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Spectro-luminescent research of 1-pyrenemethylamine interaction with nucleic acids in water

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The interactions of 1-pyrenemethylamine hydrochloride with nucleic acids (calf thymus DNA and yeast RNA) in water were studied using electron absorption and emission spectroscopy. The totality of the obtained spectral data allows us to conclude that 1-pyrenemethylamine is intercalated in DNA but not in RNA.

Keywords: intercalation, 1-pyrenemethylamine, calf thymus DNA, yeast RNA.

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Introduction

Since the middle of the 20th century, numerous biological, physical and chemical studies have been performed to find anticancer drugs whose effect is based on blocking malignant cell growth and division processes by means of binding to DNA. An intercalation, process the mechanism of which was first proposed by Leonard Lerman in 1961, is one the possible options for chemotherapeutic agent binding to DNA [1]. A direct evidence of molecule intercalation into the DNA double helix by the X-ray diffraction analysis method was published in 1987 when the structure of oligonucleotide-daunomycin (an anthracycline antibiotic) complex had been determined [2]. Aromatic molecules are introduced between DNA double helix neighboring complementary nucleobase pairs, form the $\pi-\pi$-stacking leading to partial structural changes in a polynucleotide. This, in particular, limits DNA and RNA polymerases binding to the DNA matrix [3]. The latter makes intercalants suitable for clinical therapy as antitumor drugs. Thus,daunorubicin and doxorubicin are used to treat Hodgkin lymphoma, and actinomycin D is used to treat Ewing sarcoma [4].

These antitumor substances are linearly conjugate condensed polyaromatic cycles. Pyrene, that due to its hydrophobic properties and size comparable with nucleobase pairs, is capable of entering the $\pi-\pi$-stacking-interactions and intercalating into DNA is an example of nonlinearly conjugate cycle [5]. Comparative study by the quantum-mechanical calculation and electrochemical impedance spectroscopy methods used in [6] clearly identifies that a water-soluble pyrene analogue — 1-pyrenemethylamine - is incorporated in the DNA double helix due to binding to the adenine–thymine pair. The objective of this study was to investigate the interaction between 1-pyrenemethylamine and DNA/RNA in water solution by the electronic absorption and emission spectroscopy methods at room temperature.

Experimental

Commercially available substances (1-pyrenemethylamine hydrochloride (hereinafter referred to as PMA, Figure 1), calf thymus DNA (hereinafter referred to as DNA), yeast RNA (hereinafter referred to as RNA), all made by Sigma-Aldrich) were used without additional purification. All solutions were prepared in distilled water. Spectral investigations were carried out using the equipment provided by the Shared Research Facility Center at the Department of Chemistry, Herzen University: electronic absorption spectra were measured using SF-2000 spectrophotometer („OKB Spectr“, Saint Petersburg, Russia), luminescence and luminescence excitation spectra were recorded at room temperature using the Fluorat-02-Panorama (GK „Lumex“, Saint-Petersburg, Russia) spectrofluorometer.

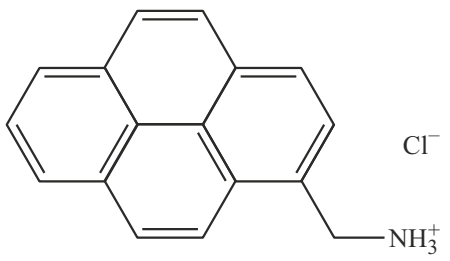

Figure 1. Structural formula of 1-Pyrenemethylamine (PMA) hydrochloride.

