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Antipyrine–gamma cyclodextrin inclusion complex: Molecular modeling, preparation, characterization and cytotoxicity studies

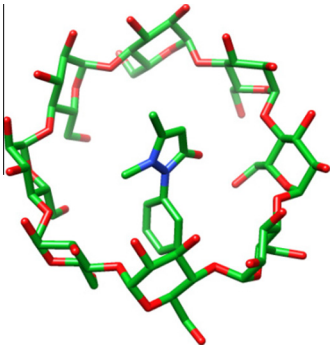
Ramesh Gannimani [a], Amanda Perumal [a], Muthusamy Ramesh [b], Karen Pillay [a], Mahmoud E. Soliman [b], Patrick Govender [a,*]

[a] Department of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, Westville, Durban 4000, South Africa
[b] Discipline of Pharmaceutical Sciences, School of Health Sciences, University of KwaZulu-Natal, Westville, Durban 4000, South Africa

HIGHLIGHTS

- A new inclusion complex for antipyrine with γ-cyclodextrin was prepared by freeze-drying method.
- Molecular modeling approach revealed most favorable conformation of inclusion complex.
- The inclusion complex was characterized by solid state analytical techniques and NMR spectroscopy.
- The complex did not exhibit toxicity up to 500 μM concentration against MDCK-1 cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Molecular docking, semi-empirical and molecular dynamics studies were conducted for α, β and γ-cyclodextrin-associated inclusion complexes of antipyrine. The results of molecular modeling were systematically analyzed to determine the stability of inclusion complexes. In preliminary computational screening, β and γ-cyclodextrin inclusion complexes of antipyrine were found to be more stable as compared to α-cyclodextrin based on docking score and binding free energies. Further, inclusion complex of antipyrine with γ-cyclodextrin was prepared by freeze drying method. Formation of the inclusion complex was investigated by solid state characterization techniques such as thermogravimetric analysis, differential scanning calorimetry, X-ray diffraction, Fourier transform infrared spectroscopy and scanning electron microscopy. The changes observed in decomposition temperature, diffractogram, vibrational frequencies and morphological appearance confirmed the formation of inclusion complex. In addition, results from ^{1}H NMR and 2D NOESY studies supported the inclusion phenomenon. The results obtained from computational studies were found to be in consistent with experimental data to ascertain the encapsulation of antipyrine into γ-cyclodextrin. The inclusion complex was found to be non-toxic toward MDCK-1 cell lines. Thus, this approach may be helpful in the formulation of drug molecules using cyclodextrins.

Introduction

Cyclodextrins (CDs) are truncated, cone-shaped cyclic host materials made up of α-D-glucopyranose units linked by α-1,4 glycosidic bonds [1]. They are commercially available in the form of α,

* Corresponding author. Tel.: +27 312607814; fax: +27 312607942.
E-mail address: govenderpt@ukzn.ac.za (P. Govender).

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β and γ with varying number of glucose units (6–8) and cavity size (5–9 Å) [2]. The α-D-glucopyranose of cyclodextrin exists in stable $^4C_1$ chair conformation, with hydrophobic ether linkages and carbon skeletons of glucose units inside and hydrophilic hydroxyl groups outside the cavity of CD [3]. This structural arrangement makes the CDs amphiphilic in nature. Cyclodextrins have the ability to form supramolecular host–guest assemblies with a variety of organic and inorganic molecules. The driving forces for this phenomena include weak van der Waals forces, hydrogen bonding and charge transfer interactions between the host and guest molecule [4]. The process is also influenced by thermodynamic factors such as enthalpy and entropy changes associated with replacement of water molecules from the CD cavity by hydrophobic molecules [4]. There is a huge interest in the cyclodextrin-based pharmaceutical formulation due to their ability to improve water solubility, stability and bioavailability of a variety of potential drugs [5]. Furthermore, CDs are biodegradable and do not require additional treatment to remove them after application [6]. The cyclodextrin derived materials can also effectively remove the organic and inorganic pollutants present in water [3,7,8]. CDs and their chemical modifications thus provide novel applications in both pharmaceutical and environmental related fields.

Antipyrine (AP; 2,3-dimethyl-1-phenyl-5-pyrazolone) is an inhibitor of cyclooxygenase and is known for anti-inflammatory, analgesic and antipyretic activity in clinical therapy [9]. Antipyrine and their derivatives have also been reported for a variety of pharmacological activities [10,11]. AP is a commonly used non-steroidal anti-inflammatory drug (NSAID) and is also one of the pharmaceutical products that contaminate water sources [12]. The most common side effects of NSAIDs include ulcer perforation and upper gastrointestinal bleeding. These side effects can be minimized by complexion with CDs, as they release the drug in a controlled manner thereby making drugs effective at lower dosages [13].

