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Unsymmetrical cationic porphyrin that forms sedimentation-unstable complexes with nucleic acids

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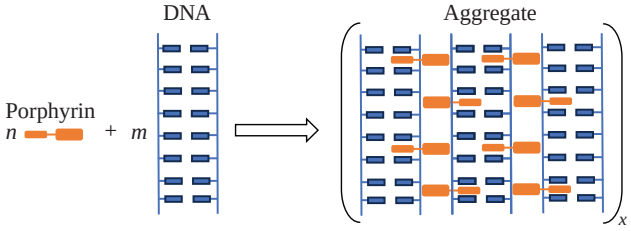
A water-soluble cationic porphyrin containing *N*-methylpyridinium fragments and an indole residue, namely, 5-[4-(1-methylindol-2-yl)phenyl]-10,15,20-tris(1-methylpyridinium-3-yl)porphyrin triiodide, has been synthesized. The porphyrin forms sedimentation unstable complexes with low and high molecular weight nucleic acids. This ability to precipitate nucleic acids can be used in laboratory, clinical and research practice for isolating nucleic acids.



Keywords: nucleic acids, DNA, cationic porphyrin, pyridinium compounds, isolation of DNA.

Nucleic acid analysis has led to a revolution in many fields, such as medicine and public health, genetics, forensics, food testing, environmental monitoring, *etc*. Improvement in nucleic acid (NA) analysis methods, development of total microanalysis systems, also known as 'laboratory on chip' necessitates the creation of more efficient analytical components and alternative chemical approaches for the isolation and study of DNA.[1] The first stage of any analysis is the extraction of nucleic acid from the sample.[2] The success of NA analysis largely depends on the extraction method, the purity, and the degree of defragmentation of NAs. At the same time, complex analysis, such as sequencing and genotyping, requires the maximally preserved NA sample. Isolation of NAs after the cell lysis stage involves the separation of DNA from impurities.[3] These issues become particularly complex in the case of high molecular weight DNA samples, since NA is quite easily subjected to defragmentation even with intense stirring of the solution. Separation of DNA from impurities (proteins, lipids, sugars and other substances) is carried out either by extraction of hydrophobic substances with organic solvents/surfactants, or by extraction of NA, for example, on silica particles or magnetic particles. Extraction methods require repetition to achieve the necessary degree of NA purification, which entails an increase in the duration of the analysis and an increase in the risk of contamination. Sorption of NAs on particles is also not a panacea, since such treatment often results in the formation of a sparingly soluble conglomerate of particles with NAs. In addition, difficulties arise in separating NAs from particles, leading to additional washing and concentration operations. Sorption methods lead to defragmentation of NA and, as a rule, are not suitable for high molecular weight NA. Alternative approaches to DNA extraction have been proposed, such as gel electrophoresis followed by electroelution from excised gel pieces.[4] However, this approach is difficult to implement in microanalysis. The polyanionic nature of NA ensures the water solubility of this biopolymer; the obvious solution is to neutralize the charges of the phosphate backbone to release NA. DNA aggregation, in which multiple strands are attracted to each other and form multiple condensed mesophases of complex structure, can be caused by multivalent counterions[5] that shield electrostatic repulsion between charged DNA strands and provide effective attraction.[6–8] DNA aggregation was studied by the action of polycations such as spermine and spermidine, chitosan, polyethylenimine, poly(L-lysine).[9–12] However, the formation of such polyplexes is mostly used for vector delivery of genetic material.[13] Polycations are not used for NA isolation due to the fact that all polycationic polymers interfere with further DNA analysis, and the destruction of polyplexes is very problematic. In addition, cationic polymers can effectively interact with proteins and other macromolecules, which does not allow achieving the required degree of NA purification. Therefore, the question of a fast, simple and reliable method for deposition of NA remains relevant and unresolved to date. Analyzing our own and published data on the different mechanisms of interaction of NA with cationic porphyrins, a hypothesis was put forward about the possibility of constructing a porphyrin compound capable of NA precipitating. It is known that cationic porphyrins are able to interact with NA forming: (1) an external complex due to the electrostatic interaction of cationic groups with the anionic phosphate backbone of DNA, (2) an external complex due to embedding into DNA grooves; (3) intercalation complex. All these complexes are water soluble and do not cause NA precipitation. For DNA precipitation, it is necessary for the porphyrin to interact with several DNAs, for example, according to the scheme presented in Figure 1.

