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Effect of microscopic environment on the self-stacking binding of porphyrin to DNA

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Abstract

The interaction model of meso-tetrakis(4-*N*-ethylpyridiumyl) porphyrin (TEPyP) and calf thymus DNA (ctDNA) was assumed by the change of UV–vis spectroscopy, fluorescence and resonance light-scattering (RLS). The changed absorption spectral properties of TEPyP titrated with ctDNA were observed from red shifted ($\Delta\lambda = 14$ nm) and a large hypochromicity (42%). In fluorescence spectra, $\lambda_{ex,max}$ of TEPyP shifted to longer wavelength ($\Delta\lambda = 13$ nm) and a decrease around 50% of the emission intensity were shown. The signal of resonance light-scattering was enhanced by nucleic acid. The study on the inclusion interaction of TEPyP with β-cyclodextrin (β-CD) and its derivatives including hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether-β-cyclodextrin (SBE-β-CD) shown that the formation constants for β-CD, HP-β-CD and SBE-β-CD with TEPyP were 550, 1.1×10^4 and 2.0×10^5 M^{-1}, respectively, with 1:1 stoichiometry. The conformation confirmed by ^{1}HNMR technique is that the alkyl and pyrrole cyclic moiety entered into the cavity of cyclodextrins. Comparative study on the interaction of TEPyP with ctDNA was further carried out in the presence of β-CD, HP-β-CD, and SBE-β-CD. The significant decrease of the binding constants and binding numbers were observed and the interaction of DNA-bound porphyrin has been inhibited. Moreover, the stronger inclusion ability of TEPyP and CDs, the bigger influence on the interaction of TEPyP with DNA, which was due to the fact that TEPyP enter into the cavity of CDs. It may influence binding affinity of porphyrin to DNA and weaken the static electronic forces in the outside self-stacking. Typically, the negative SBE-β-CD hampers directly the binding of the negative phosphate groups of the DNA backbone and the positive TEPyP.

Keywords: Meso-tetrakis(4-*N*-ethylpyridiumyl) porphyrin; Calf thymus DNA (ctDNA); Cyclodextrin; Fluorescence; Resonance light-scattering

1. Introduction

Porphyrins and their metalloderivatives play significant role in biological processes and have been studied and applied in many aspects of artificial enzyme [1], catalyze [2], supramolecular assembly [3] and so on. In the process of interaction of porphyrins and DNA, porphyrin derivatives act as the platform for the study of nucleic acid structure and dynamics, application in photodynamic therapy (PDT) [4] and virus control [5]. Among the major binding modes for the interaction of DNA with porphyrins, intercalation is foremost and may lead to the change of conformation and structure of DNA even the breakage of DNA chains. The interaction of meso-tetrakis(4-*N*-methylpyridiumyl) porphyrin (TMPyP, Fig. 1a) with DNA has been studied extensively and has been used clinically in PDT and virus control [6]. Due to the peripheral substituents or inserted metal, groove binding has been involved. In addition, porphyrin self-assembly on DNA surface has been reported [7–9]. Circular dichroism spectroscopy [10,11] and newly developed resonance light-scattering [7,12] are the powerful methods to investigate the model. Cyclodextrins (CDs) are cyclic oligosaccharides. They have been used in many important fields on the base of their unique structure and properties. And the chemical modified CDs endowed with typical function

