Journal of Inclusion Phenomena and Macrocyclic Chemistry (2007) 58:221–226
DOI 10.1007/s10847-006-9146-6

Study on the Association Phenomenon of Cyclodextrin to Porphyrin J-aggregates by NMR Spectroscopy

HUI LI MA[1,2], JIAN JUN WU[1,3], WEN JUAN LIANG[1,4] and JIAN BIN CHAO[1,*]
[1]*School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan, 030006, P.R. China;* [2]*Shanxi Vocational Poly-tech College, Taiyuan, 030006, P.R. China;* [3]*The Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, P.R. China;* [4]*Department of Chemistry, Shanxi Datong University, Datong, P.R. China*

(Received: 18 April 2006; in final form: 11 September 2006)

Key words: *meso*-tetrakis- (*p*-sulfonatophenyl) porphyrin, J-aggregation, cyclodextrins, NMR

Abstract

The nuclear magnetic resonance (NMR) spectroscopy demonstrated that the inclusion complexes of *meso*-tetrakis-(*p*-sulfonatophenyl) porphyrin (TPPS) with β-, Hydroxypropyl-β- and Methyl-β-cyclodextrin (β-, HP-β- and Me-β-CD) are formed, which resulted in the dissociation of TPPS J-aggregates efficiently under certain acidity. There are no significant differences in binding affinities and basic complexation mechanisms between TPPS and β-cyclodextrin (β-CD) or hydroxypropyl-β-cyclodextrin (HP-β-CD), i.e. porphyrin is included through the wide side of the cavity of β-CD or HP-β-CD. Alternatively, porphyrin is included through the narrow side of the Me-β-CD cavity.

Introduction

More recently, there has been a growing interest in the use of aggregates of porphyrins and their derivatives for medicine such as tracing marker for cancer detection and photosensitizers in photodynamic therapy (PDT) of cancers [1–6]. Under appropriate ionic strength or acidity, porphyrin and their analogues can form highly ordered aggregates. There are two kinds of aggregates corresponding to the extreme cases of *face-to-face* or *edge-by-edge* geometric arrangement for the adjacent stacking monomers, entitled as J- or H-aggregates, respectively [7–9]. Excitonic splitting theory predicts blue-shifted and red-shifted bands of spectrum of aggregates with respect to the monomers, for H- and J-aggregates, respectively [10, 11]. The porphyrin tetrakis- (*p*-sulfonatophenyl) porphyrin (TPPS) is the first example of a water-soluble species leading to J-aggregates [12]. The initial protonation of pyrrole nitrogen atoms to the zwitterionic diacid form has been considered to be responsible for the subsequent growth of linear arrays of stacking monomers, stabilized mainly by a network of hydrogen bonds connecting the negative sulfonate end-groups with the protonated pyrrole nitrogen atoms [13]. After protonation, the porphine part of TPPS becomes more planar with the porphyrin ring and favors aggregation [14], so it is a famous ''star'' molecule in porphyrin aggregation study. Under acidic conditions, the absorbance spectra of TPPS are characteristic of 490 nm (B band) and 705 nm (Q band) [15, 16]. However, aggregation is often considered as a disadvantage for photosensitizing applications. For example, the photophysical properties of inclusion complexes of water-soluble meso-tetrasubstituted porphyrins with cyclodextrins (CDs) have been studied in order to obtain nonaggregated porphyrins in aqueous solutions. Therefore, understanding how aggregation affects the photophysical behavior of chromophores is not only important for their behavior in biological systems but also for the use of their condensed phases in advanced materials [17]

