf thymus DNA was added to the porphyrin solution stepwise and the spectrum of porphyrin was recorded at each step. The titration experiment was continued until the absorbance of the porphyrin solution in the Uv/Vis range re-

Regular Paper

mained constant. The starting volume of the porphyrin solution was 1800 μL, and the amount of DNA stock solution added in each step was 50 μL. The spectra was recorded within the range of 300 to 700 nm about 3 min after each addition of DNA solution. The spectra were also corrected respect to dilution effect.

To observe the salt effect on the porphyrin absorption, the titration was made by addition of aliquots of the NaCl solution into cuvette containing the porphyrin solution of appropriate concentration.

The initial volume of porphyrin solution with appropriate concentration in the cuvette was (1800 μL and the values of 50 μL of NaCl stock solution (5.0M) was added to the cell at each step of titration. The Uv/Vis spectra were recorded in the range of 300 to 700 nm about 5 min after each addition of NaCl stock solution. The obtained spectra were also corrected with respect to dilution effect.

RESULTS AND DISCUSSIONS

Effect of NaCl

The Uv/Vis spectra of TMPyP, CoTMPyP and MnTMPyP consist of a distinct Soret band of 425,436 and 462 nm, respectively .The effect of NaCl on the absorption spectra of TMPyP, CoTMPyP and MnTMPyP are shown in figures 1, 2 and 3, respectively. The absorbance at all of the spectral regions of studied porphyrins has been significantly decreased due to increasing of NaCl concentration. This hypochromocity for MnTMPyP is accompanying with blue shift while for others are not. From these results, it can be concluded that TMPyP and CoTMPyP produced ill – defined aggregates due to increasing of ionic- strength but MnTMPyP consists more define aggregate (H or J aggregate)[23-26]. It can be related to more regular and planner structure

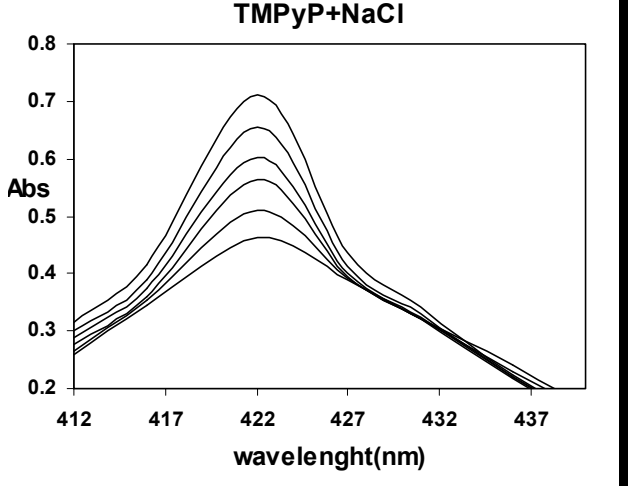


Figure 1: Corrected absorption spectra of TMPyP upon titration with NaCl in 1mM phosphate buffer, pH7.0 at 25 °C.

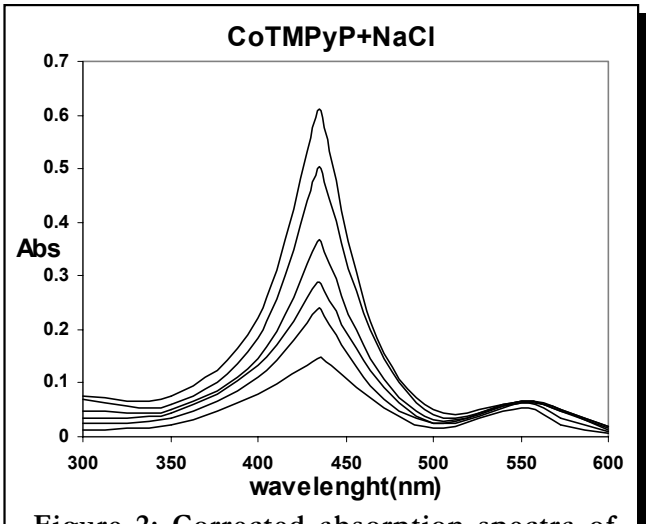


Figure 2: Corrected absorption spectra of CoTMPyP upon titration with NaCl in 1mM phosphate buffer, pH7.0 at 25°C.

