International Journal of Secondary Metabolite: Vol. 3, Issue 1, 2016, pp. 21-26

Research Article

ISSN:2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Thermodynamic interaction between PAMAM G4-NH_2, G4-OH, G3.5-COONa dendrimers and gemcitabine in water sotutions

Bartlomiej Palecz[1,*], Adam Buczkowski[1,*], Henryk Piekarski[1], Özge Kılınçarslan[2]

[1]Department of Physical Chem., Faculty of Chemistry, University of Lodz, Pomorska 165, Lodz 90-236, Poland
[2]Department of Biology, Faculty of Science and Literature, Pamukkale University, Denizli, Turkey

Received: 5 December 2015 - Accepted: 25 December 2015

Abstract: The thermodynamics of complex formation of polyamideamine dendrimers: cationic PAMAM G4-NH_2, neutral PAMAM G4-OH and anionic PAMAM G3.5-COONa with gemcitabine hydrochloride, an antitumor drug, in water at a temperature of 25^0C was studied. The investigations were carried out with the use of ITC measurements allowing one to determine the number of the drug molecules bonded by the dendrimer macromolecule, the equilibrium constants of the complex formed and the values of enthalpy and entropy of their formation. The measurement results obtained show different numbers of the drug molecules bonded by dendrimer macromolecules depending on the properties of their terminal groups.

Keywords: PAMAM dendrimers G-4, gemcitabine hydrochloride, isothermal titration calorimetry

1. Introduction

Many research centers have been investigating dendrimers (PAMAM) of G4 generation as oncological drug transporters (Buczkowski, Olesinski, Zbicinska, Urbaniak, & Palecz 2015; Buczkowski et al., 2011; Buczkowski, Urbaniak, & Palecz, 2012; Kanchi, Suresh, Priyakumar, Ayappa, & Maiti, 2015; Medina & El-Sayed, 2009; Yavuz, Pehlivan, Vural, & Ünlü, 2015). PAMAM dendrimers of the 4 and 3.5-generation are asteroid polymers with ethylenediamine core, possessing 64 terminal functional groups with different properties. Molecules of PAMAM G4-NH_2 dendrimer (M=14214 Da) are terminated with amine groups. These groups in aqueous solutions show cationic character and about 15% of them are protonated: $–NH_3^+$ (Niu, Sun, & Crooks, 2003). PAMAM G4-OH macromolecules (M=14277 Da) are terminated with hydroxyl groups –OH, while PAMAM G3.5-COONa dendrimers (M=12928 Da) are terminated with carboxylate groups that impart to them in aqueous solutions anionic character. PAMAM dendrimers terminated with hydroxyl and anionic groups are better tolerated by human organism compared with their cationic equivalents (Kesharwani, Jain, & Jain, 2014). The character of the surface groups of the dendrimers under discussion determines the interactions of these nanotransporters with the molecules of the ligands bonded. The toxicity of PAMAM nanotransporters, used in clinical examinations can be reduced by modifying their surface functional groups with fatty acid, amino acid or

*Corresponding Authors: B. Palecz, E-mail: paleczb@uni.lodz.pl
A. Buczkowski, E-mail: adam.buczkowski@chemia.uni.lodz.pl

saccharic radicals. Terminal groups can be also substituted with folic acid, a biomarker recognizable by the receptors of folic acid of tumor cells (neoplastically altered) (Vlahov & Leamon, 2012). The thermodynamic studies on the equilibriums of dendrimer-cytostatic drug complexes can contribute to more complete understanding of the interaction thermodynamics and stoichiometry of substituted less toxic dendrimers with cytostatic agents.

The ligand tested in this study is gemcitabine, 4-amino-1-(2-deoxy-2,2-difluoro-β-D-erythro-pentofuranosyl)pyrimidin-2(1H)-on, a cytostatic drug with a wide range of action, used in the treatment of inoperable pancreas cancer and neoplastic changes in breast, ovary, urinary bladder and lungs. It belongs to the group of pirymide antimetabolites, analogues of 2'-deoxycytydine with two hydrogen atoms substituted by fluorine (Figure 1). The therapeutic action of gemcitabine consists in its incorporating into the DNA helix instead of 2'-deoxycytidine during the phase S division, causing the apoptosis of the cancer-altered cell.

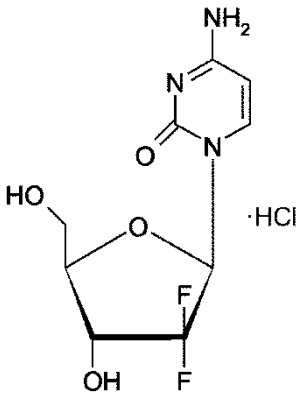

Figure 1. Gemcitabine hydrochloride.