CDs are of great importance as useful host molecules due to their characteristic structure, i.e. interior hydrophobic cavity and exterior hydrophilic groups. As a result, CDs have ability to entrap a wide variety of drugs with appropriate molecular size and polarity or chirality into their hydrophobic cavities entirely or partially under noncovalent interaction in both solution and solid state [18]. Recently, the investigation on the self-assembly using suparmolecular porphyrin-cyclodextrin complexes as building blocks gradually becomes one of the hot topics [19–23]. The current interest of the inclusion of water-soluble porphyrin with CDs lies in: (1) the modification of the porphyrin properties; (2) the hindrance of porphyrin aggregation; (3) the protection of the porphyrin macrocycle and its phenyl substituents from the active species originating during oxidation processes etc [24]. It is interested that the cavities of CDs

* Author for Correspondence. E-mail: chao@sxu.edu.cn

are favorable for loading anionic porphyrin guests and unfavorable for cationic ones [25]. However, up to our knowledge, previous studies in this area are mostly in neutral medium or in basic medium, few studies of inclusion complexes of CDs with porphyrin J-aggregates in acidic condition have been published. Based on above reviews, the investigations on the inclusive interaction of porphyrin J-aggregates with CDs in acidic condition leading to decreasing aggregate phenomena of porphyrin arose our attraction.

In a previous work, we carefully investigated the interaction between porphyrin J-aggregates and three kinds of CDs, including β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and methyl-β-cyclodextrin (Me-β-CD) using electronic spectra and fluorescence spectra [26]. The experimental results demonstrate that the disassociation of porphyrin J-aggregates occurs upon addition of β-CD, HP-β-CD and Me-β-CD and lead to decrease at 490 nm and increase at 436 nm in absorption spectra, with gradually increasing fluorescence emission. However, a detailed description of the geometry of these complexes remains uninvestigated. Nuclear magnetic resonance (NMR) spectroscopy has already been become an important tool for *in vitro* and *in vivo* studies of CDs interactions with biological macromolecules. The most obvious incentive, however, of using this precise and reliable technique for the investigation of CDs complexes is these noncovalent associations, especially the orientation of the guest molecule in the CDs cavity [27]. Therefore, in this work, we use ^{1}HNMR spectroscopy to characterize the inclusion interaction of CDs with porphyrin by which the J-aggregates were disaggregated. Further insights into the geometry of the inclusion compounds are gained by Rotational Nuclear Overhauser Effect Spectroscopy (ROESY), for detection of intermolecular Nuclear Overhauser Effects (NOEs) between the protons directly involved in the host-gust interaction.

Experimental

Reagents and apparatus

TPPS, chromatographic grade, is purchased from Alfa Aesar Reagent Company (U.S.A), and is prepared into 1×10^{-4} M stock solution and then diluted to a certain concentration when used. The β-CD (95%, purchased from Yunnan Gourmet Factory, China) is recrystallized before use. Both HP-β-CD and Me-β-CD are presented kindly by Mr. I. P. Peter of Wacker Co. (Germany) and used without further purification. The degree of substitution DS is 6.1 for HP-β-CD and 12.5 for Me-β-CD, respectively. D_2O and DCl are purchased from Cambridge Isotope Laboratories Inc. All the other reagents used here are of analytical grade, made in China. Water is doubly distilled in the whole experimental procedures.

Procedures

Typically, porphyrin solution and appropriate amount of acid are transferred into a comparison tube in turn. Appropriate volume of CDs is subsequently added and thoroughly shaken. NMR studies are carried out using D_2O as the solvent, and DCl as acidity medium. ^{1}HNMR and ROESY spectra are taken on DRX300 spectrometer (Bruker, Switzerland), using at 300.13 MHz with 10 μs as the 90° pulse width. All experiments are performed at room temperature.

