Properties of multicomponent system DNA – phthalocyanine metallocomplexes - surfactants

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Abstract. DNA interaction with metallodiphtalocyanine with different metals (Cu, Ni, Co) and cationic azobenzene containing surfactant was investigated. The influence of concentrations of components and solution ionic strength on complex formation was regarded. The investigation shows the binding of metallodiphtalocyanines with DNA in a solution. The possibility of the formation of multicomponent system (DNA, surfactant and metallophthalocyanine) was examined.

Keywords: phthalocyanines, azobenzene containing surfactant, DNA packaging, multicomponent system

1. Introduction
The metallized diphtalocyanines (Pc) represent a new type of biologically active compounds. They have a wide range of applications from systems for gas analysis to microelectronic devices, where Pc are used as "building blocks" in MOSFET (metal-oxide-semiconductor-field-effect-transistor), and catalysts for redox reactions [1]. Some attempts were made to use them as cytotoxic and cytostatic drugs as well as photosensitizers for photodynamic therapy [2]. Such exotic applying as a catalytic treatment was also used [3]. Polycations and amphiphilic compounds can induce DNA packaging in a solution. Photosensitive surfactants in this case can produce the reversible DNA condensation [4, 5]. Such self-assembling systems can be used in nanotechnology. The introducing of such components as PC into gene vector may provide the additional opportunity for multicomponent systems in therapy and creation of new applications.

The aim of the study is to examine the properties of compounds under study, to analyze DNA-PC and DNA-surfactant interaction and to regard the opportunity to form the multicomponent system DNA-Pc-surfactant.

2. Experimental section.

2.1. Materials.
In our work we used the metallized homonuclear diphtalocyanines (PC) with Ni, Co and Cu as coordinating centers. PC compounds were preparing in Prof. A. Sengul group (Zonguldak Karaelmas University, Turkey). Cationic azobenzene containing surfactant was synthesized in group of Prof. S. Santer, Potsdam University. Calf thymus DNA with molecular mass 6,5, 10 and 10,5 MDa (Sigma), NaCl (chemically pure), DMSO (99%) were used.
The distance between the planes is 7.3 Å, the distance between Me-centres is 11.06 Å. The molecular mass and chemical formulas of these compounds are:

\[ \text{MM}=2408 \text{ for } C_{152}H_{112}Ni_{2}N_{16}O_{8}, \text{ MM}=2408.48 \text{ for } C_{152}H_{112}Co_{2}N_{16}O_{8}, \text{ and MM}=2417.71, \text{ for } C_{152}H_{112}Cu_{2}N_{16}O_{8}. \]

Chemical formula of surfactant AzoTMAB is \[ \text{CH}_3(\text{CH}_2)_{3}(\text{H}_4\text{C}_6\text{N}=\text{NC}_6\text{H}_4)\text{O}(\text{CH}_2)_{6}\text{N}^+(\text{CH}_3)_{3}\text{Br}^- \], molecular weight is \( M=476.59 \). The structure and scheme of photoinduced isomerization is shown on Fig. 2.

2.2. Methods.

The systems were investigated by viscometry (low gradient Zimm-Crothers type viscometer; the specific viscosity \( \eta_r^{-1} \) of DNA solutions at constant DNA concentration was examined), spectrophotometry (spectrophotometer SF-56, Russia; the optical density of solutions \( D \) was examined), circular dichroism (autodichrograph Jobin Ivon Mark IV; the difference in extinction coefficient of DNA \( \Delta \varepsilon \) for right and left light polarization was examined).

2.3. Preparation of solutions.

The phthalocyanines were dissolved in DMSO. Then solutions were filtered and gently mixed with water. The DNA solution in water was mixed with NaCl solution and filtered. DNA concentration was determined by spectral method [6].