The aim of the study was to determine the number of gemcitabine molecules bonded by the macromolecules of the dendrimers under investigation and the thermodynamic parameters describing the formation of these complexes in aqueous solutions at a temperature of 298.15 K.

2. Materials and Methods

2.1. Materials

Dendrimers with a core of ethylenediamine: PAMAM G4-NH_2 (Sigma-Aldrich, 10% solution in methanol), PAMAM G4-OH (Sigma-Aldrich, 10% solution in methanol), PAMAM G3.5-COONa (Sigma-Aldrich, 10% solution in methanol), gemcitabine hydrochloride (GEM) (Sigma-Aldrich, ≥98%), water distilled three times, deionized and out-gassed.

2.2. Isothermal Titration Calorimetry (ITC)

Calorimetric measurements were carried out by means of an isothermal titration calorimeter VP-ITC from MicroCal (USA). The calorimeter consists of a 1.4274 ml measurement cell filled with an aqueous degassed PAMAM with a concentration of 40 μM. This solution is titrated with a degassed aqueous solution of gemcitabine with a concentration of 40 mM with 3 μl aliquots for 15 seconds at a temperature of 298.15 K. The solution of titrant was injected in 600 sec. intervals at a stirring rate of 416 rpm. There were also carried out measurements of dilution the PAMAM solutions with the same concentration with water and dilution of aqueous gemcitabine solutions in water. The complementing measurements

were carried out to calculate the effects of receptor-ligand interactions (Buczkowski & Palecz, 2014; Buczkowski et al., 2012; Buczkowski, Urbaniak, & Palecz, 2013).

3. Results and Discussion

The interactions between PAMAM G-4 dendrimers and gemcitabine hydrochloride (GEM) in aqueous solutions were examined by means of an isothermal calorimeter for ITC titration at a temperature of 298.15 K. To determine the energetic effects of the interactions of dendrimers with gemcitabine hydrochloride molecules (Buczkowski & Palecz, 2014; Buczkowski et al., 2012; Buczkowski, Urbaniak, & Palecz, 2013), the energetic effects of the dilution of dendrimer aqueous solutions with water and the dilution of aqueous solution of gemcitabine in water (Figure 2) were subtracted from the energetic effects of titration measurements of aqueous solutions of PAMAM G4-NH_2, G4-OH and G3.5-COONa with aqueous drug solutions.

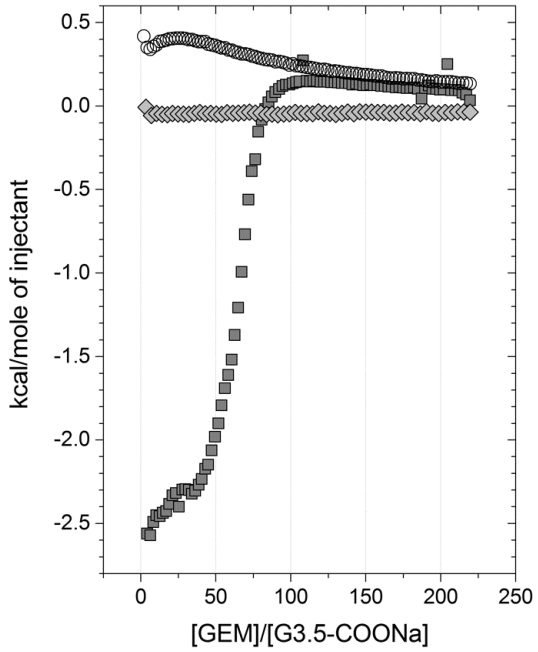

Fig. 2. Typical thermogram describing the energetic effects of the titrations between gemcitabine hydrochloride in the syringe and PAMAM G3.5-COONa dendrimer in the cell (□) and corresponded to them thermal effects of dilution of the drug (○) and the dendrimer (◊).

The pH values of aqueous gemcitabine hydrochloride solutions were also determined at a temperature of 298.15 K, using a pH meter from CERKO (equipped with an EUROSENSOR combination pH electrode). From them we calculated the acidic constant of GEM hydrochloride in water, $pK_a = 3.95$.

Using the program *"One set of sites" (Origin v.7* for isothermal titration calorimeter VP-ITC from MicroCal) we determined the thermograms showing the direct interactions of the dendrimer tested with GEM (Table 1 and Figure 3).

Table 1. Thermodynamic parameters characterizing the interactions between PAMAM dendrimers and gemcitabine molecules.

