SPECTROSCOPIC FEATURES OF PHEOPHORBIDE-a BINDING TO POLY-L-LYSINE

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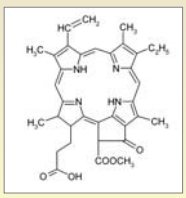
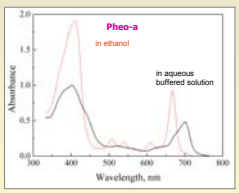
The spectroscopic features of Pheophorbide-*a* (Pheo-*a*) binding to model polycationic polypeptide poly-L-lysine (pLL) in buffered aqueous/ethanol solutions of low and near-physiological ionic strength have been studied by spectroscopic methods.

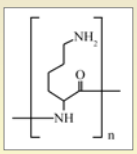
The Pheophorbide-a from Frontier Scientific Inc. (Logan, Utah, USA) and poly-L-lysine hydrobromide (Mol. weight is 30,000 - 70,000) from Sigma-Aldrich were used without further purification.

Pheophorbide-*a* (Pheo-*a*)

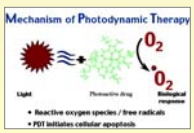




poly-L-lysine (pLL)



- macrocyclic anionic chlorine derivative
- selectively accumulates in tumor cells
- extended planar aromatic structure
- high extinction coefficient in the red region where the transparency of tissues to light increases considerably
- **photosensitizer for PDT of cancer**
- G-quadruplex binder

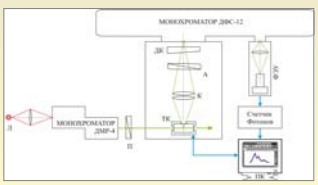


cationic polypeptide with antimicrobial action

Conformations:
- random coil (at low and neutral pH)
- α-helix (at pH > 10.6)
- β-sheet (at pH > 10.6 after heating)

The spectroscopic properties of Pheo–*a* and its complexes with poly-L-lysine have been studied using absorption and polarized fluorescence spectroscopy.

Absorption measurements: SPECORD M40 spectrophotometer (Carl Zeiss, Jena).
Fluorescence measurements: spectrofluorimeter based on the DFS-12 monochromator (LOMO, 350-800 nm, 5 Å/mm), photon counting.
Fluorescence excitation: linearly polarized beam of He-Ne laser (λ_{exc} = 633 nm) attenuated by neutral density filter.



Binding of the dye to pLL was studied in titration experiments where Pheo-a sample was added with increasing amounts of the concentrated biopolymer stock solution containing the same dye concentration, whereupon fluorescence intensities and polarization degree were measured. The several series of experiments were performed at different dye concentrations in the range $3 \cdot 10^{-6}$ - $1.95 \cdot 10^{-5}$ M.

The aim of titration experiments was to obtain the dependences of the fluorescence intensity and polarization degree characteristics on the molar *P/D* ratio.

Thermodynamical parameters of cooperative binding were estimated by **Schwarz's method** [*Schwarz G. Eur. J. Biochem., 1970,* ***12****: 442-453]* **– see Fig. 5.**

Absorption and fluorescence spectra

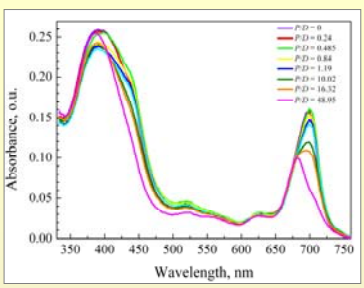


Fig. 1. Absorption spectra of **Pheo-a** in a free state and bound to **poly-L-lysine** at different *P/D* ratios in 1 mM Na-cacodylate **buffer** with 2.4% of ethanol, C_{dye} = 3 μM, path 2 cm.

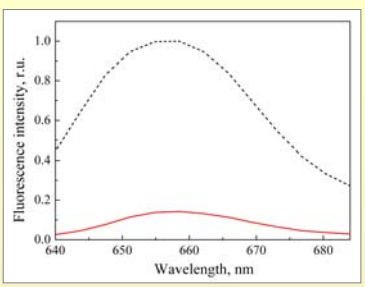
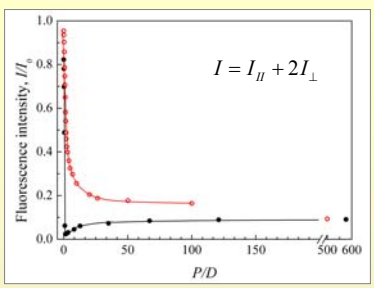


Fig. 2. Fluorescence spectra of **Pheo-a** in a **free state** (*P/D* = 0, black dashed line) and **bound to pLL** (*P/D* = 500, red line) in buffered solution with 1 mM Na^+, C_{dye} = 1 μM.