The long term exposure of AP through water contamination exerts toxic effects causing damage to lungs and mucosa [14]. Recently, chlorination of water, use of activated persulfate and hydroxide radicals were discussed for the degradation of AP or phenazone-like compounds in the water [15–17]. However, the use of cyclodextrin-derived polymers and materials could be an interesting strategy for the treatment of AP contamination as they are harmless to nature. Hence, understanding the host–guest relationship between AP and CDs will help in designing suitable CD-based material for water treatment. The study of AP inclusion complexes is thus significant in view of both pharmaceutical and environmental interests.

The inclusion complex of AP with β-CD in the solid state has previously been studied and it was suggested that the existence of AP in a betainic state which forms a weak inclusion with β-CD in the solution state [18]. Furthermore the formation of inclusion complex was characterized by thermal analysis [18]. However, to the best of our knowledge the role of γ-CD in the formation of inclusion complex with AP has not been described. In this study, we detail the molecular modeling of inclusion complexes of AP with α, β and γ-cyclodextrins, using semi-empirical AM1 calculation methods. In addition, the synthesis, characterization and cytotoxicity of AP/γ-CD inclusion complex has been discussed in detail.

Materials and methods

Computational studies

Preparation of 3D structures of α, β and γ-cyclodextrins and antipyrine

The crystal structures of α-CD (PDB code: 2ZYM) [19], β-CD (PDB code: 3CGT) [20] and γ-CD (PDB code: 2ZYK) [21] were extracted from protein data bank (PDB). The structure of AP was obtained from Pubchem (CID: 2206). The missing hydrogen atoms to CDs and atomic charges to CDs as well as AP were added using CHIMERA software package [22]. These structures were used as a starting point to perform the computational studies.

Molecular docking

The complexes of AP with α, β and γ-CD were generated from molecular docking approaches. Molecular docking was carried out using AutoDock software which employs Lamarckian genetic algorithm [23]. The Lamarckian aspect is an added feature that allows individual conformations to search their local conformations. AutoDock uses a grid based method to allow rapid evaluation of the binding free energy. The solution of AutoDock is based on the energy scores of final docking energy (FDE) and the estimated final energy of binding (EFEB). The energies comprise van der Waals, electrostatic interactions, the loss of entropy and the number of hydrogen bonds. To perform molecular docking, CDs were defined as a receptor and AP was defined as a ligand. The binding site was located using GRID and the GRID was defined from the centroid of the cavity of CDs. The top ranked complex of AP with α-CD, β-CD and γ-CD were separately stored for AM1 and AMBER studies.

Semi-empirical calculation

The complexes of AP with three different CDs were optimized by AM1 method using Gaussian03 software packages [24]. Analytical frequencies were also computed at the same level to characterize the optimized structures as minima or transition states (one negative frequency) on the potential energy surface. Partial atomic charges were estimated by performing Mulliken population analysis.

Molecular dynamics simulations

Molecular dynamics (MD) simulations were carried out using AMBER 12 software packages running under GPU version of PMED engine [25]. The atom types were modeled using ANTECHAMBER module of AMBER software packages [26]. The FF99SB force field was used to describe the solvent system [27]. The hydrogen atoms were added to the α-CD, β-CD and γ-CD using LEAP module incorporated in AMBER. The system was neutralized by the addition counter ions either (Na^+ or Cl^-). The system was embedded within an orthorhombic box (8 Å) composed of TIP3P water molecules [28]. Periodic boundary conditions were maintained. The long range electrostatic interactions were treated based on particle mesh Ewald method [29]. A restraint potential of 500 kcal/mol A^2 was applied to the solute. Initial energy minimization was performed for 2500 steps using steepest descent algorithm. Further, 2500 steps of unrestrained conjugate gradient minimization were carried out. Canonical ensemble (NVT) MD simulations were then carried out for 50 ps, with gradual heating from 0 to 300 K with harmonic restraints of 5 kcal/mol A^2 was applied to all solute atoms and a Langevin thermostat with a random collision frequency of 1/ps. The systems were subsequently equilibrated at 300 K in the NPT ensemble for 500 ps, during which no restraints were imposed and a Berendsen barostat was used to maintain the pressure at 1 bar. The bonds of all hydrogen atoms were constrained using SHAKE algorithm [30]. For MD runs, SPFP precision model was used [31]. Production MD runs were performed for 10 ns in an isothermal isobaric (NPT) ensemble using a Berendsen barostat with a target pressure of 1 bar. The trajectories were saved and analyzed in every 1 ps using CPPTRAJ module integrated with amber 12.