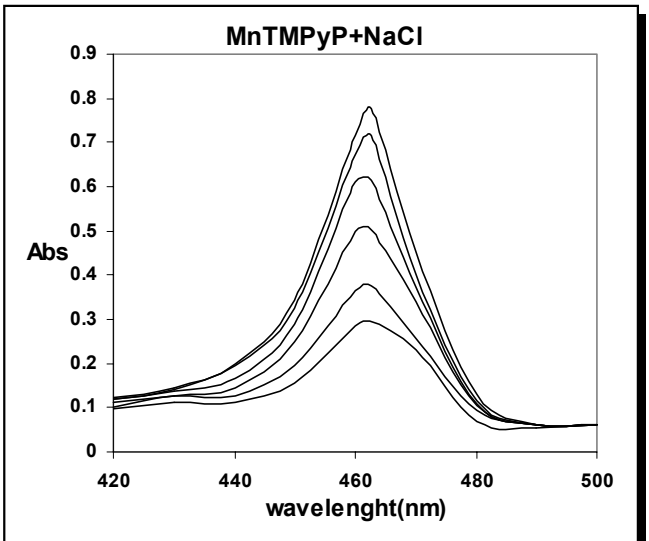


Figure 3: Corrected absorption spectra of MnTMPyP upon titration with NaCl in 1mM phosphate buffer, pH7.0 at 25°C.

Regular Paper

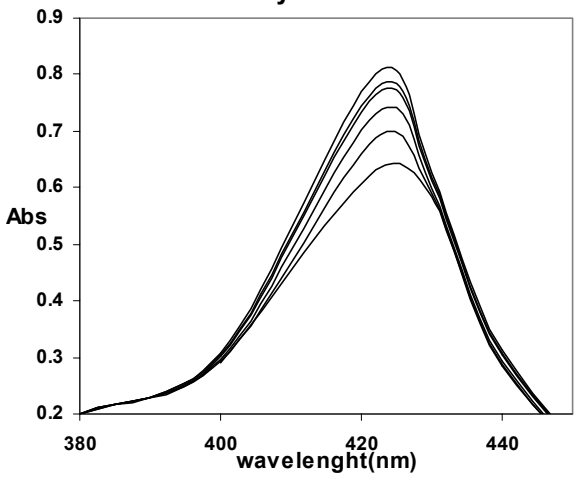


Figure 4: Corrected absorption spectra of TMPyP upon titration with DNA in 1mM phosphate buffer, pH7.0 at 25°C.

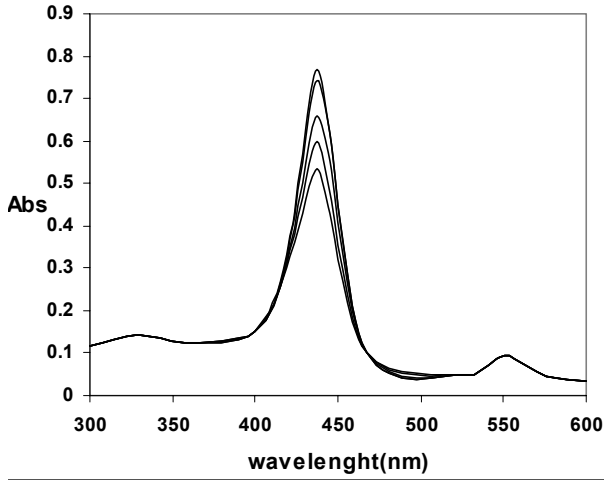


Figure 5: Corrected absorption spectra of CoTMPyP upon titration with DNA in 1mM phosphate buffer, pH7.0 at 25°C.

of MnTMPyP.

Binding of porphyrins to DNA

Spectral data analysis

The general feature of TMPyP, CoTMPyP and MnTMPyP spectra at various, DNA concentration at 25°C, were shown in figures 4, 5 and 6, respectively. The spectra of these figures consist of distinct isosbestic points that can be represents a simple equilibrium. The hypochromicity among with small red shift has been observed in Soret band of porphyrins due to increasing of DNA concentration. This can be represent the out side binding mode of porphyrins to groove of double chain of DNA.

In order to analysis the spectral data of porphyrins at various concentration of DNA in titration experiments, the 50 wavelengths showing suitable absorbance variations upon addition of DNA were selected from spectrum of porphyrin. The values of absorbance of these selected wavelengths at various DNA concentrations were analyzed in order to calculate equilibrium formation constants using SQUAD software.

This program is designed to calculate the best values for the stability constants of the proposed equilibrium model by employing a non-linear least square approach[27, 28].