To fulfill the interaction according to Figure 1, the porphyrin should contain cationic groups consisting of three peripheral substituents that ensure the electrostatic interaction of the porphyrin with the negatively charged surface of the NA and one peripheral substituent capable of intercalation interaction with another NA. From a series of monoheteryl-substituted cationic porphyrins,[14–16] 5-[4-(1-methylindol-2-yl)phenyl]-10,15,20-tris(1-methylpyridinium-3-yl)porphyrin triiodide meets the specified requirements. This porphyrin was obtained herein for the first time. The first synthesis step was the

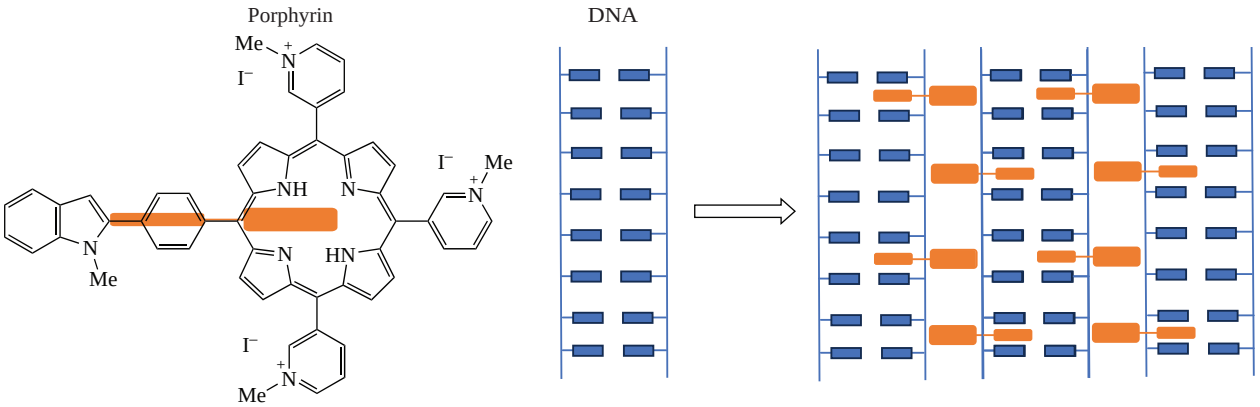
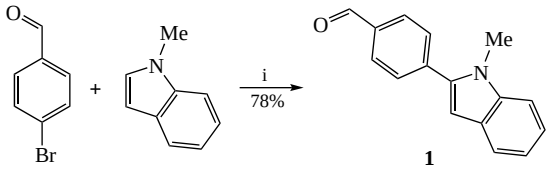


