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Interaction between PAMAM-NH_2 G4 dendrimer and paracetamol in aqueous solution

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ABSTRACT

Interactions between PAMAM-NH_2 G4 dendrimer and paracetamol in aqueous solutions were examined by UV spectroscopy, equilibrium dialysis, ^{1}H NMR spectroscopy and calorimetric techniques. The results of equilibrium dialysis show that PAMAM-NH_2 G4 dendrimer can combine 18 paracetamol molecules with equilibrium constant $K = 280 \pm 40$. Using the method of calorimetric titration (ITC), it has been found that the paracetamol addition to dendrimer is an exothermic process. Based on the model of two active sites in a dendrimer molecule, the results of equilibrium dialyses were described and it was calculated that a molecule of dendrimer has about three sites strongly combining paracetamol ($K_1 = 2100 \pm 670$) and about 15 sites that weakly combine the drug ($K_2 = 130 \pm 30$). The measurements of paracetamol solubility in aqueous solutions of PAMAM-NH_2 G4 dendrimer confirm the paracetamol addition to this dendrimer.

1. Introduction

Dendrimers are synthetic oligomeric macromolecules with a tree-like brunched structure that can be used as carriers of biological molecules including drugs [1,2].

Polyamidoamine (PAMAM) dendrimer of the fourth generation (G4) with an ethylenediamine core on account of its size (diameter: 4.5–5 nm) [3–6], globular shape [7] and the presence of functional groups analogous to those occurring in biomolecules [8] (124 amide groups, 62 tertiary amine groups, 64 superficial primary amine groups) [9] constitutes a convenient model of dendritic nanocarriers. The arrangement of steric superficial groups in G4 dendrimers enables both the combination of molecules by terminal functional groups and the penetration of dendrimer molecules by ligands [9,10].

Our previous studies on the interactions of PAMAM-NH_2 G4 dendrimer with oncological drug 5-fluorouracil [11–13] have confirmed the diversity of active sites on the surface and in the internal cavities of the dendrimer macromolecules. Therefore we decided to study the interactions of the dendrimer with paracetamol as a model ligand with a more developed molecule than that of 5-fluorouracil, possessing functional groups (amide group, phenol ring) analogous to those occurring in biomolecules.

The aim of the present study was to thermodynamically characterize the interactions between a PAMAM-NH_2 G4 macromolecule and steric, weak acidic molecules of paracetamol in aqueous solutions and quantitatively assess the resultant equilibria of the dendrimer active site saturation. For our study paracetamol has been chosen which is not an oncological medicament but is a popular anti-inflammatory and analgesic drug. This compound is a derivative of acetamide, substituted with a hydroxyl group in position 4. The concentration of a saturated paracetamol solution in water at 25 °C, according to various authors, amounts to 98.6 mM ± 0.2 mM [14] or 110 mM ± 8 mM [15]. The hydroxyl group of paracetamol can split off a proton [16,17]. The acidic dissociation constant, pK_a, of this compound amounts to 9.7 [18] or 9.78 [19].

The solubility of weak organic acid—ibuprofen in water in the presence of PAMAM-NH_2 G4 dendrimer was also examined. Ibuprofen (which is not an oncological medicament) is a sparingly water-soluble anti-inflammatory drug, $S = (270 \pm 20)$ μM [20], a steric derivative of propionic acid, $pK_a = 5.2$ [21]. The interactions of this drug with PAMAM dendrimers of integer generations have been largely investigated [21–23].

2. Material and methods

2.1. Materials

PAMAM-NH_2 G4 dendrimer (10% solution in methanol, molarity 5 mM, m.w. ~14 kDa) with ethylenediamine core, (±)-ibuprofen

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(m.w. = 0.206 kDa, ≥98%), paracetamol (m.w. = 0.151 kDa, ≥98%), deuterium oxide (99.9 atom %D), water distilled three times and degassed, and benzoylated dialysis tubing (MWCO 2 kDa) were from Sigma-Aldrich.

2.2. Measurements of drug solubility

A 50 μM initial aqueous solution of dendrimer was prepared from its stock 5 mM methanol solution. Methanol was repeatedly removed from the initial solution by evaporation to 1/3 initial volume followed by making-up with water to the initial volume. This solution was used to prepare a series of PAMAM-NH_2 G4 dendrimer solutions with concentrations from 2.5 to 45 μM. Four samples were prepared for each of the compositions tested. The dendrimer solutions were left above the sediment of a crystalline drug (ibuprofen or paracetamol) for a week at room temperature (20 °C).

The dendrimer solutions saturated with the drug were diluted and the drug concentration was spectrophotometrically determined (Specord 50, Analytic Jena) in relation to corresponding dendrimer solutions with the same dilution. The difference in the absorbance of the dendrimer–drug solution and dendrimer solution (background) was converted into the drug concentration using the molar absorption coefficient independently determined. The molar absorption coefficient measured for ibuprofen in water at wavelength $\lambda_{max} = 222$ nm within the drug concentration range from 4 to 100 μM is $\varepsilon_{max} = (8680 \pm 190)\ M^{-1}\ cm^{-1}$. The literature value of the ibuprofen molar absorption coefficient at wavelength $\lambda_{max} = 222$ nm is $\varepsilon_{max} = 9570\ M^{-1}\ cm^{-1}$ [24]. The molar absorption coefficient of paracetamol measured at wavelength $\lambda_{max} = 343$ nm within the drug concentration range from 10 to 350 μM is $\varepsilon_{max} = (9680 \pm 70)\ M^{-1}\ cm^{-1}$, while its literature values at wavelength $\lambda_{max} = 243$ nm is $\varepsilon_{max} = 10{,}080\ M^{-1}\ cm^{-1}$ [25].