Results and discussion

^{1}HNMR spectra of the inclusion of TPPS J-aggregate and CD

Three kinds of CDs, including β-CD, HP-β-CD and Me-β-CD are added into the J-aggregate system of TPPS, respectively, to investigate the influences of CDs on the J-aggregates and the models of the inclusion complexes of CDs with TPPS. If CDs and TPPS J-aggregates form inclusion assemblies, two kinds of changes in chemical shifts of protons could be expected on the ^{1}HNMR spectra of their mixtures: first, a change in the TPPS part of the spectrum, generated by the increase of the monomeric complexed form at the expense of the aggregated form; second, a change in the cyclodextrin part of the spectrum due to the magnetic anisotropy of the phenyl group inside the cyclodextrin cavity and porphyrin ring near the cyclodextrin. Figure 1 shows the ^{1}HNMR spectra of porphyrin in D_2O and without CDs (a), and of TPPS J-aggregates in the absence (b) and presence of CDs (c–e), respectively, at $[D^+] = 0.9$ M. The ^{1}HNMR chemical shifts of different β-CD in the absence and presence of TPPS J-aggregates are listed in Table 1.

Chemical shift variations of specific host or guest nucleus could provide evidence for the formation of inclusion complexes in solution, since significant changes in microenvironment are known to occur between the free and bound states. Information about the interaction of TPPS J-aggregates and CDs from ^{1}HNMR is primarily inferred from the changes in chemical shifts and line shape.

From Table 1, it can be seen that only H-3 protons, the located inside the cavity close to wide end, for the three kinds of β-CDs, are obviously shifted to up-field. In contrast, H-5 protons, inside of the β-CD close to narrow end, and H-6 proton (for β-CD and Me-β-CD) are shifted to down-field. But the chemical shift of H-6 located on the cavity rim at the narrow end of the molecule for HP-β-CD is unaffected, while the signals of H-2 and H-4 protons for HP-β-CD, as well as the signal of H-2 proton for β-CD, on the outer surface unchanged. Down-field shift appeared on H-4 proton for β-CD. In addition, it is needed to note that during the inclusive process the chemical shift values for the