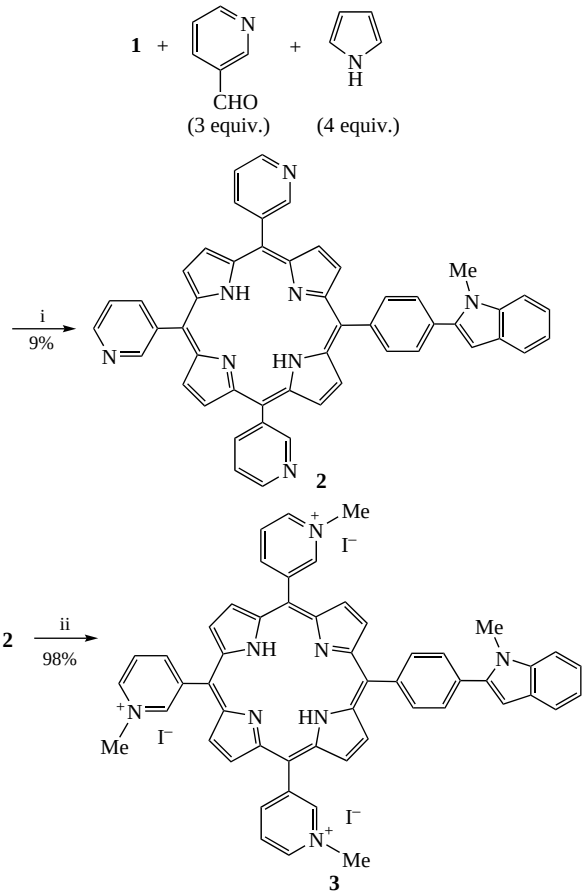
Figure 1 Interaction of porphyrin with NAs. Double-stranded DNA is shown in blue; porphyrin containing an intercalating substituent is shown in orange.



Scheme 1 *Reagents and conditions*: i, $PdCl_2$, AcONa, DMA.

palladium-catalyzed coupling of *p*-bromobenzaldehyde with *N*-methylindole leading to 2-(4-formylphenyl)-1-methylindole **1** (Scheme 1).

The subsequent mixed-aldehyde condensation of pyridine-3-carbaldehyde and compound **1** in a 3 : 1 ratio with pyrrole really afforded the required unsymmetrical porphyrin **2**, although in a 9% yield (Scheme 2). Water-soluble porphyrin **3** containing an indole residue was obtained by quaternization of pyridine fragments of porphyrin **2** with methyl iodide in DMF in 98%



Scheme 2 *Reagents and conditions*: i, TFA, xylene, air oxygen. ii, MeI, DMF.

yield (see Scheme 2). The structure of the synthesized porphyrin was confirmed by classical methods.

The validity of the hypothesis about the ability of this porphyrin to cause NA precipitation was tested by interacting the **3** with a representative short-chain oligonucleotide poly[d(GC)2], salmon sperm DNA (ssDNA) and calf thymus DNA (ctDNA). At the first stage of the work, the interaction of the synthesized porphyrin **3** with the listed NA methods of adsorption spectroscopy was studied. A 16 nm bathochromic shift of the Soret band and a decrease in its optical density by 44% are recorded when titrating a solution of **3**-poly[d(GC)2] in UV-VIS spectra. As the oligonucleotide content in the analyzed solution increases, a drift of the baseline is recorded, which indicates a violation of the optical transparency of the solution [Figure 2(*a*)]. In the case of ssDNA, a sharp decrease in the optical density of porphyrin was recorded, while an increase in the amount of ssDNA, after adding the 5th dose, practically did not lead to a change in the optical density, which can be seen when analyzing the spectrum in the region of 260 nm [Figure 2(*b*)]. The solution becomes optically opaque. Titration of a solution of **3** with NA with an even higher molecular weight (ctDNA) almost immediately led to the formation of a visible precipitate.

The resulting solutions of **3** with oligonucleotide and ssDNA were cooled to a temperature of +6–8 °C, which caused precipitation. To clarify the nature of the interaction of the **3** with NA, further studies were carried out with poly[d(GC)2], since these solutions are more segmentation stable and can be studied using solution chemistry methods. Table 1 presents information on the fluorescence lifetime of **3** in TRIS and in TRIS solutions with the addition of poly[d(GC)2]. The kinetics of **3** fluorescence quenching is described by a biexponential dependence with two components. The short-lived component is due to the mixing of