This program is completely general in scope, having the capability to refine stability constants for the general complex $M_m M'_l H_j L_n L'_q$, where m,l,n,q≥ 0 and J is positive for protons, negative (for hydroxyl ions) or zero . The algorithm employed in SQUAD and their relationships to each other have been described previously[31,32].Our input data for analysis of porphyrin- DNA system were absorbance at 50 different wavelength of 15 pophyrin spectra .These 15 spectra are correspond to 15 various concentrations of DNA.

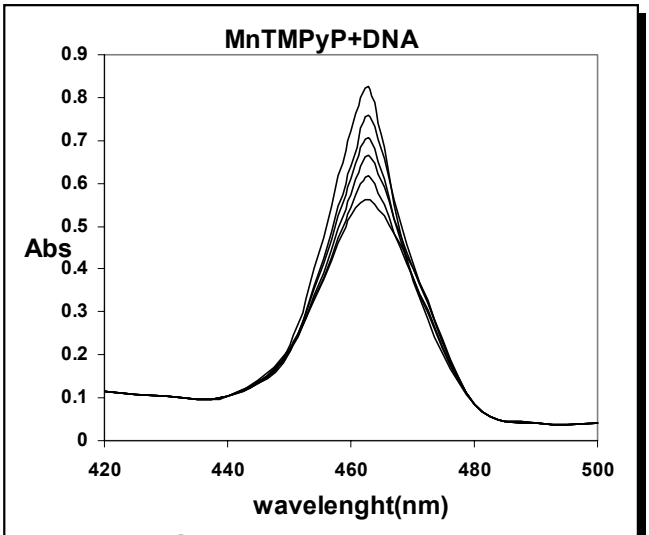


Figure 6: Corrected absorption spectra of MnTMPyP upon titration with DNA in 1mM phosphate buffer, pH7.0 at 25°C.

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TABLE 1: Thermodynamic parameters for binding of TMPyP to DNA in 1mM phosphate buffer, pH7.0 at 25°C.

$t^{\circ}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} KJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ} KJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	1.025 ± 97.967	0.061 ± -33.622	0.046 ± -103.356	0.467 ± -237.878
25	1.030 ± 57.666	0.074 ± -32.882	0.046 ± -103.356	0.459 ± -236.371
30	1.036 ± 28.238	0.088 ± -31.633	0.046 ± -103.356	0.486 ± -236.592
35	1.028 ± 14.149	0.072 ± -30.385	0.046 ± -103.356	0.435 ± -236.804
40	1.023 ± 6.771	0.060 ± -28.959	0.046 ± -103.356	0.423 ± -237.576
45	1.030 ± 3.811	0.079 ± -27.901	0.046 ± -103.356	0.473 ± -237.168

TABLE 2: Thermodynamic parameters for binding of CoTMPyP to DNA in 1mM phosphate buffer, pH7.0 at 25°C.

$t^{\circ}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} KJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ} KJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	1.042 ± 43.757	0.102 ± -31.657	0.081 ± -74.639	0.363 ± -146.621
25	1.036 ± 19.099	0.087 ± -30.142	0.081 ± -74.639	0.351 ± -149.244
30	1.049 ± 10.254	0.121 ± -29.080	0.081 ± -74.639	0.343 ± -150.285
35	1.054 ± 6.149	0.133 ± -28.497	0.081 ± -74.639	0.339 ± -149.739
40	1.059 ± 4.771	0.151 ± -27.881	0.081 ± -74.639	0.328 ± -149.315
45	1.057 ± 2.575	0.145 ± -26.864	0.081 ± -74.639	0.332 ± -150.165

TABLE 3: Thermodynamic parameters for binding of MnTMPyP to DNA in 1mM phosphate buffer, pH7.0 at 25°C.

$t^{\circ}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} KJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ} KJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	1.049 ± 7.097	0.117 ± -27.224	0.112 ± -36.856	0.219 ± -32.858
25	1.052 ± 5.258	0.126 ± -26.945	0.112 ± -36.856	0.199 ± -33.243
30	1.054 ± 4.374	0.134 ± -26.933	0.112 ± -36.856	0.211 ± -32.734
35	1.049 ± 3.2449	0.123 ± -26.611	0.112 ± -36.856	0.207 ± -33.248
40	1.045 ± 2.575	0.155 ± -26.441	0.112 ± -36.856	0.215 ± -33.260
45	1.052 ± 2.192	0.135 ± -26.438	0.112 ± -36.856	0.203 ± -32.747

The outputs are the logarithm of equilibrium formation constant, $\log k_{ij}$, for formation of $(DNA)_i$ $(Porphyrin)_j$ is defined with respect to the equation(2)

$$i\,DNA + j\,Porphyrin \leftrightarrow (DNA)_i(Porphyrin)_j \quad (1)$$

$$k_{ij} = \frac{[(DNA)_i(prophyrin)_j}{[DNA]^i[porphyrin]^j} \quad (2)$$

The values of uncertainty in $\log k_{ij}$ are also calculated by the program.

