Abstract

The aim of the study was to investigate the effects of selected drugs used in anticancer therapy: 5-fluorouracil (5-FU), 5-iodouracil (5-IU), tegafur (TF), azidothymidine (AZT), fludarabine monophosphate (F-ara-AMP) and two aromatic amino acids: L-α-tyrosine (Tyr) and L-α-tryptophan (Trp) with PPI G4 dendrimer in an aqueous medium. The spectroscopic results of the studies on the above-mentioned oncological drugs and amino acids indicate an increase in solubility of these ligands in water in the presence of the PPI G4 dendrimer. The only exception in this trend is observed for the highly water-soluble fludarabine monophosphate. The increase in the solubility of guest molecules in solution with the increase of receptor concentration can be explained by the formation of ligand-receptor supramolecular complexes. Under conditions of the large excess of ligand to the PPI G4 dendrimer, the hydrophobicity (logP) of the ligand affects the increase of the number of its molecules bound with the PPI G4 macromolecules, which is especially evident when comparing structurally similar ligands: $n_{TF} \approx n_{5-IU} > n_{5-FU}$ and $n_{Trp} > n_{Tyr}$.

Studies performed using equilibrium dialysis indicate that the PPI G4 macromolecule binds from 10 to 30 ligand molecules with weak acidic properties (tegafur, 5-fluorouracil, 5-iodouracil and azidothymidine) or zwitterionic L-α-tryptophan molecules. For fludarabine monophosphate with strong acidic properties, the number of molecules bound with the PPI G4 macromolecule is higher (n = 80) compared to ligands with weaker acidic properties (n = 10-30). This is due to the strong electrostatic interaction between the positively charged surface amino groups of the PPI G4 dendrimer and the negatively charged phosphate residues of this drug. The ligand-active site binding constants K of the PPI G4 macromolecule determined for studied ligands by dialysis indicate that the ligand which interacts the strongest with the PPI G4 dendrimer is 5-fluorouracil, whose molecules are bound with the PPI G4 macromolecule by two types of active sites with different affinity for this drug.

The results of the isothermal calorimetric titrations at 25^0C indicate that the binding process by PPI G4 macromolecules of the following ligand molecules: fludarabine monophosphate, tegafur, 5-fluorouracil, 5-iodouracil, azidothymidine and L-α-tryptophan is thermodynamically spontaneous at 25ºC in an aqueous environment (the calculated values of Gibbs free energy for all tested ligands are negative ($\Delta G < 0$)). Fludarabine monophosphate is the strongest bound to the PPI G4 dendrimer as compared to the other ligands tested. The

stability of the resulting complexes is confirmed by the strong exothermic complexing effect and the high binding constant of this drug. The least exothermic effect of the interactions of PPI G4 macromolecules was found for L-α-tryptophan. Standard values of the binding entropies for the studied drugs by the PPI G4 dendrimer indicate an increase in the order of the system ($\Delta S < 0$), which may indicate the attachment of ligand molecules mainly to the surface of the PPI G4 macromolecule. A positive standard entropy value accompanying the binding of L-α-tryptophan by the G4 PPI dendrimer indicates an increase in system disorder during complexation.

The results of ^{1}H NMR titrations of the PPI G4 dendrimer with selected ligands in a heavy water environment indicate that fludarabine monophosphate interacts strongly with both the surface groups and the other functional groups of the PPI G4 dendrimer. ^{1}H NMR studies indicate that 5-fluorouracil and tegafur interact with the surface of the PPI G4 dendrimer macromolecule stronger than with its internal functional groups. L-α-Tryptophan is bound to the dendrimer functional groups located under the surface of the dendrimer macromolecule.