European Journal of Pharmaceutical Sciences 107 (2017) 126–137

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Follicle-stimulating hormone encapsulation in the cholesterol-modified chitosan nanoparticles via molecular dynamics simulations and binding free energy calculations

Mohammad Yahyaei[a], Faramarz Mehrnejad[a,*], Hossein Naderi-manesh[b,*], Ali Hossein Rezayan[a]

[a] Department of Life Sciences Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran
[b] Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Keywords:
Cholesterol modified chitosan
Follicle-stimulating hormone
Molecular dynamics
Nanogel
Self-assembly

ABSTRACT

Follicle-stimulating hormone (FSH) is widely applied in the modern ovarian stimulation techniques. However, it must be administered daily because of its short half-life. Recently, the cholesterol (CS) modified chitosan (CTS) nanogels have attracted significant interest as promising controlled release protein delivery because of their ability to minimize the aggregation and irreversible denaturation of proteins. Herein, we report a molecular dynamics (MD) simulation investigation on the molecular mechanisms of FSH encapsulation in the CS-CTS nanogels. The MD simulations have been performed using the GROMACS software for up to 200 ns simulation time. Furthermore, the binding free energy has been calculated by the molecular mechanics [MM] with Poisson-Boltzmann [PB] and surface area solvation (MM/PBSA) method by using the g_mmpbsa tool. Our findings suggest that the main driving force of the formation of the CS-CTS nanogels is the hydrophobic interactions between the CS–CS moieties in water. The results have also indicated that the CS-CTS nanogel formation can occur through the hydrogen bonding in addition to the hydrophobic interactions. The obtained data demonstrate that the FSH encapsulation into the CS-CTS nanogels is a gradual process driven by the hydrophobic interactions between the hydrophobic patch of FSH and the hydrophobic nanodomains of the nanogel. Our results also reveal that except in the hydrophobic patch region, the flexibility of FSH was reduced in the presence of the nanogel. This study provides the elucidation of the nanogel–FSH interactions at the molecular level and presents new perspective for the ideal design and applications of the CS-CTS nanogel in protein delivery.

1. Introduction

Protein drugs have a biomedical activity that shows them as potential therapeutics, in particular, antibiotic and anticancer activities (Craik et al., 2013; Frokjaer and Otzen, 2005; Guillard et al., 2015; Pearlman and Wang, 2013; Zakas et al., 2017; Zhan et al., 2015). In addition, protein drugs have low bioavailability, high susceptibility to cleavage by proteases and colloidal instability because of aggregation (Vermonden et al., 2012). Moreover, due to the short half-life and instability of protein drugs in plasma, patients have to use frequent injections to preserve the effective concentration within the therapeutic window (Lewis and Illum, 2010; Nakai et al., 2012). Therefore, sustained-release formulation of peptide and protein drugs is a good way to reduce frequency and painless injections (Hirakura et al., 2010; Kakizawa et al., 2010). Over the past decade, various controlled-release delivery systems, such as liposomes, microspheres, cross-linked hydrogels, and nanoparticles, have been evaluated as methods to increase the peptide and protein delivery (Fujita et al., 2012; Nakai et al., 2012; Seebeck et al., 2006; Vermonden et al., 2012). It has also been stated in previous investigations that delivery systems such as hydrogel nanoparticles (nanogel), consisting of a hydrophobic group modified water-soluble polymers have attracted growing as promising carriers in the protein sustained-release (Hasegawa et al., 2009; Li et al., 2015; Nakai et al., 2012). On the other hand, the nanogels can minimize the aggregation and irreversible denaturation of proteins by trapping them in a hydrated polymer network (Li et al., 2015; Sasaki and Akiyoshi, 2010; Sawada and Akiyoshi, 2010).