The results show that the best fitting corresponds to 1:1 complex model at all studied temperatures with sum of squares of reduced error between 10^{-3}-10^{-4}. These results are in good agreement with the existence of isosbestic points that corresponds to a simple equilibrium between free prophyrin and 1:1 conjugate of DNA: Prophyrin.

The estimated equilibrium constants for the formation of 1:1 complexes between DNA and TMPyP, CoTMPyP and MnTMPyP at various temperatures are listed in TABLES 1, 2 and 3, respectively.

Thermodynamics of DNA :porphyrin interactions

The energetic of DNA: porphyrin equilibrium can be conveniently characterized by three familiar thermodynamic parameters; standard Gibbs free energy, ΔG^{o}, enthalpy, ΔH^{o} and entropy, ΔS^{o}, changes. The ΔG^{o} can be calculated from equilibrium con-

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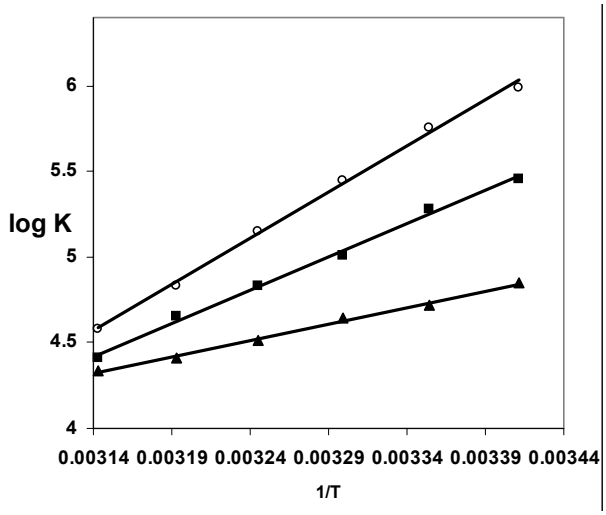

Figure 7: The vant Hoff plot for binding of TMPyP (▲), CoTMPyP (■) and MnTMPyP (o) to DNA in 1mM phosphate buffer, pH7.0 at 25°C.

stant, K, of the reaction using the familiar relationship, $\Delta G^\circ = -RT\ln K$ in which R and T referring to the gas constant and the absolute temperature, respectively. If heat capacity change of reaction is negligible, the Van't Hoff equation

$$\frac{d\ln k}{d(\frac{1}{T})} = \frac{-\Delta H^\circ}{R} \quad (3)$$

gives a linear plot of lnK versus 1/T. The ΔH° can be calculated from the slope, $\Delta H^\circ/R$, and the ΔS° from the intercept, $\Delta S^\circ/R$ or from equation (4)

$$\Delta S^\circ = \frac{(\Delta H^\circ - \Delta G^\circ)}{R} \quad (4)$$

The van't Hoff plots for binding of TMPyP, CoTMPyP and MnTMPyP to DNA in the phosphate buffer are shown in figure 7 and their calculated thermodynamic parameters are listed in TABLES 1, 2 and 3, respectively.

CONCLUSIONS

All of the studied porphyrins show strong electrolyte effect and increasing of NaCl concentration induces self-aggregation of porphyrins. However, MnTMPyP forms more define aggregate respect to others.

The hypochromicity among with small red shift has been observed in Soret band of porphyrins due to increasing of DNA.The thermodynamic parameters shows the following order for binding affinity of porphyrins at all of the studied temperatures:

MnTMPyP > CoTMPyP > TMPyP

The higher affinity of MnTMPyP and CoTMPyP can be related to formation of axial binding between metal of porphyrin and fundamental groups of DNA such as phosphate groups. This axial binding increases binding affinity. However the binding process of all porphyrins is exothermic and the order of exothermicity is the same as binding affinity. The higher affinity of MnTMPyP can be related to its more planer structure that is related to the size of Mn ion.

