

Spectroscopic studies on binding of novel pheophorbide - phenazine conjugate to synthetic polynucleotides

O. Ryazanova¹, V. Zozulya¹, I. Voloshin¹, V. Karachevtsev¹, L. Dubey², and I. Dubey²

¹*Dept. of Molecular Biophysics, B. Verkin Institute for Low Temperature Physics and Engineering of NAS of Ukraine, Kharkov, Ukraine*

²*Dept. of Synthetic Bioregulators, Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine*

Presenting Author: ryazanova@ilt.kharkov.ua

Porphyrins are macrocyclic compounds possessing by unique photophysical properties and having the great potential for application in nanotechnology, biology and medicine. Pheophorbide-*a* (Pheo-*a*) is well-known anionic porphyrin derivative which selectively accumulates in tumor cells. It has a high extinction coefficient in the red region of spectrum where the transparency of tissues to light increases considerably, that determines widespread using of Pheo-*a* as a photosensitizer for PDT of tumor. Novel conjugate formed by pheophorbide-*a* with intercalative amino-phenazinium dye (Pheo-Pzn) was synthesized. Its spectroscopic properties, as well as binding affinity to synthetic polynucleotides of different secondary structure (double-stranded poly(A)·poly(U), poly(G)·poly(C) and four-stranded poly(G)) were studied using absorption, polarized fluorescence and fluorescent titration techniques. Investigations were carried out in 2 mM Na⁺ cacodylate and phosphate buffered solutions (pH6.9) containing 2 -10% of ethanol, 0.5 mM EDTA and 0.1 M NaCl. The measurements were performed in a wide range of molar polymer-to-dye ratios (P/D) by registering the changes in the intensity and polarization degree of the pheophorbide fluorescence. Spectroscopic properties of the conjugate-polynucleotide complexes were compared with those for free constituents. It was found that in aqueous solution formation of internal heterodimer with stacking of the chromophores was occurred, manifesting itself by strong fluorescence quenching (in tens of thousands times in comparison with ethanol solution). External binding of Pheo-Pzn to poly(A)·poly(U) and poly(G)·poly(C) is apparently realised without the dimer destruction: only insignificant rise of the emission was registered along with increase of fluorescence polarisation degree up to 0.15. The binding to four-stranded poly(G) differs substantially from that for double-stranded polynucleotides. Substantial blue shift of absorption bands, 10 nm red shift of fluorescence band, 50-fold rise of pheophorbide emission, and increase in its fluorescence polarization degree up to $p = 0.3$ evidences the heterodimer disintegration and intercalation of pheophorbide moiety into the intermolecular G-quadruplex. So the conjugate studied can be used as fluorescent probe for recognition of G-quadruplex structure.