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Aggregation of two carboxylic derivatives of porphyrin and their affinity to bovine serum albumin

Yao-Bing Yin, Yi-Nong Wang, Jian-Biao Ma *

Key Laboratory of Functional Polymer Materials (Ministry of Education), Nankai University, Tianjin 300071, PR China

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Abstract

Aggregation of two porphyrin derivatives with carboxylic groups, 4-oxo-4-((4-(10,15,20-triphenyl-21*H*,23*H*-porphin-5-yl)phenyl)amino)-butanoic acid (MAC) and 4,4′,4″,4‴-[21*H*,23*H*-porphine-5,10,15,20-tetrayltetrakis(4,1-phenyleneimino)]tetrakis(4-oxo-butanoic acid) (TA4C), and their affinity to bovine serum albumin were investigated via absorption spectrometry, ^{1}H NMR and fluorescence spectrometry. MAC and its complexes with β-cyclodextrin could form aggregates in an aqueous solution while TA4C was self-associated loosely. From the absorbance profiles of MAC in the titration of bovine serum albumin, hypochromicity was observed without any shift of the maximum absorbance wavelength. In both absorption spectra of TA4C in aqueous solutions and in solid state, three Q bands appeared in the visible region. In the measurements of absorption and fluorescence spectra upon titration of BSA, some spectral changes of TA4C were observed. The whole procedure of titration could be divided into three successive stages. The three-banded profiles of TA4C might be explained according to a loose dimer model.

Keywords: Aggregation; Binding modes; Three-banded Q bands; Fluorescence; Absorption spectrum

1. Introduction

Aggregation of porphyrin derivatives is known to play an important role in biological events, such as photosynthetic light energy conversion, oxygen transport, and biological catalysis. Their affinity to biological molecules is often involved in the transportation and metabolism of porphyrins in human bodies. It is found that the formation of porphyrin aggregates in aqueous solutions is often affected by the chemical structure of porphyrins, ionic strength, temperature, pH and surfactants [1]. The affinity of some porphyrins to bovine serum albumin or human serum albumin was studied [2–6]. Especially, much attention was paid on protoporphyrin IX [7], deuteroporphyrin [8] and other carboxylic porphyrins [9,10] in the past decades. Moreover, some cyclodextrin derivatives could bind water-soluble porphyrins [11,12] so as to preclude the porphyrin–porphyrin aggregation [13].

On the other hand, three Q bands as an interesting spectral characteristic of tetracarboxyphenylporphine (TCPP) in an aqueous solution occurred in the visible region [14]. The influence of substituents, monoprotonation and steric hindrance on the three-banded phenomena was observed. The three-banded Q bands existed in the UV–vis spectra of some monoprotonated porphyrins [15], 5,15-di(4-hydrocyphenyl)-10,20-di(4-hexadecyloxyphenyl)porphyrin [16], five tri-(*N*-methyl-4-pyirdiniumyl) porphyrins with an amino acid or peptide side-chain on the fourth meso aromatic substituent [17] and four protoporphyrin IX derivatives with 2-aminoglycosamide group [18] in aqueous media. In the absorption spectrum of tetra-(*p*-*N*,*N*-diphenylamino)phenylporphine ($TDPAPPH_2$) in chloroform solution, three Q bands also occurred due to non-planar distortion of the porphine ring originated from big steric hindrance [19]. Unfortunately, the nature and the significance of three-banded profile of different porphyrins are not clear up to date.

In the present study, an attempt is to gain more insight into the aggregation behavior of some porphyrins and their interaction with biological molecules. Two porphyrin derivatives with butanoic acid groups, 4-oxo-4-((4-(10,15,20-triphenyl-21*H*,23*H*-porphin-5-yl)phenyl)amino)butanoic acid (MAC) and 4,4′,4″,4‴-[21*H*,23*H*-porphine-5,10,15,20-tetrayltetrakis(4,1-phenyleneimino)]tetrakis(4-oxo-butanoic acid) (TA4C), as illustrated in Chart 1, were studied via absorption spectrometry, ^{1}H NMR and fluorescence spectrometry. The effect of

* Corresponding author. Tel.: +86 22 23507746; fax: +86 22 23502749.
E-mail address: jbma@nankai.edu.cn (J.-B. Ma).

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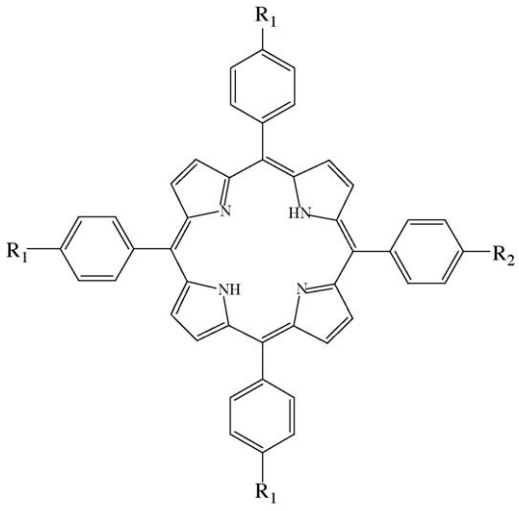

MAC R_1=H, R_2=- $NHCOCH_2CH_2COOH$

TA4C R_1=R_2=-$NHCOCH_2CH_2COOH$

Chart 1. Structures of two porphyrin derivatives with carboxylic groups.