The hypochromicity among with small red shift has been observed in Soret band of porphyrins due to increasing of DNA concentration. This can be represent the out side binding mode of porphyrins to groove of double chain of DNA. This kind of binding is usually occurred in AT reach of DNA chain.

ACKNOWLEDGEMENTS

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REFERENCES

[1] R.J.Fiel, J.C.Howard, E.H.Mark, N.Datta-Gupta; Nucleic Acids Res., **6**, 3039 **(1979)**.
[2] D.A.Musser, N.Datta-Gupta, R.J.Fiel; Biochem. Biophys.Res.Commun., **97**, 918 **(1980)**.
[3] M.J.Carvlin, N.Datta-Gupta, R.J.Fiel; Biochem. Biophys.Res.Commun., **108**, 66 **(1982)**.
[4] R.J.Fiel, T.A.Beerman, E.H.Mark, N.Datta-Gupta; Biochem.Biophys.Res.Commum., **108**, 1067 **(1982)**.
[5] G.Bock, S.Harnett; 'Photosensitizing Compounds, Their Chemistry, Biology and Clinical Use', Wiley, Chichester, UK **(1989)**.
[6] D.Kessel; 'Photodynamic Therapy of Neoplastic Disease', CRC Press, Boca Raton, **2**, **(1990)**.
[7] T.J.Dougherty; Photochem.Photobiol., **58**, 895 **(1993)**.
[8] R.Bonnett; Chem.Soc.Rev., **24**, 19 **(1995)**.
[9] E.D.Sternberg, D.Dolphin, C.Bruckner; Tetrahedron,

54, 4151 **(1998)**.
[10] J.C.Dabrowiak, B.Ward, J.Goodisman; Biochomistry, **28**, 3313 **(1989)**.
[11] R.T.Wheelhouse, D.Sun, H.Han, F.X.Han, L.H. Hurley; J.Am.Chem.Soc., **120**, 3261 **(1998)**.
[12] T.Uno, K.Hamasaki, M.Tanigawa, S.Shimbayshi; Inorg.Chem., **36**, 1676 **(1997)**.
[13] R.Lauceri, T.Campagna, A.Contino, R.Purrello; Angew.Chem., Int.Ed.Engl., **35**, 215 **(1996)**.
[14] N.V.Anantaha, M.Azam, R.D.Sheardy; Biochemistry, **37**, 2709 **(1998)**.
[15] R.F.Pasternack, E.J.Gibbs; Metal Ions in Biological System, **33**, 367 **(1997)**.
[16] G.Pratviel, J.Bernadou, B.Meunier; Metal Ions in Biological Systems, **33**, 399 **(1997)**.
[17] R.F.Pasternack, E.J.Gibbs; Seminars in Hematology, **26(2)**, 77 **(1989)**.
[18] L.G.Marzilli; New J.Chem., **14**, 409 **(1990)**.
[19] M.A.Sari, J.P.Battioni, D.Dupre, J.B.Lepecq; Biochemistry, **29**, 4205 **(1990)**.
[20] L.E.Borissevitch, C.M.Shirley, J.Gandini; Photochem. Photobiology B, Biology, **43**, 112 **(1998)**.
[21] R.F.Pasternack, E.J.Gibbs, J.J.Villafranca; Biochemistry, **22**, 2406 **(1983)**.
[22] A.Harriman, G.J.Porter; Chem.Soc.Faraday Trans.II, **75**, 1532 **(1979)**.
[23] J.M.Ribo, J.Crusats, J.A.Farrera, M.L.Valero; J.Chem. Soc.Chem.Commun., 681 **(1994)**.
[24] D.C.Barber, R.A.Freitag-Beeston, D.G.Whitten; J. Phys.Chem.A, **95**, 4074 **(1991)**.
[25] R.F.Pasternack, P.R.Huber, P.Boyd, G.Engasser, L. Francesconi, E.Gibbs, P.Fasella, G.Cerioventuro, L.D. Hinds; J.Am.Chem.Soc., **94**, 4511 **(1972)**.
[26] A.K.Bordbar, A.Eslami, S.Tangestaninejad; J. Porphyrins and Phthalocyanines, **6**, 225 **(2002)**.
[27] L.Zekan, I.Nagypal, D.Leggett; J(edt) PSEQUAD:A Comprehensive Program for the Evaluation of Potentiometric/or Spectrophotometric Equilibrium Data using Analytical Derivatives, in Computational Methods, Plenum Press, New York **(1991)**.
[28] D.J.Leggett, W.A.E.MeBryde; Anal.Chem., **47**, 1065 **(1975)**.