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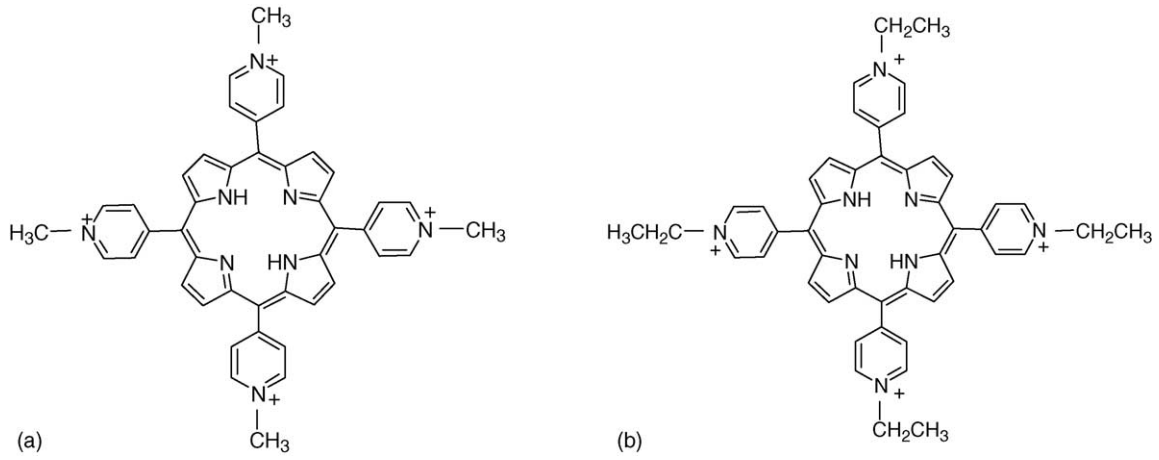

Fig. 1. Chemical structure of TMPyP and TEPyP.

groups exploit wider foreground in the application. The application and development of cyclodextrin and its derivatives in the fields related to porphyrin macrocyclic molecular mainly involve in biological artificial enzyme [13,14], photosensitized drugs [15,16], determine of gas molecular and metal ion by probe [17], and supramolecular assembly [18,19], etc. In particular, cyclodextrins supramolecular were introduced to study and confirm the system of DNA-bound porphyrin supramolecular recently. The array of *trans*-bis(*N*-methylpyridinium-4-yl)diphenylporphyrin on the DNA template was disassembly in the presence of excess β-CD[20]. However, the electrostatic binding model of meso-tetrakis[*p*-(trimethylammoniumyl)phenyl]porphyrin (TAPP) with DNA was inhibited only by negative SBE-β-CD and the neutral CD did not influence the binding[21].

The interactions of water-soluble, cationic porphyrin with DNA have been the subject of many recent investigations. The understanding of the interaction will lead porphyrin and their metal derivations to medical applications to the inhibition of AID virus HIV and photodynamic therapy of tumors. Three major binding modes have been involved in the interaction between porphyrins and DNA, namely intercalation, outside groove binding and outside self-stacking along the DNA surface. Most researches are limited to TMPyP and its derivatives; however, the binding mode is highly dependent on the substituent groups of the porphyrin, metal center, the type of DNA and the ionic strength of the medium, that is, the physical and chemical properties of porphyrin may remarkably vary with the increasing of the length of carbonic chain. In this paper, TEPyP was synthesized, and analyses of its solution properties, interaction with ctDNA in the absence and presence of β-CD and its derivatives were first carried out in detail. The results show that TEPyP formed intermolecular polymers in aqueous solution and cyclodextrins are able to interfere with the self-aggregation of TEPyP. In addition, TEPyP bound to ctDNA with outside self-stacking, the addition of β-cyclodextrins has decreased the apparent binding constants and binding numbers and is inefficient to the self-stacking binding of TEPyP and ctDNA.

2. Experimental

2.1. Reagents

Reagents, meso-tetrakis(4-pyridyl) porphyrin (TpyP) and bromoethane were purchased from Aldrich Chem. Co. and Beijing Reagents Company, respectively. The reagent TMPyP used for control experiment was purchased from Fluka. β-CD (Yu-nan Gourment Factory, China) was purified by recrystallization in double distilled water. HP-β-CD (average MW = 1657), had a degree of substitution (D.S. = 9.0). SBE-β-CD (D.S. = 2.5) was synthesized based on the literature method [22] and has been successfully used in our previous research [23].

