PEGYLATION OF CYTOSINE NUCLEIC ACID BASE: EXPERIMENT AND MODELING

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Introduction.

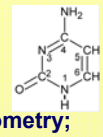
Biophysics is a multidisciplinary branch of science, which embraces and combines achievements in different areas to solve a definite problem.
In our present work, to address a question of
PEGylation of Cytosine DNA nitrogen base,
we attract opportunities of experimental mass spectrometric technique and computer modeling.

Organic polymers polyethylene glycols (PEG), polydispersed polyethers which chains are built from different number (n) of monomers $HO-(CH_2-CH_2-O)_n-H$, find versatile applications as green solvents, cryoprotectos, excipients, components of nanosomes, hydrogels, biobetters, and as PEGylation agents. PEGylation, that is binding of PEGs to biomolecules or nanoparticles, is used for drug delivery, in particular in elaboration of anti-SARS-CoVID vaccines.

Cytosine (Cyt), a DNA nitrogen base, was selected as a relatively small molecule accessible for the planed experiments.

In our previous investigations, we have demonstrated that molecular mechanism of action of PEGs consists in their wrapping over both positively and negatively charged monoatomic ions and amino acids ions [1].
Here we apply the same approach to study PEGylation of Cyt.

[1] V.G. Zobnina, M.V. Kosevich, V.V. Chagovets, O.A. Boryak.
Study of nanocomposites of amino acids and organic polyethers by means of mass spectrometry and molecular dynamics simulation.
In: Nanomaterials Imaging Techniques, Surface Studies, and Applications. Springer Proceedings in Physics 146 (Springer, New York 2013).
https://doi.org/10.1007/978-1-4614-7675-7_22



Three aspects of the problem are considered:
1 - experimental observation of Cyt – PEG interactions by means of electrospray mass spectrometry;
2 - modeling of interaction of protonated Cyt with small PEG_n oligomers in the gas phase and liquid methanol droplet;
3 - modeling of complexes behaviour under electrospray conditions.

Task 1. Experiment.

Formation of stable complexes of PEG-400 oligomers (labeled as M_n) with a set of Cyt derivatives – Cyt, m^1Cyt, m^5Cyt, Br^5Cyt was demonstrated by means of electrospray mass spectrometry. As an example, in the spectrum of PEG-Br^5Cyt system shown in Fig.1 the presence of a set of $M_n \cdot Br^5Cyt \cdot H^+$ noncovalent complexes was detected. Characteristic isotopic distribution of $^{79}Br/^{81}Br$ unambiguously evidenced the inclusion of Br^5Cyt into the clusters.

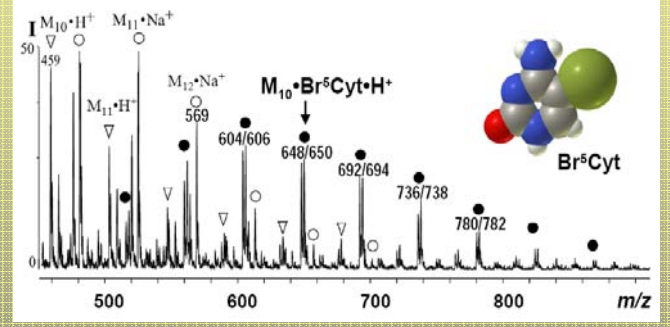
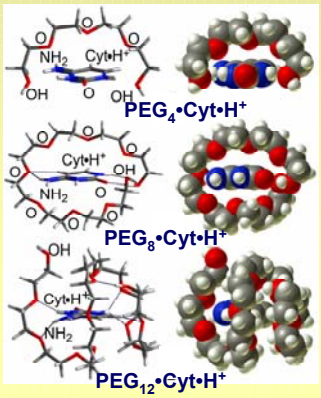


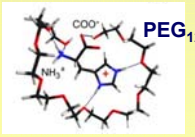
Fig. 1. Positive ion electrospray mass spectrum of PEG-Br^5Cyt system. ● - $M_n \cdot Br^5Cyt \cdot H^+$ clusters, ∇ - $M_n \cdot H^+$, o – $M_n \cdot Na^+$.

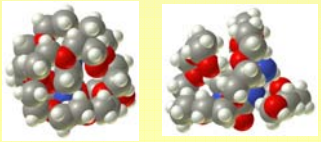
Task 2. Molecular dynamics modeling.

Interaction of neutral or protonated Cyt with small PEG oligomers in the gas phase was modeled by molecular dynamics (CHARMM force field). Similar to other studied systems, wrapping of the PEG chains over the nitrogen base occurs with time of modeling. It is shown that $M_n \cdot Cyt \cdot H^+$ clusters in the gas phase (vacuum) adopt a compact structure with a quasi-cyclic or quasi-helical self-organization of a polymeric chain around the protonated base.

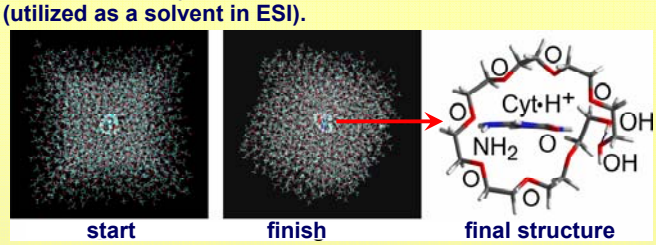


Interestingly, in contrast to Histidine amino acid, which appears in plane with PEG_n ring, Cyt is orthogonal to the PEG_n ring plane.





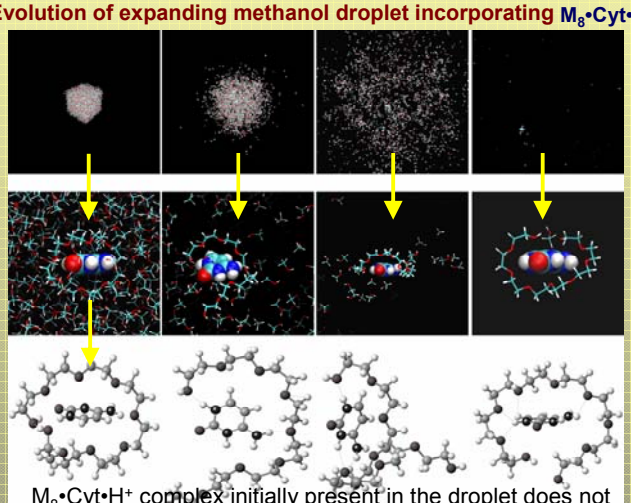
Dynamics of $M_8 \cdot Cyt \cdot H^+$ complex in a droplet of methanol (utilized as a solvent in ESI).



It is established that the compact structure of $M_8 \cdot Cyt \cdot H^+$ complex characteristic of its gas phase state is preserved in liquid methanol medium.

Task 3. Simulation of electrospray desolvation event.

Evolution of expanding methanol droplet incorporating $M_8 \cdot Cyt \cdot H^+$



$M_8 \cdot Cyt \cdot H^+$ complex initially present in the droplet does not disintegrate in the process of droplet evaporation.
The clusters recorded on the ESI mass spectra reflect adequately clusters present in solution.