PAMAM dendrimer	N	K	ΔH [cal mol^{-1}]	ΔS [cal K^{-1} mol^{-1}]	ΔG [cal mol^{-1}]
G4-NH_2	25 ± 8	1050 ± 380	-1650 ± 750	8.3	-4120 ± 1800
G4-OH	20 ± 9	670 ± 450	-1200 ± 680	8.9	-3860 ± 1200
G3.5-COONa	44 ± 6	12200 ± 1000	-2800 ± 400	9.3	-5580 ± 500

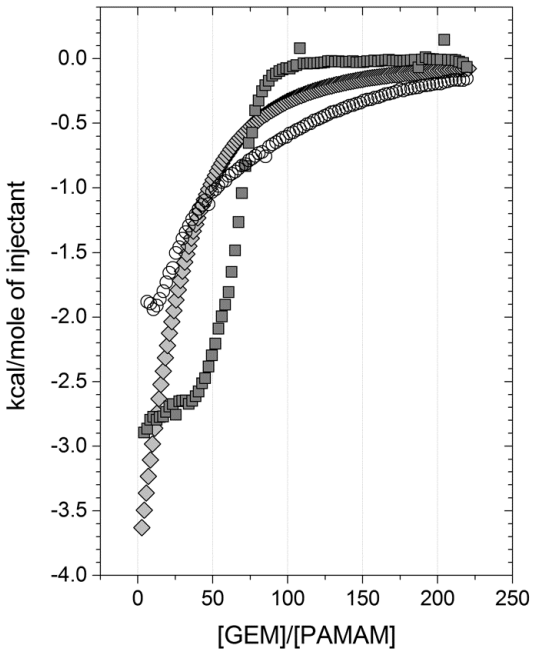

Figure 3. Thermal effect of the interaction between PAMAM dendrimer and gemcitabine hydrochloride (GEM) in water corrected with the dilution effects and calculated per one mole of the drug for: (○) G4-OH, (◊) G4-NH_2 and (□) G3.5-COONa.

The following thermodynamic parameters were also calculated: the number of drug molecules added to the macromolecule of the dendrimers tested (N), constants of dendrimer-drug complex formation (K), molar enthalpy (ΔH) and entropy (ΔS) of bonding drug molecules by PAMAM nanotransporters. The values of molar enthalpy and entropy obtained were used to calculate the molar free enthalpy (ΔG) of the interaction between the receptor and ligands. The thermodynamic parameters characterizing the interactions between PAMAM macromolecules and GEM molecules are listed in Table 1.

The parameter values determined show that the macromolecules of three PAMAM dendrimers bond GEM molecules in aqueous medium at a temperature of 25°C (ΔG<0). The number of added gemcitabine hydrochloride molecules is the highest for anionic PAMAM G3.5-COONa (N = 44 ± 6) compared to cationic PAMAM G4-NH_2 (N = 25 ± 8) and hydroxyl, neutral PAMAM G4-OH (N = 20 ± 9). The constants of GEM bonding with the active sites of dendrimer macromolecules increase in the sequence: G4-OH < G4-NH_2 < G3.5-COONa. This indicates that the electrostatic interactions of the anion-cation type are privileged as forces stabilizing the structure of the PMAM-GEM supramolecular complexes formed in relation to other types of cohesion forces: hydrogen bonds and interactions of polarized functional groups of dendrimer and ligand with unlike fractional charges.

The calculated values of thermodynamic functions of GEM bonding by the active sites in PAMAM macromolecules show that the saturation of active sites is driven in this case by both the advantageous entropy ($\Delta S>0$) and enthalpy ($\Delta H<0$) effects. The entropy factor describing the addition of GEM to the active sites of dendrimers is constant within the measurement uncertainty limits for G4-NH_2 and G4-OH dendrimers. It indicates an increase in the degree of disorder accompanying the formation of supramolecular dendrimer-drug bonds. The enthalpy factor is exothermal in the case of bonding GEM by the three PAMAM dendrimer tested. This is indicated by the domination of direct interactions of charged and polar functional groups of dendrimer and GEM over the effects of partial dehydration of these groups preceding supramolecular association. The enthalpy factor particularly well differenciates the interactions between the ligand (GEM) and the dendrimer tested. The enthalpy effects accompanying th formation of GEM-PAMAM bonds become more exothermal in the following order: G4-OH < G4-NH_2 < G3.5-COONa. This sequence probably reflects the increase in the contribution of electrostatic interactions of GEM with anionic macromolecules of PAMAM G3.5-COONa compared with cationic PAMAM G4-NH_2 and neutral PAMAM G4-OH. The resultant enthalpy effects of interactions between dendrimers and gemcitabine and practically constant (within measurement uncertainty limits) entropy effects are observed in the form of advantageous free enthalpy of the bonding process (ΔG). The values of free enthalpies of bonding calculated from enthalpy and entropy effects confirm the trend towards a particularly advantageous bonding of gemcitabine hydrochloride with the macromolecules of anionic PAMAM G3.5-COONa compared with cationic PAMAM G4-NH_2 and neutral hydroxyl PAMAM G4-OH in aqueous medium at a temperature of 25°C.