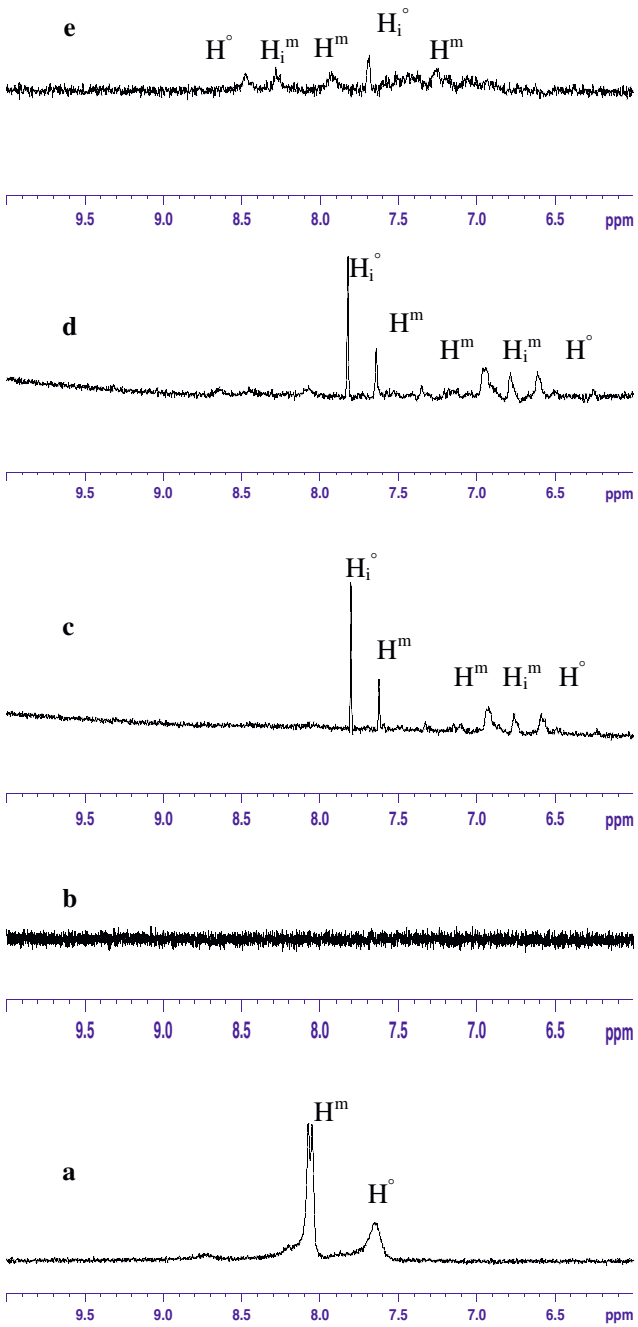


Figure 1. ^{1}HNMR spectra of TPPS in neutral D_2O without CD (a), TPPS J-aggregate in D_2O/DCL medium (b), TPPS J-aggregate in the presence of β-CD (c), TPPS J-aggregate in the presence of HP-β-CD (d), TPPS J-aggregate in the presence of Me-β-CD (e). (both [TPPS] and [CDs]: 1.0×10^{-3}M; $[D^+] = 0.9$ M).

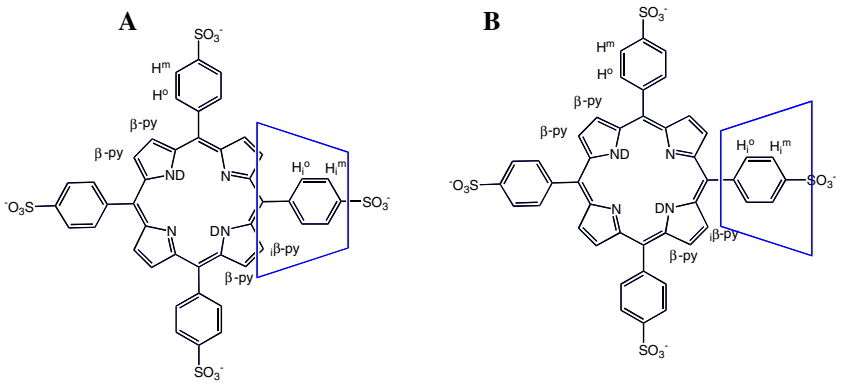
anomeric proton are also showed downshift for both β-CD and HP-β-CD. The down-field shift for the anomeric proton in CDs can be attributed to the deshielding effect from the porphin ring. The up-field shifts of the protons located within the β-CDs cavity (i.e. H-3), as well as the unchanged chemical shift observed for those at the exterior of CDs (e.g. H-2, H-4) evidenced the existence of an interaction between monomer porphyrin and the interior of the CDs cavity, resulting in a removal of TPPS J-aggregate equilibrium toward to monomer.

It is well known that the magnetic anisotropy of an aromatic ring results in an up-field ^{1}H-chemical shift of protons located above (or below) the π-electron cloud. Therefore, the up-field shift observed for H-3 proton of β-CDs can be explained that water is replaced by the hydrophobic aromatic benzene rings of TPPS J-aggregate inside the cavity. In addition, the fact could be production of the magnetic anisotropy shielding induced by the "ring-current" effects produced by at least one of the phenyl rings of TPPS J-aggregate inside the CDs cavity. Therefore, shifts to higher fields of the protons located within the CDs cavity (especially H-3) indicated that a hydrophobic interaction is existed between TPPS and the CDs [27].

Because of the higher shielding effect on H-3 proton, with respect to H-5 (and H-6), it can be assumed that TPPS J-aggregate preferentially is inserted from the secondary hydroxyl side of β-CD and HP-β-CD, as depicted in Scheme 1(A).

Unfortunately, the changes of H-1 (including the anomeric proton), H-2 and H-4 protons of Me-β-CD, in the absence of J-aggregates of TPPS, could not be determined because of the overlapping with other signals. It is interesting to note that the change of the chemical shift of H-6 proton lying on the cavity rim of Me-β-CD was the most prominent, followed by the H-5 proton and by the H-3 proton on the inner surface of the cavity of the secondary hydroxyl group side. The fact that slight larger shift of the H-6 proton in the Me-β-CD inclusive complex may be due to some conformational changes of the primary hydroxyl groups, as well as the polarity change of the cavity induced by the particularly interaction of methyl groups and guest molecule [28]. Therefore, it is hypothesized that TPPS J-aggregate penetrated into the cavity through the more accessible primary face of Me-β-CD, as show as Scheme 1(B).

