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tRNA conjugation with chitosan nanoparticles: An AFM imaging study

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ABSTRACT

The conjugation of tRNA with chitosan nanoparticles of different sizes 15,100 and 200 kDa was investigated in aqueous solution using multiple spectroscopic methods and atomic force microscopy (AFM). Structural analysis showed that chitosan binds tRNA *via* G-C and A-U base pairs as well as backbone PO_2 group, through electrostatic, hydrophilic and H-bonding contacts with overall binding constants of $K_{Ch\text{-}15\text{-}tRNA} = 4.1\ (\pm 0.60) \times 10^3\ M^{-1}$, $K_{Ch\text{-}100\text{-}tRNA} = 5.7\ (\pm 0.8) \times 10^3\ M^{-1}$ and $K_{Ch\text{-}200\text{-}tRNA} = 1.2\ (\pm 0.3) \times 10^4\ M^{-1}$. As chitosan size increases more stable polymer-tRNA conjugate is formed. AFM images showed major tRNA aggregation and particle formation occurred as chitosan concentration increased. Even though chitosan induced major biopolymer structural changes, tRNA remains in A-family structure.

1. Introduction

Chitosan is a biodegradable and biocompatible cationic polysaccharide prepared by alkaline deacetylation of chitin [1–5]. The protective effect of chitosan on DNA packing and transfection is well known [6–9]. Chitosan has shown great potential for gene and drug delivery *in vitro* [10–14]. Chitosan forms conjugations with DNA and RNA *via* ionic interactions and causes DNA compaction and particle formation [15–18]. As a biocompatible and biodegradable cationic polymer, chitosan has major application in drug delivery systems [19–21]. Even though the conjugation of chitosan with DNA is well investigated [14–16], little is known about the effect of chitosan on RNA structure and function. Therefore, it was of interest to study chitosan conjugation with tRNA, using multiple spectroscopic methods and microscopic imaging.

The structural analysis of chitosan-DNA conjugates at pH 5.5–6.5, was carried out, using multiple spectroscopic methods and AFM images. The tRNA binding sites and the effects of chitosan conjugation on tRNA aggregation and particle formation is reported here.

2. Materials and methods

2.1. Materials

Purified chitosans 15,100 and 200 KDa (90% deacetylation) were from Polysciences Inc. (Warrington, USA) and used as supplied. tRNA from Baker's yeast was purchased from Sigma Chemical Co., and used as supplied. The absorbance at 260 and 280 nm was used, in order to check the protein content of tRNA solution. The A_{260}/A_{280} ratio was 2.2 showing that the tRNA was sufficiently free from protein [22]. Other chemicals were of reagent grade and used without further purification.

2.2. Preparation of stock solutions

Stock tRNA solution was prepared by dissolving 10 mg of tRNA in 1 ml of 10 mM Tris–HCl buffer (pH 7.2 ± 0.2) at room temperature with occasional stirring to ensure homogenization. Final concentration of the stock tRNA solution was determined spectrophotometrically at 260 nm, using molar extinction coefficient of $\lambda_{260} = 9250\ cm^{-1}\ M^{-1}$ (expressed as molarity of phosphate groups) [23]. The UV absorbance at 260 nm of a diluted solution (1/250) of tRNA used in our experiments was 0.925 (path length = 1 cm), with a final molar concentration of the stock tRNA solution at 25 mM. An appropriate amount of chitosan was dissolved in 2% acetic acid and then the solution was adjusted to pH 5.5–6.5. Chitosan preparation was similar to our previous report [19].

Abbreviations: ch, chitosan; AFM, atomic force microscopy; FTIR, Fourier transform infrared; CD, circular dichroism.

