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Encapsulation of testosterone by chitosan nanoparticles

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ABSTRACT

The loading of testosterone by chitosan nanoparticles was investigated, using multiple spectroscopic methods, thermodynamic analysis, TEM images and modeling. Thermodynamic parameters showed testosterone-chitosan bindings occur mainly *via* H-bonding and van der Waals contacts. As polymer size increased more stable steroid-chitosan conjugates formed and hydrophobic contact was also observed. The loading efficacy of testosterone-nanocarrier was 40–55% and increased as chitosan size increased. Testosterone encapsulation markedly alters chitosan morphology. Chitosan nanoparticles are capable of transporting testosterone *in vitro*.

1. Introduction

Polymers are extensively used for the delivery of active pharmaceutical drugs. They can form a matrix or membrane that can control the release of a drug over a prolonged period of time. Among the potential natural cationic polymers used in drug delivery, chitosan has attracted major interest due to its unique chemical properties [1–5]. Chitosan is a nontoxic, biodegradable and biocompatible polysaccharide of b(1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine [6]. Chitosan and its derivatives have the desired properties for safe use as a pharmaceutical drug delivery tool. This has accelerated research activities worldwide on chitosan micro and nanoparticles as drug delivery vehicles. Chitosan nanoparticles were used for delivery of therapeutic proteins, peptides and small drug molecules [7–9].

Testosterone is the main androgenic hormone which controls many physiological processes such as, sexual functions and secondary sex characteristics, muscle protein metabolism, plasma lipid and bone metabolism [10]. Since a substantial part of steroids is bound to serum proteins *in vivo,* the potential application of serum proteins in steroid delivery has been reviewed [11]. Synthetic polymers are used as potential nanocarriers to deliver steroids *in vitro* and *in vivo* [12,13]. Chitosan and its derivatives were also tested as delivery tools for transporting steroids [14,15]. Therefore, it was of interest to examine the potential application of chitosan nanoparticles for testosterone delivery *in vitro* using spectroscopic, thermodynamic and microscopic analysis.

The loading of testosterone by chitosan-15 and chitosan–100 kDa was determined using multiple spectroscopic analysis, thermodynamic parameters, TEM imaging and molecular modeling. Structural information regarding testosterone-chitosan interactions and the effects of polymer size and hydrophobicity on the steroid loading are presented here.

2. Experimental section

2.1. Materials

Purified chitosans 15 and 100 kDa (90% deacetylation) were from Polysciences Inc. (Warrington, USA). Testosterone or 17β-hydroxy-4-androsten-3-one was from Steraloids Inc. and used as supplied. Other chemicals were of reagent grades and purified before sample preparation.

2.2. Preparation of stock solutions and testosterone-chitosan-conjugates

Solutions of testosterone (in ethanol/H_2O 50/50%) 120 μM was prepared and diluted to various concentrations in 10 mM Tris-HCl (pH 7.4). Chitosan was dissolved in acidic solution (0.1 M HCl) (pH 5–6) and diluted to various concentrations using 10 mM Tris-HCl. Testosterone-chitosan conjugates were prepared by the addition

Abbreviations: ch, chitosan; test, testosterone; TEM, transmission electron microscopy; FTIR, Fourier transform infrared.

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of different chitosan concentrations (1–60 μM) to a testosterone solution (60 μM). The characterization of each steroid-polymer conjugate by multiple spectroscopic methods and TEM imagings is described below.

2.3. Transmission electron microscopy

The TEM images were recorded using a Philips EM 208S microscope operating at 180 kV. The morphology of the conjugates of testosterone with chitosan-15 and 100 kDa in aqueous solution at pH 7.4 were monitored using transmission electron microscopy. One drop (5–10 μL) of the freshly-prepared mixture [chitosan solution (60 μM) + testosterone solution (60 μM)] in Tris–HCl buffer (24 ± 1 °C) was deposited onto a glow-discharged carbon-coated electron microscopy grid. The excess liquid was absorbed by a piece of filter paper and a drop of 2% uranyl acetate negative stain was added before drying at room temperature.

2.4. UV spectroscopy

The UV–vis spectra were recorded on a Perkin-Elmer Lambda spectrophotometer with a slit of 2 nm and scan speed of 400 nm min^{-1}. Quartz cuvettes of 1 cm were used. The absorbance measurements were performed at pH 7.4 by keeping the concentration of testosterone constant (60 μM), while altering chitosan concentrations (1 μM–60 μM).

