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Synthesis of Water−Soluble Non−Aggregated Porphyrin−β−Cyclodextrin Tetraconjugate

A. Lyubimtsev,[a@] E. Kudrik,[a] D. Tyurin,[a] N. Zheglova,[a] and S. Syrbu[b]

Dedicated to Academician Aslan Yu. Tsivadze on the ocassion of his 75th Birthday

[a]*Research Institute of Macroheterocycles, Ivanovo State University of Chemistry and Technology, 153000 Ivanovo, Russia*
[b]*G.A. Krestov Institute of Solution Chemistry of Russian Academy of Sciences, 153045 Ivanovo, Russia*
[@]*Corresponding author E-mail: alexlyubimtsev@mail.ru*

*The novel porphyrin–β-cyclodextrin (Por-β-CD) conjugate **3** was synthesized and investigated. Addition of four β-CD units to the porphyrin macrocycle leads to formation of non-aggregated water-soluble covalently linked conjugate, which does not demonstrate any self-assembled species even at high concentrations. New conjugate **3** forms inclusion complexes with ruthenium, simulating antitumor drug NAMI-A. The formation of stable inclusion complex was detected by UV-Vis spectroscopy.*

Keywords: Porphyrin, β-cyclodextrin, conjugate, inclusion complex, ruthenium.

Синтез водорастворимого неагрегированного порфирин−β−циклодекстрин тетраконъюгата

А. Любимцев,[a@] Е. Кудрик,[a] Д. Тюрин,[a] Н. Жеглова,[a] С. Сырбу[b]

[a]*НИИ макрогетероциклических соединений, Ивановский государственный химико-технологический университет, 153000 Иваново, Россия*
[b]*Институт химии растворов им. Г.А. Крестова РАН, 153045 Иваново, Россия*
[@]*E-mail: alexlyubimtsev@mail.ru*

Предложен простой воспроизводимый метод синтеза мезо-тетра(β-циклодекстринофенил)порфирина. Показано, что введение четырех остатков β-циклодекстрина на периферию порфиринового макроцикла приводит к водорастворимому неагрегированному коньюгату, который не образует других самоорганизующихся систем даже при высоких концентрациях. С применением электронной спектроскопии исследовано образование комплексов включения синтезированного коньюгата с комплексом рутения, моделирующим противоопухолевый препарат NAMI-A.

Ключевые слова: Порфирин, β-циклодекстрин, конъюгат, комплекс включения, рутений.

Introduction

The construction of supramolecular systems based on two or more molecules with different properties is a very attractive approach in the building multifunctional materials devoted to applications in different fields. One of these systems intensively studied in the recent years is the conjugates of porphyrins (Por) with cyclodextrins (CD).[1-12] The specific biological (tendency to accumulate in cancer tissues) and spectroscopic (high molar absorption, strong fluorescence and good quantum yields) properties of porphyrins predetermine their medical applications mostly focused on photodynamic diagnostic and photodynamic therapy (PDT).[13-16] The photoactivity of many photosensitizers in PDT is affected by their poor solubility and aggregation, which leads to a decrease in their singlet oxygen production. The considerable use of CDs as individualized entities in medicine is undeniably and have a long history.[17] Never-

theless, in the last few years because of the unique capability of forming inclusion complexes in the inner cavities and many other favorable physicochemical and biological properties, CDs and their derivatives have been applied in drug delivery systems to enhance the solubility, stability and absorption.[18-21] More recently, covalently linked porphyrin-cyclodextrin (Por-CD) conjugates have become new attractive supramolecular systems for the study of biological processes and biomimetic systems and application as drug carriers with recognition and controlled release properties. Moreover, the presence of porphyrin unit in conjugate with cyclodextrin gives the possibility of additional accurate spectroscopic detectability of the supramolecular systems before and after complexation with guests into cyclodextrin cavities.[1-12,19,21]

The several porphyrin derivatives covalently linked with cyclodextrins were synthesized and investigated.[2-4,6-12,20,22-34] Most of these conjugates contain one or two cyclodextrin units on periphery of porphyrin macrocycle. In general, as evidenced from experimental data, the cyclodextrin containing porphyrins with one and two cyclodextrin fragments are formed supramolecular complexes due to deep inclusion of unsubstituted phenyl groups in the CD cavity. Such inclusion complexes in water showed decreasing of intensities of main bands in UV-Vis spectra and, as a result, decreasing singlet oxygen quantum yield if compared with corresponding solution in organic solvents.[3-4,7,11,20,24] Only few publications contain synthetic date about tetraconjugates CD's with porphyrins.[10,23,25-26,30-31] Moreover, the synthetic approaches to prepare most of Por-CD conjugate base on relative difficult ways containing mostly multistep transformations of the cyclodextrins and/or using expensive or synthetically uneasy porphyrin precursors.