2.3. Equilibrium dialysis

On account of the low water-solubility of ibuprofen, the measurements of equilibrium dialysis were only carried out for the mixtures of PAMAM-NH_2 G4 dendrimer and paracetamol using Harvard Apparatus dialyzers within the range of the molar ratio of the drug to dendrimer from 5/1 to 490/1 as in previous studies [11,13]. The molecular weight cut off of the semi-permeable membranes used was 2 kDa. For each composition investigated, 6 independent dialyses were carried out and their results were averaged. The duration of each dialysis performed was one day and the concentration of dendrimer in each sample was 40 μM. Once the dialyses were terminated, samples of both dialyzed solutions were taken and paracetamol was determined as in the case of solubility measurements.

2.4. ^1H NMR spectroscopy

A 2.8 mM initial PAMAM-NH_2 G4 dendrimer solution in heavy water was prepared by evaporating a sample of the methanol solution of dendrimer to dryness at room temperature (20 °C) and dissolving the residue in heavy water. As in the case of measurements by the method of dialysis, ^1H NMR examinations were only carried out for the mixtures of paracetamol and dendrimer due to the low water-solubility of ibuprofen. The concentration of the stock paracetamol solution used to prepare the paracetamol–dendrimer mixture in heavy water was 20 mM. The concentration of PAMAM-NH_2 G4 dendrimer in all the samples was 140 μM. The molar ratio of the drug to dendrimer in the mixtures investigated ranged from 10/1 to 200/1. Each ^1H NMR spectrum was recorded (Bruker Avance III 600 MHz) for the given sample sixteen times and averaged.

2.5. Isothermal titration calorimetry (ITC)

Two independent titrations were carried out by the technique of calorimetric titration under isothermal conditions (VP-ITC instrument, MicroCal, USA). First, 1.4275 ml of a 20 μM aqueous PAMAM-NH_2 G4 dendrimer solution was titrated injecting 285 μl of a 50 mM paracetamol solution, 5 μl each time for 50 s at 800 s intervals. Next, the titration of water (in a cell) was carried out with paracetamol solution of the same concentration. The thermal effects accompanying the dendrimer dilution with water were within the error limits and therefore have been neglected in calculations.

3. Results and discussion

3.1. Measurements of drug solubility

The solubility measurements of ibuprofen in water in the presence of PAMAM-NH_2 G4 show a linear increase in the drug solubility with increasing dendrimer concentration (Fig. 1) within the polymer concentration range from 2.5 μM to 50 μM, as in previous studies [23] in more concentrated dendrimer solutions. The dependence of ibuprofen solubility on the dendrimer concentration is described with the straight line equation: $y = (81 \pm 2)x + (220 \pm 50)$, ($R^2 = 0.9960$). The coefficients of this straight line have a physical sense. The free term (220 μM ± 50 μM) is similar to the ibuprofen solubility in pure water (240 μM) [26], (270 ± 20) μM [20]. The slope of the straight line ($n = 81 \pm 2$) may be interpreted as a number of drug molecules combined by one dendrimer molecule [27]. The average number of ibuprofen molecules combined with a dendrimer macromolecule in the polymer solutions with a concentration ranging from 2.5 μM to 50 μM determined in this way confirms the number of ibuprofen molecules (n = 78) combined by the dendrimer determined by the group of Kannan [22].

The solubility measurements of paracetamol in water in the presence of PAMAM-NH_2 G4 dendrimer approximately indicate a linear increase in the paracetamol solubility with an increasing dendrimer concentration within the polymer concentration range from 2.5 μM to 50 μM. The dependence of the drug solubility on the dendrimer concentration (Fig. 2) is described by the straight line equation: $y = (30 \pm 15)x + (89{,}100 \pm 400)$, ($R^2 = 0.2212$). This dependence approximately shows a linear character, which is due to the enormous dilution (500×) of the saturated paracetamol solutions investigated, taken for the determination of the drug absorbance. The free term (89.1 mM) is similar to the solubility of paracetamol in pure water (98.6 mM) [14]. The slope ($n = 30 \pm 15$) may be interpreted as a number of the drug molecules combined by one dendrimer

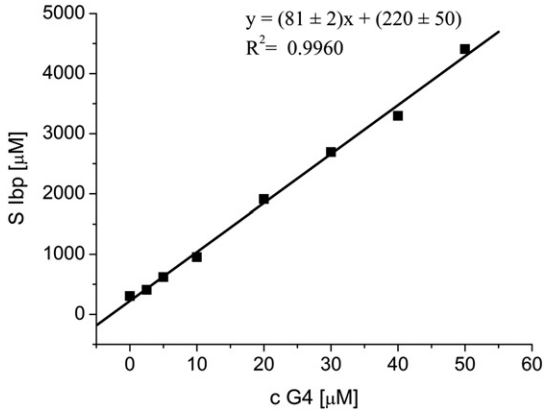

Fig. 1. Dependence of ibuprofen solubility on the concentration of PAMAM-NH_2 G4 dendrimer solution.