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Chitosan nanoparticles conjugate with trypsin and trypsin inhibitor

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ABSTRACT

Chitosan-protein conjugates are widely used in therapeutic drug delivery. We report the bindings of chitosan nanoparticles with trypsin (try) and trypsin inhibitor (tryi), using thermodynamic analysis and multiple spectroscopic methods. Thermodynamic parameters ΔS, ΔH and ΔG showed chitosan-protein bindings occur mainly *via* H-bonding and van der Waals contacts with trypsin inhibitor forming more stable conjugate than trypsin. As chitosan size increased more stable polymer-protein conjugate was formed. Chitosan complexation induces more perturbations of trypsin inhibitor structure than trypsin with reduction of protein alpha-helix and major increase of random structure. The negative value of ΔG indicates spontaneous protein-chitosan complexation at room temperature. Chitosan nanoparticles can be used to transport trypsin and trypsin inhibitor.

1. Introduction

Among the potential natural cationic polymer available for use in drug delivery, chitosan has attracted major interest due to its unique chemical properties (Samal et al., 2012; Shukla, Mishra, Arotiba, & Mamba, 2013). Chitosan and its derivatives have the desired properties for safe use as pharmaceutical drug delivery tools. This has prompted accelerated research activities worldwide on chitosan micro and nanoparticles as drug delivery vehicles. Chitosan is a biocompatible, biodegradable and versatile polymer and forms strong protein conjugates (Shukla et al., 2013; Acheco et al., 2011; Amidi, Mastrobattista, Jiskoot, & Hennink, 2010; Gan & Wang, 2007). Chitosan nanoparticles were used for delivery of therapeutic proteins, peptides and small drug molecules (Acheco et al., 2011; Amidi et al., 2010). The effect of hydrophobicity on polymer-protein interactions is well investigated (Bekale, Agudelo, & Tajmir-Riahi, 2015a; Bekale, Agudelo, & Tajmir-Riahi, 2015b; Long et al., 2015; Zhang, Yang, & Guo, 2011; Boerisa, Micheletto, Lionzo, Pesce da Silveira, & Picó, 2011; Kasimova, Velázquez-Campoy, & Nielsen, 2011).

The differences in the hydrophobicity of trypsin and trypsin inhibitor are determined and their effects on the enzymatic activity have been reported (Hedstrom, Lin, & Fast, 1996; Nakajima & Kikuchi, 1996; Chaudhuri, Das, & Sinha, 1993; Fodor et al., 2005). Trypsin a water soluble globular protein is a proteolytic enzyme that cleaves peptide bonds at the carboxylic groups of arginine and lysine (Malmsten & Larsson, 2000). The enzymatic activity and degradation of trypsin in the presence of polymer and peptides are well studied (Hahn Berg, Muller, Arnebrant, & Malmsten, 2001; Mansson, Frenning, & Malmsten, 2013; Huang, Kwok, & Liang, 2008). Trypsin inhibitors are classified as small proteins or polypeptides that exhibit inhibitory activity against trypsin and can lead to certain diseases in animals and humans (Nishida et al., 2000). The inhibitory role of trypsin inhibitors comes from their bindings to trypsin and other proteins, causing major protein structural changes (Jasti et al., 2014). Trypsin inhibitor with a large hydrophobic region show different affinity than trypsin towards ligand interactions. Recent study has shown the effect of synthetic polymer and protein interactions on the stability and secondary structures of trypsin and trypsin inhibitor (Chanphai & Tajmir-Riahi 2016; Chanphai, Bekale, & Tajmir-Riahi, 2015).

We report the thermodynamic and spectroscopic analysis for trypsin and trypsin inhibitor conjugation with chitosan nanoparticles in aqueous solution at physiological conditions. Structural information regarding protein-polymer interactions and the effect of protein hydrophobicity on the protein-chitosan conjugation are presented here.

Abbreviations: Ch, chitosan; Try, trypsin; Tryi, trypsin inhibitor; FTIR, fourier transform infrared.

