Aggregation of phthalocyanine derivatives in liquid solutions and human blood

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Abstract

The aggregation of the copper (II) 4,4′,4″,4‴-tetrasulfonated phthalocyanine anion (Cu(tsPc)−4) has been studied in aqueous solutions, DMSO and in human blood by UV–VIS absorption spectroscopy and resonance Raman spectroscopy (RRS). The vibrational mode ν4 (1530 cm−1) has been used as a probe in RRS. It has been shown that the dimerization equilibrium constant K is shifted significantly towards monomeric forms when human blood is added to the solution. The life-time of the singlet excited state S1 of (Cu(tsPc)−4) in aqueous solution has been estimated to be shorter than 500 fs using femtosecond pump-probe absorption spectroscopy.

Keywords: Phthalocyanine derivatives; Photodynamic therapy; Femtosecond pump-probe absorption spectroscopy; Resonance Raman spectroscopy

1. Introduction

Phthalocyanine derivatives have a potential use as photosensitizers in photodynamic therapy (PTD) of cancer and other medical applications [1,2]. They may overcome the major drawbacks of the photosensitizers based on the porphyrine-type materials: skin photosensitivity, low selectivity for tumor tissue and long period of clearance from the body. We are interested in photochemical and photophysical properties of phthalocyanine derivatives because they play a crucial role in evaluation of the photodynamic activity and their application in PDT therapy.

It is well known that the photochemical mechanisms of deactivation of the excited electronic states strongly depend on the degree of aggregation and may determine the mechanisms of photooxidations. The aggregation in solutions may decide whether the sensitizer reacts directly with another chemical entity (e.g. with human tissue in PDT applications) to yield transient radicals which can react further with oxygen (type I of photooxidation) or the sensitizer excited to triplet state interacts with oxygen by the energy transfer to produce an electronically excited singlet state of oxygen which reacts further with the chemical species susceptible to oxidation (type II of photooxidation). The type II of photooxidation is regarded as more efficient in PDT as the singlet oxygen in the excited state is very reactive and toxic leading to the necrosis of the human tissue.

This is a reason why we are interested in the aggregation of phthalocyanine derivatives in aqueous and DMSO solutions. However, the photochemistry of phthalocyanine in natural biological environment may differ significantly from the photochemistry in aqueous or DMSO solutions. As the photosensitizer is distributed to the tissue through blood we have also studied the photochemistry of this sensitizer in human blood.

In this paper we wish to concentrate on the aggregation of copper (II) phthalocyanine-3,4′,4″,4‴-tetrasulfonic anions (Cu(tsPc)−4) in aqueous solutions, in DMSO and in human blood by UV–VIS absorption spectroscopy and resonance Raman spectroscopy (RRS). The vibrational normal mode ν4 of Cu(tsPc)−4 has been used as a probe in RRS measurements. The aim of this paper is to determine the dimerization constant K for Cu(tsPc)−4 in aqueous solutions and in DMSO as well as to estimate the influence of the human blood on the dimeric aggregation.

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2. Experimental

Spectrograde DMSO, copper (II) phthalocyanine-3,4',4'',4'''-tetrasulfonic acid, tetrasodium salt were purchased from Aldrich. They were used without further purification. Water was distilled before preparing the solutions.

2.1. Raman resonance scattering

Raman spectra were measured using the Ramanor U1000 spectrometer (Jobin Yvon) and Spectra Physics 2017–04S argon ion laser operating at 465.8 nm, 472.7 nm, 496.5 nm and 514 nm at powers 10 mW, 4 mW, 125 mW and 125 mW, respectively. The Raman spectra in the lattice region 15–200 cm⁻¹ and for the vibrational normal mode ν₄ of Cu(tsPc) at 1527 cm⁻¹ were measured. Spectra were recorded at 294 K. The spectral slit width was 6 cm⁻¹ in the full temperature range, which corresponds to the 500 μm mechanical slit of the spectrometer. A λ/4 wave plate was used to change the linear polarization into the circular one to avoid the different sensitivities of the gratings to the polarization. The interference filter has been used to purify the laser line by removing additional natural emission lines that interfere with the Raman lines, especially in the case of solid samples.

