tRNA conjugation with chitosan nanoparticles: An AFM imaging study

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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 PO2 group, through electrostatic, hydrophilic and H-bonding contacts with overall binding constants of $K_{\text{CG}}$-$\text{tRNA} = 4.1 \times 10^5 \text{ M}^{-1}$, $K_{\text{AS}-\text{tRNA}} = 5.7 \times 10^7 \text{ M}^{-1}$ and $K_{\text{CA}-\text{tRNA}} = 1.2 \times 10^4 \text{ M}^{-1}$. 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.

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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 $\lambda_{260} = 9250 \text{ cm}^{-1} \text{ M}^{-1}$ [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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2.3. FTIR spectroscopic measurements

Infrared spectra were recorded on a FTIR spectrometer (Impact 420 model, Digilab), equipped with deuterated triglycerine 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 PO₂⁻ stretching vibrations such as 1697 (guanine), 1660 (thymine), 1607 (adenine), 1528 (cytosine),
Fig. 2. Circular dichroism of the free tRNA and its chitosan conjugates for chitosan-15 (A), chitosan-100 (B) and chitosan-200 kDa (C) in aqueous solution with 1.25 mM DNA concentration and 2.5, 5 and 10 μM chitosan concentrations at pH 5.5–6.5.

1237 cm⁻¹ (PO₂⁻ asymmetric) and 1083 (PO₂⁻ symmetric) versus the polymer concentrations were used for monitoring chitosan binding to tRNA polar groups [25,26].

2.4. CD spectroscopy

CD spectra of tRNA and its chitosan complexes were recorded at pH 5.5–6.5 with a Jasco J-720 spectropolarimeter. For measurements in the Far-UV region (200–320 nm), a quartz cell with a path length of 0.01 cm was used. Three scans were accumulated at a scan speed of 50 nm per minute, with data being collected at every nm from 200 to 320 nm. Sample temperature was maintained at 25 °C using a Neslab RTE-111 circulating water bath connected to the water-jacketed quartz cuvettes. Spectra were corrected for buffer signal and conversion to the Mol CD (Δε) was performed with the Jasco Standard Analysis software. The chitosan concentrations used in our experiment varied from 2.5 to 10 μM with the final tRNA concentration of 1.25 mM.

2.5. UV spectroscopy

The UV–vis spectra were recorded on a PerkinElmer 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 5.5–6.5 by keeping the concentration of tRNA constant (60 μM), while increasing chitosan concentrations (3–80 μM). The binding constants of chitosan-tRNA adducts were obtained according to the published method [16,27].

2.6. Atomic force microscopy

Chitosan-tRNA complexes at a ratio of 1:150 and final chitosan concentration of 10 μM were prepared in acetate buffer pH 6.5. For each sample, a freshly cleaved mica disk was covered with a 10 μl aliquot, rinsed with around three milliliters of ultrapure water and dried with Nitrogen. A Pico-AFM (Molecular Imaging, Phoenix, AZ) was operated in acoustic mode with 300 kHz frequency silicon
cantilevers (RTESP, Bruker, CA). The scanning speed was kept at 1 Hz and all the images were treated using the software Gwyddion (http://gwyddion.net/).

3. Results and discussion

3.1. FTIR spectra of chitosan-tRNA conjugates

The main spectral features of chitosan-tRNA complexes are presented in Fig. 1A–C. Major alterations of tRNA-in-plane vibrations [24–28] were observed, upon polymer-tRNA complexation (Fig. 1A–C). At low and high chitosan concentrations (30–60 µM), major intensity variations and shifting were observed for the guanine band at 1697, uracil at 1660, adenine at 1607, cytosine at 1528 and the PO2 band at 1237 cm−1 (asymmetric stretch) and 1083 cm−1 (symmetric stretch) (Fig. 1). A major shifting was observed for guanine band at 1697 to 1690–1685, uracil band from 1660 to 1659–1655, phosphate band at 1237 to 1233–1229 and 1083 to 1075–1066 cm−1 (Fig. 1A–C complexes 60 µM). The positive features in the difference spectra of chitosan-tRNA conjugates were also observed in the region 1700–1500 cm−1 for 1700–1685 (G), 1662–1640 (U) and 1240–1220 (PO2) and 1080–1070 cm−1 (PO2) (Fig. 1A–C, diffs 30 and 60 µM). These spectral changes are due to shifting and increase in intensity of tRNA vibrations as results of chitosan interactions with G-C and A-U base pairs and the backbone phosphate group. The overall spectral shifting observed for the tRNA vibrations is attributed to the hydrophilic binding of chitosan to guanine and adenine N7, uracil O2 and backbone PO2 groups. It should be noted that these interactions are hydrophilic via tRNA polar groups and the positively charged NH2 groups. The spectral changes observed were more pronounced for ch-200 than ch-100 and ch-15 kDa (Fig. 1).