The binding constants of trypsin-protein adducts were obtained according to the method described by Connors [16,17].

2.5. FTIR spectroscopy

Infrared spectra were recorded on a FTIR spectrometer (Impact 420 model), equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter, using AgBr windows. Solution of steroid was added dropwise to the chitosan solution with constant stirring to ensure the formation of homogeneous solution and to have testosterone contents 15, 30 and 60 μM with a final chitosan concentration of 60 μM. Spectra were collected after 2 h incubation of polymer and steroid solutions at room temperature, using hydrated films. Interferograms were accumulated over the spectral range 4000–600 cm^{-1} with a nominal resolution of 2 cm^{-1} and 150 scans. The difference spectra [(chitosan solution + testosterone solution) – (chitosan solution)] were generated using chitosan band around 900 cm^{-1}, as standard [18].

2.6. Docking

The docking studies were carried out with ArgusLab 4.0.1 software (Mark A. Thompson, Planaria Software LLC, Seattle, WA, http://www.arguslab.com). The chitosan structure was obtained from the literature [19] and the testosterone three-dimensional structure was generated from PM3 semi-empirical calculations using Chem3D Ultra 11.0. The docking runs were performed on the ArgusDock docking engine using regular precision with a maximum of 150 candidate poses. The conformations were ranked using the Ascore scoring function, which estimates the free binding energy. Upon location of the potential binding sites, the docked complex conformations were optimized using a steepest decent algorithm until convergence, with a maximum of 20 iterations. Chitosan donor groups within a distance of 3.5 Å relative to the testosterone were involved in complex formation.

3. Results and discussion

3.1. TEM analysis of testosterone-chitosan conjugates

We determined the morphological dynamics of chitosan nanoparticles upon steroid conjugation. The effect of steroid-chitosan interactions on the shape of chitosan nanoparticle was determined by using transmission electron microscopy. The shapes of uncomplexed Ch-15 and Ch-100 kDa alongside with their testosterone conjugates are shown in the TEM micrographs (Fig. 1). TEM micrographs show that uncomplexed chitosan had a markedly different shape depending on its size; Ch-15 has spherical-shaped, while Ch-100 is needle-shaped with smooth surface and narrow size distribution of about 90 nm [20–22]. Similar differences were observed for AFM images of Ch-15 and Ch-100 kDa where the result is attributed to the degree of polymer aggregation, as chitosan size increases [23]. However, marked differences were observed in the morphology of the testosterone–chitosan aggregates. TEM

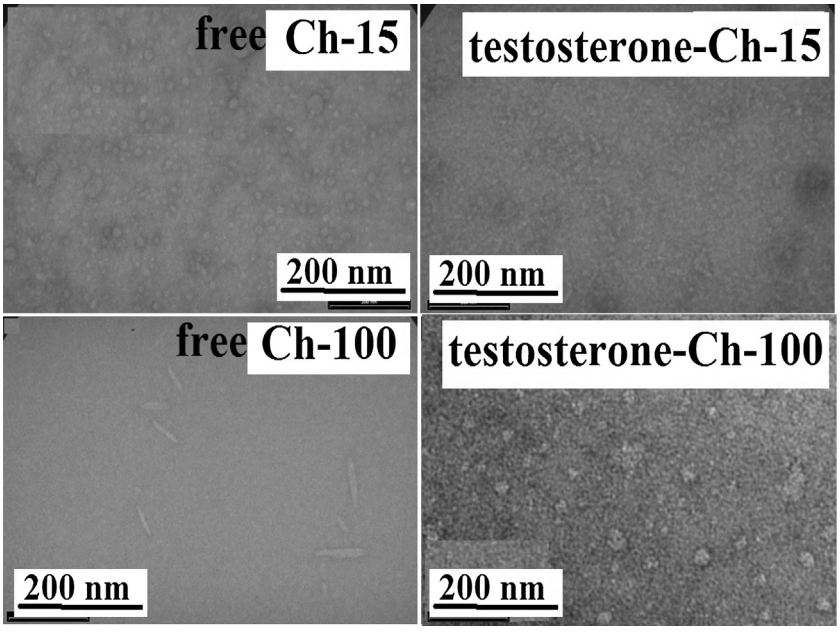

Fig. 1. TEM micrographs free chitosans Ch-15 and Ch-100 kDa and their testosterone conjugates.