Reagent grade TPyP (24 mg, 0.038 mol) and freshly distilled bromoethane (2 ml, 21.4 mmol) were added to 15 ml DMF solution. After refluxing for 2 h, the solution was cooled to room temperature, filtered and the filter cake was washed thoroughly with $CHCl_3$. The solution and recrystallization were carried out in a water/acetone (1:2) mixture to give 24 mg (85.9%) of water-soluble TEPyP (its chemical structure was given in Fig. 1b). ^{1}H NMR (D_2O), δ_H: 9.20 (d, 8H, *m*-H-pyridyl), 8.99–8.88 (b, 8H, H-pyrrole), 8.80 (d, 8H, *o*-H-pyridyl), 1.78 (t, 12H, $-CH_3$). λ_{max} (nm, H_2O): 421 (Soret), 518, 557, 582. Anal. Calcd. for $C_{48}H_{46}N_8Br_4{\cdot}4H_2O$: C 51.17, H 4.83, N 9.95. Found: C 50.92, H 4.86, N 9.90.

CtDNA was purchased from Beitai Biochemical Co., Chinese Academy of Sciences, Beijing, and used without further purification. CtDNA was dissolved in 10 ml 0.1 M NaCl solution of double distilled water and set for a week at near 4 °C. Concentrations were obtained using $\varepsilon_{DNA} = 6600\ mol^{-1}\ cm^{-1}$ L at 260 nm.

2.2. Apparatus

All absorption was performed with UV-265 spectrophotometer (Shimadzu). Fluorescence and RLS measurements were conducted on F-4500 spectrofluorimeter (Hitachi) and the bandwidths were set at 10 and 2 nm, respectively. RLS

measurements were performed by using "synchronous scan" model. Namely, the excitation and emission were preset at the same wavelength. The pH meter (E-201-C) was made in the factory of magnetic spectrograph in Shanghai. ^{1}H NMR spectra and elementary analysis were measured with DKX-300 MHz Bruker instrument and Elementar Analysensysteme GmbH VarioEL instrument, respectively. All experiments were carried out at $20 \pm 1\,^{\circ}$C.

2.3. Method

A 1 mL aliquot of the stock solution (5.0×10^{-5} mol L^{-1}) of TEPyP was transferred into a 10 mL volumetric flask and an appropriate amount of 0.01 mol L^{-1} β-CD and 0.001 mol L^{-1} HP-β-CD (or SBE-β-CD) was added. The pH was fixed with Tris–HCl buffer solution (pH = 7.61). The mixed solution was diluted to final volume with double distilled water and shaken thoroughly, and determined after 30 min at $20 \pm 1\,^{\circ}$C. DNA was added with minim injector. The spectra were recorded or fluorescence intensities were measured. D_2O solution unbuffered was used for NMR experiment. The concentrations of β-CD (or HP-β-CD) and TEPyP were fixed at 3 and 3 mM, respectively.

3. Results and discussion

3.1. The interaction of TEPyP and ctDNA

3.1.1. UV–vis

The absorption spectral change of TEPyP titrated with ctDNA is shown in Fig. 2 and the absorbance change at the Soret band (422.2 nm) is given in Fig. 3. It can be seen that λ_{max} shifted to longer wavelength ($\Delta\lambda = 14$ nm). The hypochromicity was determined by the equation: $H = ((A_f - A_b)/A_f) \times 100$, where A_f and A_b represent the Soret absorbances of free and bound porphyrins, respectively. A large hypochromicity (42%) is shown. The intensity of the λ_{max} of TEPyP decreased together with a red shift at the initial step ($r > 0.4$), and then the intensity of the red shifted peak increased with further DNA additions ($r < 0.4$), here r is the molar ratio of TEPyP to ctDNA. However, no characteristic isosbestic point was observed in Fig. 2. It was suggested that the binding processes for TEPyP were more than two steps.

3.1.2. Fluorescence spectra

Fluorescence excitation and emission spectra of TEPyP were recorded in the absence and presence of DNA. The maximum excitation and emission wavelength were 430 and 666 nm in the absence of ctDNA. With the addition of ctDNA, $\lambda_{ex,max}$ shifted to longer wavelength ($\Delta\lambda = 13$ nm) and a decrease (around 50%) of the intensity of emission was observed. However, when r is larger than 0.4, the decreased fluorescence intensity enhanced remarkably again (Fig. 4).