3.2. CD spectra of tRNA-chitosan conjugates

The CD spectra of the free tRNA and its chitosan conjugates are shown in Fig. 2. The CD spectrum of the free tRNA composed of four major peaks at 209 (negative), 222 (positive), 237 (negative) and 267 nm (positive) (Fig. 2). This is consistent with CD spectra of double helical tRNA and DNA in A conformation [25,29–31]. Upon
chitosan conjugation (2.5 to 10 μM), no major shifting of CD bands were observed, which is indicative of tRNA remaining in A conformation (Fig. 2). However, major intensity changes were observed for the CD bands due to tRNA aggregation and particle formation (Fig. 2 A–C). This is consistent with AFM images of chitosan-tRNA conjugates that will be discussed furtheron.

3.3. Stability of chitosan-tRNA complexes by UV spectroscopy

The chitosan-tRNA binding constants were determined as reported in Materials and Methods [16,27]. An increasing polymer concentration resulted into an increase in UV light absorption at 260 nm (Fig. 3). This is consistent with a reduction of base stacking interaction due to chitosan complexation (Fig. 3A–C). The double reciprocal plot of 1/(A – A₀) vs 1/(chitosan concentration) is linear and the binding constants (K) were estimated from the ratio of the intercept to the slope (Fig. 4A–C), where A₀ is the initial absorbance of the free tRNA at 260 nm and A is the recorded absorbance of complexes at different chitosan concentrations. The overall binding constants are K_{Ch-15-tRNA} = 4.1 × 10^3 M⁻¹, K_{Ch-100-tRNA} = 5.7 × 10^3 M⁻¹, and K_{Ch-200-tRNA} = 1.2 × 10^4 M⁻¹ (Fig. 4A–C). It should be noted that chitosan-15 forms weaker conjugation due to its smaller size and the limited number of charged NH₂ groups, while chitosan-200 with more cationic imine groups forms more stable tRNA complexes. The binding constants estimated are mainly...
ascribed to the chitosan-PO$_2$ interaction, which is largely ionic and can be dissociated easily in aqueous solution [25,32,33].

3.4. AFM images and ultrastructure of chitosan-tRNA adducts

The three different chitosan polymers used in this study form nano-aggregates in acetate buffer at pH 6.5 as already reported by us previously [16]. Addition of tRNA to the chitosan samples led to phase separation except for the 15 kDa chitosan. In that case with ch-15 kDa, the mica surface was covered by a thin layer of chitosan-tRNA complexes (Fig. 5D and E). The complexes formed thread-like structures with a height around 0.4 nm and a width around 25 nm. The threads are most likely an artifact of the high concentration of complexes on the surface and single spherical particle are still visible, especially in the phase image (Fig. 5E). The observed dimensions of the complexes are very similar to structures obtained with lipids and polymer [28,32,33]. tRNA aggregation and particle formation was also observed for synthetic polymers [34]. However, the effect of chitosan on tRNA particle formation is rather different from those of chitosan-DNA conjugates [16].

4. Conclusions

(a) The chitosan-tRNA conjugates form via hydrophilic and electrostatic interactions.
(b) Chitosan-tRNA bindings induce tRNA aggregation and particle formation.
(c) Chitosan-200 forms more stable complexes than chitosan-100 and chitosan-15 kDa.
(d) Chitosan binding did not alter tRNA conformation.
(e) Chitosan nanoparticles induce less alterations of tRNA structure than DNA [16].

Acknowledgments

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