Chitosan (CTS), the second most naturally abundant polysaccharide, is a typical cationic linear polysaccharide (Kumar et al., 2004; Yang et al., 2014). CTS can be modified by conjugation with various hydrophobic groups to form the amphiphilic polymer, which are able to self-assemble into the nanogel. In recent years, many efforts have been made to develop some hydrophobically modified chitosan as drug delivery systems (Agnihotri et al., 2004; Bernkop-Schnürch and

* Corresponding authors.
E-mail addresses: mehrnejad@ut.ac.ir (F. Mehrnejad), naderman@modares.ac.ir (H. Naderi-manesh).

http://dx.doi.org/10.1016/j.ejps.2017.07.007
Received 23 December 2016; Received in revised form 2 June 2017; Accepted 7 July 2017
Available online 08 July 2017
0928-0987/

Dünnhaupt, 2012; Chen et al., 2015). Kim et al. also showed that the deoxycholic acid-modified CTS could self-aggregate in aqueous media and formed complex with the plasmid (Kim et al., 2001). Liu et al. studied the loading and activity of trypsin in a nanogel based on the linoleic-acid modified CTS. The nanogel-encapsulated trypsin has shown higher thermal stability compared to the free form (Liu et al., 2005). Yuan et al. investigated the cholesterol hydrophobically modified CTS for drug delivery to ocular surface and observed a sustained release of cyclosporine A, as the model drug, from the CS-CTS nanogel (Yuan et al., 2006). Li et al. indicated that the conformation of BSA was not significantly changed in the interaction with the nanogel based on the cholesterol modified CTS (CS-CTS) (Li et al., 2012). Many studies have been carried out to show that the hydrophobically modified CTS could self-assemble into the nanoparticles in aqueous environment and had highly potential to be applied as sustained release systems for hydrophobic drugs (Guo et al., 2013; Hu et al., 2006; Quiñones et al., 2012).

FSH is a member of the glycoprotein hormone family that is used in the modern ovarian stimulation techniques (Fan and Hendrickson, 2005; Groen et al., 1996). The glycoprotein has an unstable structure and a short half-life of approximately 3 to 4 h in blood (Leao and Esteves, 2014; Practice Committee of American Society for Reproductive Medicine, 2008). Therefore, the clinical protocol in the ovarian stimulation in human and animal commonly relies on daily FSH injection (Bo and Mapletoft, 2014; Taketani et al., 2010; Tribulo et al., 2012). On the other hand, the discomfort, expensive and stressful of daily injections is a burden to patients (Janat-Amsbury et al., 2009; Loutradis et al., 2010). The development of a biodegradable system that will result in sustained, slow release of FSH over several days would be a useful alternative to the repeated injections protocol for the ovarian stimulation in patients. Hence, the design of self-assembled systems based on chitosan has received more attention in recent years because it is a biodegradable and biocompatible molecule. Consequently, the hydrophobically modified CTS systems have beneficial chemical and biological properties that make them suitable molecules for the FSH formulation.

In the last decade, various studies have demonstrated that molecular dynamic simulations help researchers to better study the structural and dynamical changes with atomic-scale resolution that are difficult to observe in experimental works (Brunk and Rothlisberger, 2015; Karplus and McCammon, 2002). On the other hand, MD simulation is a powerful technique, and a great complement to experiments that allows us to avoid costly trial-and-error experiments (Betz et al., 2016; van Gunsteren et al., 2013). For these reasons, MD simulations have been widely applied in drug delivery systems to investigate the drug encapsulation in the molecular details (Carr et al., 2017; Lin et al., 2014; Panczyk et al., 2013). Ahmad et al. applied MD simulations to investigate the interactions between the quaternary ammonium palmitoyl glycol CTS and propofol. The self-assembly process was rapid and the propofol resides were located at the interface between the hydrophobic core and the hydrophilic surface of the micelles (Ahmad et al., 2010). Chiu et al. studied the self-assembly of the N-palmitoyl CTS (NPCS) by the coarse-grained MD simulation method and showed that the intra- and intermolecular hydrophobic interactions were the main driving forces to allow NPCS to form nanoparticles (Chiu et al., 2010). Wang et al. used the salicylic acid grafted CTS oligosaccharide for the paclitaxel delivery. Their study revealed that the salicylic acid block created a hydrophobic core to entrap paclitaxel (Wang et al., 2013). Shan et al. investigated the interactions between doxorubicin (DOX) and ten hydrophobic acid modified CTS oligosaccharides. They indicated that the hydrophobicity and aromaticity of the compounds played significant role in the micelle stability and the DOX loading process (Shan et al., 2014).