Due to the phenyl protons are found to be more highly affected by the environment and charge of the porphyrin, the ^{1}H-NMR spectra of the TPPS monomer species (Figure 1(a)), therefore, show that the *ortho*-protons (H^o) of TPPS (1.0×10^{-3} M) appear as a broad shoulder in neutral D_2O. While the *meta*-protons (H^m) appear as a well-defined sharp doublet, the broadening of H^o is ascribed to self-aggregation of TPPS [25]. Under concerned conditions for investigation of J-aggregation of TPPS and dissociation of CDs to J-aggregates, the concentration of TPPS is too low (μmol/L level) to give any significant signal in NMR spectroscopy. Therefore, the researchers usually had to increase the tested concentration of porphyrin to ca. mmol/l scale to produce observable signal [19]**.** The J-aggregate should be the most probable form appeared in strong acidic medium under so high concentration of TPPS. In other words, the proton exists in an environment of the dimmer or other higher aggregated species. Thus the ^{1}HNMR spectrum of J-aggregate is not observable because of inhomogeneous broadening of the resonances [29], as show in Figure 1(b). In this case, a fast exchange



Scheme 1. Possible relative host–guest orientations of cyclodextrins inside the inclusion complex with TPPS J-aggregate.

between different forms could lead to broadening and loss of signal [30]. If aggregation equilibrium could be broken, the ^{1}HNMR signals of monomer of TPPS should be observable in principle.

Upon addition of CDs into TPPS J-aggregates, obviously changes appeared in the spectra of TPPS J-aggregate, as shown in Figure 1(c–e). New ^{1}HNMR signals of TPPS occurred in parallel with the presence of CDs. It is noteworthy that the signal of *ortho*-H protons of TPPS showed downfield shift upon addition of CDs, as well as the signal of *meta*-H protons of TPPS appeared up-field shift due to the shielding effect of the porphyrin core. The line sharpening occurred, suggesting the presence of a more rigid complex [31]. Moreover, three sets of new peaks occurred at about 6.5–7.0 ppm in the spectra of TPPS upon addition of β-CD (Figure 1(c)) and HP-β-CD (Figure 1(d)), respectively. While upon addition of Me-β-CD (Figure 1(e)), these peaks in the spectra of TPPS appeared at about 8.0–8.8 ppm. Comparison of the chemical shift of the protons (H-3, H-5) inside of the CDs cavity (cf. Table 1) before and after the interaction has a notable change. It is further deduced that the inclusion complexes of TPPS in J-aggregates and CDs are formed under experimental conditions. Moreover, the dissociation of J-aggregation of TPPS is appeared and the monomer species of TPPS are reproduced.

ROESY spectra

A ROESY experiment is suitable for obtaining information about the spatial proximity between atoms of the host and guest molecules by observing the intermolecular dipolar cross-correlations. The intensities of the cross peaks are proportional to $1/r^6$, where r represents the mean distance between the protons in dipolar interaction [32]. The NOEs is a manifestation of cross-relaxation between two nonequivalent nuclear spins that are close enough, e.g. < 5 Å (through space) [33].

In the present study, to gain further information on the inclusion complexation mode and additional insights into the geometry, ROESY spectra were acquired for the complexes. Figure 2 shows the two-dimensional ROSEY spectra of the complexes of the different β-CDs with TPPS in J-aggregates.