2.2. Pump-probe femtosecond absorption spectroscopy

The pump-probe femtosecond absorption system used for measurements is presented in Fig. 1. The femtosecond laser is pumped with the cw solid state laser (yttrium vanade crystal doped with Nd). Its second harmonic (532 nm, power 5.5 W) is used as a pumping source for the Ti: Sapphire femtosecond laser working in the mode-locking regime. The femtosecond laser is tunable in a very broad range but we use only 796-nm wavelength for further amplification. The pulse with the energy of 250 nJ, the pulse duration of 80 fs and the repetition rate of 82 MHz is amplified in the regenerative amplifier. The small part of the seed pulse is sent by the beamsplitter to the spectrum analyzer in the frequency domain to monitor the quality of the mode-locking. The regenerative amplifier is pumped by the solid state laser (Nd:YLF) working with the repetition rate of 1 kHz, the pulse duration is 250 ns. The pulse mode is achieved by Q-switching with an optoacoustic device. The laser emits at 527 nm (second harmonic from LBO nonlinear crystal). After amplification the energy of the pulse is 1 mJ, 1 kHz, 796 nm. The pulse duration is measured with the single shot autocorrelator and was found to be 96 fs. The pulse interacts nonlinearly with the KTP crystals (type I of phase matching) to generate SHG and THG. The second harmonic is used as a pump beam. The fundamental beam (796 nm) passes through the delay line, the half wave plate, dichroic polarization analyzer, lens, the sapphire plate, where the white continuum (WC) is generated in the broad range of 398–1000 nm and it is focused onto the flowing sample. The WC beam is used as a probe beam. The probe and pump beams are focused onto the flowing sample to ensure complete replacement of the sample between each laser shot. Care is taken to compensate for group velocity dispersion. The time delay for the spectra is determined by an optical delay line controlled by a motor-driven translational stage. The radiation from the sample enters the entrance slit of a 0.5 m single dispersion spectrometer (1800 g/mm grating). The dispersed radiation is detected by a multichannel (256×1024) charge-coupled device detector back illuminated, UV enhanced attached to the exit slit of the
Fig. 2. The difference absorption signal as a function of the time delay (negative signal corresponds to photobleaching).

Fig. 3. Absorption spectra of Cu(tsPc)$^{-4}$ in aqueous solutions at 294 K, optical path = 0.2 mm [3].
In Table 1 we present the peak positions and the band widths for the VV and VH Raman spectra. The band position and the band widths are nearly the same for the VV and VH spectra. 

![Image](https://example.com/image.png)

Fig. 4. VV and VH resonance Raman spectra of the ν4 mode of Cu(tsPc)4− in aqueous solution (c = 0.01 mol/dm3).

![Image](https://example.com/image.png)

Fig. 5. Absorption spectra of Cu(tsPc)4− in DMSO liquid solution at 294 K, optical path = 0.2 mm [3].

Fig. 6 shows that the band shape of the normalized intensity at approximately 1538 cm−1 strongly depends on Cu(tsPc)4− concentration while that at approximately 1527 cm−1 remains practically the same. Significant deviation from isotropic alignment suggested by the depolarization ratio of the band at 1538 cm−1 and strong dependence of the band intensity on concentration of the solution provide a clear evidence that the band at 1538 cm−1 must correspond to the structure that has much less isotropic alignment than the structure corresponding to the depolarized band at 1527 cm−1.

Table 1

<table>
<thead>
<tr>
<th>VV</th>
<th>VH</th>
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<tbody>
<tr>
<td><strong>Band I</strong></td>
<td><strong>Band II</strong></td>
</tr>
<tr>
<td>Max peak position [cm−1]</td>
<td>1527</td>
</tr>
<tr>
<td>Band width [cm−1]</td>
<td>13.5</td>
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</table>

In view of the results presented in Fig. 5 we have checked the influence of the concentration on the Raman band shape of the ν4 mode of Cu(tsPc)4− in aqueous solutions. 

Additional analysis using the least square fitting procedure. The similar band widths for the VV and VH spectra indicate that the contribution from the reorientation relaxation is negligible as a mechanism of band broadening that is rather obvious for such a large molecule like Cu(tsPc)4−. Striking behavior is revealed when we compare the depolarization ratios for both bands of the ν4 mode. Although both bands represent the same vibrational normal mode ν4 of Cu(tsPc)4− the depolarization ratios are significantly different: 0.10 for the band at 1527 cm−1 and 0.33 for the band at 1538 cm−1. It indicates that the band at 1538 cm−1 must correspond to the structure that has much less isotropic alignment than the structure corresponding to the depolarized band at 1527 cm−1.

In Table 1 we present the peak positions and the band widths for both bands after deconvolution of the band shape into two Lorentzian bands using the least square fitting procedure. The similar band widths for the VV and VH spectra indicate that the contribution from the reorientation relaxation is negligible as a mechanism of band broadening that is rather obvious for such a large molecule like Cu(tsPc)4−. Striking behavior is revealed when we compare the depolarization ratios for both bands of the ν4 mode. Although both bands represent the same vibrational normal mode ν4 of Cu(tsPc)4− the depolarization ratios are significantly different: 0.10 for the band at 1527 cm−1 and 0.33 for the band at 1538 cm−1. It indicates that the band at 1538 cm−1 must correspond to the structure that has much less isotropic alignment than the structure corresponding to the depolarized band at 1527 cm−1.

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Cu
Fig. 7 shows the low frequency Raman spectra for
Cu
Fig. 8 shows the resonance Raman spectra of the
mode \( \nu_4 \) of Cu(tsPc)
-4 in aqueous solution as a function of Cu(tsPc)
-4 concentration: (a) 0.05 mol/dm
3; (b) 0.01 mol/dm
3; (c) 0.005 mol/dm
3; and (d) 0.001 mol/dm
3.

1538 cm
-1 must correspond to the Cu(tsPc)
-4 dimer, whereas the band at 1527 cm
-1 to the monomer.