Therefore, our strategy was to introduce four β-cyclodextrin fragments to available 5,10,15,20-tetra(4'-carboxyphenyl)porphine by synthetically simple two-step procedure with formation of HPLC pure non-aggregated water-soluble porphyrin-β-cyclodextrin conjugate. By UV-Vis spectroscopy the interaction of this conjugate with $Na[RuCl_4(DMSO)_2]$ complex,[35] which is isostructural with well-known antitumor drug NAMI-A,[36] was also investigated.

Experimental

β-Cyclodextrin and all the solvents and basic materials were commercially available and used without further purification. 5,10,15,20-Tetrakis(4'-carboxyphenyl)porphine (**1**)[37] and $Na[RuCl_4(DMSO)_2]$[35] were prepared and purified according to the procedure reported previously. UV-Vis spectra were recorded with a Shimadzu UV-1800 spectrophotometer. HPLC were carried out on Shimadzu Prominence instrument equipped with a SPD-20A UV-Vis detector at 414 nm. Analytical (5μm, 4.6×250 mm) Repro-Sil-Pur Basic C18 column was used. MALDI-TOF mass spectra were recorded using Shimadzu Biotech Axima Confidence instrument with 2,5-dihydroxybenzoic acid (DHB) as a matrix.

Synthesis of chloroanhydride of 5,10,15,20-tetrakis(4'-carboxyphenyl)porphine (2). 5,10,15,20-Tetrakis(4'-carboxyphenyl)porphine (**1**) (200 mg, 0.25 mmol) was dissolved in 5 mL of freshly distilled thionyl chloride. The obtained mixture was heated under stirring at 30 °C for 6 h. The excess of $SOCl_2$ was evaporated under vacuum and traces of thionyl chloride were complete remove by vacuum evaporation with benzene. Compound **2** was used without further purification.

Porphyrin-β-CD conjugate (3). To solution of 1.36 g (1.2 mmol) of β-cyclodextrin in 30 ml of dry pyridine 100 mg (0.12 mmol) of chloroanhydride **2** was added during 2 h in the inert atmosphere at 60 °C. Then reaction mixture was heated under stirring at 60 °C for 3 h. The solvent was evaporated under reduced pressure. The title compound was purified by column chromatography on Al_2O_3 using gradient water-methanol mixture as eluent. Yield: 547 mg (44 % calcd. from starting porphyrin **1**). The individuality of conjugate **3** was confirmed using HPLC. MS (MALDI TOF) (DHB+KI) *m/z* ($C_{216}H_{302}N_4O_{144}K$) calcd.: 5294.54. Found: 5293.42.

Results and Discussion

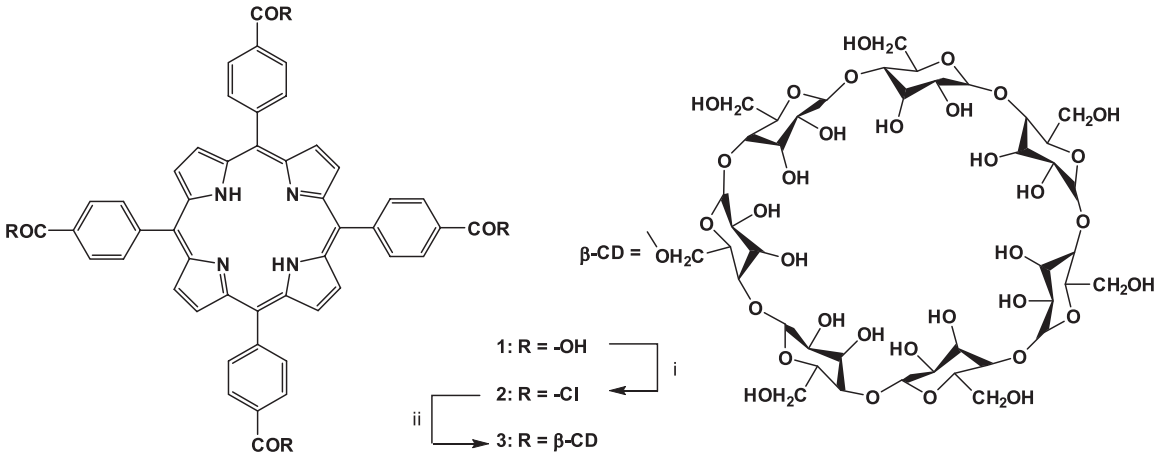
The novel porphyrin-β-cyclodextrin conjugate **3** was prepared in two steps (Scheme 1). Firstly, 5,10,15,20-tetra(4'-carboxyphenyl)porphine (**1**) was transform to corresponding chloroanhydride **2** by reaction with excess of the thionyl chloride. Then the porphyrin **2** was reacted with an excess of β-CD to give the conjugate **3**. The Por-β-CD **3** was purified using column chromatography. The purity of conjugate **3** was confirm using HPLC. MALDI TOF spectra were recorded in both positive- and negative-ion modes with DHB-matrix and without matrix. In these cases, molecular peak corresponding to conjugate **3** and peaks of starting compounds (**1**, **2** and β-CD) were not detected. For improving ionization the KI was added to DHB matrix and peak corresponding to $[M+K]^+$ was observed. In addition, many peaks were observed with *m/z* values from 5000 to 1200. The ^1H NMR spectrum measured in D_2O contains broad signals in both areas typical for porphyrin and cyclodextrin protons. Quantitative interpretation of spectrum was not possible because of exchange between conjugate protons with deuterated solvent. Behaviors MS MALDI TOF and ^1H NMR spectra will be studied in further in details.