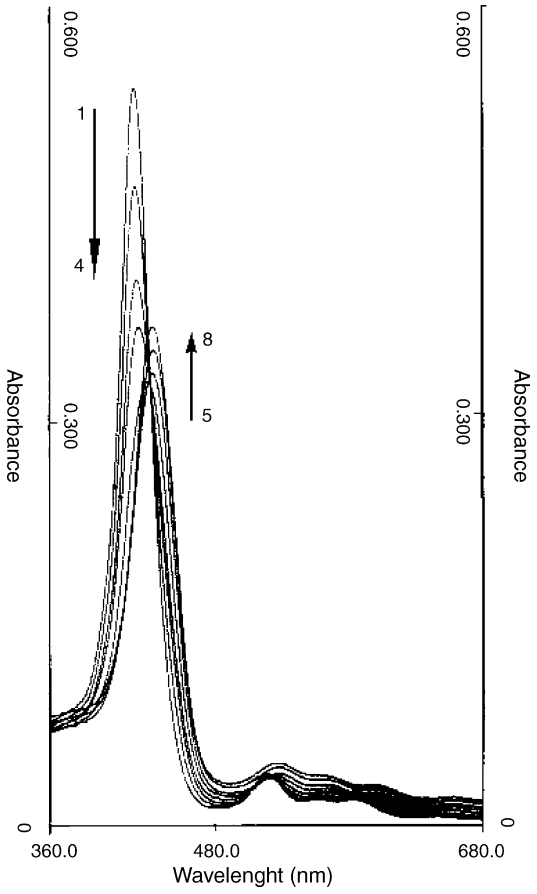

Fig. 2. The absorption spectral change of TEPyP titrated with ctDNA. The concentration of TEPyP was 5×10^{-6} mol L^{-1}. The concentration of ctDNA from 1 to 8 was 0, 5.0, 6.2, 8.3, 10.0, 12.5, 16.7 and 50.0×10^{-6} mol L^{-1}.

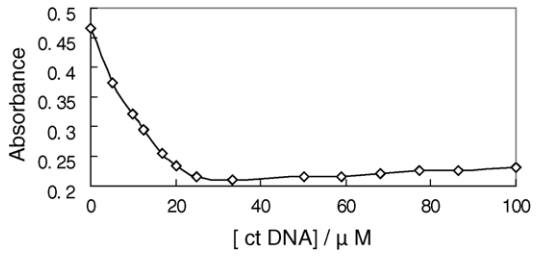

Fig. 3. The absorption change of TEPyP at 422 nm with the increasing concentration of ctDNA.

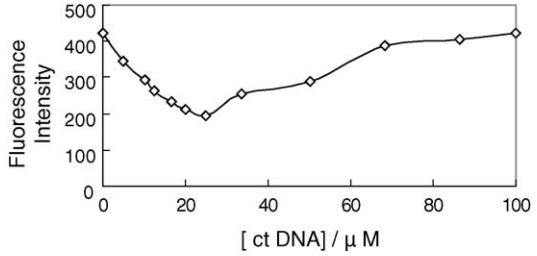

Fig. 4. The effect of increasing concentration of ctDNA on the fluorescence intensity of TEPyP.

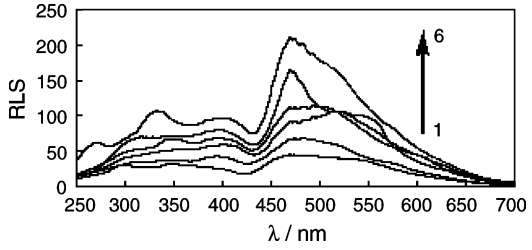

Fig. 5. The influence of ctDNA on the resonance light-scattering of TEPyP: (1–6) [ctDNA] ($\times 10^{-6}$ mol L^{-1}) is: 0, 22.0, 88.0, 110.0, 132.0 and 220.0.

3.1.3. Resonance light-scattering

Resonance light-scattering measurement is sensitive method and used to detect extended porphyrin aggregation from now on. The light-scattering profiles of TEPyP in the absence and presence of ctDNA were shown in Fig. 5. Porphyrin had relatively little scattering profile and the minima peak appeared at 438 nm near Soret maximum, at which the photons were absorbed. An enhanced RLS signal was observed with the addition of ctDNA. We could draw the conclusion from the changed spectral that extended aggregation of the porphyrin molecular on the DNA template.