Although the interactions between hydrophobically modified CTS protein delivery systems and proteins have been experimentally studied, a few studies have focused on the details of the molecular mechanisms of the protein encapsulation into the chitosan nanogels (Bekale et al., 2015; Gan and Wang, 2007; He et al., 2017; Ramezanpour et al., 2016; Sarkar et al., 2017; Thewalt and Tieleman, 2016). Therefore, MD simulation tool can be used in the protein delivery systems to provide valuable information about the various forces involved in protein-carrier interactions (Angelov et al., 2014; Zhang et al., 2016). Moreover, the present MD research may prove useful for designing a new controlled drug delivery system that can be used to stabilize FSH formulations.

In this study, we have used MD simulation to investigate the self-assembly behavior of the CS-CTS chains and the conformational changes in FSH in the presence of the nanogel. Besides, the focus of our study was to determine the main driving force and detailed molecular information for the complexation between FSH and the CS-CTS nanogel.

2. Computational methods

2.1. Preparation of the initial models

All MD simulations were performed using the GROMACS simulation package (Hess et al., 2008; Van Der Spoel et al., 2005). The Initial structure of CTS (degree of deacetylation 80%, 10 monomers) was taken from Glycam Biomolecule Builder (Kirschner et al., 2008). The CS-CTS structure with the 20% substitution degree of cholesterol was generated using HyperChem v8.0.7. The GROMACS topology of CS-CTS was generated by the Dundee PRODRG 2.5 server (beta) (SchuÈttelkopf and Van Aalten, 2004) and the Automatic Topology Builder server (Canzar et al., 2013; Malde et al., 2011). The initial structure of CS-CTS was geometrically optimized using the polymer consistent force-field (PCFF) (Maple et al., 1988). The smart algorithm (cascade of steepest descent, conjugate gradient, and quasi-Newton methods) with 50,000 steps has been applied for the geometry optimization. The convergence thresholds for the maximum energy and maximum force changes were $4.187 \times 10–4$ kJ/mol and $20.935 \times 10–2$ kJ/mol·nm, respectively. Atomic charges have been determined by PCFF force field (Machackova et al., 2013). All force field parameters for the CS-CTS chain were defined based on the GROMOS53a6 parameter set (Oostenbrink et al., 2005). The starting structure for the MD simulations of FSH was obtained from the protein databank (PDB code 1XWD; 2.92 Å resolutions) (Fan and Hendrickson, 2005). The force field parameters for FSH were obtained from the GROMOS 53a6 GLYC force field (Pol-Fachin et al., 2012; Pol-Fachin et al., 2014). FSH and the CS-CTS chain were put into a cubic box with a box size of 5.0 nm. Two modeled systems were solvated with simple point charge (SPC) water model (Berendsen et al., 1987). For a neutral simulation cells, water molecules were replaced with sodium and chloride ions. To release conflicting contacts, each system was energy minimized with the steepest descent algorithms of 50,000 steeps. Afterwards, the equilibration phase was conducted in two phases. The first phase was conducted under 100 ps of NVT ensemble (constant number of particles, volume, and temperature). In the second phase, the equilibration of pressure was conducted under 1000 ps of NPT ensemble (constant number of particles, pressure, and temperature). After completion of the two-equilibration phases, the MD simulation was carried out for 200 ns. Temperature and pressure were controlled at 300 K and 1 bar by the V-rescale (Bussi et al., 2007) and Parrinello–Rahman methods (Parrinello, 1981), respectively. Periodic boundary conditions were applied in all dimensions. The Lennard-Jones interactions were considered with a 0.9/1.4-nm twin-range cut off. The short-range electrostatic interactions were computed to1.0 nm. The long-range interactions were calculated with the Particle Mesh Ewald (PME) algorithm (Darden et al., 1993) [49]. Bond length was constrained through the LINCS (Linear Constraint Solver) algorithm (Hess et al., 1997).