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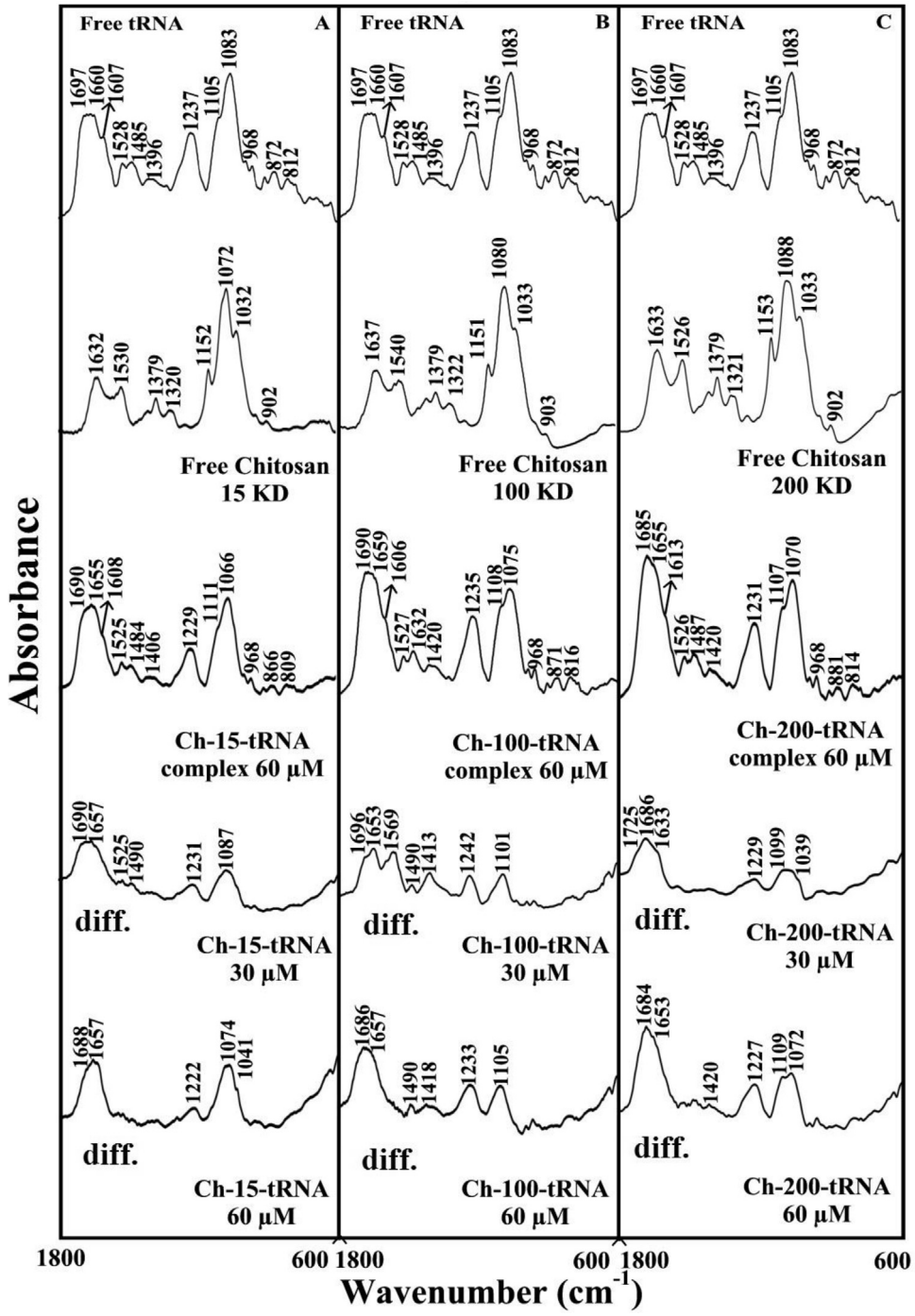

Fig. 1. FTIR spectra and difference spectra [(tRNA solution + chitosan solution) – (DNA solution)] in the region of 1800–600 cm-1 for the free tRNA and its chitosan-15 (A), chitosan-100 (B) and chitosan-200 kDa (C) complexes in aqueous solution at pH 5.5–6.5 with various polymer concentrations and constant tRNA concentration (12.5 mM).

2.3. FTIR spectroscopic measurements

Infrared spectra were recorded on a FTIR spectrometer (Impact 420 model, Digilab), equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter, using AgBr windows. Solution spectra were recorded in hydrated film on AgBr windows with resolution of 2 cm^{-1} and 100 scans. The concentrations of chitosan used in infrared spectroscopic measurements were 30 and 60 µM, with a final tRNA concentration of 12.5 mM at pH 5.5–6.5. The water subtraction was carried out with 0.1 M NaCl and Tris–HCl solution used as a reference at pH 6.5 [24] A good water subtraction was achieved as shown by a flat baseline around 2200 cm^{-1} where the water combination mode is located. This method is a rough estimate, but removes the water content in a satisfactory way. The difference spectra [(tRNA solution + polymer solution) – (tRNA solution)] were obtained, using the sharp tRNA band at 968 cm^{-1} as internal reference. This band, which is due to ribose C—C stretching vibrations, exhibits no spectral changes (shifting or intensity variation) upon chitosan-tRNA complexation, and canceled out upon spectral subtraction. The spectra are smoothed with Savitzky–Golay procedure (24). The relative intensity of several peaks of tRNA in-plane vibrations related to A-U, G-C base pairs and the PO_2^- stretching vibrations such as 1697 (guanine), 1660 (thymine), 1607 (adenine), 1528 (cytosine),