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Protein adsorption onto polysaccharides: Comparison of chitosan and chitin polymers

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ABSTRACT

Chitosan (CHS) and chitin (CHT) biopolymers have found many applications in the field of controlled-release drug delivery systems. Herein, molecular dynamics (MD) simulation and binding free energy calculations were used to investigate the potentials of CHS and CHT polymers for the controlled release of follicle-stimulating hormone (FSH). The results indicated that FSH conformation did not change in the presence of CHS and CHT. In addition, FSH-polymer interactions caused stability of the 3_{10}-helix structure of the alpha subunits of FSH (FSHα). Both the biopolymers interacted with the protein mainly through the hydrophobic forces. CHS has more affinity for FSH when compared with CHT. Furthermore, in both systems, the affinity of polymers for FSHα was more than that for beta subunits of FSH (FSHβ). The results suggested that the polysaccharides might improve the controlled-release FSH delivery.

1. Introduction

In recent years, significant attention has been directed towards the study on interaction between biodegradable carriers based on natural polysaccharides and therapeutic proteins (Amidi, Mastrobattista, Jiskoot, & Hennink, 2010; Costa, Silva, Sarmento, & Pintado, 2017; Gaber et al., 2017; Gan & Wang, 2007; Song et al., 2017). These studies have indicated the crucial role of increased research on development of novel carriers and provided fundamental information on biological aspects of protein-carrier formulation. Among the potential natural polysaccharides that are used in drug delivery systems, chitin (CHT) and its derivative, chitosan (CHS), have attracted great attention due to their biocompatibility, biodegradability, non-immunogenicity and non-toxicity (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Yang et al., 2014). Several studies have shown the potential applications of CHS and CHT-based carriers for the delivery of therapeutic peptides and proteins (Kim et al., 2017; Kondiah et al., 2017; Lancina, Shankar, & Yang, 2017; Li et al., 2017; Marciello, Rossi, Caramella, & Remuñán-López, 2017; Omer et al., 2016; Song et al., 2017; Yuan, Jacquier, & O'Riordan, 2017; Zhang, Pan, Dong, & Li, 2017). Therefore, understanding the interactions between CHS and CHT polysaccharides and proteins at a molecular level is fundamental to generate the polysaccharide-based FSH delivery systems with high bioactivity.

Molecular dynamics (MD) simulation has emerged as a powerful tool for understanding the inter-molecule interactions at the atomic level. The technique can be used to monitor the behaviour and properties of proteins in the process of interaction with polymers (Borhani & Shaw, 2012; De Vivo, Masetti, Bottegoni, & Cavalli, 2016; Thewalt & Tieleman, 2016). Therefore, by using MD simulation, the authors conducted a primary screening from large biopolymer libraries in order to select and design of suitable polymeric nanocarriers for peptide and protein delivery (Durrant & McCammon, 2011; Ramezanpour et al., 2016). Gokaraa et al. applied MD simulations in the study on interactions between CHS oligomers and human serum albumin (HSA). Their results indicated that the binding of CHS oligomers has no significant effects on the secondary structure of HSA. They predicted the CHS binding site in the structure of HSA. Interestingly, the results were similar to the experimental data (Gokara, Kimavath, Podile, & Subramanyam, 2015). In another study, Salar et al. showed that trypsin was stable in the presence of CHS nanoparticles. They indicated that the nonpolar interactions were considered as the most important forces for the formation of stable nanoparticle-trypsin complex (Salar, Mehrnejad, Sajedi, & Arough, 2017). Alaa El-Din et al. revealed the potential utilization of the αB-crystalline domain/CHS complex as a therapeutic agent for crystallinopathy (Gawad & Ibrahim, 2013). In our previous study, MD simulation was used to assess the interaction between Follicle-stimulating hormone (FSH) and the cholesterol modified CHS. The results demonstrated that the flexibility of FSH was reduced in the presence of cholesterol modified CHS. In addition, hydrophobic interactions were the main driving force in the process of FSH-cholesterol

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modified CHS complexation (Yahyaei, Mehrnejad, Naderi-manesh, & Rezayan, 2017).

FSH is a member of the glycoprotein hormone used in superovulation processes (Fan & Hendrickson, 2005). It has an unstable structure with a half-life of approximately 3–4 h in blood (Leão & Esteves, 2014). Therefore, one of the main challenges in superovulation processes is repeated injection of FSH (Janát-Amsbury, Gupta, Kablitz, & Peterson, 2009; Loutradis, Vlismas, & Drakakis, 2010). The development of a biodegradable polymer as controlled-release system of FSH would be a useful alternative to the repeated FSH injections for superovulation in patients. It seems that CHT and CHS biopolymers are good candidates for designing of biodegradable system to provide controlled-release system of FSH.

In our view, understanding the biophysical basis of the interaction between FSH and the CHS or CHT biopolymers provides an insight into the potential benefits of the structural and dynamical properties of CHT and CHS-based FSH controlled release carriers. In this work, MD simulation was conducted to investigate the possible changes in the structure and dynamics of FSH during interaction with CHS and CHT biopolymers. In addition, characterization of the binding patterns and kinetics of the complexation between FSH and the biopolymers is presented herein. This study provides basic data for clarification of the effect of CHS and CHT on FSH morphology and secondary structure.