As showed as the ROESY spectra (Figure 2(a)), it is demonstrated that both H^o and H^m phenyl protons of TPPS gave the cross peaks with inner H-3 and H-5 protons of β-CD, respectively. Furthermore, it is noteworthy that in HP-β-CD (Figure 2(b)), the *m*-H phenyl proton of the TPPS J-aggregate has a correlation with both inner H-3 and H-5 protons of cavity. However, in Me-β-CD (Figure 2(c)), the *m*-H phenyl proton of the TPPS J-aggregate has a correlation with both inner H-3 and H-5 protons of cavity. In addition, the ^{1}HNMR

Table 1. ^{1}H Chemical shifts corresponding to CDs in the presence and absence of J-aggregates of TPPS

CDs	β-CD$_S$			HP-β-CD$_S$			Me-β-CD$_S$		
	δ_F	δ_J	$\Delta\delta$	δ_F	δ_J	$\Delta\delta$	δ_F	δ_J	$\Delta\delta$
H-1	4.26	4.27	0.01	4.29	4.29	0.00	*a*	4.20	*a*
H-1*	5.01	5.02	0.01	5.03	5.04	0.01	*b*	4.96	*b*
H-2	3.02	3.02	0.00	3.05	3.05	0.00	*a*	3.01	*a*
H-3	3.47	3.45	−0.02	3.47	3.43	−0.04	3.42	3.41	−0.01
H-4	2.85	2.86	0.01	2.87	2.87	0.00	*a*	2.76	*a*
H-5	3.22	3.24	0.02	3.21	3.24	0.03	3.15	3.17	0.02
H-6	3.24	3.25	0.01	3.27	3.27	0.00	3.17	3.21	0.04

$\Delta\delta = \delta_J$-δ_F, δ_F and δ_J present ^{1}H Chemical shifts (ppm) of CDs in the absence and presence of TPPS J-aggregates, respectively; $[D^+] = 0.9$ M.
H-1*: The anomeric proton of H-1.
a: The signal could not be determined due to the overlapping with other signals.
b: The signal could not be detected.

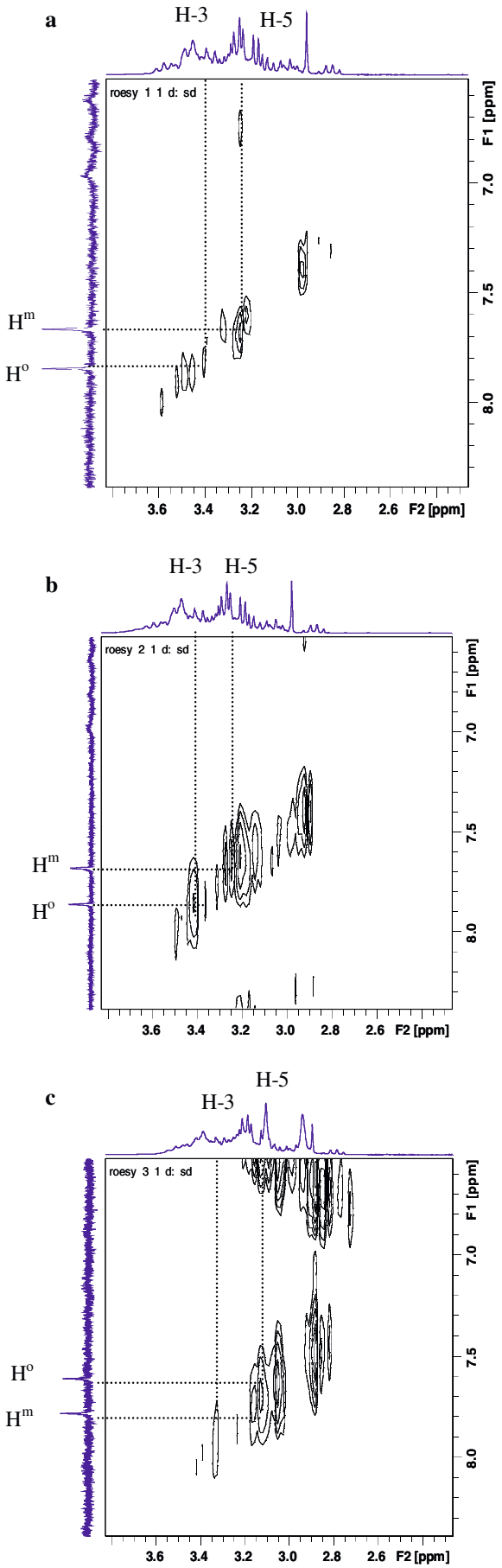


Figure 2. ROESY spectra of TPPS J-aggregates with β-CD (a), HP-β-CD (b) and Me-β-CD(c).