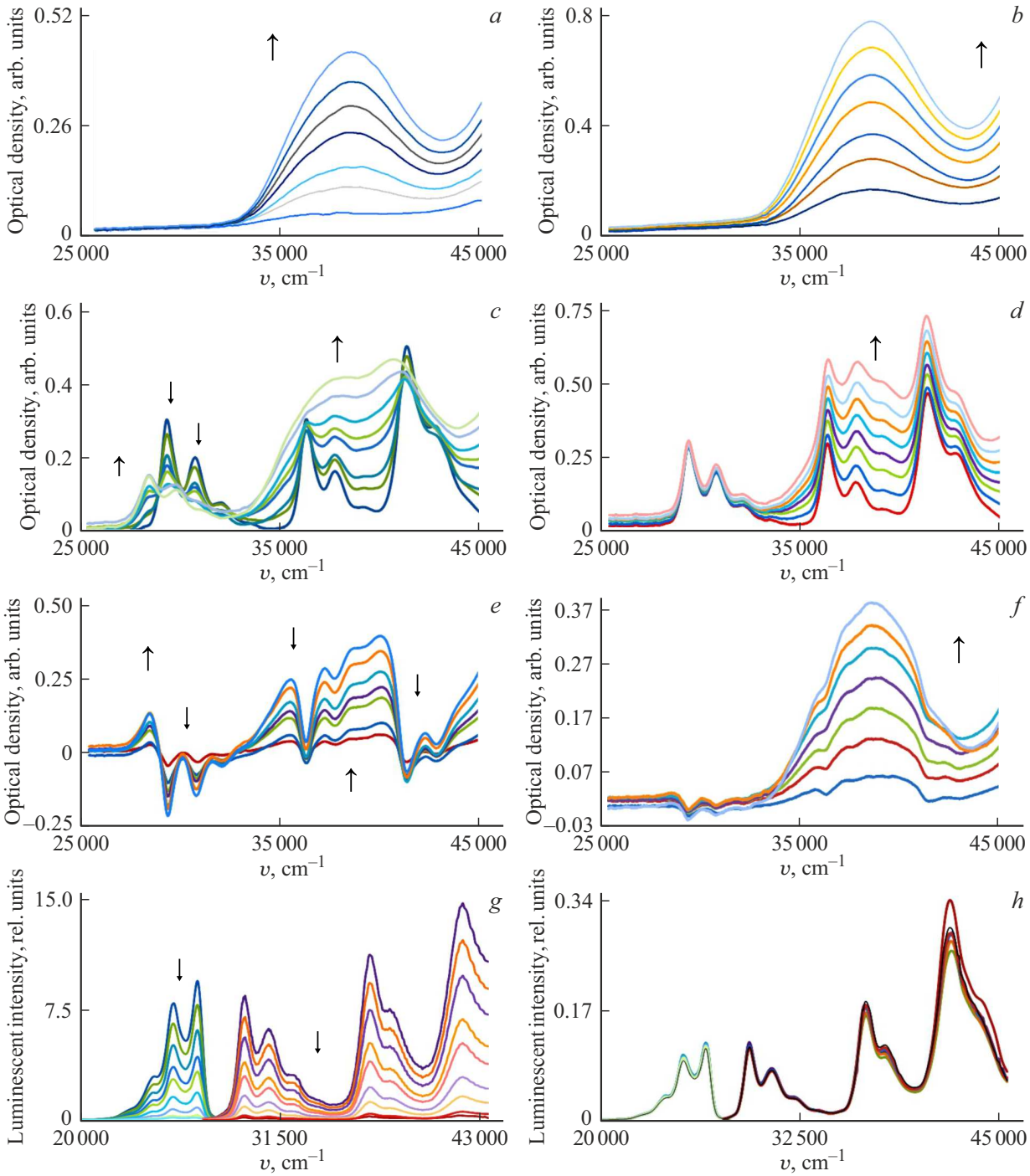

Figure 2. Absorption spectra of DNA (*a*), RNA (*b*), PMA with DNA (*c*), PMA with RNA (*d*), differential spectrum of PMA-DNA (*e*), differential spectrum of PMA-RNA (*f*). luminescence excitation spectrum and luminescence spectra of PMA with DNA (*g*), PMA with RNA (*h*). In case of increase in the nucleic acid concentration, spectral changes are shown by the arrows.