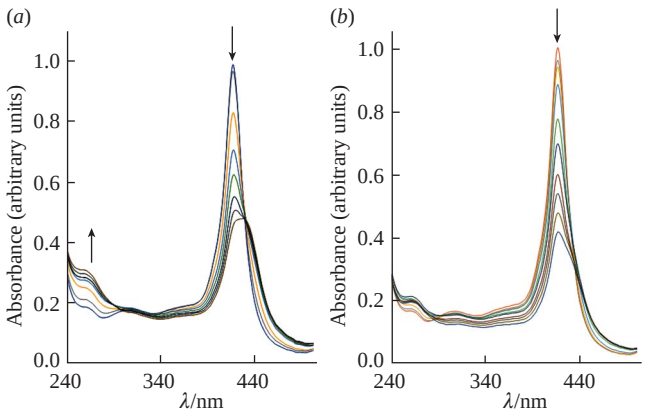


Figure 2 Changes in the UV-VIS spectrum of compound **3** (10^{-5} M, 2 ml) solution in TRIS upon addition of NA with aliquot volume 5 ml: (*a*) poly[d(GC)2] (0–1.6×10^{-5} M); (*b*) ssDNA (0–1.5×10^{-5} M).

Table 1 Fluorescence lifetime of compound **3** in TRIS at 298 K (λ_{ex} = 450 nm).

System	τ1	τ2	τ3	A1	A2	A3	τ_{av}/ns	χ2
3	0.65	6.21		51	49		5.66	1.889
3–poly[d(GC)2]	0.149	8.31	12.9	29	63	8	9.01	1.097

energetically similar S1 and CT states, in which an electron is transferred from the porphyrin core to the electron-deficient pyridinium group.[17] The average fluorescence lifetime of **3** is about 5–6 ns, which is typical of water-soluble porphyrins.[18,19] The fluorescence kinetics of **3** is described by a three-exponential relationship, and the average fluorescence lifetime increased by a factor of ⩾1.6 upon binding to the oligonucleotide (Table 1). This indicates a change in the nature of the fluorophore microenvironment. The short-wave component (τ1) decreased almost 5 times, which indicates the direct participation of *N*-methylpyridinium groups of **3** in electrostatic interaction. The values of the medium-lived (τ2) and long-lived (τ3) components of **3** in a solution with poly[d(GC)2], exceeding the fluorescence lifetime of an individual porphyrin, indicate the shielding of the porphyrin from water molecules (fluorescence quencher) in complex with poly[d(GC)2]. It is impossible not to note the well-known fact that during the intercalation of porphyrins in NA, the fluorescence of porphyrins is effectively quenched in the presence of guanine due to electron transfer from the nucleobase to photoexcited porphyrin S1–S0.[20] For example, intercalation of TMPyP4 between base pairs in poly[d(GC)2] reduces fluorescence lifetime of porphyrin from 5 to 2.5 ns.[21]

In the analyzed example, the interaction of compound **3** with poly[d(GC)2], on the contrary, leads to an increase in lifetime (see Table 1), *i.e.* the macrocyclic ring of porphyrin itself does not intercalate between pairs of nucleobases of NA. This is consistent with our proposed interaction scheme (see Figure 1).

The presence of aggregation initiated by the interaction of **3** with poly[d(GC)2] was confirmed by the results of changes in the kinematic viscosity of the poly[d(GC)2] and **3**–poly[d(GC)2] solutions, which amounted to 0.8754 and 0.9011 cP, respectively. The interaction of poly[d(GC)2] with **3**, as measured by the Zetasizer Nano particle size analyzer, leads to an increase in hydrodynamic diameter from an average of 539 to 1325–2500 nm.

To conclude, a water-soluble cationic porphyrin containing *N*-methylpyridinium fragments and an indole residue is capable of specific interaction with NA leading to precipitation of NA. In the future, it is advisable to study the structure and properties of the resulting complexes, as well as to evaluate the influence of **3** on the possibility of performing PCR and other analyses of NAs as part of complexes with porphyrin.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.06.018.

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