3. Results and discussions.

The absorption spectra of PCs in DMSO and water solutions with different NaCl concentrations at \( \lambda >400 \text{ nm} \) out of DNA absorption region are presented on Fig. 3. One can see two main bands with approximately similar positions of maximum (Fig. 3 a shows spectra normalized to the absorption at 619 nm). The difference in relative intensity of bands and noticeable shift of long-wave band for CoPC spectrum indicate that metals influence on the electron system of chromophores. The principal differences in PCs spectra remain in water solution with NaCl (see Fig 3 b, c): the short-wavelength band is playing a more prominent role for NiPC, the long-wavelength band has a higher intensity for CoPC. Both bands bring roughly equal contributions into spectrum for CuPC. NaCl concentration (from 0.005 M to 1 M) does not change significantly this picture (see Fig. 3d).
NiPC \(3.32 \times 10^{-5}\) M
CoPC \(3.24 \times 10^{-5}\) M
CuPC \(3.14 \times 10^{-5}\) M

D PCs in DMSO

CoPC in 0.15 M NaCl
CoPC in 1 M NaCl
CoPC in 0.0035 M
CoPC in DMSO

\(\lambda\), nm

Fig. 3. MePC absorption spectra at DMSO (a), in water solution with 0.15 M NaCl (b), 1 M NaCl (c); CoPC spectra in DMSO and at different NaCl concentrations (d). Spectra (a) and (d) is normalized on absorption at definite \(\lambda\).

After the mixing of DNA and PC solutions spectra of phtalocyanines have changed without great transforming of their shape (Fig. 4). The hypochromic effect can be explained with the formation of stack structure outside DNA after the binding of PC molecules via phosphate groups. Indeed, DNA secondary structure doesn't change during the interaction as it can be shown from unchanged CD spectra (Fig. 5)

Fig. 4. Absorption spectra of PCs: NiPC (a), CoPC (b) and CuPC (c) in complexes with DNA.
The binding of PCs with DNA in a solution with low NaCl concentration causes DNA shrinkage (Fig. 6). We use viscometry for the investigation of change in the volume of DNA molecular coil (it is known that the change in specific viscosity of DNA solutions reflects the swelling or shrinking of molecular coil). It was shown that with the increase of PC concentration in DNA solution macromolecule decreases its volume for all compounds under study. We can conclude that the binding is realized just after the mixing of the solutions and does not change during time (in our experiments the systems were measured after the preparation and in 1 day storage at 4°C).

We check the possibility of the formation of multicomponent system DNA + PC + surfactant in a solution. We use cationic azobenzene containing surfactant. It is well known that the reversible light-induced trans-cis isomerization of azobenzene containing compounds can be controlled via typical UV absorption spectra (Fig. 7). It is known that only azo - group is responsible for the spectral properties of surfactants under study. Trans isomer produces spectrum with maximum at 353 nm, same solution after UV-irradiation shows another spectrum with two bands at \( \lambda \geq 280 \) nm typical for cis-isomer. Similar shape of spectra is registered for surfactant-DNA solution at \( \lambda > 300 \) nm out of DNA adsorption band (Fig.8). Nevertheless, small blue shift of band maximum and disappearance of the "shoulder" at wavelength more than 400 nm during trans-isomer of surfactant binding to DNA are observed. UV irradiation of DNA-surfactant solutions leads to the typical spectra fixed for cis-isomer of free surfactant in a solution with same differences (red shift hyperchromic effect for the band at 350-400 nm).
nm). Moreover, same spectrum is observed if we use the preliminary irradiated surfactant (cis-isomer) for complex formation. It was shown [4] that DNA induces surfactant association on its chain with the formation of micelle-like state (pseudamicelles). The binding is not destroyed by trans-cis isomerization. After the investigation of DNA-surfactant solutions we prepare more complex systems containing DNA, surfactant and metal complexes.

Fig. 7. The absorption spectra of the initial solutions of surfactants (trans-AzoTAB) in 0.005M NaCl and after UV irradiation at 365 nm for 2 minutes (cis-AzoTAB) (before irradiation - line geometric shapes).