3.1.4. The binding mode

It has been reported in several articles that TMPyP are capable to intercalate into DNA. Comparative the changed spectroscopy properties of TMPyP bound to ctDNA provided by literature [24] to that of TEPyP bound to ctDNA concluded from the experiment, the interaction mechanism of TMPyP and TEPyP is different. (1) With the increasing concentration of ctDNA, no characteristic isosbestic point was observed for TEPyP, which is not consistent with TMPyP. (2) The RLS intensity of TEPyP enhanced remarkably in the presence of ctDNA. However, DNA could not increase the RLS intensity of TMPyP. On the other hand, the observed spectroscopic properties of TEPyP in the presence of DNA is consistent with those of TAPP and cationic bis-porphyrin with various lengths of diamino alkyl linkage reported by Huang et al. [25,26] and Yamakawa [27], respectively. Then, their binding mode were assigned to outside self-stacking on the DNA surface.

3.2. The inclusion of TEPyP with CDs

Above critical concentration, the dimerization and polymerization of water-soluble porphyrins is great drawback for the further application. The aggregation behavior of TEPyP in aqueous solution could be observed on the basis of the titration of UV–vis absorption. The Beer's law was no longer obeyed above concentration of 6 μM. Nevertheless, the addition of CDs to the neutral buffer solution of TEPyP resulted in a good linear comparative with the absence of CDs, which suggested that β-CD and SBE-β-CD act as receptor to inclusion TEPyP and suppress aggregation of guest molecular.

The binding constant K of the TEPyP-CDs complexes were determined from the change of fluorescence intensity. When the Benesi–Hildebrand treatment was applied, good linear were obtained from the plot of $1/(F - F_0)$ versus 1/[CD] for β-CD, HP-β-CD and SBE-β-CD, which assumed a 1:1 (host:guest) stoichiometry. The K values for β-CD, HP-β-CD and SBE-β-CD with TEPyP were 550, 1.1×10^4 and 2.0×10^5 M^{-1}, respectively. The results showed that the β-CD derivatives were prior to the native β-CD for the inclusion of TEPyP, which may be explained by the fact that the chemically modified cyclodextrins are endowed with specially functional groups. Then, the solubility and flexibility of cyclodextrins have been improved in the great degree comparative with native β-CD. Moreover, the strongest inclusion capacity of SBE-β-CD was because that SBE-β-CD is negatively charged, while TEPyP is positively charged. And electrostatic forces play important roles in process of weak inclusion interaction expect for the general weak molecular interaction such as, hydrophobic interaction, hydrogen bonding van der Waals forces and the relief of high energy water from the CDs cavity and so on.

^{1}H NMR spectra of β-CD and TEPyP/β-CD in D_2O were shown in Fig. 6. The chemical shifts data for the inclusion complex were different from these for the free compounds. Comparative with H-1, -2, -4 located on the exterior of β-CD, the protons of β-CD located within or near the cavity (H-3, -5, -6) shows remarkably large shift (−0.085, −0.087, −0.052 ppm) in the mixture. As far as the guest molecule,