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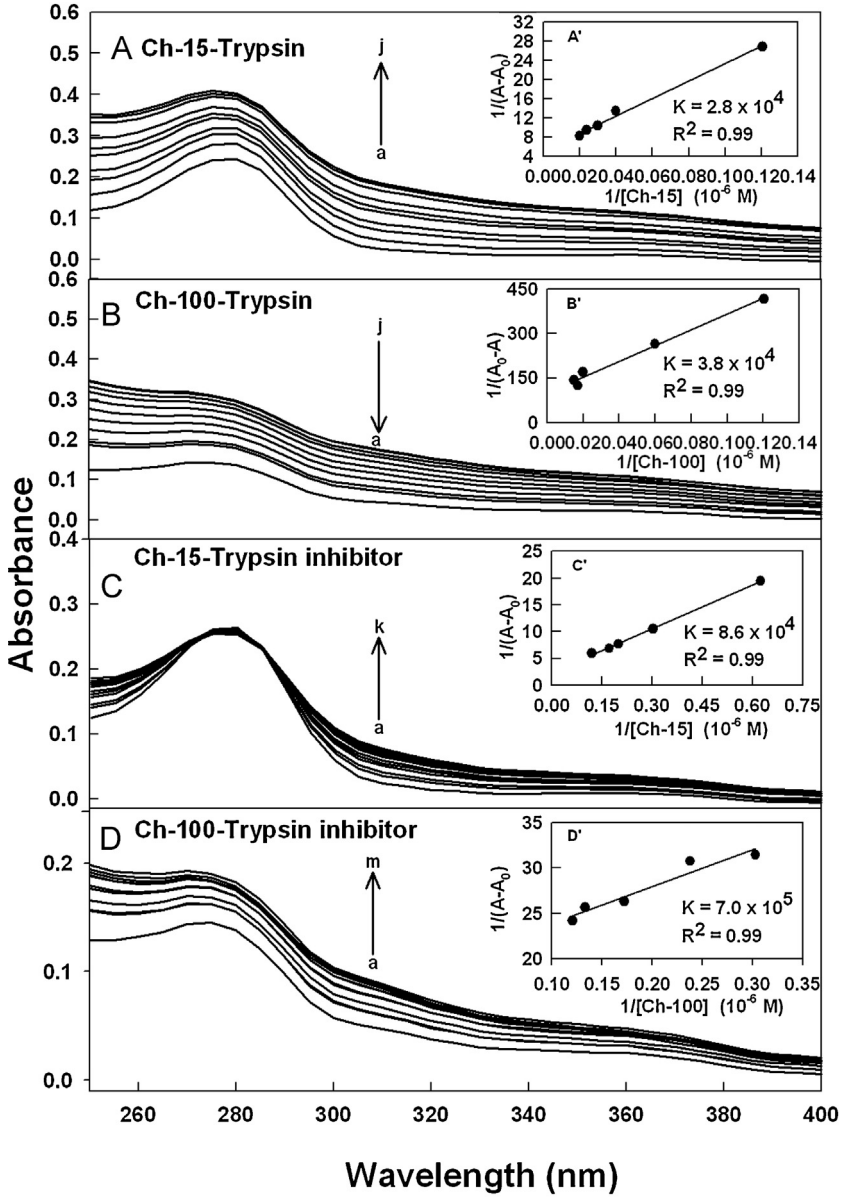

Fig. 1. UV–vis spectra of trypsin its chitosan complexes (A,B) and trypsin inhibitor (C,D) with free protein 60 μM and their complexes with chitosan-15 and chitosan–100 kDa at 1, 5, 10, 20 and 30, 40, 50, 60 and 70 μM (b–j) and chitosan-15 and chitosan-100k Da at 1, 5, 10, 20 and 30, 40, 50, 60, 70 and 80 μM (b–k **chitosan-15**) and 1, 5, 10, 20 and 30, 40, 50, 60, 70, 80. 90 and 100 μM (b–m **chitosan 100k**). Inset: plot of $1/(A - A_0)$ vs (1/chitosan concentration) and binding constant (K) for protein-polymer complexes.

Table 1
Variations of the binding constants for trypsin and trypsin inhibitor conjugation with Chitosan-15 and Chitosan–100 kDa, at different temperatures.

Complexes	Temperature (K)	Apparent constants, K_{app}
Ch-15-Trypsin	298.15	2.84×10^4
	308.15	4.08×10^3
	318.15	2.19×10^3
Ch-100-Trypsin	298.15	3.77×10^4
	308.15	2.64×10^4
	318.15	2.02×10^4
Ch-15-Trypsin inhibitor	298.15	8.59×10^4
	308.15	5.26×10^4
	318.15	1.82×10^4
Ch-100-Trypsin inhibitor	298.15	7.00×10^5
	308.15	2.23×10^5
	318.15	4.61×10^4

2. Experimental section

2.1. Materials

Trypsin from bovine pancreas (MW = 23.8 kDa) and trypsin inhibitor type-1S (MW = 20.1 kDa) from glycine, max soyabean were purchased from Sigma Chemical Company (St.-Louis, MO) and used as supplied. Purified chitosans 15 and 100 (90% deacetylation) were from Polysciences Inc. (Warrington, USA). Other chemicals were of reagent grades.

2.2. Preparation of stock solutions

Solutions of trypsin (in H_2O) and trypsin inhibitor (in ethanol/H_2O 25/75%) 120 μM were prepared and diluted to various concentrations in 10 mM Tris–HCl (pH 7.4). Chitosan was dissolved