spectra and 2D ROESY spectra for both β-CD and HP-β-CD exhibited very similar in certain range. The ^{1}HNMR and 2D ROESY spectra for the inclusive complexation of β-CDs and TPPS J-aggregate are measured under certain acidic condition, instead of neutral or basic condition. Therefore, this similarity should be mainly induced by specific solvent effect under acidic condition.

In conclusion, the fact is that the NOEs occurred between the phenyl protons (*o*-H, *m*-H) of TPPS and H-3, H-5 protons of cavity. These results coupled with the ^{1}HNMR experiments demonstrated that the same functional groups of the TPPS are affected, in similar proportion, in the presence of β-CD and HP-β-CD. We could conclude that the mechanism of inclusion is similar for both β-CD and HP-β-CD, and confirmed the hypothesis that J-TPPS is embedded in the cavity through the primary side of Me-β-CD.

In terms of the intensities of the signals corresponding to the cross-peaks between the phenyl protons of TPPS and H-3, H-5 protons inner CDs cavity, it is interested that the intensities of the cross-peaks for TPPS with Me-β-CD are the strongest, follow by that of TPPS with HP-β-CD, then by that of TPPS with β-CD. Therefore, it is deduced that complex capacity of TPPS with CDs obey the following order: Me-β-CD > HP-β-CD > β-CD. These findings are agreed with the previous reports [26].

For the TPPS J-aggregate and CDs complexes, strong intermolecular cross-magnetizations between the H-3, H-5 protons located inside the CDs cavity and the *m*-H, *o*-H phenyl protons are observed. This observation demonstrated that the inclusion complexes of theses groups are formed in the hydrophobic cavity.

Conclusions

NMR spectra provided the evidence that CDs inclusive of β-CD, HP-β-CD and Me-β-CD can efficiently disassociate the porphyrin J-aggregates and lead to reproduce the TPPS monomer species under certain acidity. There are some similarities in binding affinities and basic complexation mechanisms for β-CD and HP-β-CD, while the difference occurs for Me-β-CD due to the steric effects.

Acknowledgments

This work is financially supported by the Natural Science Foundation of China and the Natural Science Foundation of Shanxi Province, and authors enjoy very much the referee's kind suggestions and comments.