Titration procedure: portions of 10 μl 0.06% DNA or RNA solution ($\sim 10^{-3}$ mol/l solution calculated as the average molar mass of nucleotide) were added to 2 ml of PMA solution (concentration $\sim 10^{-5}$ mol/l), the components were mixed and absorption, luminescence and luminescence excitation spectra were recorded consecutively (Figure 2).

Results and discussion

Figure 2, *a, b* shows DNA and RNA absorption spectra that are similar to each other by the presence of peaks at 38450 cm^{-1} because they contain nucleobases (adenine, cytosine, thymine, guanine and uracil) — heteroaromatic compounds.

PMA is a polycyclic aromatic molecule, therefore, its electronic absorption spectrum has a series of structured bands corresponding to the resonant transitions $0 \rightarrow 0$ with pronounced high intensity peaks. PMA absorption spectrum in water (Figure 2, *c*) is represented by bands corresponding to the dipole-allowed $\pi-\pi^*$-transitions S_4, S_3 and S_2 with peaks at 41408 (S_4), 36364 (S_3) and 29369 (S_2); 26695 cm^{-1} — S_1-transition is symmetry-allowed, but is dipole-forbidden with low intensity, that's why it is not visible in Figure 2, *c, d* (the spectrum is given in supplementary materials).

During PMA solution titration by the DNA solution, oppositely directed changes in the absorption spectra were observed (Figure 2, *c*):

1) at 38450 cm^{-1} — increased absorbance is attributable to the increase in DNA concentration that has intrinsic absorption in the same range because it contains aromatic heterocyclic substances — nucleobases;

2) at 29400–31750 cm^{-1} — reduced absorbance;

3) at 27800–28570 cm^{-1} — increased absorbance, appearent bathochromic shift of the absorption band.

The presence of 9 isosbestic points (43103, 42735, 42194, 41152, 36496, 36232, 32680, 30121 and 23986 cm^{-1}) indicates the only PMA-DNA interaction product was formed. This interaction may be seen more clearly on the differential spectra obtained by subtracting the one-component PMA solution (with zero DNA concentration) spectrum from the PMA-DNA mixture spectra. The resulting differential spectra (Figure 2, *e*) do not coincide with the pure DNA solution spectra (Figure 2, *a*), which clearly indicates the interaction between PMA and DNA nucleobases.

After recording the absorption spectra, luminescence spectra (at 21277–26316 cm^{-1}) and luminescence excitation spectra (at 26316–43478 cm^{-1}) of the same PMA-DNA solutions were immediately recorded (Figure 2, *g*). During titration of the PMA solution by the DNA solution, quenching of vibrationally-structured luminescence and luminescence-excitation spectra was observed. Thus, the electronic both absorption and emission spectroscopy methods proved the fact of PMA intercalation into calf thymus double-stranded DNA molecules that had been earlier identified by other methods [6].

Similar spectral studies were carried out by titration of the PMA solution with the RNA solution. As shown in Figure 2, *d*, an increased solution optical absorbance was observed at 38462 cm^{-1}, but at 29412 cm^{-1} the solution optical absorbance remained almost unchanged. Differential spectra (Figure 2, *f*) recorded by subtracting the pure PMA spectrum from the RNA-PMA mixture spectra appeared to be similar to the pure RNA absorption spectra (Figure 2, *b*). Therefore, PMA does not interact with RNA because RNA is predominantly single-stranded, and the its nucleobases are not involved in the $\pi-\pi$-stacking interaction. The luminescent experiment leads to the same conclusion (Figure 2, *h*). Increased RNA concentration in the PMA solution neither lead to any change in the vibrationally-structured luminescence spectra nor in the luminescence excitation spectra.

The study of interaction between PMA and nucleic acids in water solution by the electronic absorption and emission spectroscopy methods revealed the fact of PMA intercalation into calf thymus double-stranded DNA and the absence of interaction between PMA and yeast single-stranded RNA.

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Conflict of interest

The authors declare no conflict of interest.

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