To provide further evidence that the assignment is correct we have monitored the low frequency Raman spectra in the region of 15–200 cm
-1 as a function of concentration. It is well known that the low frequency Raman spectra are very sensitive to the details of the structure generated in the sample where the phonon peaks signalize existence of translationally ordered crystal structures whereas the absence of any phonon peak indicates that the glassy phase has been formed. In contrast, liquid isotropic phases show the Rayleigh wing indicating that there is no ordered alignment.

Fig. 7 shows the low frequency Raman spectra for Cu(tsPc)
-4 in aqueous solutions at 294 K as a function of concentration. One can see that at low concentrations of Cu(tsPc)
-4 (from 2 \times 10^{-6} to 10^{-4} mol/dm
3) we observe the Rayleigh wings typical for isotropic liquid phase. However, at higher concentrations the phonon peaks begin to appear that are clearly visible at \( c = 0.05 \) mol/dm
3. It clearly indicates that in contrast to diluted solutions, the highly concentrated aqueous solutions of Cu(tsPc)
-4 are partially organized and they contain crystal like domains even in the liquid phase. It is known [4–6] that the crystal polymorphs of Cu(tsPc)
-4 form ring-stacked columns close enough in space to overlap efficiently between the \( \pi \)-electronic clouds. It is usually assumed that the aggregation in phthalocyanine molecules arises from the presence of the strong \( \pi \)-\( \pi \) interactions that stabilize columnar aggregates [6].

We have shown [7] that the peripheral substitution at the benzene groups has a tremendous influence on the interactions between the Cu(tsPc)
-4 molecules that perturbs the vibrational state energies and electronic excited state energies leading to larger force constants for vibrations (at least for the \( \nu_4 \) mode) and indicating stronger \( \pi \)-\( \pi \) interactions or/and interactions between the sulfono groups of the adjacent Cu(tsPc)
-4 molecules.

So far we presented the results for the Cu(tsPc)
-4 aggregation in DMSO and aqueous solutions. However, the aggregation of phthalocyanines in natural biological environment may differ significantly. The aggregation photosensitizer plays a crucial role in evaluation of its PDT activity since the role of deactivation by internal conversion to the ground state for dimer greatly exceeds that of the monomer and no useful photochemistry from the photobiological point of view can be expected.

Fig. 8 shows the resonance Raman spectra of the mode \( \nu_4 \) of Cu(tsPc)
-4 in the human blood and for comparison, in aqueous solution. One can see that the equilibrium constant \( K \) for dimerization is significantly shifted toward monomeric forms when compared with the aqueous solution. This result is very promising for the photodynamic therapy since it indicates that in the natural biological environment there exist more monomer phthalocyanine molecules that can be excited to their triplet states. The excited triplet states may interact with oxygen producing an electronically excited singlet state of oxygen that can further react with the human tissue leading to necrosis in the PDT therapy.

It is well known that the photochemical mechanisms of deactivation of the excited electronic states strongly depend on the degree of aggregation and may determine...
the mechanisms of photooxidations. The photochemistry of Cu(tsPc)$^{4+}$ in liquid solutions, crystals and glasses has been studied in our recent paper [3]. In most photochemical reactions higher excited states are not involved in the reactions. They are usually deactivated rapidly to the lowest excited state, which deactivates to the ground state via emission, internal conversion or undergoes reactions competing with the deactivation. However, it has been found [8] that in some compounds (including porphyrines) the higher excited states are involved in photochemical reactions. Therefore, it was essential to determine the life-times of the higher excited states of Cu(tsPc)$^{4+}$ in aqueous solutions and determine how they depend on the degree of aggregation. Using femtosecond pump-probe absorption spectroscopy we have estimated that the lifetime of the singlet excited state $S_n$ of (Cu(tsPc)$^{4+}$) for the Q transition in aqueous solution is shorter than 500 fs. More details have been presented in Ref. [9].

4. Conclusions

We have studied the aggregation of copper (II) phthalocyanine-3,4,4',4'''-tetrasulfonic anion (Cu(tsPc)$^{4+}$) in DMSO, aqueous solutions and in human blood by UV–VIS absorption spectroscopy, resonance Raman spectroscopy, and pump-probe femtosecond spectroscopy. We have analyzed the difference absorption signal $\Delta A = A - A_0$ as function of a time delay between the pump and the probe pulses with femtosecond resolution. We have estimated that the lifetime of the higher excited singlet state $S_n$ for the Q electronic transition is shorter than 500 fs.

We have found that Cu(tsPc)$^{4+}$ in aqueous solutions exhibits a pronounced tendency to form ring stacked dimers (or polymers) in contrast to DMSO solution where Cu(tsPc)$^{4+}$ molecules exist almost entirely as monomers. The dimerization equilibrium constants $K$ in aqueous and DMSO solutions were found to be $8.75 \times 10^4$ and $1.50 \times 10^3$ at 294 K, respectively. For Cu(tsPc)$^{4+}$ in human blood the dimerization equilibrium is shifted significantly towards monomeric forms. This result is important for photodynamic therapy application, because the degree of aggregation plays an important role in the photochemical mechanisms of photooxidation as one of the main features of PDT sensitizers is the ability to generate singlet, excited electronic state of oxygen that is lower for dimers than for monomers.

Acknowledgments

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References