References

1. P.J. Meadows, E. Dujardin, S.R. Hall, and S. Mann: *Chem. Commun.* 3688 (2005).

2. R.F. Pasternack, C. Fleming, S. Herring, P.J. Collongs, J. de Paula, G. Decatro, and E.J. Gibbs: *J. Biophys*. **79**, 550 (2000).
3. L. Zhang, J. Yuan, and M.H. Liu: *J. Phys. Chem. B* **107**, 12768 (2003).
4. L. Zhang, Q. Lu, and M.H. Liu: *J. Phys. Chem. B* **107**, 2565 (2003).
5. S.M. Andreda and S.M.B. Costa: *J. Fluoresc*. **12**, 77 (2002).
6. S.C. Doan, S. Shanmygham, D.E. Aston, and J.L. McHale: *J. Am. Chem. Soc*. **127**, 5885 (2005).
7. A.V. Udal'tsov, M. Tosoka, and G. Kaupp: *J. Mol. Struct*. **660**, 15 (2003).
8. P. Kubát, K. Lang, K. Prochazkova, and P. Anzenbacher Jr.: *Langmuir* **19**, 422 (2003).
9. L.M. Scolaro, A. Romeo, M.A. Castriciano, and Micali N: *Chem. Commun*. 3018 (2005).
10. A. Mishra, R.K. Behera, P.K. Mishra, and G.B. Behera: *Chem. Rev*. **100**, 1973 (2000).
11. G. Pescitelli, S. Gabriel, Y. Wang, J. Fleischhauer, R.W. Woody, and N. Berova: *J. Am. Chem. Soc*. **125**, 7613 (2003).
12. O. Ohno, Y. Kaizu, and H. Kobayashi: *J. Chem. Phys*. **99**, 4128 (1993).
13. N. Micali, A.R. Lauceri, R. Purrello, F. Mallama, and L.M. Scolaro: *J. Phys. Chem. B* **104**, 9416 (2000).
14. G. Deluca, A. Romeo, and L.M. Scolaro: *J. Phys. Chem. B* **109**, 7149 (2005).
15. D.L. Akins, H.-R. Zhu, and C. Guo: *J. Phys. Chem*. **98**, 3612 (1994).
16. D.L. Akins, H.-R. Zhu, and C. Guo: *J. Phys. Chem*. **100**, 5420 (1996).
17. H. García-Ortega, J.L. Bourdelande, J. Crusats, Z. El-Hachemi, and J.M. Ribó: *J. Phys. Chem. B* **108,** 4631 (2004).
18. S. Simova and H.-J. Schneider: *J. Chem. Soc., Perkin. Trans*. **2**, 1717 (2000).
19. J. Mosinger, M. Deumié, K. Lang, P. Kubát, and D.M. Wagnerová: *J. Photochem. Photobiol. A: Chem*. **130**, 13 (2000).
20. Y. Liu, P. Liang, Y. Chen, Y.-M. Zhang, J.-Y. Zheng, and H. Yue: *Maromoleculaes* **38**, 9095 (2005).
21. K. Kano, H. Kitagishi, S. Tamura, and A. Yamada: *J. Am. Chem. Soc*. **126**, 15202 (2004).
22. K. Kano, R. Nishiyabu, T. Yamazaki, and L. Yamazaki: *J. Am. Chem. Soc*. **125**, 10625 (2003).
23. F. Venema, A.E. Rowan, and R.J.M. Nolte: *J. Am. Chem. Soc*. **118**, 257 (1996).
24. J.M. Ribó, J.-A. Farrera, M.L. Valero, and A. Virgili: *Tetrahedron* **51**, 3705 (1995).
25. K. Kano, R. Nishiyabu, T. Asada, and Y. Kuroda: *J. Am. Chem. Soc*. **124**, 9937 (2002).
26. J.J. Wu, H.L. Ma, H.S. Mao, Y. Wang, and W.J. Jin: *J. Photochem. Photobiol. A: Chem*. **173**, 296 (2005).
27. H.-J. Schneider, F. Hachet, and V. Rüdiger: *Chem. Rev*. **98**, 1755 (1998).
28. M. Fathy and M. Sheha: *Pharmazie* **55**, 513 (2000).
29. Y. Ikeda, S. Motoune, T. Matsuka, H. Arima, F. Hirayama, and K. Uekama: *J. Pharma. Sci*. **91**, 2390 (2002).
30. N.C. Maiti, S. Mazumdar, and N. Periasamy: *J. Phys. Chem. B* **102**, 1528 (1998).
31. M.Y. Choi, J.F. Pollard, M.A. Webb, and J.L. Mchale: *J. Am. Chem. Soc*. **125**, 810 (2003).
32. I. Correia, N. Bezzenine, N. Ronzani, N. Platzer, J.-C. Beloeil, and B.-T. Doan: *J. Phys. Org. Chem*. **15**, 647–659 (2002).
33. S.H. Toma, M. Umi, S. Nikolaou, D.M. Tomazela, M.N. Eberlin, and H.E. Toma: *Inorg. Chem*. **43**, 3521 (2004).