2. Computational methods

2.1. Preparation of the initial models

All MD simulations were performed using the GROMACS simulation package, versions 5.0.1 (Hess, Kutzner, Van Der Spoel, & Lindahl, 2008; Van Der Spoel et al., 2005) containing the GROMOS 53A6 force field (Ooestenbrik, Soares, Van Der Vegt, & Van Gunsteren, 2005). The model to simulate the CHS and CHT was a 10-mer polysaccharide chain (Fig. S1). The starting structure for the MD simulations of FSH was obtained from the protein databank (PDB code 1XWD; 2.92 Å resolutions) (Fan & Hendrickson, 2005) (Fig. S1). In this study, only the conformation of a single domain of FSH was applied as the starting conformation for the MD simulations. Three systems FSH, FSH/CHS, and FSH/CHT were prepared. In each system, FSH with CHS or CHT was put into a cubic box with a box size of 5.0 nm. All the systems were solvated with simple point charge (SPC) (Berendsen, Grigera, & Straatsma, 1987).

The total charges of the simulation cells were neutralized by the sodium and chloride ions. Each system was firstly energy minimized with the steepest descent algorithms of 50000 steeps. Afterwards, the equilibration phase was conducted in two phases. The first phase was conducted under 1000 ps of NVT ensemble (constant number of particles, volume, and temperature) to stabilize the temperature of the simulation systems at 298 K. In the second phase, equilibration of pressure was conducted under 1000 ps of NPT ensemble (constant number of particles, pressure, and temperature) to stabilize pressure of the simulation system at 1 bar. After completion of the two-equilibration phases, the MD simulations were carried out for 200 ns. The temperature and pressure close to the intended values (300 K and 1 bar) by the V-rescale (Bussi, Donadio, & Parrinello, 2007) and Parrinello–Rahman methods (Parrinello & Rahman, 1981), respectively. Periodic boundary conditions were applied in all dimensions. Lennard-Jones interactions were handled with a cutoff distance with a 0.9/1.4-nm twin-range cut off. The short-range electrostatic interactions were calculated with a distance to1.0 nm. The long-range electrostatic interactions were computed with Particle Mesh Ewald (PME) algorithm (Darden, York, & Pedersen, 1993). All Bond length has been constrained through the LINCS (Linear Constraint Solver) algorithm (Hess, Bekker, Berendsen, & Fraaije, 1997). Further details of the MD simulation can be found in the supplementary data.

2.2. Binding free energy calculations

To study the binding free energy FSH to CHT and CHS, the molecular mechanics Poisson Boltzmann surface area (MM/PBSA) analysis was performed using g_mmpbsa tool of GROMACS (Baker, Sept, Joseph, Holst, & McCammon, 2001; Kumari, Kumar, Consortium, & Lynn, 2014). The MM-PBSA method for calculation of binding energy has been calculated using Eq. (1):

$$\Delta G_{binding} = G_{complex} - (G_{FSH} + G_{biopolymer}) \quad (1)$$

where $G_{complex}$, G_{FSH} and $G_{nanoparticle}$ are the free energies of complex, FSH and biopolymer in solvent, respectively. The free energies were estimated by:

$$G_x = E_{MM} + G_{solvation} \quad (2)$$

$$E_{MM} = E_{bonded} + E_{non-bonded} = E_{bonded} + (E_{vdw} + E_{elec}) \quad (3)$$

where G_x is the complex, FSH, or biopolymer. E_{MM} is the average molecular mechanics potential energy in vacuum and expressed as the sum of the internal interaction (bonded), the electrostatic (ele) and van der Waals (vdW) interaction energies. ΔE_{bonded} is always taken as zero.

The solvation free energy ($G_{solvation}$) was estimated as the sum of electrostatic solvation free energy (G_{polar}) and apolar solvation free energy$G_{non-polar}$:

$$G_{solvation} = G_{polar} + G_{non-polar} \quad (4)$$

where G_{polar} was calculated with the Poisson-Boltzmann (PB) equation and $G_{non-polar}$ is estimated from the solvent-accessible surface area (SASA) as equation following:

$$G_{non-polar} = \gamma SASA + b \quad (5)$$

where the values of empirical constants γ are as follows:

$\gamma = 0.02267$ Kj/Mol/Å^2 or 0.0054 Kcal/Mol/Å^2

b = 3.849 Kj/Mol or 0.916 Kcal/Mol

3. Results and discussion

3.1. Protein structural consequence

FSH is a glycoprotein hormone consisting of two subunit: FSHα (91 amino acids) and FSHβ (111 amino acids) which are arranged together with non-covalent links (Fan & Hendrickson, 2005). The two subunits of the protein are slightly wound around each other. On the other hand, αL2, βL1and βL3 form one end of the FSH structure and αL1, BL2 and αL3 form the other end (Fox, Dias, & Van Roey, 2001). According to reports, the α-subunit is significantly more hydrophobic than the β-subunit (Loureiro, de Oliveira, Torjesen, Bartolini, & Ribela, 2006). In this study, all analyses were done separately for each subunit.

Firstly, the root mean square deviation (RMSD) analysis was applied to investigate the structural features of FSH interacting with CHT/CHS. Herein, the RMSD results over the last 50 ns of simulation time were focused on. The averages of RMSD for FSHα were 0.31 ± 0.03, 0.23 ± 0.01 and 0.19 ± 0.02 nm in water, the CHT and CHS systems, respectively (Fig. 1).

The finding suggest that the conformation of FSHα in the CHT and CHS systems is more constrained than in pure water, at least on the time scale sampled in this study. The averages of RMSD values for FSHβ in the CHT and CHS systems were 0.28 ± 0.02 and 0.29 ± 0.01 nm, respectively, indicating the minimum deviation of water system (0.22 ± 0.03 nm) (Fig. 1). This result shows that FSHβ retained its native conformation after adsorption onto CHT and CHS polymers.

To identify the effective residues included in maintenance of the FSH structure, the root mean square fluctuation (RMSF) of the Cα atoms of residues was analyzed (Fig. 2). As shown, the RMSF of residues exhibits the same pattern in all the three systems. In addition, the major differences were observed in the loops: αL1, αL2 and αL3 of FSHα and