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Comparative study of molecular recognition of folic acid subunits with cyclodextrins

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ABSTRACT

The complexation of pteroic acid and pterine, subunits of folic acid, with native cyclodextrins (α–, β–, and γ–CDs) was studied in solution (UV–vis), and in the solid state (thermal analysis, IR and Raman). UV–vis titrations at pH = 7.4 provided data regarding stoichiometry of the formed complexes as well as their associations constants. Stability of the complexes increases in the series: γ–CD < β–CD < < α–CD for pterine, and γ–CD < α–CD < < β–CD for pteroic acid. The mode of complexation was further exploited by molecular modeling studies (docking studies, PM6) showing additionally changes in conformation of pteroic acid upon complexation. Comparison of the association constants of the complexes of pterine and pteroic acid with native cyclodextrins with data obtained for analogous complexes with folic acid shows that all folic acid complexes are less stable than those formed from its subunits.

1. Introduction

Folic acid (pteroylglutamic acid, FA, Fig. 1a) has a complex molecular structure which generally can be divided into two main subunits: pteroic acid (PTRA) and pterine (PTN). PTRA is known to be ricin toxin chain A (RTA) inhibitor (Bai, Monzingo, & Robertus, 2009; Burnett, Henchal, Schmaljohn, & Bavari, 2005). RTA is one of the most poisonous naturally occurring substances and thus has been a candidate for biological weapon (Burnett et al., 2005). The improvement of PTRA solubility in water would facilitate its use in the field of biodefense. PTRA and PTN are used as anchors in miscellaneous drug delivery systems. PTRA has been also used as a conjugate with nitroheterocyclic phosphoramidates alkylating agents for targeting folate receptor (FR) overexpressing cancer cells (Steinberg & Borch, 2001). FR targeting is also realized with PTRA-^{111}Indium-diethylenetriaminepentaacetic acid conjugate (Ke, Mathias, & Green, 2005). PTRA is applied in drug delivery system for idarubicin, synthetic analogue of daunorubicin (Jain et al., 2011), as conjugate for histone deacetylase (HDAC) inhibition (Sodji, Kornacki, McDonald, Mrksich, & Oyelere, 2015), and for the development of ligands for cancer imaging (Nakhaei et al., 2017). PTN is bound by dihydropteroate synthase (DHPS), which plays an important role in bacterial folate synthesis and is the target for the sulfonamide antibiotics. In contrary to compounds binding to *p*-amino benzoic acid (*p*ABA) binding site of DHPS, those binding to PTN binding site, are supposed to overcome sulfonamide resistance (Hevener et al., 2010; Yun et al., 2012). Therefore, several PTN–sulfonamide conjugates (Zhao et al., 2016), as well as PTN-based fluorescent probes for DHPS screening (Zhao et al., 2011) were developed and tested.

Unfortunately, the formation of efficient drug delivery systems based on PTRA and PTN is limited due to their low solubility in water [0.175 mg/ml (10^{-3} m/dm^3) and 0.253 mg/ml ($8*10^{-4}$ m/dm^3), respectively]. Keeping in mind that PTRA and PTN are subunits of FA and inspired by our recent studies involving complexation of folic acid (FA, Fig. 1a) with native cyclodextrins (Ceborska et al., 2012; Ceborska, Zimnicka, Wszelaka-Rylik, & Troć, 2016; Zimnicka et al., 2014), knowing that such treatment leads to increase in the solubility of the studied compound, we decided to perform analogues complexation with PTRA and PTN. Moreover, comparison of the data obtained for PTRA, PTN and FA would improve our knowledge of complex-type compounds of folate-like moieties, which is especially significant due to the FA importance. FA is involved in several important biochemical processes in human body, such as synthesis of purines and pyrimidines, and replication and methylation of DNA (Weinstein et al., 2003). It is employed in numerous drug delivery systems (DDS) for anticancer therapies (Ceborska, 2017; Sudimack & Lee, 2000) as it is taken up by folate receptor-positive cancer cells. Native cyclodextrins (CDs, Fig. 1b) are cyclic oligosaccharides built from six (α−CD), seven (β−CD) or eight (γ−CD) glucopyranose units, widely known to form inclusion-type host-guest complexes with various bio-relevant molecules both in solution (Brewster, 2007; Zhang & Ma, 2013) and in the solid state (Ceborska, 2014). The complexation with CDs improves the bioavailability, solubility in water and stability of the guest molecule (Loftsson