β-cyclodextrin on aggregation of MAC in an aqueous medium was investigated. The spectral characteristic of the three Q bands was observed and discussed to elucidate the aggregation of TA4C in an aqueous solution and the binding of TA4C to BSA.

2. Experimental

2.1. Materials

Monomethoxy poly(ethylene glycol) was supplied by Sigma Chemical Company. Bovine serum albumin (BSA) was purchased from Institute of Hematology, Chinese Academy of Medical Sciences. All chemicals were of analytic grade and purified prior to use. 5,10,15,20-tetrakisphenylporphyrin (TPP) was prepared according to the previous literature. 5,10,15,20-Tetrakis(4-aminophenyl)porphyrin (*p*-TAPP) [20] and 5-(4-aminophenyl)-10,15,20-triphenylporphyrin (*p*-MAPP) [21,22] were prepared from the corresponding nitro-substituted benzaldehyde and pyrrole [23] via the condensation and successive reduction with $SnCl_2$ in 12 mol dm^{-3} HCl–acetic acid (1:1, v/v) at 65 °C. TA4C and MAC were synthesized via esterification of *p*-TAPP and *p*-MAPP with succinic anhydride.

2.2. Instruments

Proton NMR spectra were measured on a Varian UNITY Plus-400 spectrometer. Reflective solid UV–vis spectra were recorded with a JASCO U-570 spectrophotometer. Ultraviolet–visible (UV–vis) spectra were obtained on a SHIMADZU UV-2101PC spectrophotometer where the temperature of the solutions was maintained by the use of a constant-temperature circulation pump (Tulabo F12) and a variable-temperature cell holder (Hitachi). Steady-state fluorescence spectra were measured using JY FluoroMax-P spectrofluorometer after the samples were equilibrated for 1 h.

2.3. Spectra titration

A stock solution was prepared by dissolving TA4C in phosphate buffer at pH 7.4. A stock solution of MAC was prepared by successive dissolution of MAC into THF, addition of distilled water, and removement of THF via evaporation in vacuo. The quartz cuvettes were cleaned in 3 mol dm^{-3} nitric acid overnight and washed with distilled water, ethanol, distilled water, and the sample solution before any measurement. Both the porphyrins fluorescence and absorbance were titrated with protein solutions. All experiments were carried out at pH 7.4 in phosphate buffer containing 0.01 mol dm^{-3} NaCl at 25 °C. The concentration of porphyrin derivatives maintains 1×10^{-6} mol dm^{-3}.

3. Results and discussion

3.1. Beer's law

The Beer's law titrations of the TA4C at pH 7.4 (Fig. 1) and MAC at pH 7.4 in the presence and the absence of β-CD were performed respectively at a suitable wavelength. As seen in Fig. 1, the photophysical properties of TA4C poorly adhere to the Beer's law in the present case, indicating that molecules of TA4C form aggregates even in a dilute aqueous solution. Remarkable deviation from the Beer's law is observed for MAC both in the presence and the absence of β-CD (plots not shown). In fact, it is difficult to measure the spectral absorption of MAC because it is per se insoluble in distilled water. Although β-cyclodextrin as protective molecules can preclude the porphyrin–porphyrin interaction [13], deviation from the Beer's law is significant for MAC in the presence of β-CD. The result implies that the tetraphenylporphyrin with only one butanoic acid group predominantly trends to aggregate in aqueous media owing to its poor hydrophilicity. It is in line with the previous results that the increased lipophilicity of porphyrins with long nonpolar alkyl

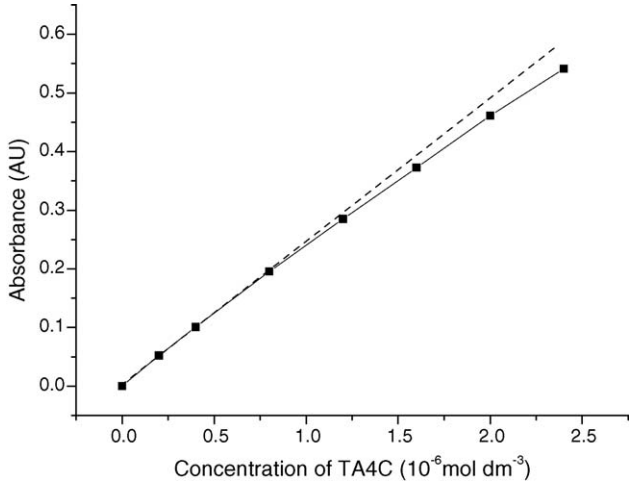

Fig. 1. Beer's law titration of TA4C.