We prepare solutions with DNA, surfactant and PCs in 0.005 M NaCl. We also check the influence of DMSO on spectra of compounds because the initial solution of PCs contains this solvent and finally our systems under investigations have 10% DMSO. It is clear from the experimental data that DMSO does not influence significantly on spectral properties of compounds. Fig. 9 demonstrates that the order of the addition of components into solution does not influence on the result. Indeed, absolutely similar spectra were observed for [(DNA+surfactant)+PC] and [(DNA+PC)+surfactant] systems for trans- and cis-isomers of surfactant.

Fig. 8. Spectra of free surfactant (line with symbols) in a complex with DNA before and after 2 min UV irradiation (365 nm). C (DNA) = 0.003%, 0.006%, 0.009%, 0.012%, 0.015%, 0.018%; z = 0.44, 0.22, 0.15, 0.11, 0.09, 0.07.

Fig. 9. Cis- and trans-Azo in complexes with DNA in a solution with PC and control bi-component systems. Concentrations of components and the way of system preparation indicate on Figures.
Finally, we can conclude that our experimental results show that the compounds CuPC, NiPC, CoPC bind to DNA in solution. The presence of DNA stabilizes PC solution and prevents precipitation of drugs. DNA-PC binding does not change the secondary structure of macromolecules. Drugs interact with DNA via the attraction to the phosphate groups outside of the helix. The state of PC compounds (chromophores) during the binding with DNA depends on the type of metal ion inside the PC rings, as it is evidenced by the variation in the absorption spectra in the long-wave area.

The interaction of DNA with photosensitive cationic surfactant in solution was regarded. It was shown that the binding of pre-UV-irradiated surfactant to DNA and the irradiation of DNA-trans-surfactant complexes lead to the same results. The preparation and investigation of multicomponent systems demonstrate that metal complexes bind to DNA in the presence of surfactants and DMSO. The formation of DNA-surfactant-metal complexes system does not depend on the order of addition of the components into solution. UV irradiation of complex system causes trans-cis transition of surfactant as well as in DNA-surfactant solution and in free surfactant in 0.005 M NaCl. Thus our experiments show the principle possibility to create the multicomponent systems with DNA-surfactant-metal complexes.

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References.

1. Şengül A, Doğan HZ, Altundal A, Özkaya AR, Salih B, Bekaroğlu Ö., Synthesis, interface (Au/M2Pc2/p-Si), electrochemical and electrocatalytic properties of novel ball-type phthalocyanines, Dalton Trans. 2012 Jul 7;41(25):7559-72.
2. HUANG Jinling, CHEN Naisheng, HUANG Jiandong, LIU Ersheng, XUE Jinping, YANG Suling, HUANG Ziqiang, SUN Jiancheng, Metal phthalocyanine as photosensitizer for photodynamic therapy (PDT) -- Preparation, characterization and anticancer activities of an amphiphilic phthalocyanine ZnPcS2P2, SCIENCE IN CHINA (Series B), April 2001, Vol. 44 No. 2, pages 113-122.
3. A. A. Chernonosov, L. I. Solov’eva, E. A. Luk’yanets, D. G. Knorre, O. S. Fedorova, Dimeric Fe-Co Phthalocyanine Complex as a Reagent for the Selective Damage of Nucleic Acids, Macrocycles, 2011, 4(2), pages 135-137.
4. PHYSICAL REVIEW E 84, 021909 (2011) DNA compaction by azobenzene-containing surfactant Yuriy Zakreveskyy, Alexey Kopyshev, Nino Lomadze, Elena Morozova, Ludmila Lysyakova, Nina Kasyanenko, and Svetlana Santer.
5. Anne-Laure M. Le Ny and C.Ted Lee. Jr., Photoreversible DNA Condensation Using Light-Responsive Surfactants, Journal of American Chemical Society, 2006, 128 (19), pp 6400-6408
6. Spirin A. S., Spectrophotometric determination of total amount of nucleic acids, Biochemistry (USSR), volume 23, issue 5, page 656, 1958