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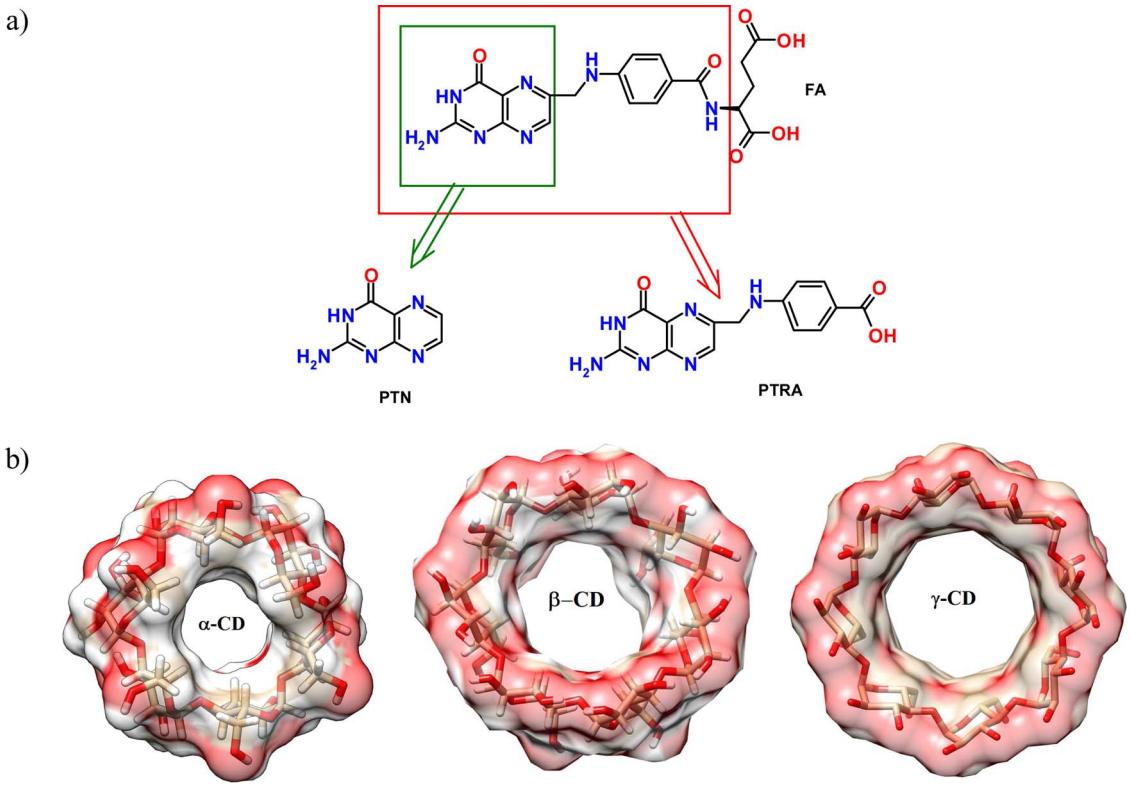

Fig. 1. Molecular structures of a) guests: folic acid (FA), pterine (PTN), pteroic acid (PTRA), and b) hosts:α−, β− and γ−CD molecules.

& Brewster, 2010).

The complexes formation was studied in solution by ultraviolet-visible spectroscopy (UV–vis), and in the solid state by differential scanning calorimetry (DSC), thermogravimetry (TG), infrared (IR) and Raman spectroscopy techniques. Association constants of all of the formed complexes were determined in phosphate buffered saline (PBS, pH = 7.4) upon UV titration experiments. Experimental data was supported by molecular modeling studies, including initial docking studies followed by their optimization at the PM6 semiempirical quantum level of theory. The obtained results were subsequently compared to the data gathered previously for FA. The purpose of this work is to study in detail complexation process of PTRA and PTN with native CDs. Formation of the host-guest associates leads to the increased solubility in water that may influence further application of these molecules as platforms in miscellaneous drug delivery systems. Moreover, this process would enable getting insight into the complexation of FA with native cyclodextrins through studying the inclusion process of FA subunits: PTRA and PTN. The data about stoichiometry of the obtained complexes as well as their stability and 3-D architecture should facilitate understanding of the factors governing host/guest interactions in this kind of associates. Moreover, the comparison of the data obtained for PTRA and PTN with data obtained for FA would additionally improve that knowledge.

2. Materials and methods

2.1. Materials

PTN was obtained from Sigma Aldrich, PTRA from Aurum Pharmatech, α−, β−, and γ−CDs were obtained from Cyclolab (Hungary) and were used without further purification.

2.2. Preparation of solid-state complexes

The water content in cyclodextrins determined during TG-DSC measurements (12% for α−CD, 12.48% for β−CD, and 9.04% for γ−CD) was taken under consideration during preparation of cyclodextrin solutions.

2.2.1. PTRA/native CDs complexes

To the solution of PTRA in water [3.1 mg (0.01 mmol) per 20 mL H_2O] solution of α−CD in water [10.9 mg (0.01 mmol) per 5 mL H_2O] was added and thus obtained solution was stirred at RT for 1 h. The solvent was evaporated and the resulting solid was dried under vacuum. The procedure was repeated for β− [25.5 mg (0.02 mmol) per 5 mL H_2O] and γ−CDs [28.3 mg (0.02 mmol) per 5 mL H_2O].

2.2.2. PTN/native CDs complexes

To the solution of PTN in water [1.6 mg (0.01 mmol) per 20 mL H_2O] solution of α−CD in water [21.8 mg (0.02 mmol) per 5 mL H_2O] was added and thus obtained solution was stirred at RT for 1 h. The solvent was evaporated and the resulting solid was dried under vacuum. The procedure was repeated for β− [12.75 mg (0.01 mmol) per 5 mL H_2O] and γ−CDs [14.15 mg (0.01 mmol) per 5 mL H_2O].

2.3. Methods

2.3.1. Ultra-visible spectroscopy (UV–vis)

The UV–visible absorption spectra of FA, PTN, PTRA, and their respective complexes with α−CD, β−CD, and γ−CDs were recorded using Evolution 220 UV/vis spectrometer (Thermo Scientific) in the range 250–500 nm. All measurements were performed in phosphate-buffered saline (PBS, pH = 7.4). Molar absorption coefficients of PTRA, PTN, and their respective complexes with α−, β−, and γ−CDs were derived from experimental data with HypSpec program (Gans, Sabatini, & Vacca, 1996, Gans, Sabatini, & Vacca, 1999; Gans, Sabatini, & Vacca, 2000).

2.3.1.1. Evaluation of association constants (K_{as}) of native CDs with PTRA and PTN. UV–vis titrations. Change in absorption of guest (PTRA and