4. Conclusions

The interactions of the PAMAM dendrimers investigated with gemcitabine hydrochloride (GEM) have an exothermal character ($\Delta H<0$) and spontaneously proceed ($\Delta G<0$), facilitating the formation of receptor-ligand complex. Based on the results obtained, it may be assumed that the driving force of PAMAM-GEM complex formation consists of enthalpy and entropy effects. The PAMAM dendrimers investigated are capable to transport up to several dozen GEM molecules. The addition of the molecules of this drug to the macromolecule of PAMAM G3.5-COONa is particularly advantageous (about 45 drug molecules transported). This seems to result from relatively strong electrostatic interactions, having a character of ionic pairs, proceeding between dissociated terminal groups, $–COO^–$, of this dendrimer and partly protonated molecules of gemcitabine hydrochloride in an aqueous medium. Therefore macromolecules of anionic PAMAM G3.5-COONa dendrimer are the optimal carrier of anticancer drug, gemcitabine hydrochloride in aqueous medium.

Acknowledgments

Research was funded by the National Science Centre of Poland according to the grant decision: DEC-2012/07/B/ST4/00509 (OPUS).

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

5. References

Buczkowski, A., Olesinski, T., Zbicinska, E., Urbaniak, P., & Palecz , B. (2015). Spectroscopic and calorimetric studies of formation of the supramolecular complexes of PAMAM G5-NH_2 and G5-OH dendrimers with 5-fluorouracil in aqueous solution. *Int J Pharm., 490,* 102-111.

Buczkowski, A., & Palecz, B. (2014). Study of the interactions of PAMAM-NH_2 G4 dendrimer with selected natural amino acids in aqueous solutions. *J. Chem. Thermodyn., 70*, 95-100.

Buczkowski, A., Sekowski, S., Grala, A., Palecz, D., Milowska, K., Urbaniak, P., et al. (2011). Interaction between PAMAM-NH_2 G4 dendrimer and 5-fluorouracil in aqueous solution. *Int J Pharm., 408*, 266-270.

Buczkowski, A., Urbaniak, P., & Palecz, B. (2012). Thermochemical and spectroscopic studies on the supramolecular complex of PAMAM-NH_2 G4 dendrimer and 5-fluorouracil in aqueous solution. *Int J Pharm., 428*, 178– 182.

Buczkowski, A., Urbaniak, P., & Palecz, B. (2013). Interaction between PAMAM-NH_2 G4 dendrimer and paracetamol in aqueous solution. *J. Mol. Liq., 186*, 70-75.

Kanchi, S., Suresh, G., Priyakumar, U. D., Ayappa, K. G., & Maiti, P. K. (2015). Molecular dynamics study of the structure, flexibility, and hydrophilicity of PETIM dendrimers: a comparison with PAMAM dendrimers. *Journal of Physical Chemistry B, 119*, 12990-13001.

Kesharwani, P., Jain, K., & Jain, N. K. (2014). Dendrimer as nanocarrier for drug delivery. *Prog. Polym. Sci., 39*, 268-307.

Medina, S. H., & El-Sayed, M. E. H. (2009). Dendrimers as carriers for delivery of chemotherapeutic agents. *Chem. Rev., 109*, 3141–3157.

Niu, Y., Sun, L., & Crooks, R. M. (2003). Determination of the intrinsic proton binding constants for poly(amidoamine) dendrimers via potentiometric pH titration. *Macromolecules, 36*, 5725-5731.

Vlahov, I. R., & Leamon, C. P. (2012). Engineering folate-drug conjugates to target cancer: from chemistry to clinic. *Bioconjugate Chemistry, 23*, 1357-1369.

Yavuz, B., Pehlivan, S. B., Vural, I., & Ünlü, N. (2015). In Vitro/In Vivo Evaluation of Dexamethasone - PAMAM Dendrimer Complexes for Retinal Drug Delivery. *J. Pharm. Sci., 104*, 3814-3823.