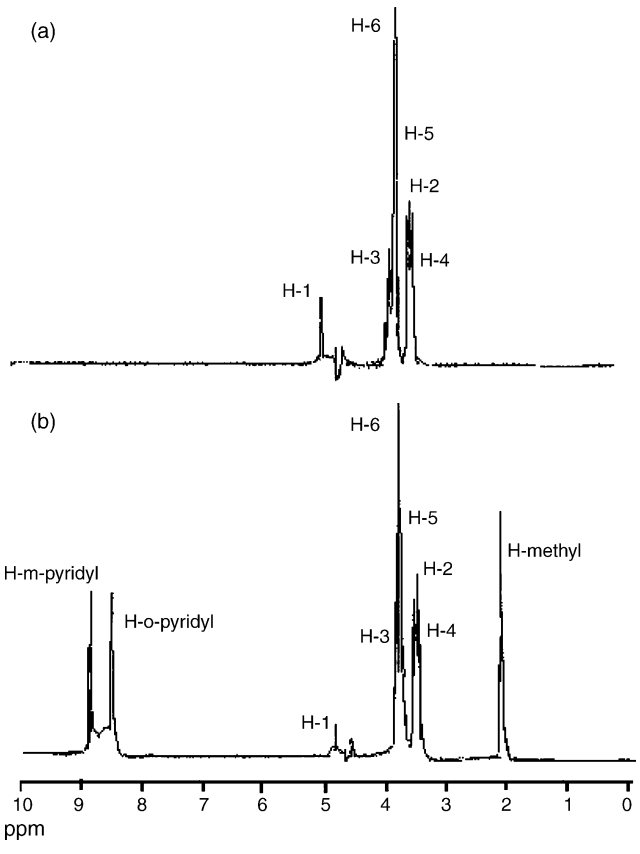

Fig. 6. ^{1}H NMR spectra (300 MHz, 20 °C, DO_2) of: (a) β-CD (3 mM); (b) TEPyP-β-CD (3:3 mM).

the more shields protons of *m*-H, *o*-H of pyridyl and methyl moiety showed apparently downfield shift (0.016, 0.020, 0.015 ppm) in the presence of β-CD, which provided further analytical results that the inclusion complex was formed between TEPyP and β-CD.

3.3. Change of microscopic environment by cyclodextrins

It is well known that the binding constants and binding number for porphyrin interaction with nucleic acid can provide valuable information and were determined by the quench of fluorescence according to the equation [25]

$$\frac{\lg(F_0 - F)}{F} = \lg K + n \lg[\mathrm{DNA}]$$

where F and F_0 are the fluorescence intensity in the absence and presence of DNA, respectively. K represents the binding constant, n is the binding number and [DNA] the concentration of added DNA. Instead of fluorescence intensity (F) with absorbance (A), the equation can be used to investigate electron absorbance spectroscopy. Then, K and n were calculated from the slope and intercept, respectively. Table 1 showed the K and n values of the interaction of TEPyP with ctDNA in the absence and presence of cyclodextrins. In addition, the results of control experiment with TMPyP were listed in Table 1.

It is noted that with the addition of these cyclodextrins, the binding constants and binding numbers were remarkably decreased. The effect of β-CD, HP-β-CD and SBE-β-CD on the interaction of TEPyP with DNA exhibited significant difference. It is well known that cyclodextrins possess unique hydrophobic cavity, which may influence binding affinity of porphyrin to DNA and weaken the static electronic forces in the outside self-stacking. Typically, the negative phosphate groups of hamper directly the binding of the negative phosphate groups of the DNA backbone and the positive TEPyP.

Table 1
The binding constants and n values for TEPyP and TMPyP measured by absorption and fluorescence in the absence and presence of CDs

	Water	β-CD	HP-β-CD	SBE-β-CD
TEPyP				
A				
K	4.84×10^5	6.18×10^4	1.00×10^4	2.11×10^3
n	1.05	0.90	0.80	0.74
r	0.998	0.990	0.992	0.979
F				
K	5.54×10^7	6.27×10^6	4.73×10^6	3.62×10^4
n	1.43	1.29	1.32	0.96
r	0.998	0.993	0.995	0.993
TMPyP				
A				
K	4.27×10^5	3.02×10^5	1.12×10^5	3.80×10^4
n	1.14	1.11	1.05	1.00
r	0.995	0.996	0.995	0.999
F				
K	9.94×10^7	5.75×10^7	2.73×10^7	1.17×10^5
n	1.55	1.44	1.43	0.99
r	0.991	0.993	0.989	0.992

4. Conclusion

The results of experiment expect to provide useful and valuable information for the study of supramolecular chemistry. The conclusion could be draw from the results that the supramolecular assembly of DNA-bound porphyrin has been disassembled, which due to that cyclodextrins admitting porphyrin into their cavities partly and the process weaken the static electronic forces in the outside self-stacking.

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