Fluorescence titration study



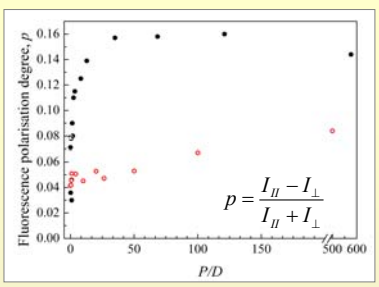


Fig.3. Dependence of relative fluorescence intensity, I/I_0, and fluorescence polarization degree, *p*, of **Pheo-a** on *P/D* upon titration by **pLL** in aqueous buffered solution with 5.6% of ethanol and 1 μM Na^+ (●), 0.15 M Na^+ (○), C_{dye}=19.5 μM λ_{exc} = 633 nm, λ_{obs} = 660 nm

Melting study

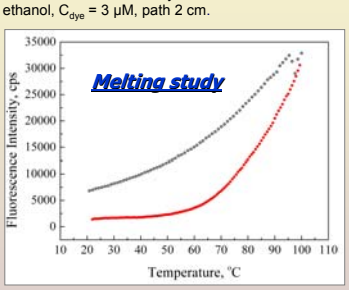


Fig. 4. Temperature dependence of the fluorescence intensity upon ***Pheo-a*-pLL** complex (*P/D* = 500) dissociation (●) and association (○) in aqueous buffered solution with 5.9 % of ethanol and 1 mM Na^+, C_{dye} = $1.95 \cdot 10^{-5}$ M, λ_{exc} = 633 nm, λ_{obs} = 660 nm.

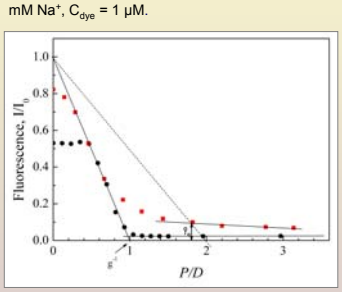


Fig. 5. Fluorescence titration curves of **Pheo-a** with **pLL** in aqueous buffered solutions with 1 mM Na^+: ● - solution with 6% of ethanol, C_{dye} = 19.5 μM; ■ - solution with 2.4% of ethanol, C_{dye} = $3 \cdot 10^{-6}$ μM.

Thermodynamical parameters of binding

A number of binding sites per monomer unit of pLL: $g = 1.00$

The cooperative binding constants:

$$K = \frac{1}{\bar{\gamma}_0 \cdot C_T}$$

For solutions of low (1 mM Na) ionic strength with 6 % of ethanol, C_{dye} = $1.95 \cdot 10^{-5}$ M, **$K \approx 2.1 \cdot 10^6$ M^{-1}**

$$K = q \cdot K^+$$

K^+ - a binding constant of isolated (non-aggregated) ligand molecule

The cooperativity parameter: q ≈ 5000.

CONCLUSIONS

Anionic Pheophorbide-*a* exhibits a strong binding affinity to polycationic matrix of poly-L-lysine due to highly cooperative interaction.

At low *P/D* ≤ 10 Pheo-a forms continuous stacking associates on the exterior of polycationic pLL matrix. The melting experiment evidences the great thermal stability of Pheo-pLL complexes, which dissociation begin at T = 50 °C, but it is not completed even at 100 °C. The complex formation is a reversible.

At high P/D values it is suggested that the dye binds to pLL in dimeric form.

The self-stacking of Pheo-*a* on the pLL exterior is characterized by strong quenching of the dye fluorescence. The residual emission intensity is near 2%. The aggregation of Pheo-*a* is accompanied by blue shift and hypochromisity of visible absorption bands. Their magnitudes are different for aggregates and dimers.

It is supposed that binding of Pheo-*a* induces the biopolymer adjustment to pheophorbide stacks that can results in the changes in the pLL conformation from disordered coil to ordered linear or helical structure.

The strong tendency of Pheo-*a* to self-stacking upon the electrostatic interaction with proteins can reduce the dye photodynamical activity due to decrease of singlet oxygen quantum yield as results of formation of externally bound dye dimers or multimers.

XXV ISSSMC, September 21-24, 2021, Kyiv, Ukraine