Por-β-CD **3** is poorly soluble in neat DMF and MeOH, but shows very good solubility in water in comparison with porphyrin **1**, which is good soluble in DMF and buffer solutions at p*H*>7 and insoluble in water. The absorption spectrum (Figure 1) of conjugate **3** in both DMF (not shown in Figure 1) and water is identical and characterized by the intense Soret band at near 411 nm and weaker *Q*-bands with maxima in the region 505–635 nm.

As the concentration was increased, the all absorption bands also increased. Lambert-Beer's law was obeyed for the conjugate **3** in water even in the high concentrations (from $1.01\cdot10^{-5}$ to $14.4\cdot10^{-5}$ M) (Figure 1). This finding reveals that the porphyrin chromophore retains its spectroscopic properties upon attachment of four β-CD macrocycles, ruling out any intra- or intermolecular interactions between the subunits of the conjugate in water and organic solvents. In addition, no self-assembled species by change of concentration or solvents were found.

We have investigated also the interaction of Por-β-CD **3** with four equivalents of $Na[RuCl_4(DMSO)_2]$ which is isoelectronic with well-known ruthenium containing antitumor drug NAMI-A using UV-Vis spectroscopy.

As can been seen from Figure 2, at the addition of $Na[RuCl_4(DMSO)_2]$ into solution of **3** the intensity of conjugate's Soret band is only slightly decreased, and the new band at 355 nm is formed. At the same time the band at 396 nm, typical for initial Ru-complex, disappears. This



Scheme 1. Synthesis of porphyrin-β-cyclodextrin conjugate **3**: i) $SOCl_2$, 30 °C, 6 h; ii) β-cyclodextrin, Py, 60 °C, 5 h.

