

## Spectroscopic Studies on Binding of Cationic Pheophorbide-a Derivative to Double-stranded and Quadruplex Polynucleotides

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Pheophorbide-a (Pheo) is an anionic porphyrin derivative. It is widely used as a photosensitizer in photodynamical therapy of tumors because of its high photosensitizing activity in vitro and in vivo [1, 2]. Modification of Pheo with the trimethylammonium group was carried out to obtain a cationic dye derivative (CatPheo) capable of polyanion binding. The interaction of CatPheo with double-stranded poly(A)·poly(U) and poly(G)·poly(C) as well as with four-stranded poly(G) homopolymer was investigated in buffered aqueous solutions (pH6.9) of low ionic strengths (2mM Na<sup>+</sup>) by methods of absorption and polarized fluorescence spectroscopy in a wide range of molar phosphate-to-dye ratios, P/D. Determination of type of the complexes formed by CatPheo with the polynucleotides and definition of binding characteristics were carried out.

It was revealed two mechanisms of CatPheo binding to the polynucleotides: (i) chromophore intercalation between the nucleic bases; (ii) formation of the external complexes via electrostatic attraction of cationic dye to polynucleotide backbones. It was shown that (ii) type is predominant for double-stranded polynucleotides. However, in the case of four-stranded poly(G) containing systems it competes with the intercalation binding mechanism. Fluorescent technique was revealed to be efficient for recognition of the type of complex formed, because of CatPheo emission intensity increases upon its intercalation and quenches strongly upon the external stacking-association. It was established that for poly(G) at low P/D values (P/D < 5) the outside cooperative binding was predominated being accompanied with CatPheo associations due to chromophore stacking; while the P/D increase results in disintegration of these external complexes and prevalence of the intercalative binding mechanism. It was confirmed by rise in fluorescence polarization degree under P/D increase.

Simulation of the external binding to DNA on a system containing the single-stranded polyphosphate was carried out under the same conditions. It was established that at low P/D values CatPheo forms continuous stacking associates on the polyanionic matrix, and at large P/D it binds to polyphosphate in the dimer form. The increase in the solution ionic strength reduces the electrostatic binding efficiency due to CatPheo and Na<sup>+</sup> ions competition. However, even at the physiological ionic strength this interaction type gives essential contribution into the complex formation. The thermodynamic characteristics of external complex formation were estimated by Schwarz's method [3].

The improved photodynamical activity for CatPheo in comparison with that for Pheo is expected because of a good water solubility of CatPheo and the efficient dye binding with polyanionic biopolymers. Besides, the strong CatPheo binding with nucleic acids probably allows to use this porphyrin derivative in anticancer applications for targeting of G-quadruplexes of telomeric DNA.

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[2] B. Roeder, *Lasers Med. Sci.* 5 (1990) 99-106.

[3] G. Schwarz, *Eur. J. Biochem.* 12 (1970) 442-453.