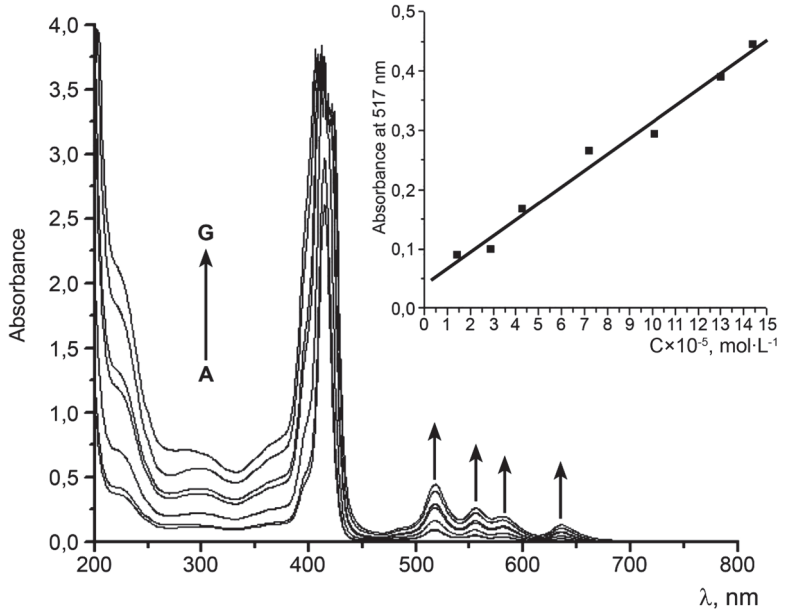


Figure 1. UV-Vis spectra of conjugate **3** at different concentrations in water. Concentration range: (A) $1.01 \cdot 10^{-5}$ M, (B) $1.44 \cdot 10^{-5}$ M, (C) $2.89 \cdot 10^{-5}$ M, (D) $4.33 \cdot 10^{-5}$ M, (E) $7.22 \cdot 10^{-5}$ M, (F) $13.0 \cdot 10^{-5}$ M, (G) $14.4 \cdot 10^{-5}$ M. Inset: Lambert-Beer law plot.

fact clearly demonstrates formation of host-guest complex and Ru-complex encapsulated in all four β-CD units. Incorporation of guest molecules is relatively slow reaction and can be kinetically investigate. The rate of reaction linearly depends on the Ru complex concentration and complex 1:4 is finally formed. Inclusion complex is thermodynamically stable and K_{eq} value is too high for its possible determination using spectral methods. These observations demonstrate that synthetically available **3** can be useful platform for searching new complex systems, containing both porphyrin unit and drugs, encapsulated into β-CD moieties.

Conclusions

In summary, we have synthesized a new non-aggregated water-soluble porphyrin-β-cyclodextrin tetraconjugate by means of a nucleophilic substitution of chlorine atoms in the chloroanhydride **2** with good yield. This conjugate can encapsulate $Na[RuCl_4(DMSO)_2]$ molecule used as a model of anticancer drug NAMI-A. The present work not only provides a very simple way for the synthesis of non-aggregated CD containing porphyrins, but also shows a possibility for use of these compounds for the drug delivery systems.

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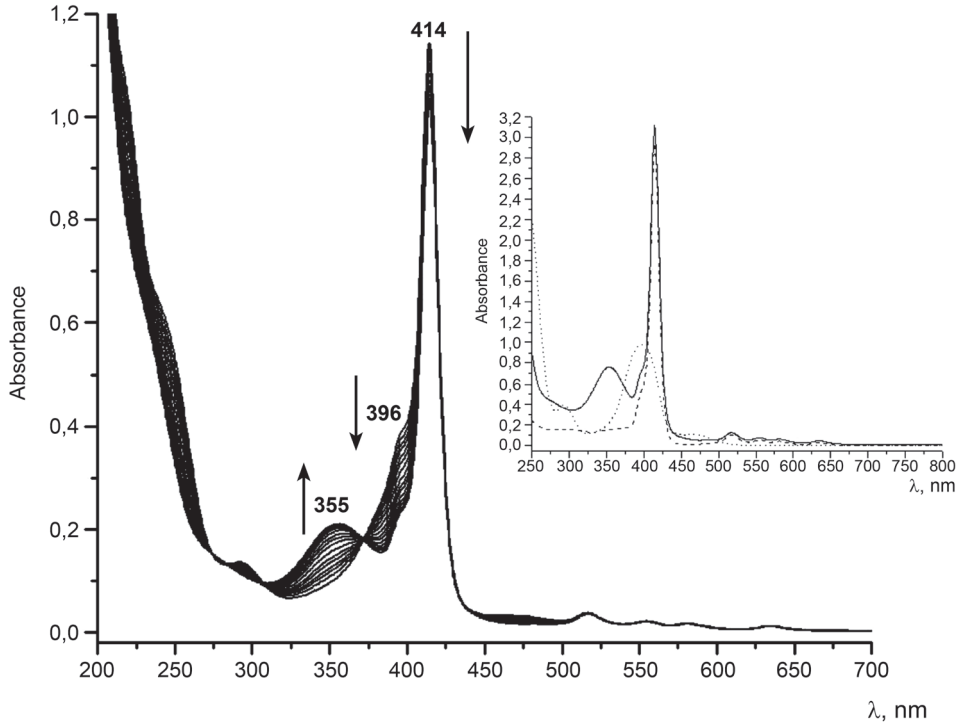

Figure 2. UV-Vis spectral changes during the reaction of conjugate **3** (16.7 μM, 1 eq) with $Na[RuCl_4(DMSO)_2]$ (66.8 μM, 4 eq). Inset: UV-Vis spectra of conjugate **3** (dashed line), $Na[RuCl_4(DMSO)_2]$ (dotted line) and inclusion complex (solid line) in water.

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